EXAMINING THE POTENTIAL ROLE OF BIOGENIC AMINES IN VAGINAL MICROBIAL ECOLOGY AND GYNECOLOGICAL HEALTH

by

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DEDICATION:

When I was 5, my mom sent my brothers and me out to the alley behind SavALot to look through the trash for food; my dad was gone, and we hadn't eaten in days. By age eight, I had seen one of my seven brothers' overdose, my sister die, and my mom diagnosed as bipolar and with PTSD. As a teenager, when my friends were filling out college applications, my primary goals were to be fed, to be safe, and to protect my younger siblings. At 19, there were times I was homeless; fear of where I was going to sleep and when I was going to eat, crippled me. But then, strangers opened up their backyard "flat" to me: 300 sq. ft. complete with a toilet and a sink. I worked two full-time jobs, an overnight shift in the bakery, and an evening shift as a caregiver, while attending a local community college. I was safe but so alone and so hungry. When I was 21, I started at Point Loma Nazarene University; I worked two jobs and interned in a research lab. I was still hungry, and I could have drowned; but I was surrounded by professors and peers that saw more in me than I ever could; they held me up. When I was 24, I started MSU; I flourished.

I dedicate this dissertation to all the previous iterations of me. Once upon a time, you didn't know how to dream; instead, you were a shadow of who you are now. You lived your life in constant fear, shame, and hunger; you worked until you were bone-weary, and now here you are, finally able to rest. You will never eat trash again.

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ABSTRACT

Bacterial vaginosis is the most common vaginal disorder amongst reproductive-aged women affecting nearly 1/3 of all US women. Bacterial vaginosis is associated with an increased risk of adverse sexual and reproductive health outcomes including an increased sexually transmitted infection acquisition risk. Characteristic features of bacterial vaginosis include an increase in vaginal pH (>4.5) that is associated with a depletion of vaginal *Lactobacillus* spp., and replacement by various strict and facultative anaerobes. The depletion of *Lactobacillus* spp. is an important feature as the production of lactic acid by vaginal *Lactobacillus* species is considered the major barrier to infection. Women with bacterial vaginosis have reduced lactic acid and higher concentrations of biogenic amines. Biogenic amines may be important biomolecules bridging important microbiological and clinical characteristics that connect shifts in the vaginal microbiome to common features of bacterial vaginosis. Herein, we utilized 16S rRNA gene amplicon sequencing combined with metabolomics to evaluate the association between biogenic amines and the most prevalent bacterial (*Chlamydia trachomatis*) and viral (Human papillomavirus) sexually transmitted infection. We further assessed the effect of biogenic amines upon specific growth properties of vaginal lactobacilli. We observed that women who were HPV positive or had *Chlamydia trachomatis* mono- or *Chlamydia trachomatis* /*Mycoplasma genitalium* co-infection had higher concentrations of biogenic amines when compared to women who were uninfected. Growth assays demonstrated that biogenic amines adversely affected the growth of the major vaginal lactobacilli. Putrescine was associated with increased doubling times and longer lag times for all tested species, while cadaverine increased the doubling times of all except *L. iners*, Exposure to biogenic amines was generally associated with reduced production of lactic acid, Collectively, these data provide valuable evidence that biogenic amines negatively affect the growth of vaginal *Lactobacillus* species, *in vitro*, and are associated not only with bacterial vaginosis but also with HPV and *C. trachomatis* mono- and co-infection. Taken together, these data provide a more refined understanding of the potential impact of biogenic amines upon the vaginal microenvironment and increased susceptibility to bacterial vaginosis, vaginal dysbiosis, and bacterial and viral STIs.

CHAPTER ONE

GENERAL INTRODUCTION

Vaginal *Lactobacillus* Species

For over a century, vaginal *Lactobacillus* spp. have been recognized as essential components in reproductive and sexual vaginal health[1]. Utilizing wet lab bacteriology experiments in 1892, Albert Döderlein was the first to describe and associate the presence of bacilli within vaginal secretions as an indication of "normal" and contrast that with the absence of vaginal bacilli that was associated with elevated pH, the presence of staphylococci and streptococcus, and a creamy, gray-white discharge, "*leukorrhea*"[1-3]. The subsequent ~ 130 years of research are complex, as focus shifted towards the cause and implications of leukorrhea, and early studies were crude and often questionable; many involving the direct inoculation of vaginal secretions, or pure isolates, into virgins, volunteers, or wards of the state[1, 4-7]. Further complicating matters, the fastidious nature of many vaginal bacteria did not allow for an inclusive understanding of the vaginal microenvironment – until 1980, it was thought a single *Lactobacillus* species, *L. acidophilus*, colonized the vaginal tract, with the more common *L. iners,* not being discovered until 1999[8]. Despite these limitations, the paradigm has primarily remained the same: vaginal microbiota dominated by lactobacilli are associated with positive reproductive and sexual health outcomes.

The application of molecular methods to the study of the vaginal microenvironment and its relationship to sexual and reproductive health has undoubtedly refined and extended

our understanding of the vaginal microbiota. One such method is the sequencing of portions of the bacterial 16S rRNA gene, a gene including highly conserved regions, allowing for identification and amplification, and nine variable regions, enabling assignment of species-specific taxonomy[9, 10]. Using 16S rRNA gene amplicon sequencing, we now know that the vaginal microbiota of asymptomatic, reproductive-aged women can be categorized into one of five major microbial community state types (CSTs)[11]. Four of these CSTs are dominated by *Lactobacillus* species – *L. crispatus* (I), *L. gasseri* (II), *L. iners* (III), and *L. jensenii* (V), and associated with varying levels of favorable health outcomes (**reviewed in Chapter 2**). CST IV is depauperate for *Lactobacillus* spp., associated with an increased vaginal pH, and characterized by an outgrowth of facultative and strict anaerobes such as *Gardnerella, Prevotella, Atopobium* and *Dialister* species. These features are also attributes of the vaginal disorder, bacterial vaginosis (BV), leading to the recently proposed labeling of CST IV as "molecular BV"[12].

Bacterial Vaginosis:

Bacterial vaginosis (BV) is the most common vaginal disorder amongst reproductive-aged women, having been estimated to affect nearly 1/3 of all US women[13]. Signs and symptoms of BV include an increase in vaginal pH (>4.5), a creamy gray vaginal discharge, the presence of superficial squamous cells with peripheral clumps of bacteria (clue cells), and an amine or "fishy" odor[14]. Symptoms may present alone or in any combination of the above, although the clinical diagnosis of BV, per the Amsel criteria, requires the simultaneous manifestation of three of the four markers[15]. However,

approximately 40% of women with BV are asymptomatic – they present with three of the four clinical signs yet do not report symptoms upon direct questioning[16].

BV is associated with a myriad of adverse health outcomes (reviewed in **Chapter 2**) that briefly include increased risk of acquiring viral and bacterial STIs, as well as complications during pregnancy and increased risk of pelvic inflammatory disease. While the exact etiology of BV remains unknown, several molecular studies based on 16S rRNA gene amplicon sequencing have defined both BV and molecular BV bacterial communities as deplete of *Lactobacillus* spp. and characterized by a diverse, high relative abundance of obligate and facultative anaerobes such as *Gardnerella, Prevotella,* and *Atopobium* species[11, 17, 18]. The application of metabolomics, the study of small molecular compounds produced during metabolism[19], to the vaginal environment has identified several metabolites associated with BV[20-24]. These metabolites include the biogenic amines (BAs), putrescine and cadaverine, which have been specifically linked to the malodor characteristic of BV[21, 22, 25, 26].

Biogenic Amines:

Biogenic amines are small, cationic molecules primarily produced from specific amino acid decarboxylation reactions[27, 28]. The biogenic amines, tyramine and cadaverine are produced from the decarboxylation of tyrosine and lysine, respectively. Putrescine can be formed through the decarboxylation of ornithine via ornithine decarboxylase, and also from arginine through the agmatinase decarboxylation pathway[27, 29]. Spermidine and spermine are higher order amines and biosynthesized from putrescine[27, 30]. Trimethylamine

can be formed through the reduction of trimethylamine oxide via trimethylamine oxide reductase, and from choline via choline-trimethylamine lyase[31]. BAs can be produced by both mammals and prokaryotes, although mammalian cells can only produce putrescine, spermine, and spermidine[30].

In bacteria, the production of biogenic amines may represent a mechanism of acid-stress resistance in response to environmental acidity[27, 28, 32]. The decarboxylation of amino acids involves the consumption of an intracellular proton, which increases the pH of the cytoplasm relative to the environment, generating a membrane potential[33]. These reactions are also coupled to an electrogenic antiport system, for each amino acid that is converted to its respective BA, a positive charge is translocated across the membrane and a proton removed from the cytoplasm[32, 34]. Thus, the presence of BAs may also represent a means by which bacteria obtain metabolic energy [28, 33-35].

To date, the only vaginal bacteria reported to produce a biogenic amine are *Mobiluncus mulieris* and *M. curtisii* which are reportedly able to produce trimethylamine[36]. However, a genomic analysis of vaginal isolates sequenced as part of the human microbiome project indicated *Escherichia coli* and bacteria of the genera *Prevotella, Dialister, Veillonella, Janthinobacterium, Peptostreptococcus, Parvimonas, Megasphaera, and Leptotrichia* were also likely capable of producing agmatine, cadaverine, putrescine, trimethylamine and/or tyramine[21]. Curiously, with the exception of *L. iners*, each of the other three major *Lactobacillus* species found in the vagina also possess genes suggesting a capacity to produce putrescine, the significance of which remains to be determined.

Biogenic Amines and the Vaginal Environment:

Within the vaginal microenvironment, high concentrations of the BAs putrescine, cadaverine and trimethylamine have been linked to the clinical sign malodor of BV[36-41]. In addition to BV, these BAs have been reported to be in higher concentrations in women with CST IV microbiota than those dominated by vaginal *Lactobacillus* spp.[20-22, 24] and have been correlated to the increased relative abundances of various CST-IV bacteria, including *Gardnerella, Dialister, Prevotella,* and *Atopobium* spp. [21, 23, 37, 38]. An older study of *Lactobacillus* species relevant to the food industry found that while spermine and spermidine promoted *Lactobacillus* species growth, putrescine and cadaverine significantly reduced growth[39] leading us to hypothesize that BAs may play a direct role in modulating the vaginal microbiota promoting the transition from a *Lactobacillus* spp. dominant microbiota to that of the low-*Lactobacillus* spp., CST IV (Figure 1).[21, 26]

Furthermore, BAs have been shown to enhance the virulence of various pathogens by mediating resistance to antibiotics[40, 41], providing protection against oxygen byproducts[42], and altering susceptibility to host innate defenses. This includes the sexually transmitted infective agent *Neisseria gonorrhea*, which in addition to increased growth, displays increased resistance to LL-37 with increasing concentrations of BAs [27, 43, 44]. LL-37 is a cathelicidin-related antimicrobial peptide that typically has well described bactericidal and bacteriostatic activity against a range of urogenital pathogens and STI, including *N. gonorrhea*, but not against vaginal lactobacilli[45, 46]. Further, it has also recently been shown that *N. gonorrhea* is protected against the bactericidal activity of lactic acid when provided with putrescine or one of its amino acid precursors, arginine[44]. Thus, given

the association of BAs with BV, molecular BV (CST IV), their potential role in enhancing the virulence of agents of sexually transmitted infection, as well as their potential roles in the reduction of environmental acidity and increased resistance to host immunological defenses, we hypothesize that BAs may be a major factor underscoring the increased risk of STI associated with CST-IV/(molecular) BV.

To assess these hypotheses, we sought to 1) evaluate the association of BAs with the most common bacterial and viral STIs and 2) to evaluate whether exogenous BAs directly influence growth properties of vaginal *Lactobacillus* spp.

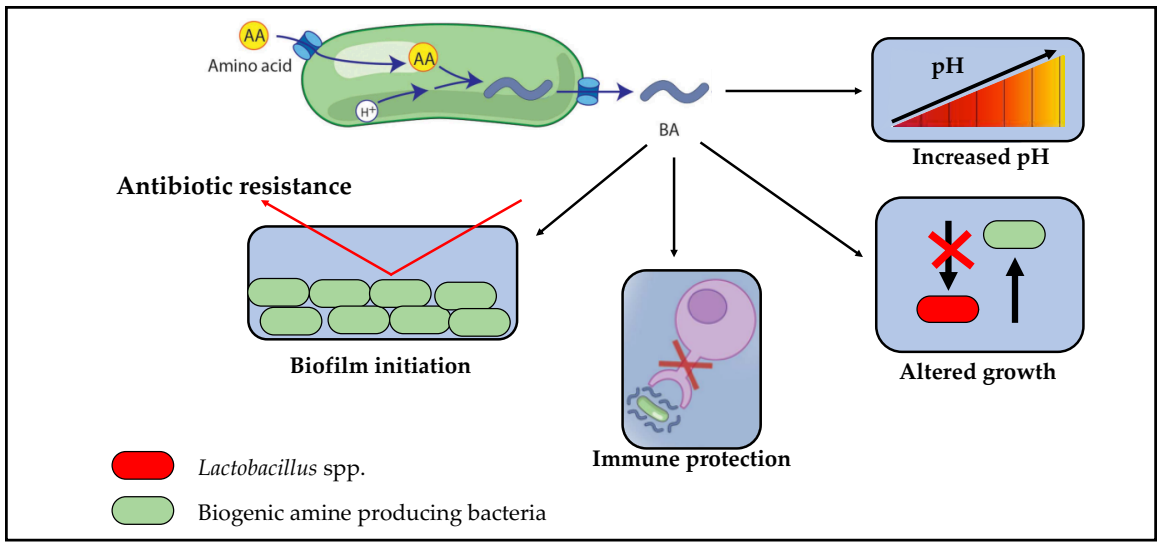

Figure 1. Proposed model of biogenic amines and how they may impact the vaginal environment and increase susceptibility to vaginal dysbiosis. We propose that biogenic amine production leads to vaginal increased pH, decreases the competitiveness of vaginal *Lactobacillus* spp., and increases pathogen resistance by promoting antibiotic resistance and protection against host innate defenses, cumulatively increasing host susceptibility to colonization by potentially pathogenic and anaerobic bacteria.

Thesis Aims:

The aims of this thesis were to provide an overview of the vaginal microbiome with a focus on advancements made using molecular methods; to investigate the association between the vaginal microbiota, metabolome, including biogenic amines, and positive detection of the most common viral (human papillomavirus (HPV)) and bacterial (*Chlamydia trachomatis* (CT) and *Mycoplasma genitalium*) STIs and to evaluate the direct effect of vaginal biogenic amines upon the growth of vaginal *Lactobacillus* species.

Chapter 2 is a review of the history of the association between vaginal *Lactobacillus* spp. with a focus on advancements made using molecular techniques. This review summarizes available data on *Lactobacillus* spp. mediated protection, the prevalence of vaginal microbiota compositions, and their associations with behavioral and clinical outcomes. In **Chapter 3**, we utilized liquid- and gas-chromatography mass-spectrometry based metabolomics, as well as 16S rRNA gene amplicon sequencing, to evaluate the association between the vaginal microbiota, metabolome, and cervical HPV detection. In **Chapter 4,** we similarly describe the association between vaginal microbiota composition, the vaginal metabolites and women who have been clinically diagnosed with *C. trachomatis* mono- and *C. trachomatis / M. genitalium* co-infection. In **Chapter 5**, we utilized a targeted liquid-chromatography mass-spectrometry based method to quantify the physiological concentrations of select biogenic amines and evaluated their effect upon vaginal *Lactobacillus* spp. using traditional growth curves. Finally, the observations presented within this thesis, as well as their implications for future research, will be synthesized and discussed in **Chapter 6**.

REFERENCES

1. Döderlein A. Das scheidensekret und seine bedeutung fur puerperalfieber. . Zentralblatt fur Bakteriology. 1892:11:699.

2. Kaplan AFLaB. A Study of Döderlein's Vaginal Bacillus. The Journal of Infectious Diseases.Vol. 38:pp. 333-40.

3. Thomas S. Döderlein's Bacillus: Lactobacillus Acidophilus. The Journal of Infectious Diseases. 1928;43(3):218–27.

4. Vahle. Ueber das Vorkommen von Streptococcen in der Scheide Gebarender. Zeit f Geb u Gyn. 1896;xxxv:92-214.

5. Menge. Ueber ein bacterienfeindliches Verhalten der Seheidensekrete Nichtschwangerer. Deutsche med Wochenschrift. 1894;46-48.

6. Transactions of the American Gynecological Society. 1898;23.

7. The American Journal of Obstetrics and Diseases of Women and Children. 1896;XXXIV.

8. Falsen E, Pascual C, Sjoden B, Ohlen M, Collins MD. Phenotypic and phylogenetic characterization of a novel Lactobacillus species from human sources: description of Lactobacillus iners sp. nov. Int J Syst Bacteriol. 1999;49 Pt 1:217-21.

9. Woese CR. Bacterial evolution. Microbiol Rev. 1987;51(2):221-71.

10. Woese CR, Stackebrandt E, Macke TJ, Fox GE. A phylogenetic definition of the major eubacterial taxa. Syst Appl Microbiol. 1985;6:143-51.

11. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011;108 Suppl 1:4680-7.

12. McKinnon LR, Achilles SL, Bradshaw CS, Burgener A, Crucitti T, Fredricks DN, et al. The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. AIDS Res Hum Retroviruses. 2019;35(3):219-28.

13. Peebles K, Velloza J, Balkus JE, McClelland RS, Barnabas RV. High Global Burden and Costs of Bacterial Vaginosis: A Systematic Review and Meta-Analysis. Sex Transm Dis. 2019;46(5):304-11.

14. Spiegel CA, Amsel R, Eschenbach D, Schoenknecht F, Holmes KK. Anaerobic bacteria in nonspecific vaginitis. N Engl J Med. 1980;303(11):601-7.

15. Chen KC, Amsel R, Eschenbach DA, Holmes KK. Biochemical diagnosis of vaginitis: determination of diamines in vaginal fluid. J Infect Dis. 1982;145(3):337-45.

16. Klebanoff MA, Schwebke JR, Zhang J, Nansel TR, Yu KF, Andrews WW. Vulvovaginal symptoms in women with bacterial vaginosis. Obstet Gynecol. 2004;104(2):267-72.

17. Fettweis JM, Serrano MG, Sheth NU, Mayer CM, Glascock AL, Brooks JP, et al. Species-level classification of the vaginal microbiome. BMC Genomics. 2012;13 Suppl 8:S17.

18. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med. 2005;353(18):1899-911.

19. Clish CB. Metabolomics: an emerging but powerful tool for precision medicine. Cold Spring Harb Mol Case Stud. 2015;1(1):a000588-a.

20. Borgogna J-LC, Yeoman CJ. The Application of Molecular Methods Towards an Understanding of the Role of the Vaginal Microbiome in Health and Disease. The Human Microbiome. Methods in Microbiology2017. p. 37-91.

21. Nelson TM, Borgogna JL, Brotman RM, Ravel J, Walk ST, Yeoman CJ. Vaginal biogenic amines: biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis? Frontiers in physiology. 2015;6:253.

22. Yeoman CJ, Thomas SM, Miller ME, Ulanov AV, Torralba M, Lucas S, et al. A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. PLoS One. 2013;8(2):e56111.

23. Vitali B, Cruciani F, Picone G, Parolin C, Donders G, Laghi L. Vaginal microbiome and metabolome highlight specific signatures of bacterial vaginosis. Eur J Clin Microbiol Infect Dis. 2015;34(12):2367-76.

24. Srinivasan S, Morgan MT, Fiedler TL, Djukovic D, Hoffman NG, Raftery D, et al. Metabolic signatures of bacterial vaginosis. MBio. 2015;6(2).

25. Srinivasan S, Morgan MT, Fiedler TL, Djukovic D, Hoffman NG, Raftery D, et al. Metabolic signatures of bacterial vaginosis. mBio. 2015;6(2):10.1128/mBio.00204-15.

26. Nelson TM, Borgogna JC, Michalek RD, Roberts DW, Rath JM, Glover ED, et al. Cigarette smoking is associated with an altered vaginal tract metabolomic profile. Sci Rep. 2018;8(1):852.

27. Shah P, Swiatlo E. A multifaceted role for polyamines in bacterial pathogens. Mol Microbiol. 2008;68(1):4-16.

28. Kanjee U, Houry WA. Mechanisms of acid resistance in Escherichia coli. Annu Rev Microbiol. 2013;67:65-81.

29. King CC, Jamieson DJ, Wiener J, Cu-Uvin S, Klein RS, Rompalo AM, et al. Bacterial vaginosis and the natural history of human papillomavirus. Infect Dis Obstet Gynecol. 2011;2011:319460.

30. Pegg AE. Mammalian polyamine metabolism and function. IUBMB Life. 2009;61(9):880-94.

31. Craciun S, Balskus EP. Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. Proceedings of the National Academy of Sciences. 2012;109(52):21307-12.

32. Barbieri F, Montanari C, Gardini F, Tabanelli G. Biogenic Amine Production by Lactic Acid Bacteria: A Review. Foods. 2019;8(1):17.

33. Molenaar D, Bosscher JS, ten Brink B, Driessen AJ, Konings WN. Generation of a proton motive force by histidine decarboxylation and electrogenic histidine/histamine antiport in Lactobacillus buchneri. J Bacteriol. 1993;175(10):2864-70.

34. Marcobal A, De las Rivas B, Landete JM, Tabera L, Munoz R. Tyramine and phenylethylamine biosynthesis by food bacteria. Crit Rev Food Sci Nutr. 2012;52(5):448-67.

35. Pereira CI, Matos D, San Romao MV, Crespo MT. Dual role for the tyrosine decarboxylation pathway in Enterococcus faecium E17: response to an acid challenge and generation of a proton motive force. Appl Environ Microbiol. 2009;75(2):345-52.

36. Cruden DL, Galask RP. Reduction of Trimethylamine Oxide to Trimethylamine by Mobiluncus Strains Isolated from Patients with Bacterial Vaginosis. Microbial Ecology in Health and Disease. 1988;1(2):95-100.

37. Yeoman CJ, Thomas SM, Miller MEB, Ulanov AV, Torralba M, Lucas S, et al. A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. PloS one. 2013;8(2):e56111.

38. Sobel JD, Karpas Z, Lorber A. Diagnosing vaginal infections through measurement of biogenic amines by ion mobility spectrometry. European journal of obstetrics, gynecology, and reproductive biology. 2012.

39. Guirard BM, Snell EE. Effect of Polyamine Structure on Growth Stimulation and Spermine and Spermidine Content of Lactic Acid Bacteria. J Bacteriol. 1964;88:72-80.

40. Kwon DH, Lu CD. Polyamine effects on antibiotic susceptibility in bacteria. Antimicrob Agents Chemother. 2007;51(6):2070-7.

41. Kwon DH, Lu CD. Polyamines induce resistance to cationic peptide, aminoglycoside, and quinolone antibiotics in Pseudomonas aeruginosa PAO1. Antimicrob Agents Chemother. 2006;50(5):1615-22.

42. Chattopadhyay MK, Tabor CW, Tabor H. Polyamines protect Escherichia coli cells from the toxic effect of oxygen. Proc Natl Acad Sci U S A. 2003;100(5):2261-5.

43. Goytia M, Shafer WM. Polyamines can increase resistance of Neisseria gonorrhoeae to mediators of the innate human host defense. Infect Immun. 2010;78(7):3187-95.

44. Gong Z, Tang MM, Wu X, Phillips N, Galkowski D, Jarvis GA, et al. Arginine- and Polyamine-Induced Lactic Acid Resistance in Neisseria gonorrhoeae. PLoS One. 2016;11(1):e0147637.

45. Smeianov V, Scott K, Reid G. Activity of cecropin P1 and FA-LL-37 against urogenital microflora. Microbes Infect. 2000;2(7):773-7.

46. Shafer WM, Qu X, Waring AJ, Lehrer RI. Modulation of Neisseria gonorrhoeae susceptibility to vertebrate antibacterial peptides due to a member of the resistance/nodulation/division efflux pump family. Proc Natl Acad Sci U S A. 1998;95(4):1829-33.

CHAPTER 2

THE APPLICATION OF MOLECULAR METHODS TOWARDS AN UNDERSTANDING OF THE ROLE OF THE VAGINAL MICROBIOME IN HEALTH AND DISEASE

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CHAPTER

3 The Application of Molecular Methods Towards an Understanding of the Role of the Vaginal Microbiome in Health and Disease

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1 INTRODUCTION

The microbial communities (*microbiota*) that colonize the human vagina are unique from those observed among all other species (Barfod et al., 2013; Swartz et al., 2014) including our closest evolutionary relatives (Yildirim et al., 2014) and are unique among each of our other microbial ecosystems (*microbiomes*) including that of the skin, mouth, gut, *inter alia* (Consortium, 2012). Specifically, the healthy human vagina exhibits a uniquely low microbial species α-diversity (Swartz et al., 2014; Yildirim et al., 2014). Whereas in most other microbial ecosystems, low species α-diversity is commonly associated with dysbiosis and disease, it is instead high species α-diversity of the vagina that corresponds to a myriad of morbidities. For instance, low α-diversity of the skin microbiome is associated with atopic dermatitis (Kong et al., 2012) and psoriasis (Statnikov et al., 2013), while in the gut, low species α-diversity has been linked to obesity (Turnbaugh et al., 2009), inflammatory bowel disease (Huttenhower, Kostic, & Xavier, 2014) and Types 1 (Kostic et al., 2015) and 2 diabetes (Qin et al., 2012). Conversely, high α-diversity in the vagina is often associated with bacterial vaginosis (BV) (DiGiulio et al., 2015), cervical intraepithelial neoplasia (CIN) (Kong et al., 2012) and preterm birth (Statnikov et al., 2013). The low microbial α-diversity of the healthy human vagina results from both a low microbial richness and skewed evenness wherein *Lactobacillus* spp. are numerically dominant (Miller, Beasley, Dunn, & Archie, 2016; Yildirim et al., 2014). *Lactobacillus* spp. have been enumerated at 10^7–10^8 CFU/g of vaginal fluid (Andreu, Stapleton, Fennell, Hillier, & Stamm, 1995) and comprise 60%–99.9% of the total human vaginal microbiota in healthy reproductive-aged women (Ravel et al., 2011; Yildirim et al., 2014).

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Lactobacillus spp. ferment by-products of vaginal glycogen to produce lactic acid (Spear et al., 2014); the production of lactic acid is a classic form of niche construction (Yeoman et al., 2011) that results in acidification of the vaginal environment (pH < 4.5) leading to limitations on the colonizing potential and outgrowth of other microbes (O'Hanlon, Moench, & Cone, 2011). By contrast, *Lactobacillus* spp. are often seen to represent <1% of the total vaginal microbiota among other mammalian species and are only marginally increased in our closest evolutionary relative, the chimpanzee, wherein *Lactobacillus* spp. still comprise <3.5% of the vaginal microbiota (Miller et al., 2016; Yildirim et al., 2014). Although other lactic acid bacteria including *Aerococcus*, *Abiotrophia*, *Allofustis*, *Facklamia*, *Globicatella* and *Streptococcus* species are common to the vaginal microbiota of other mammalian species (Yildirim et al., 2014), their role does not appear to be similar as low pH and microbial diversity are unique human attributes (Miller et al., 2016; Spear et al., 2012; Swartz et al., 2014; Yildirim et al., 2014). The enrichment of *Lactobacillus* spp. and resulting acidic environment appears to be hallmarks of an evolved mutualistic relationship between the human host and *Lactobacillus* spp., wherein the microbes protect the human host against potentially pathogenic bacteria in exchange for a nutrient rich, anoxic environment (Danielsson, Teigen, & Moi, 2011). The contextual nature of this relationship is perhaps best seen in the stark contrast that exists between the healthy human vaginal microbiome and that associated with acute acidosis of ruminants. Both the healthy human vagina and acidotic rumen are characterized by greater relative abundances of *Lactobacillus* spp., elevated levels of lactic acid, a low pH, and a corresponding low species α-diversity (Emmanuel, Madsen, Churchill, Dunn, & Ametaj, 2007; Nagaraja & Titgemeyer, 2007). However, in the case of acute ruminal acidosis these features are associated with severe disease involving inflammation, reduced barrier function, septicemia and often death (Emmanuel et al., 2007; Nagaraja & Titgemeyer, 2007). By contrast, acidified lactate has been seen to modulate inflammation of the vaginal epithelium (Aldunate et al., 2015), and our immune system appears permissive of *Lactobacillus* spp. enrichment in the vagina with the levels of multiple proinflammatory cytokines, including interleukin (IL)-1α, IL-1β and IL-8 all inversely correlated with *Lactobacillus* spp. abundance (Anton, Rid, Mylonas, Friese, & Weissenbacher, 2008; Spandorfer, Neuer, Giraldo, Rosenwaks, & Witkin, 2001; Spear, Zariffard, Cohen, & Sha, 2008; Wennerholm et al., 1998).

Much of the research that has led to our contemporary understanding of the vaginal microbiome has focused on BV. BV is the most common vaginal disorder among reproductive-aged women, having been estimated to affect ~30% of all US women between the ages of 14–49 (Allsworth & Peipert, 2007). BV is associated with a reduction in vaginal *Lactobacillus* spp. (Nugent, Krohn, & Hillier, 1991), and clinical manifestations include an increase in vaginal pH (>4.5), a creamy grey vaginal discharge, the presence of superficial squamous cells with peripheral clumps of bacteria (clue cells) and an amine or "fishy" odour (Amsel et al., 1983). Signs and symptoms may present alone or in any combination of the above although the clinical diagnosis of BV, per the Amsel criteria, requires the simultaneous manifestation of three of the

four markers (Amsel et al., 1983). Additionally, women with BV often report pain, itching or burning in or around the vagina (Hillier & Holmes, 1999). While diagnosis alone is cause for discomfort and disconcertion, women diagnosed with BV have additionally been shown to have increased risks of acquiring urinary tract infections (Sumati & Saritha, 2009), various bacterial (Bautista et al., 2017; Brotman, Klebanoff, et al., 2010; Wiesenfeld, Hillier, Krohn, Landers, & Sweet, 2003) and viral STIs (Cherpes, Hillier, Meyn, Busch, & Krohn, 2008; Cohen et al., 2012; Gillet et al., 2011; Ross et al., 2005), and when cooccurring with pregnancy, BV exacerbates the risks of preterm delivery, miscarriage and postpartum endometritis (Dingens, Fairfortune, Reed, & Mitchell, 2016; Leitich et al., 2003). Although recent studies have shown women may persist in a *Lactobacillus*-depauperate state, it is generally accepted that this bacterial genera plays an important role in the preservation of vaginal health.

2 *LACTOBACILLUS*-MEDIATED PROTECTION

2.1 *LACTOBACILLUS* spp. OF THE VAGINAL MICROBIOME

While ubiquitous in nature, *Lactobacillus* spp. have been shown to inhabit only three regions of the human body: the gut, oral cavity and the vaginal tract (Pavlova et al., 2002). Moreover, while over 100 *Lactobacillus* spp. have been identified (Hammes & Hertel, 2015), only four species are commonly observed as dominant members of the vaginal microbiota: *L. crispatus, L. iners*, *L. gasseri* and *L. jensenii* (Ravel et al., 2011; Zhou et al., 2007) although several other *Lactobacillus* species (including *L. acidophilus*, *L. coleohominis* (Srinivasan et al., 2012), *L. johnsonii* (Petricevic et al., 2014), *L. plantarum* (Vásquez, Jakobsson, Ahrné, Forsum, & Molin, 2002), *L. rhamnosus* (Pavlova et al., 2002), *L. salivarius* (Gustafsson et al., 2011) *and L. vaginalis* (Ravel et al., 2011)) are also occasionally detected. *Lactobacillus* spp. are generally considered to subsist on by-products of vaginal glycogen. Although glycogen itself appears inaccessible to the vaginal *Lactobacillus* spp., glycogen is hydrolyzed to maltodextrins, maltobiose and maltose in vaginal fluid by an α-amylase activity that appears to be host encoded (Spear et al., 2014; Fig. 1). It is these, more simple, maltose-based polymers that can be metabolized by vaginal *Lactobacillus* spp. (Fig. 1). Metabolomic studies have shown the relative concentration of the maltodextrin's, maltotriose and maltopentose, along with maltose are positively correlated to the relative abundances of *L. crispatus and L. jensenii* (Srinivasan et al., 2015). The production of vaginal glycogen is directly influenced by circulating levels of oestrogen, which stimulates the deposition of glycogen in vaginal epithelial tissue (Gregoire, Kandil, & Ledger, 1971). Consequently, the abundance of *Lactobacillus* spp. is often seen to be affected by oestrogen levels (Fig. 1).

The association between *Lactobacillus* spp. dominance and vaginal health has long been recognized; beginning in 1892, when Döderlein systematically examined

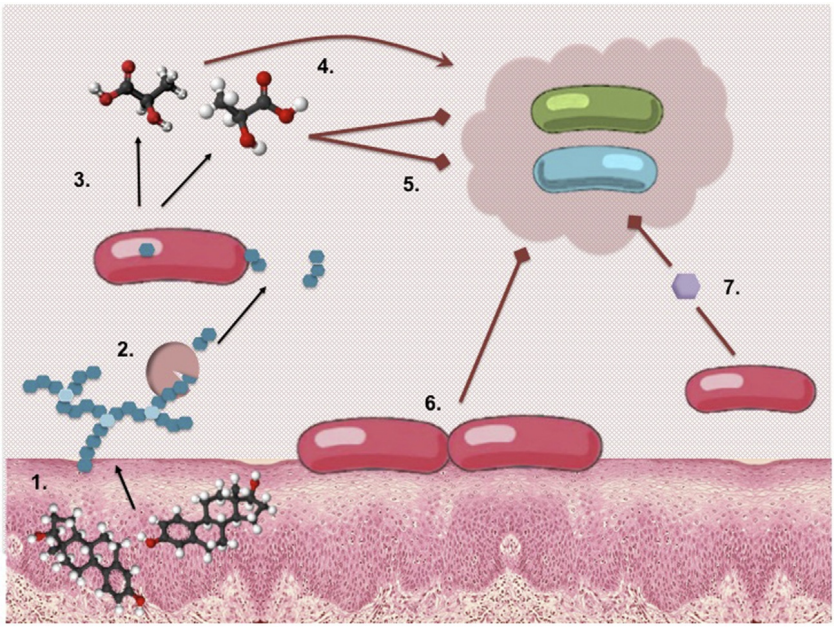

FIG. 1

Overview of the *Lactobacillus* species-mediated prevention of pathogenic invasion. Lactobacillus species preserve vaginal health, and their abundance is influenced by oestrogen. (1) Oestrogen levels stimulate the deposition of glycogen in vaginal epithelial tissue. Although *Lactobacillus* spp. cannot subsist on glycogen, a host-encoded α-amylase degrades glycogen to produce maltose and maltooligomers (2). *Lactobacillus* species consume maltose and maltooligomers and produce D- and/or L-lactic acid (3) acidifying the vaginal milieu (4). Acidification of the vagina creates protonated lactate which is antimicrobial and prevents the outgrowth of other microbial species (5). *Lactobacillus* spp. also adhere to vaginal epithelial cells excluding potential pathogens (6) and produce antimicrobial compounds known as bacteriocins (7).

the bacteriology of vaginal secretions from pregnant women, delineating secretions as either normal or abnormal based on colour, consistency, texture and the presence/absence of gas bubbles and mucus (Döderlein, 1892). Exclusive to the normal vaginal secretions, Döderlein described what initially came to be known as 'Döderlein's bacilli'—a nonspore-forming, lactic acid-producing, Gram-positive bacilli that we contemporarily know as belonging to the genus *Lactobacillus* (Döderlein, 1892; Thomas, 1928). Döderlein thought the bacilli to be the sole colonizer of normal vaginal fluid during pregnancy and revealed it to have antagonistic effects towards pathogens by means of lactic acid production both in vitro and in vivo (Döderlein, 1892). Conversely, Döderlein noted abnormal vaginal secretions as a thin, homogenous, greyish-white discharge deplete of bacillus, and instead replete with bacteria of varying morphologies and associated with an elevated vaginal pH (Döderlein,

1892). Thus, for over a century, it has generally been accepted that the presence of vaginal *Lactobacillus* spp. is indicative of reproductive health and underpinned by their lactic acid-mediated acidification of the vaginal milieu. This paradigm has been confirmed both through cultivation-dependent and modern molecular techniques, and further expanded to include the *Lactobacillus* spp. production of hydrogen peroxide (H_2O_2), bacteriocins (Stoyancheva, Marzotto, Dellaglio, & Torriani, 2014) and biosurfactants (Reid, Heinemann, Velraeds, van der Mei, & Busscher, 1999) that collectively provide one of the most important barriers against bacterial and viral pathogenic invasion (Aldunate et al., 2013; Gong, Luna, Yu, & Fan, 2014; Kaewnopparat et al., 2013; O'Hanlon et al., 2011).

2.1.1 Adhesion and competitive exclusion

Both in vitro and in vivo studies have demonstrated the ability of *Lactobacillus* spp. to adhere to host cells thus preventing the colonization of various pathogenic species. For example, the displacement and competitive exclusion of *Gardnerella vaginalis* (Boris, Suárez, Vázquez, & Barbés, 1998), *Staphylococcus aureus* (Boris et al., 1998), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Chlamydia trachomatis* (Mastromarino et al., 2014), *Candida albicans* (Parolin et al., 2015) and *Trichomonas vaginalis* by *Lactobacillus* spp. has been described (Atassi, Brassart, Grob, Graf, & Servin, 2006a; Phukan, Parsamand, Brooks, Nguyen, & Simoes-Barbosa, 2013). Mastromarino et al. (2002) observed that in the presence of vaginal *Lactobacillus* spp., the adhesion of *C. albicans* and *G. vaginalis* to vaginal epithelial cells was both reduced by 50%. Genomic analyses have revealed several molecular *Lactobacillus* factors, including putative adhesion proteins involved in mucus and fibrinogen (Fn) binding, that may play a role in the competitive exclusion of *G. vaginalis* (France, Mendes-Soares, & Forney, 2016; Mendes-Soares, Suzuki, Hickey, & Forney, 2014; Ojala et al., 2014). This includes the *L. crispatus*-encoded *Lactobacillus* epithelium adhesion protein (LEA; LACT00252), an antagonist to a *G. vaginalis* pilus-encoding gene (Yeoman et al., 2010), which was predicted to inhibit the adherence of *G. vaginalis* to epithelial cells (Ojala et al., 2014) as validated by an adhesion inhibition assay utilizing HeLa cells (Ojala et al., 2014).

Additionally, the ability of several vaginal *Lactobacillus* spp. isolates to inhibit herpes simplex virus 2 (HSV-2) viral activity has been directly linked to their adherence to epithelial cells (Mastromarino et al., 2002; Mastromarino, Cacciotti, Masci, & Mosca, 2011). Mechanistic insight into this ability has been greatly advanced through genomic and proteomic analyses of vaginal *Lactobacillus* spp. Genomic studies identified the presence of several putative adhesion proteins including many chitin-, collagen-, Fn-, mannose- and mucus-binding proteins in the various vaginal *Lactobacillus* spp. (Anukam et al., 2013; Leccese Terraf, Mendoza, Juarez Tomas, Silva, & Nader-Macías, 2014; Malik et al., 2014; Ojala et al., 2014). A study examining the vaginal proteomes of 50 Rwandan women identified 11 proteins specific to *L. crispatus* (Borgdorff et al., 2016). Of these, the authors observed a high abundance of glycolysis-related proteins, namely, glyceraldehyde-3-phosphate

dehydrogenase (GAPDH), enolase (ENO) and glucose-6-phosphate isomerase (GPI) with *L. crispatus*. Interestingly, GAPDH, ENO and GPI have been previously described as adhesive moonlighting proteins that aid in adhesion to epithelial cells, extracellular matrices, plasminogen and secreted mucin (Bergmann et al., 2003; Henderson & Martin, 2011). GAPDH, ENO and GPI have been shown to be released from the surface of *L. crispatus* after cell trauma, under conditions of alkaline stress or in the presence of the antimicrobial peptide (AMP), LL-37, which is produced by epithelial cells and acts against the cytoplasmic membrane of bacteria (Hurmalainen et al., 2007; Kainulainen et al., 2012; Sochacki, Barns, Bucki, & Weisshaar, 2011). Interestingly, ENO and GAPDH have been shown to be bound to acidic lipoteichoic acids in the cell wall of *L. crispatus* at an acidic pH but not at a neutral pH (Kainulainen et al., 2012), suggesting that vaginal acidity is important in facilitating this protective ability. This contrasts with the epithelial adherence of *G. vaginalis*, which has been shown to depend on an elevated pH (optimal ~pH 6) (Peeters & Piot, 1985), which is more consistent with the pH observed in *Lactobacillus*-depauperate microbiomes.

2.1.2 Lactic acid

Dogmatically, acidification of the vagina is mediated by the production of lactic acid by *Lactobacillus* spp. Cultivation-dependent and molecular-based studies support the production of lactic acid by *Lactobacillus* spp. as their primary protective mechanism. The average lactic acid concentrations of the healthy vagina have been reported within the range of 11–111 mM (O'Hanlon, Moench, & Cone, 2013). Metabolomic analyses have shown the relative concentrations of lactic acid within the vaginal milieu are highest in women with *Lactobacillus* spp.-dominant microbiota and decreases with reductions in *Lactobacillus* spp. abundance (Yeoman et al., 2013) and in the onset of BV (Srinivasan et al., 2015). The decrease in lactic acid has also been shown to be inversely correlated with vaginal pH (Srinivasan et al., 2015), consistent with studies showing its role in acidification of the vagina (O'Hanlon et al., 2013). Although both the vaginal epithelium and the *Lactobacillus* spp. are both capable of producing lactic acid, the role of the *Lactobacillus* spp. in producing lactic acid has been largely confirmed through studies of the lactic acid enantiomers. Vaginal epithelial cells generally only produce the L-lactic acid enantiomer, while major *Lactobacillus* spp. can encode genes for the production of both L- and D-enantiomers (Witkin et al., 2013; Table 1). Studies of the vaginal milieu have shown it comprises quantities of both L- and D-lactic acid enantiomers (Boskey, Cone, Whaley, & Moench, 2001; Witkin et al., 2013), with the ratio of L-:D-lactic acid enantiomers varying by community state type (CST), being lowest for CST-I (*L. crispatus*-dominated microbiota) and greatest for CST-III (*L. iners*-dominated microbiota) and -IV (*Lactobacillus*-depauperate microbiota) (Witkin et al., 2013). Cultivation-based studies have shown that *Lactobacillus* spp. can acidify the vagina at a rate of $\sim 10^6$ protons/bacterium/s and alter the vaginal pH by an estimated -0.56 to -0.75 pH units/h during exponential growth (Boskey, Telsch, Whaley, Moench, & Cone, 1999). The resting pH of the vagina differs by CST

Table 1 Lactic Acid Enantiomers Encoded for by Major Vaginal *Lactobacillus* spp.[a]

Producer	L-Lactic Acid	D-Lactic Acid
L. crispatus	+	+
L. gasseri	+	+
L. iners	+	−
L. jensenii	−	+

[a]*Data obtained from Witkin et al. (2013) where +/− indicates the ability to produce a given enantiomer or lack thereof.*

(Ravel et al., 2011) but is widely reported to be <pH 4.5 when *Lactobacillus* spp. are predominant and has been reported as low as pH 2.8 (O'Hanlon et al., 2013). However, the acidification of the vagina alone appears insufficient to prevent the outgrowth of many BV- and CST-IV-associated species. For instance, O'Hanlon et al. (2011) demonstrated that acidification with acetic acid does not prevent the outgrowth of common vaginal bacteria. Instead, protection is given through the combinatorial acidification and production of lactic acid that leads to the generation of protonated lactate in the vaginal milieu and it is the protonated lactate that prevents the outgrowth of potentially pathogenic bacteria (Linhares, Summers, Larsen, Giraldo, & Witkin, 2011; O'Hanlon et al., 2011; Fig. 1).

2.1.3 Bacteriocins

Bacteriocins are small, ribosomally synthesized AMPs that can be categorized into two classes: those that undergo posttranslational modification and those that remain largely unmodified (Cotter, Ross, & Hill, 2013). Traditionally, it has been thought that the ammensalistic activity of bacteriocins is limited to closely related microorganisms other than the producer due to specific antitoxin mechanisms (Cotter, Hill, & Ross, 2005; Cotter et al., 2013); however, several studies have identified both narrow- and broad-spectrum bacteriocins that can control pathogens, often without negatively affecting commensal populations (Boakes et al., 2012; Cotter et al., 2013; Rea et al., 2010). Narrow-spectrum bacteriocins have been shown to inhibit the growth of closely related *Lactobacillus* species (Klaenhammer, 1988), while broad-spectrum bacteriocins have been shown to inhibit a diverse group of bacteria including *E. coli* (McGroarty & Reid, 2011) and *G. vaginalis* (Skarin & Sylwan, 1986). Genomic studies have helped to guide more recent in vitro studies demonstrating that several *Lactobacillus* species are capable of producing bacteriocins (Fig. 1). Producers are known to include all major *Lactobacillus* species, including *L. crispatus* (Stoyancheva et al., 2014), *L. iners* (Macklaim, Gloor, Anukam, Cribby, & Reid, 2011), *L. gasseri* (Aroutcheva et al., 2001; Stoyancheva et al., 2014) and *L. jensenii* (Aroutcheva et al., 2001) as well as other commonly detected species such as *L. fermentum* (Kaur, Balgir, Mittu, Kumar, & Garg, 2013; Pascual, Daniele, Giordano, Pájaro, & Barberis, 2008), *L. rhamnosus* (Ruíz, Pascual,

Giordano, & Barberis, 2015) and *L. salivarius* (Ocaña et al., 1999a). Several studies have highlighted strain-specific variation in bacteriocin activity, for example, *L. gasseri* G3 has antimicrobial activity against *E. coli*, *K. pneumoniae* and several *Lactobacillus* spp., while *L. gasseri* G7 only exhibits antimicrobial activity against *E. coli* (Stoyancheva et al., 2014). Vaginal strains of *L. gasseri* isolated from the swabs of Lebanese women were found to exhibit antimicrobial activity, linked to the production of bacteriocins, against *E. coli*, *S. aureus*, *G. vaginalis* and *C. albicans* (Al Kassaa, Hamze, Hober, Chihib, & Drider, 2014). Additionally, in a study of 22 *Lactobacillus* spp. isolated from vaginal swabs, three strains of *L. gasseri* and one strain of *L. jensenii* were observed to be high producers of bacteriocins and to exhibit high inhibitory effects against *G. vaginalis*, while a single strain of *L. vaginalis* was evaluated as a moderate bacteriocin producer, and *L. crispatus* isolates were not observed to produce bacteriocins (Aroutcheva et al., 2001); genomic analysis has, however, shown that strains of *L. crispatus* encode for several bacteriocins including LS2, a two-component bacteriocin known to inhibit the growth of *Listeria*, *Shigella* and *Yersinia* spp. (Busarcevic & Dalgalarrondo, 2012; Ojala et al., 2014) and gassericin A (Stoyancheva et al., 2014). Gassericin A has antimicrobial activity against *K. pneumoniae* and *S. aureus* (Stoyancheva et al., 2014). *L. iners* was not detected in the study of Aroutcheva et al. (2001) due to the utilization of MRS, rather than blood agar (Falsen, Pascual, Sjödén, Ohlén, & Collins, 1999). However, genome sequencing of *L. iners* AB-1 revealed three genes thought to encode putative bacteriocin immunity proteins although complete bacteriocin biosynthetic genes were not identified (Macklaim et al., 2011). A list of described bacteriocins is shown in Table 2.

2.1.4 Hydrogen peroxide

Stemming from a cultivation-based study in 1989, it has been argued that the production of H_2O_2 by vaginal *Lactobacillus* spp. inhibits the colonization of BV-associated bacteria (Eschenbach et al., 1989). H_2O_2 is an oxidizing agent with a well-established mechanism of bacterial antagonism (Eschenbach et al., 1989). The bactericidal effect of H_2O_2 occurs through the generation of oxidizing metabolites such as the radical OH^- which causes breaks in cell DNA; this effect is notably enhanced in acidic environments by the presence of peroxidases and halides (Eschenbach et al., 1989; Klebanoff, Hillier, Eschenbach, & Waltersdorph, 1991; Strus, Brzychczy-Włoch, Gosiewski, Kochan, & Heczko, 2006). It has also been speculated that the production of H_2O_2 can both directly damage the host epithelium (Ding et al., 2004; Huycke, Abrams, & Moore, 2002) and induce cell death in bacteria (Pericone, Overweg, Hermans, & Weiser, 2000; Pridmore, Pittet, Praplan, & Cavadini, 2008). H_2O_2 acts against anaerobic species (including *Prevotella*, *Peptostreptococcus* and *Gardnerella* spp.) that lack catalase and peroxidase enzymes, which are necessary for protection against oxygen free radicals (Eschenbach et al., 1989; Rolfe, Hentges, Campbell, & Barrett, 1978; Strus et al., 2006). Molecular studies have shown that *Lactobacillus* spp. differ in their capability to produce H_2O_2 (Ocaña et al., 1999a; Song et al., 1999; Wilks et al., 2004): *L. crispatus*,

Table 2 Described *Lactobacillus* spp. Bacteriocins and Known Targets

Producer	Bacteriocin	Shown to Be Active Against	Notes
L. crispatus	LS2	*Listeria* spp. (Busarcevic & Dalgalarrondo, 2012; Ojala et al., 2014) *Shigella* spp. (Busarcevic & Dalgalarrondo, 2012; Ojala et al., 2014) *Yersinia* spp. (Busarcevic & Dalgalarrondo, 2012; Ojala et al., 2014)	
L. fermentum HV6b MTCC 10770	Fermenticin HV6b	*Bacteroides* spp. (Kaur et al., 2013) *Gardnerella vaginalis* (Kaur et al., 2013) *Mobiluncus* spp. (Kaur et al., 2013)	
L. fermentum strain L23	Bacteriocin L23	Gram-positive bacteria (Pascual et al., 2008) Gram-negative bacteria (Pascual et al., 2008) *Candida* spp. (Pascual et al., 2008)	Active over a wide range of pH (4.0–7.0) (Pascual, Daniele, Pájaro, & Barberis, 2006)
L. gasseri	Gassericin A	Gram-positive bacteria (Stoyancheva et al., 2014) Gram-negative bacteria (Arakawa et al., 2009; Gillissen, Schumacher, & Breuer-Werle, 1991; Hishinuma, Izaki, & Takahashi, 2014; Pandey, Malik, Kaushik, & Singroha, 2013) *E. coli* (Stoyancheva et al., 2014)	An operon for gassericin A is reportedly present in *L. crispatus* (Stoyancheva et al., 2014) In combination with gassericin T and glycine inhibits the growth of both Gram-positive and Gram-negative bacteria (Arakawa et al., 2009; Gillissen et al., 1991; Hishinuma et al., 2014; Pandey et al., 2013)
	Gassericin T	Gram-positive bacteria (Stoyancheva et al., 2014) Gram-negative bacteria (Arakawa et al., 2009; Gillissen et al., 1991; Hishinuma et al., 2014; Pandey et al., 2013) *E. coli* (Stoyancheva et al., 2014)	In combination with gassericin A and glycine inhibits the growth of both Gram-positive and Gram-negative bacteria (Arakawa et al., 2009; Gillissen et al., 1991; Hishinuma et al., 2014; Pandey et al., 2013)
	Acidocin LF221A	*E. coli* (Stoyancheva et al., 2014)	

Continued

Table 2 Described *Lactobacillus* spp. Bacteriocins and Known Targets—cont'd

Producer	Bacteriocin	Shown to Be Active Against	Notes
L. gasseri JV-V03	Lactocillin	*S. aureus* (Donia et al., 2014) *E. faecalis* (Donia et al., 2014) *G. vaginalis* (Donia et al., 2014) *Corynebacterium* spp. (Donia et al., 2014)	Thiopeptide, inactive against *L. jensenii* JV-V16, *L. crispatus* JV-V01 *and L. gasseri* SV-16A-US at 425 nM (concentrations of Lactocillin assessed ranged from 42 to 425 nM) (Donia et al., 2014)
L. rhamnosus strain L60	Bacteriocin L60	*Neisseria gonorrhoeae* (Ruíz et al., 2015)	In the presence of lactic acid inhibits the growth of *N. gonorrhoeae* (Ruíz et al., 2015)
L. salivarius subsp. *salivarius* 1328	Salivaricin CRL 1328	*Enterococcus faecalis* (Vera Pingitore, Hébert, Nader-Macias, & Sesma, 2009) *E. faecium* (Ocaña, Pesce De Ruiz Holgado, & Nader-Macias, 1999a) *Neisseria gonorrhoeae* (Ocaña et al., 1999a)	Disrupts proton motive force membrane potential (Vera Pingitore et al., 2009)

L. jensenii and *L. vaginalis* are frequent producers (Hillier, Krohn, Nugent, & Gibbs, 1992; Hillier et al., 2009; Ocaña, Pesce De Ruiz Holgado, & Nader-Macias, 1999b; Song et al., 1999; Wilks et al., 2004), while *L. gasseri* and *L. iners* are reportedly not (Antonio, Hawes, & Hillier, 1999; Martinez et al., 2008; Song et al., 1999; Verstraelen et al., 2009). Genomic analysis has shown *L. crispatus* encodes a pyruvate oxidase, which produces of H_2O_2 as a by-product of the conversion of pyruvate into acetate (Antonio et al., 1999; Ojala et al., 2014). Several cultivation-based studies have demonstrated the inhibitory properties of vaginal H_2O_2-producing *Lactobacillus* spp. against pathogens (Aroutcheva et al., 2001; Atassi & Servin, 2010; Eschenbach et al., 1989; Fontaine, Claydon, & Taylor-Robinson, 2009; Hawes et al., 1996; Hillier, Krohn, Klebanoff, & Eschenbach, 1992; McGroarty, Tomeczek, Pond, Reid, & Bruce, 1992; Rosenstein et al., 1997); however, the actual role H_2O_2 in inhibiting these pathogens in vivo is unclear as the anoxic environment likely limits or precludes the production of H_2O_2 (O'Hanlon et al., 2011) and many studies examining this phenomenon do not adequately discriminate between inhibitory effects due to the production of H_2O_2, lactic acid or bacteriocins (Atassi, Brassart, Grob, Graf, & Servin, 2006b; Atassi & Servin, 2010; Klebanoff et al., 1991; McLean & McGroarty, 1996; O'Hanlon, Lanier, Moench, & Cone, 2010). Agitation is a commonly employed microbiological technique to improve aeration; however, conflicting information exists from studies looking at the effect of agitation: Ocaña et al. (1999a) studied the growth kinetics of *L. crispatus* and determined

that H_2O_2 was only produced under agitation, conversely, Strus et al. (2006) reported that *L. gasseri* and *L. crispatus* are capable of producing H_2O_2 regardless of agitation. In a recent study, O'Hanlon et al. (2011) demonstrated that even if produced, H_2O_2 alone was insufficient for the inhibition of BV-associated bacteria by *Lactobacillus* spp. and that cervicovaginal fluid and semen significantly block H_2O_2 activity.

2.1.5 Immune modulation

Because microscopic examination of BV specimens does not show increases in white blood cells or overt indications of inflammation, BV has long been considered a non-inflammatory condition (Anahtar et al., 2015). It has only been through the more recent application of molecular techniques that the relationship of the microbiota of the vagina to various immune molecules has been described. In general, the immune system appears largely permissive of *Lactobacillus* spp. colonization. The levels of IL-1α, IL-1β and IL-8 all show inverse correlations with *Lactobacillus* spp. abundance and are seen to increase in BV (Anahtar et al., 2015; Hedges, Barrientes, Desmond, & Schwebke, 2006; Mattsby-Baltzer, Platz Christensen, Hosseini, & Rosén, 1998; Platz Christensen, Mattsby-Baltzer, Thomsen, & Wiqvist, 1993; Spandorfer et al., 2001; Spear, Zariffard, et al., 2008; Wennerholm et al., 1998). Among common BV-associated bacteria, *Atopobium vaginae*, but not *Prevotella bivia*, has been shown to evoke expression of these cytokines along with IL-6, and tumour necrosis factor α (TNF-α), CCL20 and hBD-2 in epithelial cell models of the vagina (Doerflinger, Throop, & Herbst-Kralovetz, 2014). Studies of *G. vaginalis* have also suggested that it alone is insufficient to induce expression of common proinflammatory IL's, including IL-8 (Valore, Wiley, & Ganz, 2006). Using the same epithelial cell model described earlier, *L. iners* was reported to induce pattern-recognition receptor signalling (PRR) activity, but not to affect IL-6 or IL-8 expression (Doerflinger et al., 2014). In fact, the presence of *L. iners* has also been associated with lower levels of IL-8 in the vaginas of healthy, asymptomatic, Belgian women (Kyongo et al., 2012). In a study of healthy Swedish women, Nikolaitchouk, Andersch, Falsen, Strömbeck, and Mattsby-Baltzer (2008) observed a strong negative correlation between *L. iners* and the proinflammatory cervical IL-1α and a moderate positive correlation between *L. iners* and secretory leukocyte protease inhibitor (SLPI). SLPI is an antiinflammatory molecule with antimicrobial properties and is commonly found in reduced levels in the vaginal tract of women with BV (Draper et al., 2000; Sallenave, 2002). Conversely, in 2014, Orfanelli et al. (2014) observed that the levels of SLPI were reduced in the presence of either *L. iners* or *G. vaginalis* and that the median levels of IL-1β, 1L-1ra and HE4, a recently identified serum biomarker of ovarian cancer (Drapkin et al., 2005), were comparable when the vaginal microbial communities were dominated by either *L. iners* or *G. vaginalis*. Finally, utilizing a combination of metaproteomic, metagenomics and in vitro approaches, Zevin et al. (2016) observed that soluble products derived from *G. vaginalis* decreased epithelial barrier integrity, but those derived from *L. iners* did not.

Various studies have evaluated the role of *L. crispatus* in modulating the host immunological defence system. Using an epithelial cell model of the vagina,

L. crispatus did not invoke PRR signalling, or the expression of AMPs, defensins or proinflammatory cytokines (Doerflinger et al., 2014). Consistently, *L. crispatus*-dominated communities have been found to lower levels of IL-1α, IL-1β and IL-8 relative to communities dominated by anaerobic bacteria or even *L. iners* (Anahtar et al., 2015). Other major *Lactobacillus* species have received less attention, though in models of the human vaginal epithelium, *L. jensenii* has been seen to induce the synthesis of IL-8 and human beta-defensin 2 mRNA (Valore et al., 2006).

Finally, AMPs of the innate immune system, including LL-37, have been shown to preferentially inactivate *Neisseria gonorrhoeae* but have little effect on *L. crispatus* (Moncla, Mietzner, & Hillier, 2012). Intriguingly, in the presence of LL-37, several moonlighting proteins involved in adhesion are released from the surface of *L. crispatus* (Kainulainen et al., 2012), suggesting the host immune system may be able to regulate its adhesion and/or motile life phases.

3 THE VAGINAL MICROBIOME

3.1 COMMUNITY STATE TYPES

Both 16S rRNA and cultivation-based studies have shown that vaginal microbiota are generally dominated by just one of four *Lactobacillus* species, namely, *L. crispatus* (CST-I), *L. gasseri* (CST-II), *L. iners* (CST-III) and *L. jensenii* (CST-V). However, as is often seen, *Lactobacillus* spp. dominance does not preclude the presence of other taxa and several molecular-based studies have identified that some reproductive-aged women, without any overt signs or symptoms of gynecologic morbidity, have vaginal microbiota depauperate for *Lactobacillus* spp. (Anukam et al., 2006; Hyman et al., 2014; Ravel et al., 2011; Srinivasan et al., 2012; Verhelst et al., 2004; Zhou et al., 2004, 2007). Ravel et al. (2011), in one of the largest surveys to date, used a 16S rRNA clustering approach to show the vaginal microbiota of reproductive-aged women could be stratified into five major CSTs that included the *Lactobacillus* spp.-depauperate CST-IV that is instead characterized by an outgrowth of diverse facultative and strictly anaerobic bacteria. Several comparative cross-sectional and longitudinal studies have shown that these CSTs vary in their prevalence, and relationship to health and disease (Brotman, He, et al., 2014; Brotman, Shardell, Gajer, Fadrosh, et al., 2014; Brotman, Shardell, Gajer, Tracy, et al., 2014; Kiss et al., 2007; Petricevic et al., 2014; Verstraelen et al., 2009).

3.2 CST-I: *L. CRISPATUS*-DOMINATED MICROBIOTA

L. crispatus (CST-I)-dominated vaginal microbiota is one of the most widely reported and detected vaginal CSTs (Brotman, He, et al., 2014; Brotman, Shardell, Gajer, Fadrosh, et al., 2014; Dareng et al., 2015; Drell et al., 2013; Forney et al., 2010; Hummelen et al., 2010; Lee et al., 2013; Martin, Zozaya,

Lillis, Miller, & Ferris, 2012; Nelson et al., 2015; Ravel et al., 2011; Smith et al., 2012; Yamamoto, Zhou, Williams, Hochwalt, & Forney, 2009; Yeoman et al., 2013; Yoshimura et al., 2011; Zhou et al., 2009) having been reported as the dominant CST across various ethnicities (Chaban et al., 2014; Jespers et al., 2012, 2015; Jin et al., 2007; Kilic, Pavlova, Alpay, Kilic, & Tao, 2001; Martínez-Peña, Castro-Escarpulli, & Aguilera-Arreola, 2013; Ravel et al., 2011; Santiago et al., 2012; Vásquez et al., 2002).

L. crispatus has the largest reported genome of all major *Lactobacillus* spp. (Mendes-Soares et al., 2014) averaging ~2.25 Mbp and encodes the ability to ferment a broad range of sugars, including fructose, galactose, glucose, maltose, mannose, trehalose and sucrose of which maltose and glucose are common breakdown products of glycogen (France et al., 2016; Spear et al., 2014). France et al. (2016), in characterizing the genomes of 15 *L. crispatus* strains, described a core and accessory genome for *L. crispatus* of 1442 and 2884 genes, respectively, with the current pan-genome comprising ~4300 genes. Importantly, the *L. crispatus* genome encodes several loci related to vaginal health (Ojala et al., 2014). These include core genome genes for the production of several bacteriocins and molecules that may be involved in the competitive exclusion of potentially pathogenic and BV-associated bacteria (France et al., 2016; Ojala et al., 2014). It is therefore little surprise that *L. crispatus* and CST-I occur most frequently among women without signs or symptoms of gynaecological morbidity (Borgdorff et al., 2014; Ravel et al., 2011; van de Wijgert et al., 2014) and have most often been associated with positive reproductive outcomes, including being protective against preterm birth (Kindinger et al., 2016; MacIntyre et al., 2015; Petricevic et al., 2014; Romero et al., 2014b; Verstraelen et al., 2009). In a prospective cohort of pregnant Caucasian women, those exhibiting a *L. crispatus*-dominated vaginal microbiota during the first trimester were found to be less likely to transition to a *Lactobacillus* spp.-depauperate (CST-IV) microbiota during the subsequent trimesters (Verstraelen et al., 2009). Further, a recent study investigating the cervical microbiota of women who had pregnancies complicated by preterm prelabour rupture of membranes, a condition occurring in ~3% of all births and 30% of all preterm births (Kacerovsky et al., 2015; McDonald, O'loughlin, Jolley, Vigneswaran, & McDonald, 1992; Mercer, 2005; Waters & Mercer, 2011), reported that women with a CST-I microbiota have a lowered risk of microbial invasion of the amniotic cavity and intraamniotic infection (Kacerovsky et al., 2015).

Women with CST-I microbiota reportedly have a reduced risk of acquiring various bacterial, fungal and viral pathogens, including causative agents of UTIs such as *E. coli* (Atassi et al., 2006a; Cadieux, Burton, Devillard, & Reid, 2009; Ghartey et al., 2014; Kalyoussef et al., 2012), and STIs such as *C. trachomatis,* human immunodeficiency virus (HIV), HSV-2 and human papillomavirus (HPV) (Borgdorff et al., 2014; Dols, Reid, Brown, et al., 2012; Gong et al., 2014; Nardini et al., 2016; Rizzo et al., 2015; van de Wijgert et al., 2014). Interestingly, the relative importance of different inhibitory mechanisms employed by *L. crispatus* appears to vary among pathogens. For example, the inhibition of *C. trachomatis* in vitro has been shown to

be dependent upon both the abundance *of L. crispatus,* an acidic pH, and the concentration of lactate, with the inhibitory activity against *C. trachomatis* being lost at pH 7 (Gong et al., 2014; Nardini et al., 2016; Rizzo et al., 2015).

Several studies have reported an inverse relationship between HIV infection and the abundance of *L. crispatus* (Dols, Reid, Brown, et al., 2012; Schellenberg et al., 2011; Spear, Sikaroodi, et al., 2008). HIV-1-infected, CST-I women also have reduced viral RNA shedding (35%), when compared to women who were not colonized with *L. crispatus* (Mitchell et al., 2013). Furthermore, *L. crispatus* demonstrates broad-spectrum fungicidal activity against several *Candida* spp. including *C. albicans* and *C. lusitaniae* (Gong et al., 2014; Parolin et al., 2015; Wang et al., 2017). *L. crispatus* not only strongly inhibits the growth and hyphal formation of *C. albicans* but may also repress the expression of hyphae-specific genes (Wang et al., 2017).

Longitudinal studies evaluating the temporal fluctuations of vaginal microbiota have found that CST-I women tend to exhibit the greatest stability, being least likely to transition to an alternative CST. When CST-I women do undergo a transition, they appear most likely to transition to a CST-III (*L. iners*-dominated) microbiota and least likely to transition to a CST-IV (*Lactobacillus*-depauperate) or dysbiotic state consistent with BV (Gajer et al., 2012; Verstraelen et al., 2009). This is consistent with the observation that *L. crispatus* is commonly isolated from BV-negative women (Dumonceaux et al., 2009; Gajer et al., 2012; Hummelen et al., 2010; Nikolaitchouk et al., 2008; Petricevic et al., 2014; Ravel et al., 2011; Schellenberg et al., 2011; Spear, Sikaroodi, et al., 2008; Srinivasan et al., 2010; Verstraelen et al., 2009; Wertz, Isaacs-Cosgrove, Holzman, & Marsh, 2008; Yamamoto et al., 2009; Yoshimura et al., 2011; Zozaya-Hinchliffe, Lillis, Martin, & Ferris, 2010) and, in general, is negatively correlated with BV-associated bacteria (Martin et al., 2012; Smith et al., 2012). Women who are colonized with *L. crispatus* in the rectum have also been shown to be less likely to have or to develop BV (Antonio, Rabe, & Hillier, 2005; El Aila et al., 2009; Marrazzo et al., 2012). *L. crispatus* does appear to be sensitive to menses as seen in a few studies examining longitudinal fluctuations of the vaginal microbiota during the menstrual cycle. These studies have reported the decreased detection and abundance of *L. crispatus* during menses (Gustafsson et al., 2011; Petricevic et al., 2014; Santiago et al., 2012; Srinivasan et al., 2010; Zhang et al., 2012) with their relative abundance declining on the order of 100-fold, with little or no concentration of *L. crispatus* being detected from most women during menses (Santiago et al., 2012).

Analysis of the *L. crispatus* genome has also provided additional insight into the vaginal ecosystem, including the possible presence of a pool of previously undiscovered vaginal plasmids and/or viruses (Ojala et al., 2014). This is in addition to the presence of 31 prophage-like regions found to be enriched in 9 vaginal isolates of *L. crispatus* (Ojala et al., 2014), consistent with previous observations of the presence of temperate phages in the bacterium (Damelin et al., 2011; Ojala et al., 2014). Ojala et al. (2014) identified distinct types of CRISPR/Cas systems predicted to belong to the Type II CRISPR/Cas system in *L. crispatus*. CRISPRs (clustered

regularly interspaced short palindromic repeats) and CRISPR-associated (Cas) genes provide a form of acquired immunity in their host bacterium by integrating short bacteriophage or plasmid sequences at the cell's CRISPR locus, thereby providing bacterial protection from phage or plasmids that have been previously encountered by the host (Rath, Amlinger, Rath, & Lundgren, 2015) and chronicling specific lytic phage types that invade microbial hosts in a given ecosystem (Deveau, Garneau, & Moineau, 2010). However, homology searches between the spacer regions and publically available plasmid and virus sequences failed to yield positive identifications, suggesting a novel pool of plasmids and/or viruses exist in the vagina. Although this likely reflects the paucity of studies looking at the vaginal viriome or plasmidome.

Several strains of *L. crispatus* have been suggested as probiotic agents by which to reduce recurrent UTIs. In 2007, a randomized, double-blind, phase 1 trial established the safety and tolerance of Lactin-V, a vaginal suppository containing *L. crispatus* CTV-05 (Czaja, Stapleton, Yarova-Yarovaya, & Stamm, 2007). *L. crispatus* CTV-05 holds potential as a probiotic as it has high adherence to epithelial cells in vitro and is capable of colonizing the vagina (Antonio, Meyn, Murray, Busse, & Hillier, 2009). Notably, Czaja et al. (2007) observed that vaginal insertion of *L. crispatus* CTV-05 is followed by a mild inflammatory response in the bladder and the vaginal mucosa in some subjects but does not cause prolonged urogenital infection. In the subsequent, randomized, placebo-controlled phase 2 trial, recurrent UTIs occurred in 7/48 (15%) of women receiving treatment with Lactin-V compared to 13/48 (27%) of women who received the placebo (Stapleton et al., 2011). Importantly, high abundance of *L. crispatus* was obtained from the vaginas of women in the treatment group but not in the placebo group (Stapleton et al., 2011). *L. crispatus* CTV-05 has also entered clinical trials as method to prevent BV (Hemmerling et al., 2009, 2010). It is of interest that the ability of *L. crispatus* CTV-05 to colonize the vagina was hindered by vaginal intercourse, semen exposure and the endogenous presence of other *L. crispatus* strains (Antonio et al., 2009).

3.3 CST-III: *L. INERS*-DOMINATED MICROBIOTA

First discovered in 1999 by Falsen et al. (1999), *L. iners* had historically been an underrecognized vaginal species due to its inability to grow on selective media commonly used to cultivate *Lactobacillus* spp. (MRS and Rogosa agar). Nevertheless, *L. iners* has been detected in women worldwide, irrespective of ethnic backgrounds (Anukam et al., 2006; Borgdorff et al., 2016; Burton & Reid, 2002; El Aila et al., 2009; Hummelen et al., 2010; Lee et al., 2013; Martinez et al., 2008; Oh, Seo, Kong, Lee, & Kim, 2015; Pandya et al., 2017; Santiago et al., 2012; Shi, Chen, Tong, & Xu, 2009; Vásquez et al., 2002). Additionally, *L. iners* has been reported to be the most dominant *Lactobacillus* spp. detected in Asian (42.7%) (Ravel et al., 2011), Black (31.4%) (Ravel et al., 2011; Zhou et al., 2007), Caucasian

(66%) (Zhou et al., 2007), Hispanic (36.1%) (Ravel et al., 2011) and Indian (38%) (Pandya et al., 2017) women.

L. iners has the smallest reported genome size, averaging ~1.3 Mbp (France et al., 2016) and a comparison of 15 *L. iners* strains identified average core and accessory genomes of 993 and 1233 genes, respectively (France et al., 2016). The current pangenome of *L. iners* is 2300 genes, one-half the size of *L. crispatus* (France et al., 2016). The role of *L. iners* in vaginal health remains enigmatic and undefined. Its presence has been strongly associated with an intermediate transient microbiota (Dumonceaux et al., 2009; Wertz et al., 2008; Zozaya-Hinchliffe et al., 2010), defined as a Nugent score of 4–6 (Ling et al., 2010; Shipitsyna et al., 2013), and studies examining the dynamic fluctuations of CSTs have found that women categorized with a CST-III (*L. iners*-dominated) microbiota are twice as likely to transition to a microbiota consistent with BV than women who have a CST-I (*L. crispatus*-dominated) microbiota (Gajer et al., 2012; van de Wijgert et al., 2014; Verstraelen et al., 2009). *L. iners* has been reported to be strongly correlated with BV-associated bacteria (Martin et al., 2012; Smith et al., 2012), has been commonly isolated from individuals clinically diagnosed with BV (Anukam et al., 2006; Burton, Cadieux, & Reid, 2003; Burton & Reid, 2002; Tärnberg, Jakobsson, Jonasson, & Forsum, 2002; Zhou et al., 2004) and, in three independent studies, was reported as the sole *Lactobacillus* spp. detected in women diagnosed with BV (Fredricks, Fiedler, & Marrazzo, 2005; Lamont et al., 2011; Verhelst et al., 2004; Wertz et al., 2008). *L. iners* has also been isolated from the vaginas of women recovering from BV (Ferris, Norori, Zozaya-Hinchliffe, & Martin, 2007; Hummelen et al., 2010; Jakobsson & Forsum, 2007; Shi et al., 2009; Verstraelen et al., 2009; Zozaya-Hinchliffe et al., 2010)—several studies investigating the vaginal composition of BV patients prior to and 1-month after metronidazole reported the detection of *L. iners* in all patients postmetronidazole treatment (Ferris et al., 2007; Jakobsson & Forsum, 2007). Interestingly, a longitudinal study, examining the vaginal microbiota of women with recurrent BV from diagnosis through remission to recurrence, reported *L.* iners-dominated remission phases that after treatment did not convert from *L. iners* to a *L. crispatus*-dominated microbiota (Lambert, John, Sobel, & Akins, 2013). Furthermore, Srinivasan et al. (2010) reported that, for some individuals, the abundance of *L. iners* increased with antibiotic treatment for BV.

A few studies have reported the detection, increase and transition to *L. iners*-dominated microbiota during menses (Gajer et al., 2012; Hickey et al., 2013; Santiago et al., 2012; Srinivasan et al., 2012, 2010). *L. iners* has also been implicated in both the development of BV during pregnancy and preterm birth (Skarin & Sylwan, 1986). A prospective cohort of pregnant Caucasian women reported that women dominated by *L. iners* during the first trimester were 10 times more likely than those with CST-I or CST-II to transition to a microbiota consistent with BV at some point during pregnancy (Petricevic et al., 2014; Verstraelen et al., 2009). Petricevic et al. (2014) reported that *L. iners* was the sole *Lactobacillus* spp. isolated from 11/13 women who delivered preterm. Conversely, the detection of *L. iners* as the sole *Lactobacillus* spp. occurred in only 16/98 women who delivered at term

(Petricevic et al., 2014), suggesting that *L. iners*-dominated microbiota might be associated with preterm delivery.

L. iners strains encode for a cholesterol-dependent cytolysin not reported in other *Lactobacillus* spp. (Macklaim et al., 2011; Mendes-Soares et al., 2014). When expressed and secreted, this toxin is thought to increase virulence as it utilizes cholesterol in host cell membranes as a receptor for binding, forming pores in epithelial cells and activating proinflammatory signalling (Macklaim et al., 2011). A subsequent meta-RNA seq analysis demonstrated that this cytolysin was upregulated sixfold under BV conditions compared to healthy conditions (Macklaim et al., 2013). Furthermore, cholesterol has been shown to be 20–43-fold reduced in the vaginal metabolome of BV-afflicted women along with other cell membrane-associated metabolites (Yeoman et al., 2013). *L. iners* isolated from women diagnosed with BV also exhibits increased expression of mucin- and glycerol transport-related enzymes (Macklaim et al., 2013). Notably, this study observed strain-specific functions attributed to *L. iners*: for example, the authors observed a locus related to carbohydrate utilization that contained a mannose-family phosphoenolpyruvate-dependent transport system that was only expressed in six of the *L. iners* strains under study (Macklaim et al., 2013). However, this study is limited as the sample included only four women (Macklaim et al., 2013). A comparative functional genomics study observed that *L. iners* strains lack integral membrane proteins, protein families involved in the acetyltransferase GNAT family (Gcn5-related *N*-acetyltransferases) and various transcriptional regulators present in most other vaginal strains (Mendes-Soares et al., 2014). However, these *L. iners* strains possess numerous ABC transport permeases absent in other vaginal strains (Mendes-Soares et al., 2014). In 2016, Borgdorff et al. (2016) observed that *L. iners*-specific proteins, GAPDH and DNA starvation/stationary phase protection protein (DPS), were significantly decreased in the cervical lavage supernatants of Rwandan women at high risk to HIV infection. Additionally, DPS, GAPDH, GPI and fructose-bisphosphate aldolase (ALDO) had a significant negative association with vaginal pH (Borgdorff et al., 2016). *L. iners* AB-1 has been shown to interfere with and to displace *G. vaginalis* biofilms, thus it was surprising that genome sequencing revealed the lack of many adhesion molecules commonly detected in other *Lactobacillus* spp. (Macklaim et al., 2011). Interestingly, one Fn-binding protein, LINAB1_0564, common to pathogenic strains of *S. aureus* was identified (Macklaim et al., 2011). In 2013, McMillan et al. assessed the ability of *L. iners* to bind immobilized Fn, a glycoprotein found in a soluble form in plasma and in an insoluble form in the extracellular matrix and attached to the surface of host cells. Their results indicate that *L. iners* AB-1 binds to human Fn and that this process is protein mediated (McMillan, Macklaim, Burton, & Reid, 2013). Genomic studies of *L. iners* strains have identified the presence of alkaline shock proteins, universal stress proteins and a cold shock protein. This is interesting as *L. iners* strains have the ability to survive both low pH (3.6–4.5) associated with a healthy vagina and the higher pH (>4.5) associated with BV conditions (Ravel et al., 2011), and the presence of these proteins may collectively aid in the ability of the organism to tolerate varying levels of pH (Macklaim et al., 2011).

The detection and dominance of *L. iners* during vulnerable periods of reproductive health, including menses, pregnancy and prior to, during and after treatment of BV (Shipitsyna et al., 2013; Srinivasan et al., 2012), have led to the suggestion that *L. iners* provides little means of protection against colonization by potentially pathogenic bacteria and thus is a risk factor for BV (Lambert et al., 2013; Verstraelen et al., 2009). Alternatively, some maintain that the frequent detection of *L. iners* in recurrent BV patients indicates that *L. iners* is capable of persisting and exploiting the vaginal environment during acute BV and is potentially important in driving the recovery of the vaginal microbiota (Macklaim et al., 2011).

3.4 CST-II: *L. GASSERI*- AND CST-V: *L.* JENSENII-DOMINATED MICROBIOTA

The occurrence of these CSTs is much less frequent, being reported by Ravel et al. (2011) to occur in 5%–6% of US individuals and being most frequent within those of white ethnicity (8.2% and 9.3%, respectively) and least within those of black ethnicity (4.8% and 1%, respectively) (Ravel et al., 2011). Both CSTs have also been reported among Japanese women. Because of their low prevalence, these CSTs are often not examined in various studies, with *L. jensenii* occasionally not being detected in vaginal samples at all (Gajer et al., 2012; Ravel et al., 2011; Smith et al., 2012). Consequently, comparatively little information currently exists on these two community states, making trends and patterns difficult to discern. Despite their low prevalence, CST-II appears stable: women with CST-II have been rarely observed transitioning to other CSTs. In a longitudinal study composed of 32 women, Gajer et al. (2012) observed no transitions from CST-II into any other CST and only two transitions from CST-III to CST-II. Women with *L. jensenii*- and *L. gasseri*-dominated CSTs have been reported to have a slightly higher pH (4.7–5.0, respectively) than women dominated by *L. crispatus* (Ravel et al., 2011). Although to a lesser extent than seen for *L. iners*, *L. gasseri* is often found associated with "intermediate" (Nugent score 4–6) microbiota (De Backer et al., 2007; Martin et al., 2012; Verstraelen et al., 2009; Wertz et al., 2008). *L. gasseri* encodes for several bacteriocins, and strain ATCC 33323 can actively displace adherent *N. gonorrhoeae* (Spurbeck & Arvidson, 2008). *L. gasseri* has also been frequently isolated from HPV+ women (Dols, Reid, Brown, et al., 2012; Gao, Weng, Gao, & Chen, 2013), and when present during the first trimester of pregnancy, there is a higher risk than seen for *L. crispatus* of transition to a BV-associated microbiota than during later trimesters (Verstraelen et al., 2009). *L. jensenii* appears to be protective, with strain ATCC 25258 having also been shown to reduce adhesion and invasion of *N. gonorrhoeae* (Spurbeck & Arvidson, 2008). For *L. jensenii*, these inhibitory properties have been linked to released surface components that inhibit *N. gonorrhoeae* interactions with endometrial epithelial cells in vitro by obstructing fibronectin-binding sites (Spurbeck & Arvidson, 2008, 2010). In the vagina, fibronectin is secreted and coats the mucosal surface (Cohen, Black, Proctor, & Sparling, 1984). *L. jensenii* plays an active role in modulating the magnitude of cytokine

responses being shown to temper the immune response of vaginal epithelial cells subjected to TLR agonists (Rose et al., 2012).

3.5 CST-IV: *LACTOBACILLUS*-DEPAUPERATE MICROBIOMES

The CST-IV microbiota is delineated by its *Lactobacillus*-depauperate state and is associated with the outgrowth of facultative and strict anaerobes including *Aerococcus*, *Atopobium*, *Corynebacterium*, *Dialister*, *Eggerthella*, *Finegolida*, *Gardnerella*, *Megasphaera*, *Mobiluncus*, *Peptoniphilus*, *Prevotella*, *Sneathia* and *Streptococcus* spp. (Ferris et al., 2004, 2007; Ravel et al., 2011; Srinivasan & Fredricks, 2009; Verhelst et al., 2004; Verstraelen et al., 2009; Zhou et al., 2004, 2007). Of these, the monospeciated *Gardnerella* (*G. vaginalis*) genus has garnered much attention over the past 60 years since being identified as the likely causative agent of BV by Gardner & Dukes (1954). Understanding the role of *G. vaginalis* in BV has been confounded by findings that this bacterium can be isolated from the vagina of 58%–68% of women that lack signs or symptoms of disease (Sautter & Brown, 1980; Totten, Amsel, Hale, Piot, & Holmes, 1982) and detected in 33%–50% of 16S rRNA gene profiling efforts (Ravel et al., 2011; Yeoman et al., 2010), including all CSTs (Ravel et al., 2011). At least some of this variation may be driven by variation in pathogenic potential among *G. vaginalis* strains. Despite representing a single species, screening of various enzymatic activities revealed that between 8 and 17 biotypes exist depending on the activities examined (Benito, Vazquez, Berron, Fenoll, & Saez-Neito, 1986; Piot et al., 1984) and that these biotypes have differing relationships to disease (Briselden & Hillier, 1990). Subsequent comparative genomic analyses have supported at least four distinct groups that appear genetically isolated (Ahmed et al., 2012). Two studies comparing the genomes of group 4 *G. vaginalis* isolates obtained from asymptomatic individuals to isolates from symptomatic BV individuals revealed variation in gene content and the sequence compositions of known virulence genes (Harwich et al., 2010; Yeoman et al., 2010). These include differences corresponding to greater adherence to epithelial cells, aggregation and biofilm formation of BV isolates (Harwich et al., 2010). Isolates obtained from BV individuals only appear able to degrade and utilize mucin (Yeoman et al., 2010), a potentially key virulence marker (McGregor et al., 1994; Roberton et al., 2005). Because 16S rRNA gene sequencing cannot readily distinguish between potentially pathogenic and nonpathogenic *G. vaginalis* or *A. vaginae* organisms, such data need to be used with caution when attempting to predict an "unhealthy" vaginal state (Gajer et al., 2012; Hyman et al., 2005; Ravel et al., 2011; Stoyancheva et al., 2014). Gajer et al. (2012) have shown that individuals may exhibit a persisting CST-IV vaginal microbiota without the onset of disease. However, even in the absence of signs and symptoms of disease, it is not clear that CST-IV could be considered a "healthy" state. The microbiology of CST-IV and its strong correlative relationship to the often employed Nugent scoring criteria (Nugent et al., 1991; Ravel et al., 2011) suggests that many studies describing an asymptomatic BV, or not measuring clinical signs and symptoms of the disease, may be describing CST-IV individuals.

Studies focusing on asymptomatic BV have shown it is associated with various reproductive and gynaecological morbidities (Gibbs, 2007), while those relying entirely on Nugent criteria have blurred the line as to whether morbidities associated with BV are related to its clinical manifestation or a *Lactobacillus*-depauperate state.

Since being defined in 2011, CST-IV microbiota have been associated with a myriad of adverse clinical morbidities. In a series of molecular-based studies, CST-IV microbiota have been associated with *T. vaginalis* infection (Brotman et al., 2012), vulvovaginal atrophy (Brotman, Shardell, Gajer, Fadrosh, et al., 2014), high-risk HPV infection (Dareng et al., 2015), a slow HPV remission rate (Brotman, Shardell, Gajer, Tracy, et al., 2014) and increasing CIN severity (a precursor to invasive cervical cancer), regardless of HPV status (Mitra et al., 2015). Women with CST-IV microbiota have reportedly higher IL-6 concentrations in cervical fluid (2- to 2.5-fold) and higher incidence of microbial invasion of the amniotic cavity, intraamniotic infection and histological chorioamnionitis (Kacerovsky et al., 2015). Postmenopausal women predominantly have CST-IV microbiota (Brotman, Shardell, Gajer, Fadrosh, et al., 2014), and among these women, this CST has been associated with signs of vulvovaginal atrophy. Specifically, women who were clinically diagnosed with mild or moderate vulvovaginal atrophy having 25-fold greater odds of being classified as CST-IV vs *L. crispatus*-dominant CST (Brotman, Shardell, Gajer, Fadrosh, et al., 2014).

It is our view that CST-IV likely represents a vulnerable state that is more susceptible to infection by agents capable of eliciting symptomatic BV, or other gynaecological disease. However, further complicating the issue is that CST-IV can be further stratified to two subgroupings CST-IVa and CST-IVb (Brotman, Shardell, Gajer, Fadrosh, et al., 2014; Gajer et al., 2012), which are also evident when using alternate microbial gene markers (Albert et al., 2015), and these subgroupings may have differing relationships to disease. Women with a CST-IV microbiota exhibit the highest vaginal pH of all CSTs (Ravel et al., 2011), and it has been hypothesized that in addition to the depauperation of *Lactobacillus* spp., increases in select biogenic amines (BAs) may contribute to this observation. Metabolomic studies have shown CST-IV exhibits characteristic increases in several BAs including cadaverine, putrescine and agmatine when compared to women with *Lactobacillus*-dominant CSTs (Nelson et al., 2015). High concentrations of the BAs putrescine and cadaverine, along with trimethylamine have been associated with the malodour characteristic of BV (Srinivasan et al., 2015; Wolrath, Boren, Hallen, & Forsum, 2002; Yeoman et al., 2013). Most BAs, with the exception of spermine and spermidine, are produced via specific amino acid decarboxylation reactions and involve the consumption of hydrogen ions and the consequential reduction of intracellular and extracellular acidity (Kanjee & Houry, 2013; Nelson et al., 2015). Although host cells are capable of producing putrescine, spermine and spermidine, which play roles in immune regulation, lipid metabolism, nucleic acid stabilization and cell division, other BAs are exclusively of microbial origin (Pegg, 2009). The production of putrescine, cadaverine and agmatine have extensively been described as being used by

various bacteria for acid-stress resistance and can protect *E. coli* at a pH of 2.5 (Large, Walk, & Whittam, 2005). A comprehensive genomic screen of the most prevalent vaginal bacteria indicates that the ability to produce BAs is limited to just a handful of species that are mainly associated with CST-IV and BV. These include members of the genus *Mobiluncus*, including *M. mulieris* and *M. curtisii*, which can produce trimethylamine (Cruden & Galask, 2009). These *Mobiluncus* species were seen to produce trimethylamine either through the reduction of TMA oxide or weakly through the reduction of choline (Cruden & Galask, 2009). Additionally, the vaginal parasite, *T. vaginalis*, has been shown to encode an ornithine decarboxylase, necessary for the production of putrescine, spermine and spermidine (Yarlett, Goldberg, Moharrami, & Bacchi, 1993). Accordingly, elevated levels of TMA and putrescine have been associated with diagnosis of BV and with trichomoniasis (Sobel, Karpas, & Lorber, 2012). Based on their observations, Nelson et al. (2015) hypothesize that these BAs may reduce vaginal acidity and thereby decreasing the primary barrier to pathogen outgrowth and/or directly impacting the growth of vaginal *Lactobacillus* spp. while favouring CST-IV-associated bacteria.

4 MODULATORS OF THE VAGINAL MICROBIOME

4.1 AGE AND PHYSIOLOGY

The composition of the vaginal microbiota is directly linked to the structural and physiological changes that occur in the vaginal environment throughout a woman's lifetime (Farage & Maibach, 2006; Farage, Miller, & Sobel, 2010). While the vaginal epithelium provides an important barrier against STIs and potentially pathogenic bacteria, structural and compositional changes remain poorly characterized, with the crux of our knowledge based upon the seminal review written by Farage and Maibach (2006) and from inferences gleaned from the epidermidis (Blaskewicz, Pudney, & Anderson, 2011). Broadly, it is well established that the physiology of the vagina changes over lifetime and that these changes are most directly linked not only to the production and concentration of oestrogen (Farage & Maibach, 2006) but also the composition of the vaginal microbiota.

4.1.1 Newborn to infancy

It has been long considered that infants are born sterile, and their initial colonization comes from either the vaginal or skin microbiota depending upon mode of delivery (Dominguez-Bello et al., 2010). However, recent studies utilizing 16S rRNA gene sequencing technology have reported the potential for a low-biomass microbiota within the human placenta that is unique from that in vagina (Amarasekara, Jayasekara, Senanayake, & Dissanayake, 2015; Collado, Rautava, Aakko, Isolauri, & Salminen, 2016; Dong et al., 2015; Doyle et al., 2014; Fichorova et al., 2015, 2011; Hecht et al., 2009; Prince et al., 2016; Wassenaar & Panigrahi, 2014). Irrespective of the source, maternal inoculation is a crucial event in the colonization and shaping of an infant's microbiome (Dominguez-Bello et al., 2010) and

this is also likely to be true of the human vagina. For the first month, an infant's vulva and vagina are influenced by residual, transplacental, maternal oestrogens that facilitate the temporary stratification of the vaginal epithelium and the production of glycogen, which is metabolized by endogenous bacteria resulting in a low vaginal pH (Farage & Maibach, 2006; Hickey et al., 2015). As the residual maternal oestrogens are metabolized, the vaginal epithelium loses its glycogen content and stratification and there is a concomitant increase in vaginal pH (Farage & Maibach, 2006; Farage et al., 2010).

4.1.2 Childhood

To date, there exists no known molecular-based study investigating the vaginal microbiota of children. Cultivation-based studies have established that the vaginal environment of young children remains similar to that of older infants: the epithelium remains thin, there is very little glycogen and the vaginal pH is alkaline or neutral (Farage & Maibach, 2006). Hammerschlag, Alpert, Onderdonk, et al. (1978) cultured bacteria from the vaginal canals of 100 children aged 2–15 years and found high colonization of diphtheroids (*Corynebacterium* spp. 78%), *Staphylococcus epidermidis* (73%) and mycoplasma. Interestingly, the occurrence of *Lactobacillus* spp. increased from a 45% prevalence in children under 2 to 88% by age 11 (Hammerschlag, Alpert, Rosner, et al., 1978).

4.1.3 Adolescence

During puberty, under the influence of adrenal and gonadal maturation, oestrogen levels begin to rise, the vaginal epithelial thickens and intracellular glycogen is produced (Farage & Maibach, 2006; Farage et al., 2010). To date, there are only two known molecular-based studies examining the vaginal microbiota during puberty. Yamamoto et al. (2009) characterized the vagina microbiota of menarcheal adolescents (ages 13–18) using 16S rRNA terminal-restriction fragment length polymorphisms (**T-RFLP**). The vaginal composition of menarcheal adolescents was similar to reproductive-aged women—four major clusters were identified which could be divided into those dominated by *Lactobacillus* spp. (namely, *L. crispatus*, *L. iners*) and those dominated by alternate lactic acid-producing bacteria, *Atopobium* and *Streptococcus* spp. In 2015, Hickey et al. (2015) characterized longitudinal changes in the vaginal and vulvar microbial communities of premenarcheal girls in relation to the Tanner scale (stage 1 = prepuberty; stage 5 = full maturity) to track physiological changes as a response to oestrogen levels as these adolescents progressed through pubertal changes. The vaginal microbiota of many premenarcheal girls separated into *Lactobacillus*-dominated clusters more than a year prior to the onset of menarche, and this *Lactobacillus* dominance was not necessarily associated with low vaginal pH (Hickey et al., 2015). *Lactobacillus* dominance occurred during the transition from Tanner stage 2–3 and prior to the onset of menarche (usually occurring in Tanner stage 3 or 4). Interestingly *G. vaginalis* was also detected in nearly one-third of the perimenarcheal vaginal microbiota and was the fourth most abundant species detected (Hickey et al., 2015).

4.1.4 Reproductive-aged women

In reproductive-aged women, the vaginal environment is considered mature—beyond the regular columnar cell arrangement of the endocervix, surface cells are stratified, squamous epithelium that forms a natural barrier against potential pathogens (Eschenbach, Thwin, et al., 2000; Farage & Maibach, 2006; Farage et al., 2010). The layers of vaginal epithelium include the basal, superbasal and *stratum corneum* cells, each of which contributes broadly to reproductive health. Basal cells serve as progenitors for the apical layers and are frequently sloughed off into the vaginal cavity, while *stratum corneum* cells contain substantial amounts of glycogen and mucin and are thought to be important mediators of immune defence (Anderson, Marathe, & Pudney, 2014; Farage & Maibach, 2006; Rose et al., 2012). Additionally, *stratum corneum* cells lack tight junctions and are permeable to water and soluble proteins (Anderson et al., 2014; Blaskewicz et al., 2011). A study investigating the generation time of vaginal cells provided evidence that a single cell layer is lost from the vaginal epithelium every 4 h (Anderson et al., 2014; Averette, Weinstein, & Frost, 1970; Patton et al., 2000). The amount of time it takes for a single cell to be exfoliated, or sloughed, may be affected by numerous factors including intercourse, use of vaginal products and hormonal status. In addition, fluctuations in oestrogen affect both thickness of vaginal epithelium and glycogen stores (Eschenbach et al., 2001; Eschenbach, Patton, et al., 2000; Farage & Maibach, 2006; Patton et al., 2000).

It is widely maintained that the exfoliation of vaginal cells is an effective means by which to eliminate pathogens adhered to the cell surface (Farage & Maibach, 2011; Hladik et al., 2007). As epithelial cells are exfoliated, their glycogen-rich contents are released into the vaginal lumen providing a nutrient source for *Lactobacillus* spp. While *Lactobacillus* spp. cannot metabolize glycogen directly, glycogen is degraded by α-amylase into polymers such as maltose and maltotriose which are then capable of being utilized by *Lactobacillus* spp. (Spear et al., 2014). Interestingly, women clinically diagnosed with BV are often found to have lower vaginal levels of α-amylase (Nasioudis et al., 2015).

4.1.5 Postmenopausal women

Relatively little information exists about the postmenopausal vaginal microbiome. From a physiological perspective, the onset of menopause is associated with decreased levels of oestrogen and the cessation of menstruation; the vaginal epithelium atrophies due to the decrease in oestrogen, the production of cervicovaginal secretions is markedly reduced and there is an increase in vaginal pH (Farage & Maibach, 2006; Farage et al., 2010; Gupta, Kumar, Singhal, Kaur, & Manektala, 2006). The vaginal microbiota are thought to once again transition from a *Lactobacillus*-dominant microbiota commonly associated with healthy, reproductive-aged women to one dominated primarily anaerobic and enteric bacteria (Ginkel, Soper, Bump, & Dalton, 1993; Larsen, Goplerud, Petzold, Ohm-Smith, & Galask, 1982). In 2014, Brotman, Shardell, Gajer, Fadrosh, et al. (2014) conducted one of the first cultivation-independent studies characterizing the composition of vaginal

microbiota of women classified by "Stages of Reproductive Aging Workshop" guidelines (STRAW) into pre-, peri- and postmenopausal groups. Interestingly, *Lactobacillus* spp. dominance was frequent among each stage of menopause (83% in premenopausal women, 83% in perimenopausal women and 54% in postmenopausal women). However, women who were perimenopausal were most likely to have vaginal microbiota dominated by *L. gasseri* or be CST-IV and women who were postmenopausal were primarily categorized as CST-IV-A (Brotman, Shardell, Gajer, Fadrosh, et al., 2014). An exploratory study conducted by Mirmonsef et al. (2015) examined the association of free glycogen and *Lactobacillus* spp. abundance in postmenopausal women. This study is important as it has been demonstrated that the epithelial cells of some postmenopausal women and ovariectomized women still produce glycogen (Ayre, 1951; Willson & Goforth, 1942). Moreover, intraepithelial glycogen content has been reported to be independent of hormonal changes (Gregoire et al., 1971) and previous work conducted by Mirmonsef et al. (2014) suggests that free glycogen in the genital fluid may be more closely associated with *Lactobacillus* levels in the genital tract than epithelial produced glycogen. Cervicovaginal lavage samples were obtained from pre- and postmenopausal women over the course of at least two clinical visits, and the abundance of *L. crispatus*, *L. iners* and *L. jensenii* within each cervicovaginal lavage sample was quantified using qPCR. Overall, this study demonstrated that free glycogen was present in both pre- and postmenopausal women and was positively correlated with the relative abundance of *Lactobacillus* spp. (Mirmonsef et al., 2014). This might explain why some studies have reported high *Lactobacillus* spp. abundance in postmenopausal women. This study was limited due to its small sample size of pre- and postmenopausal women, and postmenopausal women were only sampled twice (Mirmonsef et al., 2015).

4.2 MENSES

A few molecular-based studies reported that menses exerts a strong influence upon the stability of the vaginal ecosystem (Chaban et al., 2014; Gajer et al., 2012; Hickey et al., 2013; Jespers et al., 2012; Lambert et al., 2013; Santiago et al., 2011, 2012; Srinivasan et al., 2010). Srinivasan et al. (2010) used taxon-specific qPCR to examine the longitudinal fluctuations of vaginal bacterial communities in 22 reproductive-aged women, 8 of whom were diagnosed with BV. Women who were BV negative had vaginal communities dominated by *Lactobacillus* spp. although *G. vaginalis* was also detected. During menses, significant increases in *G. vaginalis* were observed in 81% of the monitored menstrual cycles along with an increased abundance in *L. iners*. As the abundances of both *G. vaginalis* and *L. iners* increased, there was a concomitant decrease of *L. crispatus* and *L. jensenii* (Srinivasan et al., 2010). Santiago et al. (2011) utilized culture-dependent techniques, intergenic length polymorphism analysis (tDNR-PCR) and 16S rRNA gene sequencing to characterize isolated from vaginal swabs self-collected daily from 17 nonpregnant, reproductive-aged, Caucasian women over a period of two menstrual cycles. The authors reported that variability within individuals was common and that menses had a negative

association with *L. crispatus*. However, this study was limited not only by its small sample size but because the authors first cultured bacteria from the swabs thus fastidious organisms such as *A. vaginae* or *Leptotrichia* spp. are underrepresented or absent (Santiago et al., 2011). A longitudinal analysis utilizing pyrosequencing of the V1–V2 regions of the 16S rRNA gene examined the vaginal communities of 32, healthy, reproductive-aged women who self-collected vaginal swabs, twice weekly over the course of 16 weeks (Gajer et al., 2012). During this time, some women transitioned between community states quite frequently, while others did not. Moreover, some women who were categorized into CST-I or CST-II most often experienced transitions between community states (namely, CST-III) during menses (Gajer et al., 2012). In 2012, Santiago et al. (2011, 2012) conducted a follow-up to their 2011 study using qPCR primers specific to several vaginal species. This study demonstrated that during menses, concentrations of *L. crispatus* declined, while *L. iners*, *G. vaginalis*, *A. vaginae* and *P. bivia* increased (Santiago et al., 2012). A small study conducted by Lambert et al. (2013) examined the microbial community fluctuations of isolates obtained from five women clinically diagnosed with recurrent BV using qPCR. In women with recurrent BV, the depletion of *Lactobacillus* spp. and the concomitant replacement with other species occurred during, or immediately after, menses (Lambert et al., 2013).

In a small study, Hickey et al. (2013) used pyrosequencing of the V1–V2 region of the 16S rRNA genes to determine the effect of tampons and menses upon the vaginal microbiotas of seven women and observed that while community dynamics varied during menses, these changes were largely individualized. Moreover, neither tampon nor pad use affected the vaginal microbiome (Hickey et al., 2013). Finally, in contrast to the aforementioned studies, Chaban et al. (2014) examined the stability of the vaginal microbiome of healthy Canadian women throughout a single menstrual cycle using cpn60-based microbiota analysis and observed that overall, the vaginal microbiome of most women remained stable throughout the menstrual cycle. Taken together, the evidence suggests that menses is largely associated with transitions in the community composition of the vaginal microbiota, but that these changes are largely interindividual. However, some trends can be generally applied including the reported decrease of *Lactobacillus* spp. (particularly, *L. crispatus*) and the concomitant increase of *L. iners*.

4.3 PREGNANCY

During pregnancy, the vaginal environment undergoes physiological changes to better maintain a healthy gestational period and to prepare the body for parturition—vaginal epithelia hypertrophies, connective tissues thicken and oestrogen (estradiol) levels steadily increase over the course of pregnancy (Farage & Maibach, 2006; Farage et al., 2010; Nieburgs, 1947).

Numerous studies utilizing molecular techniques have characterized the vaginal microbiota of pregnant women during various stages of pregnancy and have consistently reported the prevalence of *Lactobacillus*-dominated vaginal microbiota (Aagaard et al., 2012; Bisanz et al., 2015; DiGiulio et al., 2015; El Aila et al.,

2009; Frank et al., 2012; Hernández-Rodríguez et al., 2011; Huang et al., 2015; Husain et al., 2014; MacIntyre et al., 2015; Romero et al., 1989, 2014a, 2014b; Subramaniam et al., 2016; Verstraelen et al., 2009; Walther-António et al., 2014). Verstraelen et al. (2009) combined Gram-staining, culture-dependent and terminal-restriction fragment length polymorphism (tRFLP) techniques to demonstrate the role of *L. crispatus and L. gasseri* in maintaining the stability of the vaginal environment during pregnancy in a population of 100 Dutch women sampled once in each trimester. Hernández-Rodríguez et al. (2011) utilized PCR-DGGE and sequencing of the V3 region of the 16S rRNA gene to characterize bacterial communities isolated from vaginal swabs of healthy, pregnant, Mexican women across all trimesters ($n = 64$). *Lactobacillus* spp. were isolated from 98% of the women studied with *L. acidophilus* (78%) and *L. iners* (54%) being the most prevalent species. In 2012, Aagaard et al. (2012) utilized 16S rRNA gene sequence analysis in a cross-sectional study and observed an overall decrease in diversity and richness in pregnant women compared to nonpregnant women with pregnant women having a high relative abundance of *Lactobacillus* spp. This pattern was also reported in a retrospective case–control longitudinal study investigating differences the vaginal microbiota of healthy pregnant women from that of nonpregnant women that confirmed that the vaginal microbiota of healthy pregnant women had a higher abundance of *Lactobacillus* spp. than nonpregnant women (Romero et al., 2014b).

A cross-sectional, prospective study utilized culture dependent, 16S rRNA gene sequencing and MALDI-TOF mass spectrometry to characterize and validate the identification of *Lactobacillus* strains isolated from vaginal swabs of women at 13- and 20-week gestation (Husain et al., 2014). This showed significant differences in the vaginal *Lactobacillus* spp. obtained between ethnic groups at two points during pregnancy—in particular, African-American and Asian women were less likely to have *Lactobacillus* spp. at 20 weeks compared to women who were Caucasian (Husain et al., 2014). A small, longitudinal study conducted by Walther-António et al. (2014) assessed the vaginal microbiota of women ($n = 12$) at 8-week intervals throughout full-term uncomplicated pregnancy using sequencing of the V3–V5 region of the 16S rRNA gene and observed that a normal, healthy pregnancy is characterized by *Lactobacillus* dominance. Interestingly, eight of the women were dominated by *L. crispatus* throughout their pregnancy and two women transitioned from a *L. crispatus*-dominated microbiota to a *L. iners*-dominated microbiota between the first and second trimester (Walther-António et al., 2014). These findings are consistent with results obtained from Verstraelen et al. (2009) who demonstrated that women with *L. crispatus*-dominated microbiota are relatively stable and, in the event of a transition, are more likely to transition to *L. iners*-dominated microbiota than to any other CST. Finally, MacIntyre et al. (2015) sequenced the V1–V2 regions of the 16S rRNA gene to characterize the vaginal microbiota of a mixed British cohort ($n = 42$) who had uncomplicated, healthy pregnancies and were sampled longitudinally throughout pregnancy and 6 weeks postpartum. This study demonstrated that, while the pregnant vaginal microbiome was characteristically dominated by *Lactobacillus* spp. and low α-diversity, unlike Northern American populations, a significant number of pregnant women in the British population had vaginal microbiota

dominated by *L. jensenii* (MacIntyre et al., 2015). This is particularly interesting as a few studies have observed differences across race, and this may point to a genetict predisposition.

A few molecular-based studies have demonstrated the association between the depletion of vaginal *Lactobacillus* spp. in pregnant women and an increased risk of adverse pregnancy outcomes such as preterm birth and an increased risk of mother-to-child transmission of HIV. When the vaginal microbial communities of pregnant women who had a spontaneous preterm delivery and women who delivered full term were analysed, there were no differences in the vaginal communities of either groups (Romero et al., 2014a). In contrast, in a separate study, the diversity and composition of bacterial communities from a case–control study of pregnant women, 15 of whom had a preterm delivery, there was a strong association between the *Lactobacillus*-depauperate CST-IV and preterm delivery (DiGiulio et al., 2015). A retrospective, nested, case–control study used a combination of broad-range amplification of the 16S rRNA gene and pyrosequencing to analyse cervicovaginal lavages obtained from mothers with HIV infection in West Africa who did or did not transmit HIV to their children (Frank et al., 2012). Interestingly, consistent with previous reports, *Lactobacillus* spp. were the predominant species in both groups; however, increases in bacterial diversity were significantly associated with mother-to-child transmission of HIV. Moreover, the relative abundance of *G. vaginalis* was twofold higher in lavagaes obtained from mothers who transmitted HIV to their children shortly after birth compared to mothers who did not (Frank et al., 2012). Finally, a small pilot study ($n = 40$) evaluated differences in the vaginal microbiota of pregnant women based upon race time of delivery (preterm/full term) and BV (present/absent) (Subramaniam et al., 2016). As expected, there was a significant difference in the microbial composition of pregnant women with BV compared to women who did not have BV; however, it is notable that the authors reported visible clustering associated with BV and preterm birth (Subramaniam et al., 2016). Although due to the small sample size and blocked design this trend was not significant, it holds promise for larger studies (Subramaniam et al., 2016).

It is important to note that BV is a well-established risk factor for pregnancy complications such as miscarriages and postpartum endometritis. Moreover, women with BV during pregnancy have an almost 10-fold greater risk of spontaneous abortion and as much as 7.5-fold increased risk of preterm birth (Hay et al., 1994). Among full-term infants, exposure to BV has been associated with an increased risk of ventilation/respiratory distress at birth (1.28-fold) (Dingens et al., 2016), NICU admissions (1.4-fold), neonatal sepsis and duration of NICU stay >2 days (15% vs 6%) (Laxmi, Agrawal, Raghunandan, Randhawa, & Saili, 2011).

4.4 HOST BEHAVIOURS

Certain host behaviours have been associated with alterations of the vaginal microbiome. Behaviours like pessary use (Alnaif & Drutz, 2000; Collins, Beigi, Mellen, O'Sullivan, & Tulikangas, 2015), smoking (Alnaif & Drutz, 2000; Brotman, He, et al., 2014), douching (Brotman et al., 2008; Ness et al., 2002) or the use of

lubricants (Brotman, Ravel, Cone, & Zenilman, 2010) have been shown to increase the likelihood of having a *Lactobacillus*-depauperate CST-IV or BV-associated vaginal microbiota. Metabolomics has provided some initial insight into these associations. Smokers have a lower abundance of *Lactobacillus* spp. than nonsmokers (Borgogna et al., 2016). Smokers are 25 times more likely to have a CST-IV microbiota than CST-I (*L. crispatus*) (Brotman, He, et al., 2014); and a metabolomic analysis of the vaginal environment has shown that the vaginal concentrations of the breakdown products of cotinine and BAs are greater in smokers than nonsmokers (Borgogna et al., 2016). This may hint at the mechanism through which smoking affects the vaginal microbiome as is indicated earlier, BAs are thought to influence the vaginal microbiota towards CST-IV (Nelson et al., 2015). Similarly, in a separate metabolomic study, among all host variables, douching was determined to have the most perturbing impact on the vaginal metabolome (Yeoman et al., 2013).

4.5 SEXUAL ACTIVITY

Several studies have examined the effect of sexual activity upon the vaginal microbiome. The presence of BV-associated species is uncommon among virgin women, and intriguingly vaginal colonization with these species does not appear to be affected following sexual debut (Mitchell, Fredricks, Winer, & Koutsky, 2012). However, the preservation of the vaginal microbiota is likely to be severely challenged during unprotected sex, as the presence of semen abolishes vaginal acidity for several hours to allow an opportunity for fertilization by sperm that would otherwise be rapidly inactivated at pH 4 (Shedlovsky & Belcher, 1942; Tevi-Bénissan et al., 1997). Although, not confined to unprotected intercourse, Gajer et al. (2012) determined that sexual activity had a significant, negative effect on the stability of the composition of the vaginal microbiome; however, this effect was weak in comparison to that of CST. Nevertheless, women who engage in unprotected penile–vaginal sex were more likely to have vaginal microbiota dominated by either *G. vaginalis* (CST-IV) or *L. iners* (CST-III) after adjusting for ethnicity and oral contraceptive use (Vodstrcil et al., 2017). In a related study, Jespers et al. (2012) identified a negative association between prostate-specific antigen (a proxy for sexual activity occurring within the last 48 h) and *L. crispatus*. The same molecule was positively associated with both *L. gasseri* and *L. iners* (Jespers et al., 2012). A study examining the vaginal colonization capabilities of probiotic *L. crispatus* CTV-05 observed that females who engaged in sexual intercourse and who used condoms during the first week of the trial were less likely to become colonized (Antonio et al., 2009). Several studies suggest concordance of microbiota between sexual partners, including the sharing of *Gardnerella* oligotypes between monogamous heterosexual couples (Eren et al., 2011) and whole microbiota between monogamous lesbian couples (Marrazzo, Thomas, Fiedler, Ringwood, & Fredricks, 2010). Perhaps, not surprisingly, in studies of women (ages 16–35) who engaged in sex with other women, only a new female sex partner who had a history of BV, or sharing of sex toys were found to be significantly associated with increased risk of BV acquisition (Marrazzo et al.,

2010). Marrazzo et al. (2010) argue that this is likely due to the polymicrobial nature of BV and provides evidence against the sexual transmission of BV.

5 VAGINAL DYSBIOSIS AND MORBIDITY

The prevalence of BV has been shown to vary across racial groups, with nonwhite women repeatedly reporting higher rates (African-American 51%, Mexican Americans 31.9%) compared to white women (23.2%) (Allsworth & Peipert, 2007; Kenyon, Colebunders, & Crucitti, 2013). Women diagnosed with BV have been shown to have an ~14-fold increased risk of acquiring UTIs (Sumati & Saritha, 2009) and are more likely to test positive for *N. gonorrhoeae* (fourfold) and *C. trachomatis* (3.4-fold) STIs when compared to women without BV (Wiesenfeld et al., 2003). These relationships appear causal, as seen in a longitudinal analysis ($n = 3620$), which observed that women who presented with BV in one clinical visit were 1.7 times more likely to acquire *N. gonorrhoeae*, *C. trachomatis* or *T. vaginalis* STIs in the subsequent visit (Brotman, Klebanoff, et al., 2010). Further, a nested, case–control study demonstrated that, for every additional episode of BV, the risk of acquiring chlamydia and gonorrhoea infections increased by 13% and 26%, respectively (Bautista et al., 2017). It is interesting that, in this respect, metabolomic studies have drawn attention to the increase in vaginal BAs corresponding to the transition to a *Lactobacillus*-depauperate state (Nelson et al., 2015) and BV (Yeoman et al., 2013), as BAs have also been shown to improve the growth and virulence of several pathogens, including the STI *N. gonorrhoeae* (Goytia, Dhulipala, & Shafer, 2013; Jelsbak, Thomsen, Wallrodt, Jensen, & Olsen, 2012; Shah & Swiatlo, 2008). Women with BV have also been associated with an increased risk of acquiring viral STIs, including HSV-2 (twofold) (Cherpes, Meyn, Krohn, Lurie, & Hillier, 2003), CMV (4.5-fold) (Ross et al., 2005), HPV (1.4-fold) (Gillet et al., 2011) and HIV (1.6-fold) (Gillet et al., 2011). A longitudinal study ($n = 2236$) of HIV-1-positive females and HIV-1-uninfected males demonstrated that BV is associated with a threefold increase in female-to-male HIV-1 transmission (Cohen et al., 2012).

Perhaps of greatest concern for many, BV is a well-established risk factor for pregnancy complications including preterm delivery, miscarriage, and postpartum endometritis (Dingens et al., 2016; Leitich et al., 2003). Women with BV during pregnancy have an almost 10-fold greater risk of spontaneous abortion, and as much as 7.5-fold increased risk of delivering preterm (Leitich et al., 2003). The community composition of placenta membranes differs between term and preterm labours, and this difference is independent of delivery mode (Aagaard et al., 2014; Doyle et al., 2014; Prince et al., 2016). Specifically, placentas from preterm births exhibit reduced abundances of *Lactobacillus* spp. when compared to placentas from full-term births (Doyle et al., 2014; Fichorova et al., 2011). Additionally, a cross-sectional, nested study utilizing metagenomics found that women who experience spontaneous preterm birth harbour a placental microbiota that is unique from women who delivered full term and that this microbiota was further differentiated by the severity of chorioamnionitis (Prince et al., 2016). Notably, in contrast to the aforementioned studies,

Amarasekara et al. (2015) were only able to detect bacteria in placentas from pregnancies that were complicated by preeclampsia (12.7%).

Much interest in the vaginal microbiome has been garnered from the observation that the vaginal microbiota is the one of the earliest, if not the earliest, microbial influences on conventionally born infants (Dominguez-Bello et al., 2010). It is therefore notable that among full-term infants, exposure to BV during gestation has been associated with an increased risk of ventilation/respiratory distress at birth (1.28-fold) (Dingens et al., 2016), NICU admissions (1.4-fold), neonatal sepsis (Dingens et al., 2016) and duration of NICU stay >2 days (15% vs 6%) (Laxmi et al., 2011).

Beyond the microbiological and metabolomic changes associated with BV, recent research has identified the formation of a dense biofilm adhering to the endocervical epithelium as a common, and potentially important character of BV. Biofilms are multicellular mono- or polymicrobial communities encapsulated in an extracellular polysaccharide (EPS) matrix that are capable of self-reproduction and can be associated with infection (Jain, Gupta, Agrawal, Khare, & Jain, 2007). The physical structure of the EPS-encapsulated biofilm allows bacteria to survive otherwise inhospitable conditions and often increases their resistance to antimicrobial treatments and host immune responses. While many BV-associated bacteria have a tendency to form a biofilm (Alves, Castro, Sousa, & Cereija, 2014), the biofilm identified in BV is principally composed of *G. vaginalis* (Alves et al., 2014; Machado & Cerca, 2015). Vaginal biofilms are typically polymicrobial, *A. vaginae* is commonly observed (cooccurring in 60%–80% of vaginal biofilms) and in some studies has been reported to make up to 40% of the biofilm mass (Verstraelen & Swidsinski, 2013). Other bacterial taxa, including *Lactobacillus*, *Bacteroides*, *Streptococcus* and *Veillonella* spp., have been described, but these taxa are inconsistently present (Verstraelen & Swidsinski, 2013). The role of biofilm in the aetiology of BV remains unclear, and although it may play an important role, the overall process is likely more complicated as adherence of *G. vaginalis* to epithelial cells has been shown to depend on an elevated pH (optimal ~pH 6) above the typical vaginal pH of <4.5 (Peeters & Piot, 1985). Nevertheless, studies have shown these BV-associated biofilms tolerate higher concentrations of antibiotics (Swidsinski et al., 2014) and may therefore be important for difficult to treat or recurrent cases of BV.

Although the aetiology of BV remains enigmatic 125 years after Döderlein-related abnormal vaginal secretions to definable microbiological changes, modern molecular techniques have provided fresh hypotheses and a clearer mechanistic understanding of the vaginal microbiomes role in health and disease.

SUMMARY AND CONCLUSIONS

The human vagina harbours a unique and complex microbial ecosystem wherein the composition and function of the vaginal microbiota influences, and is influenced by, the human host. *Lactobacillus* spp. promote vaginal health and prevent the

colonization of potentially pathogenic species via competitive exclusion and the production of lactic acid, bacteriocins and biosurfactants. Modern molecular techniques, including genomic, proteomic, metagenomic, metatranscriptomic, metabolomic and immunological studies, have provided additional evidence in support of existing paradigms, have provided new mechanistic insights and occasionally reshaped our understanding of this important microbiome. *L. crispatus* (CST-I), *L. iners* (CST-III) and the *Lactobacillus*-depauperate state (CST-IV) have been relatively well characterized; however, much less is known about *L. gasseri* (CST-II) and *L. jensenii* (CST-V). Further, the utilization of proteomics and metabolomics allows for further functional insight into how these species influence and interact with host physiology. However, more in-depth studies are merited in order to better understand the dynamics between host and microbial interactions and how they influence reproductive health.

REFERENCES

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. *Science Translational Medicine*, *6*(237), 237ra65. https://doi.org/10.1126/scitranslmed.3008599.

Aagaard, K., Riehle, K., Ma, J., Segata, N., Mistretta, T.-A., Coarfa, C., et al. (2012). A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS One, 7*(6), e36466. https://doi.org/10.1371/journal.pone.0036466.

Ahmed, A., Earl, J., Retchless, A., Hillier, S. L., Rabe, L. K., Cherpes, T. L., et al. (2012). Comparative genomic analyses of 17 clinical isolates of *Gardnerella vaginalis* provide evidence of multiple genetically isolated clades consistent with subspeciation into genovars. *Journal of Bacteriology*, *194*(15), 3922–3937. https://doi.org/10.1128/JB.00056-12.

Albert, A. Y. K., Chaban, B., Wagner, E. C., Schellenberg, J. J., Links, M. G., van Schalkwyk, J., et al. (2015). A study of the vaginal microbiome in healthy Canadian women utilizing cpn60-based molecular profiling reveals distinct *Gardnerella* subgroup community state types. *PLoS One, 10*(8), e0135620. https://doi.org/10.1371/journal.pone.0135620.

Aldunate, M., Srbinovski, D., Hearps, A. C., Latham, C. F., Ramsland, P. A., Gugasyan, R., et al. (2015). Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. *Frontiers in Physiology*, *6*, 164. https://doi.org/10.3389/fphys.2015.00164.

Aldunate, M., Tyssen, D., Johnson, A., Zakir, T., Sonza, S., Moench, T., et al. (2013). Vaginal concentrations of lactic acid potently inactivate HIV. *The Journal of Antimicrobial Chemotherapy*, *68*(9), 2015–2025. https://doi.org/10.1093/jac/dkt156.

Al Kassaa, I., Hamze, M., Hober, D., Chihib, N.-E., & Drider, D. (2014). Identification of vaginal lactobacilli with potential probiotic properties isolated from women in North Lebanon. *Microbial Ecology*, *67*(3), 722–734. https://doi.org/10.1007/s00248-014-0384-7.

Allsworth, J. E., & Peipert, J. F. (2007). Prevalence of bacterial vaginosis: 2001–2004 national health and nutrition examination survey data. *Obstetrics and Gynecology*, *109*(1), 114–120. https://doi.org/10.1097/01.AOG.0000247627.84791.91.

Alnaif, B., & Drutz, H. P. (2000). Bacterial vaginosis increases in pessary users. *International Urogynecology Journal and Pelvic Floor Dysfunction*, *11*(4), 219–222. discussion 222–3.

Alves, P., Castro, J., Sousa, C., & Cereija, T. B. (2014). *Gardnerella vaginalis* outcompetes 29 other bacterial species isolated from patients with bacterial vaginosis, using in an in vitro biofilm formation model. *The Journal of Infectious Diseases*, *210*, 593–596.

Amarasekara, R., Jayasekara, R. W., Senanayake, H., & Dissanayake, V. H. W. (2015). Microbiome of the placenta in pre-eclampsia supports the role of bacteria in the multifactorial cause of pre-eclampsia. *The Journal of Obstetrics and Gynaecology Research*, *41*(5), 662–669. https://doi.org/10.1111/jog.12619.

Amsel, R., Totten, P. A., Spiegel, C. A., Chen, K. C. S., Eschenbach, D., & Holmes, K. K. (1983). Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *The American Journal of Medicine*, *74*(1), 14–22. https://doi.org/10.1016/0002-9343(83)91112-9.

Anahtar, M. N., Byrne, E. H., Doherty, K. E., Bowman, B. A., Yamamoto, H. S., Soumillon, M., et al. (2015). Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. *Immunity*, *42*(5), 965–976.

Anderson, D. J., Marathe, J., & Pudney, J. (2014). The structure of the human vaginal stratum corneum and its role in immune defense. *American Journal of Reproductive Immunology*, *71*(6), 618–623. https://doi.org/10.1111/aji.12230.

Andreu, A., Stapleton, A. E., Fennell, C. L., Hillier, S. L., & Stamm, W. E. (1995). Hemagglutination, adherence, and surface properties of vaginal Lactobacillus species. *Journal of Infectious Diseases*, *171*(5), 1237–1243.

Anton, G., Rid, J., Mylonas, I., Friese, K., & Weissenbacher, E.-R. (2008). Evidence of a TH1-shift of local vaginal inflammatory response during bacterial vaginosis. *Infection*, *36*(2), 147–152. https://doi.org/10.1007/s15010-007-7152-2.

Antonio, M. A., Hawes, S. E., & Hillier, S. L. (1999). The identification of vaginal *Lactobacillus* species and the demographic and microbiologic characteristics of women colonized by these species. *Journal of Infectious Diseases*, *180*(6), 1950–1956. https://doi.org/10.1086/315109.

Antonio, M. A. D., Meyn, L. A., Murray, P. J., Busse, B., & Hillier, S. L. (2009). Vaginal colonization by probiotic *Lactobacillus crispatus* CTV-05 is decreased by sexual activity and endogenous Lactobacilli. *Journal of Infectious Diseases*, *199*(10), 1506–1513. https://doi.org/10.1086/598686.

Antonio, M. A. D., Rabe, L. K., & Hillier, S. L. (2005). Colonization of the rectum by *Lactobacillus* species and decreased risk of bacterial vaginosis. *Journal of Infectious Diseases*, *192*(3), 394–398. https://doi.org/10.1086/430926.

Anukam, K. C., Macklaim, J. M., Gloor, G. B., Reid, G., Boekhorst, J., Renckens, B., et al. (2013). Genome sequence of *Lactobacillus pentosus* KCA1: Vaginal isolate from a healthy premenopausal woman. *PLoS One, 8*(3), e59239. https://doi.org/10.1371/journal.pone.0059239.

Anukam, K. C., Osazuwa, E., Osemene, G. I., Ehigiagbe, F., Bruce, A. W., & Reid, G. (2006). Clinical study comparing probiotic *Lactobacillus* GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. *Microbes and Infection*, *8*(12 – 13), 2772–2776. https://doi.org/10.1016/j.micinf.2006.08.008.

Arakawa, K., Kawai, Y., Iioka, H., Tanioka, M., Nishimura, J., Kitazawa, H., et al. (2009). Effects of gassericins A and T, bacteriocins produced by *Lactobacillus gasseri*, with glycine on custard cream preservation. *Journal of Dairy Science*, *92*(6), 2365–2372. https://doi.org/10.3168/jds.2008-1240.

Aroutcheva, A., Gariti, D., Simon, M., Shott, S., Faro, J., Simoes, J. A., et al. (2001). Defense factors of vaginal lactobacilli. *American Journal of Obstetrics and Gynecology*, *185*(2), 375–379. https://doi.org/10.1067/mob.2001.115867.

Atassi, F., Brassart, D., Grob, P., Graf, F., & Servin, A. L. (2006a). Lactobacillus strains isolated from the vaginal microbiota of healthy women inhibit *Prevotella bivia* and *Gardnerella vaginalis* in coculture and cell culture. *FEMS Immunology & Medical Microbiology*, *48*(3), 424–432. https://doi.org/10.1111/j.1574-695X.2006.00162.x.

Atassi, F., Brassart, D., Grob, P., Graf, F., & Servin, A. L. (2006b). Vaginal *Lactobacillus* isolates inhibit uropathogenic Escherichia coli. *FEMS Microbiology Letters*, *257*(1), 132–138. https://doi.org/10.1111/j.1574-6968.2006.00163.x.

Atassi, F., & Servin, A. L. (2010). Individual and co-operative roles of lactic acid and hydrogen peroxide in the killing activity of enteric strain *Lactobacillus johnsonii* NCC933 and vaginal strain *Lactobacillus gasseri* KS120.1 against enteric, uropathogenic and vaginosis-associated pathogens. *FEMS Microbiology Letters*, *304*(1), 29–38. https://doi.org/10.1111/j.1574-6968.2009.01887.x.

Averette, H. E., Weinstein, G. D., & Frost, P. (1970). Autoradiographic analysis of cell proliferation kinetics in human genital tissues. *American Journal of Obstetrics and Gynecology*, *108*(1), 8–17.

Ayre, W. B. (1951). The glycogen-estrogen relationship in the vaginal tract. *The Journal of Clinical Endocrinology & Metabolism*, *11*(1), 103–110. https://doi.org/10.1210/jcem-11-1-103.

Barfod, K. K., Roggenbuck, M., Hansen, L. H., Schjørring, S., Larsen, S. T., Sørensen, S. J., et al. (2013). The murine lung microbiome in relation to the intestinal and vaginal bacterial communities. *BMC Microbiology*, *13*(1), 303. https://doi.org/10.1186/1471-2180-13-303.

Bautista, C. T., Wurapa, E. K., Sateren, W. B., Morris, S. M., Hollingsworth, B. P., & Sanchez, J. L. (2017). Association of bacterial vaginosis with chlamydia and gonorrhea among women in the U.S. Army. *American Journal of Preventive Medicine*, *52*(5), 632–639. https://doi.org/10.1016/j.amepre.2016.09.016.

Benito, R., Vazquez, J. A., Berron, S., Fenoll, A., & Saez-Neito, J. A. (1986). A modified scheme for biotyping *Gardnerella vaginalis*. *Journal of Medical Microbiology*, *21*(4), 357–359. https://doi.org/10.1099/00222615-21-4-357.

Bergmann, S., Wild, D., Diekmann, O., Frank, R., Bracht, D., Chhatwal, G. S., et al. (2003). Identification of a novel plasmin(ogen)-binding motif in surface displayed α-enolase of *Streptococcus pneumoniae*. *Molecular Microbiology*, *49*(2), 411–423. https://doi.org/10.1046/j.1365-2958.2003.03557.x.

Bisanz, J. E., Enos, M. K., PrayGod, G., Seney, S., Macklaim, J. M., Chilton, S., et al. (2015). Microbiota at multiple body sites during pregnancy in a rural Tanzanian population and effects of Moringa-supplemented probiotic yogurt. *Applied and Environmental Microbiology*, *81*(15), 4965–4975. https://doi.org/10.1128/AEM.00780-15.

Blaskewicz, C. D., Pudney, J., & Anderson, D. J. (2011). Structure and function of intercellular junctions in human cervical and vaginal mucosal epithelia. *Biology of Reproduction*, *85*(1), 97–104. https://doi.org/10.1095/biolreprod.110.090423.

Boakes, S., Ayala, T., Herman, M., Appleyard, A. N., Dawson, M. J., & Cortés, J. (2012). Generation of an actagardine A variant library through saturation mutagenesis. *Applied Microbiology and Biotechnology*, *95*(6), 1509–1517. https://doi.org/10.1007/s00253-012-4041-0.

Borgdorff, H., Armstrong, S. D., Tytgat, H. L. P., Xia, D., Ndayisaba, G. F., Wastling, J. M., et al. (2016). Unique insights in the cervicovaginal *Lactobacillus iners* and L. crispatus proteomes and their associations with microbiota dysbiosis. *PLoS One*, *11*(3), e0150767–14. https://doi.org/10.1371/journal.pone.0150767.

Borgdorff, H., Tsivtsivadze, E., Verhelst, R., Marzorati, M., Jurriaans, S., Ndayisaba, G. F., et al. (2014). *Lactobacillus*-dominated cervicovaginal microbiota associated with reduced

HIV|[sol]|STI prevalence and genital HIV viral load in African women. *The ISME Journal*, *8*(9), 1781–1793. https://doi.org/10.1038/ismej.2014.26.

Borgogna, J.-L., Roberts, D., Rath, J. M., Glover, E. D., Ravel, J., Shardell, M., et al. (2016). 3: Cigarette smoking is associated with an altered metabolomic profile of the vaginal environment. *American Journal of Obstetrics and Gynecology*, *215*(6), S815. https://doi.org/10.1016/j.ajog.2016.09.004.

Boris, S., Suárez, J. E., Vázquez, F., & Barbés, C. (1998). Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. *Infection and Immunity*, *66*(5), 1985–1989.

Boskey, E. R., Cone, R. A., Whaley, K. J., & Moench, T. R. (2001). Origins of vaginal acidity: High D/L lactate ratio is consistent with bacteria being the primary source. *Human Reproduction (Oxford, England)*, *16*(9), 1809–1813.

Boskey, E. R., Telsch, K. M., Whaley, K. J., Moench, T. R., & Cone, R. A. (1999). Acid production by vaginal flora in vitro is consistent with the rate and extent of vaginal acidification. *Infection and Immunity*, *67*(10), 5170–5175.

Briselden, A. M., & Hillier, S. L. (1990). Longitudinal study of the biotypes of *Gardnerella vaginalis*. *Journal of Clinical Microbiology*, *28*(12), 2761–2764.

Brotman, R. M., Bradford, L. L., Conrad, M., Gajer, P., Ault, K., Peralta, L., et al. (2012). Association between *Trichomonas vaginalis* and vaginal bacterial community composition among reproductive-age women. *Sexually Transmitted Diseases*, *39*(10), 807–812. https://doi.org/10.1097/OLQ.0b013e3182631c79.

Brotman, R. M., Ghanem, K. G., Klebanoff, M. A., Taha, T. E., Scharfstein, D. O., & Zenilman, J. M. (2008). The effect of vaginal douching cessation on bacterial vaginosis: A pilot study. *American Journal of Obstetrics and Gynecology*, *198*(6), 628.e1–628.e7. https://doi.org/10.1016/j.ajog.2007.11.043.

Brotman, R. M., He, X., Gajer, P., Fadrosh, D., Sharma, E., Mongodin, E. F., et al. (2014). Association between cigarette smoking and the vaginal microbiota: A pilot study. *BMC Infectious Diseases*, *14*(1), 471. https://doi.org/10.1186/1471-2334-14-471.

Brotman, R. M., Klebanoff, M. A., Nansel, T. R., Yu, K. F., Andrews, W. W., Zhang, J., et al. (2010). Bacterial vaginosis assessed by gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. *Journal of Infectious Diseases*, *202*(12), 1907–1915. https://doi.org/10.1086/657320.

Brotman, R. M., Ravel, J., Cone, R. A., & Zenilman, J. M. (2010). Rapid fluctuation of the vaginal microbiota measured by Gram stain analysis. *Sexually Transmitted Infections*, *86*(4), 297–302. https://doi.org/10.1136/sti.2009.040592.

Brotman, R. M., Shardell, M. D., Gajer, P., Fadrosh, D., Chang, K., Silver, M. I., et al. (2014). Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. *Menopause (New York, N.Y.)*, *21*(5), 450–458. https://doi.org/10.1097/GME.0b013e3182a4690b.

Brotman, R. M., Shardell, M. D., Gajer, P., Tracy, J. K., Zenilman, J. M., Ravel, J., et al. (2014). Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *Journal of Infectious Diseases*, *210*(11), 1723–1733. https://doi.org/10.1093/infdis/jiu330.

Burton, J. P., Cadieux, P. A., & Reid, G. (2003). Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. *Applied and Environmental Microbiology*, *69*(1), 97–101. https://doi.org/10.1128/AEM.69.1.97-101.2003.

Burton, J. P., & Reid, G. (2002). Evaluation of the bacterial vaginal flora of 20 postmenopausal women by direct (Nugent score) and molecular (polymerase chain reaction and denaturing gradient gel electrophoresis) techniques. *Journal of Infectious Diseases*, *186*(12), 1770–1780. https://doi.org/10.1086/345761.

Busarcevic, M., & Dalgalarrondo, M. (2012). Purification and genetic characterisation of the novel bacteriocin LS2 produced by the human oral strain *Lactobacillus salivarius* BGHO1. *International Journal of Antimicrobial Agents*, *40*(2), 127–134.

Cadieux, P. A., Burton, J., Devillard, E., & Reid, G. (2009). *Lactobacillus* by-products inhibit the growth and virulence of uropathogenic *Escherichia coli*. *Journal of Physiology and Pharmacology*, *60*, 13–18.

Chaban, B., Links, M. G., Jayaprakash, T. P., Wagner, E. C., Bourque, D. K., Lohn, Z., et al. (2014). Characterization of the vaginal microbiota of healthy Canadian women through the menstrual cycle. *Microbiome*, *2*(1), 23. https://doi.org/10.1186/2049-2618-2-23.

Cherpes, T. L., Hillier, S. L., Meyn, L. A., Busch, J. L., & Krohn, M. A. (2008). A delicate balance: Risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race, and positive herpes simplex virus type 2 serology. *Sexually Transmitted Diseases*, *35*(1), 78–83. https://doi.org/10.1097/OLQ.0b013e318156a5d0.

Cherpes, T. L., Meyn, L. A., Krohn, M. A., Lurie, J. G., & Hillier, S. L. (2003). Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. *Clinical Infectious Diseases*, *37*(3), 319–325. https://doi.org/10.1086/375819.

Cohen, M. S., Black, J. R., Proctor, R. A., & Sparling, P. F. (1984). Host defences and the vaginal mucosa. A re-evaluation. *Scandinavian Journal of Urology and Nephrology. Supplementum*, *86*, 13–22.

Cohen, C. R., Lingappa, J. R., Baeten, J. M., Ngayo, M. O., Spiegel, C. A., Hong, T., et al. (2012). Bacterial vaginosis associated with increased risk of female-to-male HIV-1 transmission: A prospective cohort analysis among African couples. *9*(6), e1001251. https://doi.org/10.1371/journal.pmed.1001251.

Collado, M. C., Rautava, S., Aakko, J., Isolauri, E., & Salminen, S. (2016). Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Scientific Reports*, *6*(1), 565. https://doi.org/10.1038/srep23129.

Collins, S., Beigi, R., Mellen, C., O'Sullivan, D., & Tulikangas, P. (2015). The effect of pessaries on the vaginal microenvironment. *American Journal of Obstetrics and Gynecology, 212*(1), 60.e1–6. https://doi.org/10.1016/j.ajog.2014.07.024.

Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: Developing innate immunity for food. *Nature Reviews. Microbiology*, *3*(10), 777–788. https://doi.org/10.1038/nrmicro1273.

Cotter, P. D., Ross, R. P., & Hill, C. (2013). Bacteriocins—A viable alternative to antibiotics? *Nature Reviews. Microbiology*, *11*(2), 95–105. https://doi.org/10.1038/nrmicro2937.

Cruden, D. L., & Galask, R. P. (2009). Reduction of trimethylamine oxide to trimethylamine by *Mobiluncus* strains isolated from patients with bacterial vaginosis. *Microbial Ecology in Health and Disease*, *1*, 95–100. https://doi.org/10.3109/08910608809140187;page:string:Article/Chapter.

Czaja, C. A., Stapleton, A. E., Yarova-Yarovaya, Y., & Stamm, W. E. (2007). Phase I trial of a *Lactobacillus crispatus* vaginal suppository for prevention of recurrent urinary tract infection in women. *Infectious Diseases in Obstetrics and Gynecology*, *2007*(3), 35387–35388. https://doi.org/10.1155/2007/35387.

Damelin, L. H., Paximadis, M., Mavri-Damelin, D., Birkhead, M., Lewis, D. A., & Tiemessen, C. T. (2011). Identification of predominant culturable vaginal *Lactobacillus* species and associated bacteriophages from women with and without vaginal discharge syndrome in South Africa. *Journal of Medical Microbiology*, *60*(Pt. 2), 180–183. https://doi.org/10.1099/jmm.0.024463-0.

Danielsson, D., Teigen, P. K., & Moi, H. (2011). The genital econiche: Focus on microbiota and bacterial vaginosis. *Annals of the New York Academy of Sciences*, *1230*(1), 48–58. https://doi.org/10.1111/j.1749-6632.2011.06041.x.

Dareng, E. O., Ma, B., Famooto, A. O., Akarolo-Anthony, S. N., Offiong, R. A., Olaniyan, O., et al. (2015). Prevalent high-risk HPV infection and vaginal microbiota in Nigerian women. *Epidemiology and Infection*, *144*(01), 123–137. https://doi.org/10.1017/S0950268815000965.

De Backer, E., Verhelst, R., Verstraelen, H., Alqumber, M. A., Burton, J. P., Tagg, J. R., et al. (2007). Quantitative determination by real-time PCR of four vaginal *Lactobacillus* species, *Gardnerella vaginalis* and *Atopobium vaginae* indicates an inverse relationship between L. gasseri and L. iners. *BMC Microbiology*, *7*(1), 115. https://doi.org/10.1186/1471-2180-7-115.

Deveau, H., Garneau, J. E., & Moineau, S. (2010). CRISPR/Cas system and its role in phage-bacteria interactions. *Annual Review of Microbiology*, *64*(1), 475–493. https://doi.org/10.1146/annurev.micro.112408.134123.

DiGiulio, D. B., Callahan, B. J., McMurdie, P. J., Costello, E. K., Lyell, D. J., Robaczewska, A., et al. (2015). Temporal and spatial variation of the human microbiota during pregnancy. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(35), 11060–11065. https://doi.org/10.1073/pnas.1502875112.

Ding, S.-Z., O'Hara, A. M., Denning, T. L., Dirden-Kramer, B., Mifflin, R. C., Reyes, V. E., et al. (2004). *Helicobacter pylori* and H2O2 increase AP endonuclease-1/redox factor-1 expression in human gastric epithelial cells. *Gastroenterology*, *127*(3), 845–858.

Dingens, A. S., Fairfortune, T. S., Reed, S., & Mitchell, C. (2016). Bacterial vaginosis and adverse outcomes among full-term infants: A cohort study. *BMC Pregnancy and Childbirth*, *16*(1), 278. https://doi.org/10.1186/s12884-016-1073-y.

Döderlein, A. (1892). *Das Scheidensekret und seine bedeutung fur das puerperal-fieber.* (pp. 1–118). Leipzig, Besold.

Doerflinger, S. Y., Throop, A. L., & Herbst-Kralovetz, M. M. (2014). Bacteria in the vaginal microbiome alter the innate immune response and barrier properties of the human vaginal epithelia in a species-specific manner. *Journal of Infectious Diseases*, *209*(12), 1989–1999. https://doi.org/10.1093/infdis/jiu004.

Dols, J. A. M., Reid, G., Brown, J. M., Tempelman, H., Bontekoe, T. R., Quint, W. G. V., et al. (2012). HPV type distribution and cervical cytology among HIV-positive Tanzanian and South African women. *ISRN Obstetrics and Gynecology*, *2012*, 514146. https://doi.org/10.5402/2012/514146.

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., et al. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(26), 11971–11975. https://doi.org/10.1073/pnas.1002601107.

Dong, X.-D., Li, X.-R., Luan, J.-J., Liu, X.-F., Peng, J., Luo, Y.-Y., et al. (2015). Bacterial communities in neonatal feces are similar to mothers' placentae. *The Canadian Journal*

of Infectious Diseases & Medical Microbiology = Journal Canadien Des Maladies Infectieuses Et De La Microbiologie Medicale, *26*(2), 90–94.

Donia, M. S., Cimermancic, P., Schulze, C. J., Wieland Brown, L. C., Martin, J., Mitreva, M., et al. (2014). A systematic analysis of biosynthetic gene clusters in the human microbiome reveals a common family of antibiotics. *Cell*, *158*(6), 1402–1414. https://doi.org/10.1016/j.cell.2014.08.032.

Doyle, R. M., Alber, D. G., Jones, H. E., Harris, K., Fitzgerald, F., Peebles, D., et al. (2014). Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery. *Placenta*, *35*(12), 1099–1101. https://doi.org/10.1016/j.placenta.2014.10.007.

Draper, D. L., Landers, D. V., Krohn, M. A., Hillier, S. L., Wiesenfeld, H. C., & Heine, R. P. (2000). Levels of vaginal secretory leukocyte protease inhibitor are decreased in women with lower reproductive tract infections. *American Journal of Obstetrics and Gynecology*, *183*(5), 1243–1248. https://doi.org/10.1067/mob.2000.107383.

Drapkin, R., von Horsten, H. H., Lin, Y., Mok, S. C., Crum, C. P., Welch, W. R., et al. (2005). Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Research*, *65*(6), 2162–2169. https://doi.org/10.1158/0008-5472.CAN-04-3924.

Drell, T., Lillsaar, T., Tummeleht, L., Simm, J., Aaspõllu, A., Väin, E., et al. (2013). Characterization of the vaginal micro- and mycobiome in asymptomatic reproductive-age Estonian women. *PLoS One, 8*(1), e54379. https://doi.org/10.1371/journal.pone.0054379.

Dumonceaux, T. J., Schellenberg, J., Goleski, V., Hill, J. E., Jaoko, W., Kimani, J., et al. (2009). Multiplex detection of bacteria associated with normal microbiota and with bacterial vaginosis in vaginal swabs by use of oligonucleotide-coupled fluorescent microspheres. *Journal of Clinical Microbiology*, *47*(12), 4067–4077. https://doi.org/10.1128/JCM.00112-09.

El Aila, N. A., Tency, I., Claeys, G., Verstraelen, H., Saerens, B., Santiago, G. L. D. S., et al. (2009). Identification and genotyping of bacteria from paired vaginal and rectal samples from pregnant women indicates similarity between vaginal and rectal microflora. *BMC Infectious Diseases*, *9*(1), 167. https://doi.org/10.1186/1471-2334-9-167.

Emmanuel, D. G. V., Madsen, K. L., Churchill, T. A., Dunn, S. M., & Ametaj, B. N. (2007). Acidosis and lipopolysaccharide from Escherichia coli B:055 cause hyperpermeability of rumen and colon tissues. *Journal of Dairy Science*, *90*(12), 5552–5557. https://doi.org/10.3168/jds.2007-0257.

Eren, A. M., Zozaya, M., Taylor, C. M., Dowd, S. E., Martin, D. H., & Ferris, M. J. (2011). Exploring the diversity of *Gardnerella vaginalis* in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation. *PLoS One, 6*(10), e26732. https://doi.org/10.1371/journal.pone.0026732.

Eschenbach, D. A., Davick, P. R., Williams, B. L., Klebanoff, S. J., Young-Smith, K., Critchlow, C. M., et al. (1989). Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. *Journal of Clinical Microbiology*, *27*(2), 251–256.

Eschenbach, D. A., Patton, D. L., Hooton, T. M., Meier, A. S., Stapleton, A., Aura, J., et al. (2001). Effects of vaginal intercourse with and without a condom on vaginal flora and vaginal epithelium. *Journal of Infectious Diseases*, *183*(6), 913–918. https://doi.org/10.1086/319251.

Eschenbach, D. A., Patton, D. L., Meier, A., Thwin, S. S., Aura, J., Stapleton, A., et al. (2000). Effects of oral contraceptive pill use on vaginal flora and vaginal epithelium. *Contraception*, *62*(3), 107–112.

Eschenbach, D. A., Thwin, S. S., Patton, D. L., Hooton, T. M., Stapleton, A. E., Agnew, K., et al. (2000). Influence of the normal menstrual cycle on vaginal tissue, discharge, and microflora. *Clinical Infectious Diseases*, *30*(6), 901–907. https://doi.org/10.1086/313818.

Falsen, E., Pascual, C., Sjödén, B., Ohlén, M., & Collins, M. D. (1999). Phenotypic and phylogenetic characterization of a novel Lactobacillus species from human sources: Description of *Lactobacillus iners* sp. nov. *International Journal of Systematic Bacteriology*, *49*(Pt. 1), 217–221. https://doi.org/10.1099/00207713-49-1-217.

Farage, M., & Maibach, H. (2006). Lifetime changes in the vulva and vagina. *Archives of Gynecology and Obstetrics*, *273*(4), 195–202. https://doi.org/10.1007/s00404-005-0079-x.

Farage, M. A., & Maibach, H. I. (2011). Morphology and physiological changes of genital skin and mucosa. *Current Problems in Dermatology*, *40*, 9–19. https://doi.org/10.1159/000321042.

Farage, M. A., Miller, K. W., & Sobel, J. D. (2010). Dynamics of the vaginal ecosystem—Hormonal influences. *Infectious Diseases: Research and Treatment*, *3*, 1–15.

Ferris, M. J., Masztal, A., Aldridge, K. E., Fortenberry, J. D., Fidel, P. L., & Martin, D. H. (2004). Association of *Atopobium vaginae*, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. *BMC Infectious Diseases*, *4*(1), 5. https://doi.org/10.1186/1471-2334-4-5.

Ferris, M. J., Norori, J., Zozaya-Hinchliffe, M., & Martin, D. H. (2007). Cultivation-independent analysis of changes in bacterial vaginosis flora following metronidazole treatment. *Journal of Clinical Microbiology*, *45*(3), 1016–1018. https://doi.org/10.1128/JCM.02085-06.

Fichorova, R. N., Beatty, N., Sassi, R. R. S., Yamamoto, H. S., Allred, E. N., Leviton, A., et al. (2015). Systemic inflammation in the extremely low gestational age newborn following maternal genitourinary infections. *American Journal of Reproductive Immunology*, *73*(2), 162–174. https://doi.org/10.1111/aji.12313.

Fichorova, R. N., Onderdonk, A. B., Yamamoto, H., Delaney, M. L., DuBois, A. M., Allred, E., et al. (2011). Maternal microbe-specific modulation of inflammatory response in extremely low-gestational-age newborns. *mBio, 2*(1), e00280–10. https://doi.org/10.1128/mBio.00280-10.

Fontaine, E. A., Claydon, E., & Taylor-Robinson, D. (2009). Lactobacilli from women with or without bacterial vaginosis and observations on the significance of hydrogen peroxide. *Microbial Ecology in Health and Disease*, *9*, 135–141.

Forney, L. J., Gajer, P., Williams, C. J., Schneider, G. M., Koenig, S. S. K., McCulle, S. L., et al. (2010). Comparison of self-collected and physician-collected vaginal swabs for microbiome analysis. *Journal of Clinical Microbiology*, *48*(5), 1741–1748. https://doi.org/10.1128/JCM.01710-09.

France, M. T., Mendes-Soares, H., & Forney, L. J. (2016). Genomic comparisons of *Lactobacillus crispatus* and Lactobacillus iners reveal potential ecological drivers of community composition in the vagina. *Applied and Environmental Microbiology*, *82*(24), 7063–7073. https://doi.org/10.1128/AEM.02385-16.

Frank, D. N., Manigart, O., Leroy, V., Meda, N., Valéa, D., Zhang, W., et al. (2012). Altered vaginal microbiota are associated with perinatal mother-to-child transmission of HIV in African women from Burkina Faso. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *60*(3), 299–306. https://doi.org/10.1097/QAI.0b013e31824e4bdb.

Fredricks, D. N., Fiedler, T. L., & Marrazzo, J. M. (2005). Molecular identification of bacteria associated with bacterial vaginosis. *The New England Journal of Medicine*, *353*(18), 1899–1911. https://doi.org/10.1056/NEJMoa043802.

Gajer, P., Brotman, R. M., Bai, G., Sakamoto, J., Schütte, U. M. E., Zhong, X., et al. (2012). Temporal dynamics of the human vaginal microbiota. *Science Translational Medicine*, *4*(132), 132ra52. https://doi.org/10.1126/scitranslmed.3003605.

Gao, W., Weng, J., Gao, Y., & Chen, X. (2013). Comparison of the vaginal microbiota diversity of women with and without human papillomavirus infection: A cross-sectional study. *BMC Infectious Diseases*, *13*(1), 271. https://doi.org/10.1186/1471-2334-13-271.

Gardner, H. L., & Dukes, C. D. (1954). New etiologic agent in nonspecific bacterial vaginitis. *Science (New York, N.Y.)*, *120*(3125), 853.

Ghartey, J. P., Smith, B. C., Chen, Z., Buckley, N., Lo, Y., Ratner, A. J., et al. (2014). *Lactobacillus crispatus* dominant vaginal microbiome is associated with inhibitory activity of female genital tract secretions against *Escherichia coli*. *PLoS One, 9*(5), e96659. https://doi.org/10.1371/journal.pone.0096659.

Gibbs, R. S. (2007). Asymptomatic bacterial vaginosis: Is it time to treat? *American Journal of Obstetrics and Gynecology*, *196*(6), 495–496. https://doi.org/10.1016/j.ajog.2007.04.001.

Gillet, E., Meys, J. F., Verstraelen, H., Bosire, C., De Sutter, P., Temmerman, M., et al. (2011). Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: A meta-analysis. *BMC Infectious Diseases, 11*(1), 10. https://doi.org/10.1186/1471-2334-11-10.

Gillissen, G., Schumacher, M., & Breuer-Werle, M. (1991). Modulation of antimicrobial effects of beta-lactams by amino acids in vitro. *Zentralblatt Für Bakteriologie*, *275*(2), 223–232. https://doi.org/10.1016/S0934-8840(11)80069-1.

Ginkel, P. D., Soper, D. E., Bump, R. C., & Dalton, H. P. (1993). Vaginal flora in postmenopausal women: The effect of estrogen replacement. *Infectious Diseases in Obstetrics and Gynecology*, *1*(2), 94–97. https://doi.org/10.1155/S1064744993000225.

Gong, Z., Luna, Y., Yu, P., & Fan, H. (2014). Lactobacilli inactivate *Chlamydia trachomatis* through lactic acid but not H2O2. *PLoS One, 9*(9), e107758. https://doi.org/10.1371/journal.pone.0107758.

Goytia, M., Dhulipala, V. L., & Shafer, W. M. (2013). Spermine impairs biofilm formation by *Neisseria gonorrhoeae*. *FEMS Microbiology Letters*, *343*(1), 64–69. https://doi.org/10.1111/1574-6968.12130.

Gregoire, A. T., Kandil, O., & Ledger, W. J. (1971). The glycogen content of human vaginal epithelial tissue. *Fertility and Sterility*, *22*, 64–68.

Gupta, S., Kumar, N., Singhal, N., Kaur, R., & Manektala, U. (2006). Vaginal microflora in postmenopausal women on hormone replacement therapy. *Indian Journal of Pathology & Microbiology*, *49*(3), 457–461.

Gustafsson, R. J., Ahrné, S., Jeppsson, B., Benoni, C., Olsson, C., Stjernquist, M., et al. (2011). The *Lactobacillus* flora in vagina and rectum of fertile and postmenopausal healthy Swedish women. *BMC Women's Health*, *11*(1), 17. https://doi.org/10.1186/1472-6874-11-17.

Hammerschlag, M. R., Alpert, S., Onderdonk, A. B., Thurston, P., Drude, E., McCormack, W. M., et al. (1978). Anaerobic microflora of the vagina in children. *American Journal of Obstetrics and Gynecology*, *131*(8), 853–856.

Hammerschlag, M. R., Alpert, S., Rosner, I., Thurston, P., Semine, D., McComb, D., et al. (1978). Microbiology of the vagina in children: Normal and potentially pathogenic organisms. *Pediatrics*, *62*(1), 57–62.

Hammes, W. P., & Hertel, C. (2015). *Lactobacillus. In W. B. Whitman, F. Rainey, P. K. mpfer, M. Trujillo, J. Chun, & P. DeVos, et al.Bergey's manual of systematics of archaea and bacteria*. Hoboken, NJ: John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118960608.gbm00604.

Harwich, M. D., Alves, J. M., Buck, G. A., Strauss, J. F., Patterson, J. L., Oki, A. T., et al. (2010). Drawing the line between commensal and pathogenic *Gardnerella vaginalis* through genome analysis and virulence studies. *BMC Genomics*, *11*(1), 375. https://doi.org/10.1186/1471-2164-11-375.

Hawes, S. E., Hillier, S. L., Benedetti, J., Stevens, C. E., Koutsky, L. A., Wølner-Hanssen, P., et al. (1996). Hydrogen peroxide—Producing Lactobacilli and acquisition of vaginal infections. *Journal of Infectious Diseases*, *174*(5), 1058–1063. https://doi.org/10.1093/infdis/174.5.1058.

Hay, P. E., Lamont, R. F., Taylor-Robinson, D., Morgan, D. J., Ison, C., & Pearson, J. (1994). Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. *BMJ*, *308*(6924), 295–298. https://doi.org/10.1136/bmj.308.6924.295.

Hecht, J. L., Onderdonk, A., Delaney, M., Allred, E. N., Kliman, H. J., Zambrano, E., et al. (2009). Characterization of chorioamnionitis in 2nd-trimester C-section placentas and correlation with microorganism recovery from subamniotic tissues. *Pediatric and Developmental Pathology*, *11*(1), 15–22. https://doi.org/10.2350/07-06-0285.1.

Hedges, S. R., Barrientes, F., Desmond, R. A., & Schwebke, J. R. (2006). Local and systemic cytokine levels in relation to changes in vaginal flora. *Journal of Infectious Diseases*, *193*(4), 556–562. https://doi.org/10.1086/499824.

Hemmerling, A., Harrison, W., Schroeder, A., Park, J., Korn, A., Shiboski, S., et al. (2009). Phase 1 dose-ranging safety trial of *Lactobacillus crispatus* CTV-05 (LACTIN-V) for the prevention of bacterial vaginosis. *Sexually Transmitted Diseases*, *36*(9), 564–569. https://doi.org/10.1097/OLQ.0b013e3181a74924.

Hemmerling, A., Harrison, W., Schroeder, A., Park, J., Korn, A., Shiboski, S., et al. (2010). Phase 2a study assessing colonization efficiency, safety, and acceptability of *Lactobacillus crispatus* CTV-05 in women with bacterial vaginosis. *Sexually Transmitted Diseases*, *37*(12), 745–750. https://doi.org/10.1097/OLQ.0b013e3181e50026.

Henderson, B., & Martin, A. (2011). Bacterial virulence in the moonlight: Multitasking bacterial moonlighting proteins are virulence determinants in infectious disease. *Infection and Immunity*, *79*(9), 3476–3491. https://doi.org/10.1128/IAI.00179-11.

Hernández-Rodríguez, C., Romero-González, R., Albani-Campanario, M., Figueroa-Damián, R., Meraz-Cruz, N., & Hernández-Guerrero, C. (2011). Vaginal microbiota of healthy pregnant Mexican women is constituted by four *Lactobacillus* species and several vaginosis-associated bacteria. *Infectious Diseases in Obstetrics and Gynecology*, *2011*(4), 851485–851489. https://doi.org/10.1155/2011/851485.

Hickey, R. J., Abdo, Z., Zhou, X., Nemeth, K., Hansmann, M., Osborn, T. W., et al. (2013). Effects of tampons and menses on the composition and diversity of vaginal microbial communities over time. *BJOG: An International Journal of Obstetrics & Gynaecology*, *120*(6), 695–704. discussion 704–6. https://doi.org/10.1111/1471-0528.12151.

Hickey, R. J., Zhou, X., Settles, M. L., Erb, J., Malone, K., Hansmann, M. A., et al. (2015). Vaginal microbiota of adolescent girls prior to the onset of menarche resemble those of reproductive-age women. *mBio, 6*(2), e00097–15. https://doi.org/10.1128/mBio.00097-15.

Hillier, S. L., & Holmes, K. K. (1999). Sexually transmitted diseases. In K. K. Holmes, P. Sparling, & P. Mardh (Eds.), *Bacterial vaginosis* (3rd ed., pp. 563–586). New York: McGraw-Hill.

Hillier, S. L., Krohn, M. A., Klebanoff, S. J., & Eschenbach, D. A. (1992). The relationship of hydrogen peroxide-producing lactobacilli to bacterial vaginosis and genital microflora in pregnant women. *Obstetrics and Gynecology*, *79*(3), 369–373.

Hillier, S. L., Krohn, M. A., Nugent, R. P., & Gibbs, R. S. (1992). Characteristics of three vaginal flora patterns assessed Gram stain among pregnant women. *American Journal of Obstetrics and Gynecology*, *166*(3), 938–944.

Hillier, S. L., Nugent, R. P., Eschenbach, D. A., Krohn, M. A., Gibbs, R. S., Martin, D. H., et al. (2009). Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The vaginal infections and prematurity study group. *The New England Journal of Medicine*, *333*(26), 1737–1742. https://doi.org/10.1056/NEJM199512283332604.

Hishinuma, F., Izaki, K., & Takahashi, H. (2014). Effects of glycine and D-amino acids on growth of various microorganisms. *Agricultural and Biological Chemistry*, *33*(11), 1577–1586. https://doi.org/10.1080/00021369.1969.10859511.

Hladik, F., Sakchalathorn, P., Ballweber, L., Lentz, G., Fialkow, M., Eschenbach, D., et al. (2007). Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity*, *26*(2), 257–270. https://doi.org/10.1016/j.immuni.2007.01.007.

Huang, Y.-E., Wang, Y., He, Y., Ji, Y., Wang, L.-P., Sheng, H.-F., et al. (2015). Homogeneity of the vaginal microbiome at the cervix, posterior fornix, and vaginal canal in pregnant Chinese women. *Microbial Ecology*, *69*(2), 407–414. https://doi.org/10.1007/s00248-014-0487-1.

Hummelen, R., Fernandes, A. D., Macklaim, J. M., Dickson, R. J., Changalucha, J., Gloor, G. B., et al. (2010). Deep sequencing of the vaginal microbiota of women with HIV. *PLoS One, 5*(8), e12078. https://doi.org/10.1371/journal.pone.0012078.

Hurmalainen, V., Edelman, S., Antikainen, J., Baumann, M., Lähteenmäki, K., & Korhonen, T. K. (2007). Extracellular proteins of *Lactobacillus crispatus* enhance activation of human plasminogen. *Microbiology (Reading, England)*, *153*(Pt. 4), 1112–1122. https://doi.org/10.1099/mic.0.2006/000901-0.

Husain, S. M., Wilks, M., Mupita, M., Reddy, S. P., Hennessy, E. M., Macfarlane, A. J., et al. (2014). Diversity and stability of cultured vaginal lactobacilli in pregnant women from a multi-ethnic urban UK population. *Journal of Applied Microbiology*, *117*(1), 258–265. https://doi.org/10.1111/jam.12506.

Huttenhower, C., Kostic, A. D., & Xavier, R. J. (2014). Inflammatory bowel disease as a model for translating the microbiome. *Immunity*, *40*(6), 843–854. https://doi.org/10.1016/j.immuni.2014.05.013.

Huycke, M. M., Abrams, V., & Moore, D. R. (2002). *Enterococcus faecalis* produces extracellular superoxide and hydrogen peroxide that damages colonic epithelial cell DNA. *Carcinogenesis*, *23*(3), 529–536.

Hyman, R. W., Fukushima, M., Diamond, L., Kumm, J., Giudice, L. C., & Davis, R. W. (2005). Microbes on the human vaginal epithelium. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(22), 7952–7957. https://doi.org/10.1073/pnas.0503236102.

Hyman, R. W., Fukushima, M., Jiang, H., Fung, E., Rand, L., Johnson, B., et al. (2014). Diversity of the vaginal microbiome correlates with preterm birth. *Reproductive Sciences (Thousand Oaks, Calif.)*, *21*(1), 32–40. https://doi.org/10.1177/1933719113488838.

Jain, A., Gupta, Y., Agrawal, R., Khare, P., & Jain, S. K. (2007). Biofilms—A microbial life perspective: A critical review. *Critical Reviews in Therapeutic Drug Carrier Systems*, *24*(5), 393–443.

Jakobsson, T., & Forsum, U. (2007). *Lactobacillus iners*: A marker of changes in the vaginal flora? *Journal of Clinical Microbiology*, *45*(9), 3145. https://doi.org/10.1128/JCM.00558-07.

Jelsbak, L., Thomsen, L. E., Wallrodt, I., Jensen, P. R., & Olsen, J. E. (2012). Polyamines are required for virulence in *Salmonella enterica* serovar Typhimurium. *PLoS One, 7*(4), e36149. https://doi.org/10.1371/journal.pone.0036149.

Jespers, V., Menten, J., Smet, H., Poradosú, S., Abdellati, S., Verhelst, R., et al. (2012). Quantification of bacterial species of the vaginal microbiome in different groups of women, using nucleic acid amplification tests. *BMC Microbiology*, *12*(1), 83. https://doi.org/10.1186/1471-2180-12-83.

Jespers, V., van de Wijgert, J., Cools, P., Verhelst, R., Verstraelen, H., Delany-Moretlwe, S., et al. (2015). The significance of *Lactobacillus crispatus* and L. vaginalis for vaginal health and the negative effect of recent sex: A cross-sectional descriptive study across groups of African women. *BMC Infectious Diseases*, *15*(1), 115. https://doi.org/10.1186/s12879-015-0825-z.

Jin, L., Tao, L., Pavlova, S. I., So, J.-S., Kiwanuka, N., Namukwaya, Z., et al. (2007). Species diversity and relative abundance of vaginal lactic acid bacteria from women in Uganda and Korea. *Journal of Applied Microbiology*, *102*(4), 1107–1115. https://doi.org/10.1111/j.1365-2672.2006.03147.x.

Kacerovsky, M., Vrbacky, F., Kutova, R., Pliskova, L., Andrys, C., Musilova, I., et al. (2015). Cervical microbiota in women with preterm prelabor rupture of membranes. *PLoS One, 10*(5), e0126884. https://doi.org/10.1371/journal.pone.0126884.

Kaewnopparat, S., Dangmanee, N., Kaewnopparat, N., Srichana, T., Chulasiri, M., & Settharaksa, S. (2013). In vitro probiotic properties of *Lactobacillus fermentum* SK5 isolated from vagina of a healthy woman. *Anaerobe*, *22*, 6–13. https://doi.org/10.1016/j.anaerobe.2013.04.009.

Kainulainen, V., Loimaranta, V., Pekkala, A., Edelman, S., Antikainen, J., Kylväjä, R., et al. (2012). Glutamine synthetase and glucose-6-phosphate isomerase are adhesive moonlighting proteins of *Lactobacillus crispatus* released by epithelial cathelicidin LL-37. *Journal of Bacteriology*, *194*(10), 2509–2519. https://doi.org/10.1128/JB.06704-11.

Kalyoussef, S., Nieves, E., Dinerman, E., Carpenter, C., Shankar, V., Oh, J., et al. (2012). *Lactobacillus* proteins are associated with the bactericidal activity against *E. coli* of female genital tract secretions. *PLoS One, 7*(11), e49506. https://doi.org/10.1371/journal.pone.0049506.

Kanjee, U., & Houry, W. A. (2013). Mechanisms of acid resistance in *Escherichia coli*. *Annual Review of Microbiology*, *67*(1), 65–81. https://doi.org/10.1146/annurev-micro-092412-155708.

Kaur, B., Balgir, P. P., Mittu, B., Kumar, B., & Garg, N. (2013). Biomedical applications of fermenticin HV6b isolated from *Lactobacillus fermentum* HV6b MTCC10770. *BioMed Research International, 2013*(2), 168438. 8. https://doi.org/10.1155/2013/168438.

Kenyon, C., Colebunders, R., & Crucitti, T. (2013). The global epidemiology of bacterial vaginosis: A systematic review. *American Journal of Obstetrics and Gynecology*, *209*(6), 505–523. https://doi.org/10.1016/j.ajog.2013.05.006.

Kilic, A. O., Pavlova, S. I., Alpay, S., Kilic, S. S., & Tao, L. (2001). Comparative study of vaginal *Lactobacillus* phages isolated from women in the United States and Turkey: Prevalence, morphology, host range, and DNA homology. *Clinical and Diagnostic Laboratory Immunology*, *8*(1), 31–39. https://doi.org/10.1128/CDLI.8.1.31-39.2001.

Kindinger, L. M., MacIntyre, D. A., Lee, Y. S., Marchesi, J. R., Smith, A., McDonald, J. A. K., et al. (2016). Relationship between vaginal microbial dysbiosis, inflammation, and pregnancy outcomes in cervical cerclage. *Science Translational Medicine*, *8*(350), 350ra102. https://doi.org/10.1126/scitranslmed.aag1026.

Kiss, H., Kögler, B., Petricevic, L., Sauerzapf, I., Klayraung, S., Domig, K., et al. (2007). Vaginal *Lactobacillus* microbiota of healthy women in the late first trimester of pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology*, *114*(11), 1402–1407. https://doi.org/10.1111/j.1471-0528.2007.01412.x.

Klaenhammer, T. R. (1988). Bacteriocins of lactic acid bacteria. *Biochimie*, *70*(3), 337–349.

Klebanoff, S. J., Hillier, S. L., Eschenbach, D. A., & Waltersdorph, A. M. (1991). Control of the microbial flora of the vagina by H2O2-generating lactobacilli. *Journal of Infectious Diseases*, *164*(1), 94–100.

Kong, H. H., Oh, J., Deming, C., Conlan, S., Grice, E. A., Beatson, M. A., et al. (2012). Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Research*, *22*(5), 850–859. https://doi.org/10.1101/gr.131029.111.

Kostic, A. D., Gevers, D., Siljander, H., Vatanen, T., Hyötyläinen, T., Hämäläinen, A.-M., et al. (2015). The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host & Microbe*, *17*(2), 260–273. https://doi.org/10.1016/j.chom.2015.01.001.

Kyongo, J. K., Jespers, V., Goovaerts, O., Michiels, J., Menten, J., Fichorova, R. N., et al. (2012). Searching for lower female genital tract soluble and cellular biomarkers: Defining levels and predictors in a cohort of healthy Caucasian women. *PLoS One, 7*(8), e43951. https://doi.org/10.1371/journal.pone.0043951.

Lambert, J. A., John, S., Sobel, J. D., & Akins, R. A. (2013). Longitudinal analysis of vaginal microbiome dynamics in women with recurrent bacterial vaginosis: Recognition of the conversion process. *PLoS One, 8*(12), e82599. https://doi.org/10.1371/journal.pone.0082599.

Lamont, R. F., Sobel, J. D., Akins, R. A., Hassan, S. S., Chaiworapongsa, T., Kusanovic, J. P., et al. (2011). The vaginal microbiome: New information about genital tract flora using molecular based techniques. *BJOG: An International Journal of Obstetrics & Gynaecology*, *118*(5), 533–549. https://doi.org/10.1111/j.1471-0528.2010.02840.x.

Large, T. M., Walk, S. T., & Whittam, T. S. (2005). Variation in acid resistance among shiga toxin-producing clones of pathogenic *Escherichia coli*. *Applied and Environmental Microbiology*, *71*(5), 2493–2500. https://doi.org/10.1128/AEM.71.5.2493-2500.2005.

Larsen, B., Goplerud, C. P., Petzold, C. R., Ohm-Smith, M. J., & Galask, R. P. (1982). Effect of estrogen treatment on the genital tract flora of postmenopausal women. *Obstetrics and Gynecology*, *60*(1), 20–24.

Laxmi, U., Agrawal, S., Raghunandan, C., Randhawa, V. S., & Saili, A. (2011). Association of bacterial vaginosis with adverse fetomaternal outcome in women with spontaneous preterm labor: A prospective cohort study. *The Journal of Maternal-Fetal & Neonatal Medicine*, *25*(1), 64–67. https://doi.org/10.3109/14767058.2011.565390.

Leccese Terraf, M. C., Mendoza, L. M., Juarez Tomas, M. S., Silva, C., & Nader-Macías,-M. E. F. (2014). Phenotypic surface properties (aggregation, adhesion and biofilm formation) and presence of related genes in beneficial vaginal lactobacilli. *Journal of Applied Microbiology*, *117*(6), 1761–1772. https://doi.org/10.1111/jam.12642.

Lee, J. E., Lee, S., Lee, H., Song, Y.-M., Lee, K., Han, M. J., et al. (2013). Association of the vaginal microbiota with human papillomavirus infection in a Korean twin cohort. *PLoS One, 8*(5), e63514. https://doi.org/10.1371/journal.pone.0063514.

Leitich, H., Bodner-Adler, B., Brunbauer, M., Kaider, A., Egarter, C., & Husslein, P. (2003). Bacterial vaginosis as a risk factor for preterm delivery: A meta-analysis. *189*(1), 139–147. https://doi.org/10.1067/mob.2003.339.

Ling, Z., Kong, J., Liu, F., Zhu, H., Chen, X., Wang, Y., et al. (2010). Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. *BMC Genomics*, *11*(1), 488. https://doi.org/10.1186/1471-2164-11-488.

Linhares, I. M., Summers, P. R., Larsen, B., Giraldo, P. C., & Witkin, S. S. (2011). Contemporary perspectives on vaginal pH and lactobacilli. *American Journal of Obstetrics and Gynecology*, *204*(2), 120.e1–120.e5. https://doi.org/10.1016/j.ajog.2010.07.010.

Machado, A., & Cerca, N. (2015). Influence of biofilm formation by *Gardnerella vaginalis* and other anaerobes on bacterial vaginosis. *The Journal of Infectious Diseases*, *212*, 1856–1861.

MacIntyre, D. A., Chandiramani, M., Lee, Y. S., Kindinger, L., Smith, A., Angelopoulos, N., et al. (2015). The vaginal microbiome during pregnancy and the postpartum period in a European population. *Scientific Reports*, *5*, 8988–8989. https://doi.org/10.1038/srep08988.

Macklaim, J. M., Fernandes, A. D., Di Bella, J. M., Hammond, J.-A., Reid, G., & Gloor, G. B. (2013). Comparative meta-RNA-seq of the vaginal microbiota and differential expression by *Lactobacillus iners* in health and dysbiosis. *Microbiome*, *1*(1), 12. https://doi.org/10.1186/2049-2618-1-12.

Macklaim, J. M., Gloor, G. B., Anukam, K. C., Cribby, S., & Reid, G. (2011). At the crossroads of vaginal health and disease, the genome sequence of *Lactobacillus iners* AB-1. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(Suppl. 1), 4688–4695. https://doi.org/10.1073/pnas.1000086107.

Malik, S., Siezen, R. J., Renckens, B., Vaneechoutte, M., Vanderleyden, J., & Lebeer, S. (2014). Draft genome sequence of *Lactobacillus plantarum* CMPG5300, a human vaginal isolate. *Genome Announcements*, *2*(6), e01149–14. https://doi.org/10.1128/genomeA.01149-14.

Marrazzo, J. M., Fiedler, T. L., Srinivasan, S., Thomas, K. K., Liu, C., Ko, D., et al. (2012). Extravaginal reservoirs of vaginal bacteria as risk factors for incident bacterial vaginosis. *Journal of Infectious Diseases*, *205*(10), 1580–1588. https://doi.org/10.1093/infdis/jis242.

Marrazzo, J. M., Thomas, K. K., Fiedler, T. L., Ringwood, K., & Fredricks, D. N. (2010). Risks for acquisition of bacterial vaginosis among women who report sex with women: A cohort study. *PLoS One, 5*(6), e11139. https://doi.org/10.1371/journal.pone.0011139.

Martin, D. H., Zozaya, M., Lillis, R., Miller, J., & Ferris, M. J. (2012). The microbiota of the human genitourinary tract: Trying to see the forest through the trees. *Transactions of the American Clinical and Climatological Association*, *123*, 242–256.

Martinez, R. C. R., Franceschini, S. A., Patta, M. C., Quintana, S. M., Nunes, A. C., Moreira, J. L. S., et al. (2008). Analysis of vaginal lactobacilli from healthy and infected Brazilian women. *Applied and Environmental Microbiology*, *74*(14), 4539–4542. https://doi.org/10.1128/AEM.00284-08.

Martínez-Peña, M. D., Castro-Escarpulli, G., & Aguilera-Arreola, M. G. (2013). *Lactobacillus* species isolated from vaginal secretions of healthy and bacterial vaginosis-intermediate Mexican women: A prospective study. *BMC Infectious Diseases*, *13*(1), 189. https://doi.org/10.1186/1471-2334-13-189.

Mastromarino, P., Brigidi, P., Macchia, S., Maggi, L., Pirovano, F., Trinchieri, V., et al. (2002). Characterization and selection of vaginal *Lactobacillus* strains for the preparation of vaginal tablets. *Journal of Applied Microbiology*, *93*(5), 884–893. https://doi.org/10.1046/j.1365-2672.2002.01759.x.

Mastromarino, P., Cacciotti, F., Masci, A., & Mosca, L. (2011). Antiviral activity of *Lactobacillus brevis* towards herpes simplex virus type 2: Role of cell wall associated components. *Anaerobe*, *17*(6), 334–336. https://doi.org/10.1016/j.anaerobe.2011.04.022.

Mastromarino, P., Di Pietro, M., Schiavoni, G., Nardis, C., Gentile, M., & Sessa, R. (2014). Effects of vaginal lactobacilli in *Chlamydia trachomatis* infection. *International Journal of Medical Microbiology: IJMM*, *304*(5–6), 654–661. https://doi.org/10.1016/j.ijmm.2014.04.006.

Mattsby-Baltzer, I., Platz Christensen, J. J., Hosseini, N., & Rosén, P. (1998). IL-1beta, IL-6, TNFalpha, fetal fibronectin, and endotoxin in the lower genital tract of pregnant women with bacterial vaginosis. *Acta Obstetricia et Gynecologica Scandinavica*, *77*(7), 701–706.

McDonald, H. M., O'loughlin, J. A., Jolley, P., Vigneswaran, R., & McDonald, P. J. (1992). Prenatal microbiological risk factors associated with preterm birth. *British Journal of Obstetrics and Gynaecology*, *99*(3), 190–196. https://doi.org/10.1111/j.1471-0528.1992.tb14497.x.

McGregor, J. A., French, J. I., Jones, W., Milligan, K., McKinney, P. J., Patterson, E., et al. (1994). Bacterial vaginosis is associated with prematurity and vaginal fluid mucinase and sialidase: Results of a controlled trial of topical clindamycin cream. *American Journal of Obstetrics and Gynecology*, *170*(4), 1048–1059. discussion 1059–60.

McGroarty, J. A., & Reid, G. (2011). Detection of a Lactobacillus substance that inhibits *Escherichia coli*. *Canadian Journal of Microbiology*, *34*(8), 974–978. https://doi.org/10.1139/m88-171.

McGroarty, J. A., Tomeczek, L., Pond, D. G., Reid, G., & Bruce, A. W. (1992). Hydrogen peroxide production by *Lactobacillus* species: Correlation with susceptibility to the spermicidal compound nonoxynol-9. *Journal of Infectious Diseases*, *165*(6), 1142–1144.

McLean, N. W., & McGroarty, J. A. (1996). Growth inhibition of metronidazole-susceptible and metronidazole-resistant strains of *Gardnerella vaginalis* by Lactobacilli in vitro. *Applied and Environmental Microbiology*, *62*(3), 1089–1092.

McMillan, A., Macklaim, J. M., Burton, J. P., & Reid, G. (2013). Adhesion of *Lactobacillus iners* AB-1 to human fibronectin: A key mediator for persistence in the vagina? *Reproductive Sciences (Thousand Oaks, Calif.)*, *20*(7), 791–796. https://doi.org/10.1177/1933719112466306.

Mendes-Soares, H., Suzuki, H., Hickey, R. J., & Forney, L. J. (2014). Comparative functional genomics of *Lactobacillus spp*. reveals possible mechanisms for specialization of vaginal lactobacilli to their environment. *Journal of Bacteriology*, *196*(7), 1458–1470. https://doi.org/10.1128/JB.01439-13.

Mercer, B. M. (2005). Preterm premature rupture of the membranes: Current approaches to evaluation and management. *Obstetrics and Gynecology Clinics of North America*, *32*(3), 411–428. https://doi.org/10.1016/j.ogc.2005.03.003.

Miller, E. A., Beasley, D. E., Dunn, R. R., & Archie, E. A. (2016). Lactobacilli dominance and vaginal pH: Why is the human vaginal microbiome unique? *Frontiers in Microbiology*, *7*, 1936. https://doi.org/10.3389/fmicb.2016.01936.

Mirmonsef, P., Hotton, A. L., Gilbert, D., Burgad, D., Landay, A., Weber, K. M., et al. (2014). Free glycogen in vaginal fluids is associated with *Lactobacillus* colonization and low vaginal pH. *PLoS One*, *9*(7), e102467–11. https://doi.org/10.1371/journal.pone.0102467.

Mirmonsef, P., Modur, S., Burgad, D., Gilbert, D., Golub, E. T., French, A. L., et al. (2015). An exploratory comparison of vaginal glycogen and *Lactobacillus* levels in pre- and postmenopausal women. *Menopause (New York, N.Y.)*, *22*(7), 702–709. https://doi.org/10.1097/GME.0000000000000397.

Mitchell, C., Balkus, J. E., Fredricks, D., Liu, C., McKernan-Mullin, J., Frenkel, L. M., et al. (2013). Interaction between lactobacilli, bacterial vaginosis-associated bacteria, and HIV type 1 RNA and DNA genital shedding in U.S. and Kenyan women. *AIDS Research and Human Retroviruses*, *29*(1), 13–19. https://doi.org/10.1089/AID.2012.0187.

Mitchell, C. M., Fredricks, D. N., Winer, R. L., & Koutsky, L. (2012). Effect of sexual debut on vaginal microbiota in a cohort of young women. *Obstetrics and Gynecology*, *120*(6), 1306–1313. https://doi.org/10.1097/AOG.0b013e31827075ac.

Mitra, A., MacIntyre, D. A., Lee, Y. S., Smith, A., Marchesi, J. R., Lehne, B., et al. (2015). Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. *Scientific Reports*, *5*(1), 16865. https://doi.org/10.1038/srep16865.

Moncla, B. J., Mietzner, T. A., & Hillier, S. L. (2012). In vitro activity of cationic peptides against *Neisseria gonorrhoeae* and vaginal *Lactobacillus* species: The effect of divalent cations. *Advances in Bioscience and Biotechnology*, *2012*(03), 249–255. https://doi.org/10.4236/abb.2012.33034.

Nagaraja, T. G., & Titgemeyer, E. C. (2007). Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. *Journal of Dairy Science*, *90*(Suppl. 1), E17–38. https://doi.org/10.3168/jds.2006-478.

Nardini, P., Ñahui Palomino, R. A., Parolin, C., Laghi, L., Foschi, C., Cevenini, R., et al. (2016). *Lactobacillus crispatus* inhibits the infectivity of *Chlamydia trachomatis* elementary bodies, in vitro study. *Scientific Reports*, *6*(1), 29024. https://doi.org/10.1038/srep29024.

Nasioudis, D., Beghini, J., Bongiovanni, A. M., Giraldo, P. C., Linhares, I. M., & Witkin, S. S. (2015). α-Amylase in vaginal fluid: Association with conditions favorable to dominance of *Lactobacillus*. *Reproductive Sciences (Thousand Oaks, Calif.)*, *22*(11), 1393–1398. https://doi.org/10.1177/1933719115581000.

Nelson, T. M., Borgogna, J.-L. C., Brotman, R. M., Ravel, J., Walk, S. T., & Yeoman, C. J. (2015). Vaginal biogenic amines: Biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis? *Frontiers in Physiology*, *6*(91), 181. https://doi.org/10.3389/fphys.2015.00253.

Ness, R. B., Hillier, S. L., Richter, H. E., Soper, D. E., Stamm, C., McGregor, J., et al. (2002). Douching in relation to bacterial vaginosis, lactobacilli, and facultative bacteria in the vagina. *Obstetrics and Gynecology*, *100*(4), 765.

Nieburgs, H. E. (1947). Gestational changes in the vaginal epithelium and their relation to the sex of the foetus. *The Journal of Obstetrics and Gynaecology of the British Empire*, *54*(5), 653–655.

Nikolaitchouk, N., Andersch, B., Falsen, E., Strömbeck, L., & Mattsby-Baltzer, I. (2008). The lower genital tract microbiota in relation to cytokine-, SLPI- and endotoxin levels: Application of checkerboard DNA-DNA hybridization (CDH). *APMIS: Acta Pathologica, Microbiologica, Et Immunologica Scandinavica*, *116*(4), 263–277. https://doi.org/10.1111/j.1600-0463.2008.00808.x.

Nugent, R. P., Krohn, M. A., & Hillier, S. L. (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *Journal of Clinical Microbiology*, *29*(2), 297–301.

Ocaña, V. S., Pesce De Ruiz Holgado, A. A., & Nader-Macias, M. E. (1999a). Characterization of a bacteriocin-like substance produced by a vaginal Lactobacillus salivarius strain. *Applied and Environmental Microbiology*, *65*(12), 5631–5635.

Ocaña, V. S., Pesce De Ruiz Holgado, A. A., & Nader-Macias, M. E. (1999b). Selection of vaginal H2O2-generating Lactobacillus species for probiotic use. *Current Microbiology*, *38*(5), 279–284.

Oh, H. Y., Seo, S.-S., Kong, J.-S., Lee, J.-K., & Kim, M. K. (2015). Association between obesity and cervical microflora dominated by Lactobacillus iners in Korean women. *Journal of Clinical Microbiology*, *53*(10), 3304–3309. https://doi.org/10.1128/JCM.01387-15.

O'Hanlon, D. E., Lanier, B. R., Moench, T. R., & Cone, R. A. (2010). Cervicovaginal fluid and semen block the microbicidal activity of hydrogen peroxide produced by vaginal lactobacilli. *BMC Infectious Diseases*, *10*(1), 120. https://doi.org/10.1186/1471-2334-10-120.

O'Hanlon, D. E., Moench, T. R., & Cone, R. A. (2011). In vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid but not hydrogen peroxide. *BMC Infectious Diseases*, *11*(1), 200. https://doi.org/10.1186/1471-2334-11-200.

O'Hanlon, D. E., Moench, T. R., & Cone, R. A. (2013). Vaginal pH and microbicidal lactic acid when lactobacilli dominate the microbiota. *PLoS One, 8*(11), e80074. https://doi.org/10.1371/journal.pone.0080074.

Ojala, T., Kankainen, M., Castro, J., Cerca, N., Edelman, S., Westerlund-Wikström, B., et al. (2014). Comparative genomics of Lactobacillus crispatus suggests novel mechanisms for the competitive exclusion of Gardnerella vaginalis. *BMC Genomics*, *15*(1), 1070. https://doi.org/10.1186/1471-2164-15-1070.

Orfanelli, T., Jayaram, A., Doulaveris, G., Forney, L. J., Ledger, W. J., & Witkin, S. S. (2014). Human epididymis protein 4 and secretory leukocyte protease inhibitor in vaginal fluid: Relation to vaginal components and bacterial composition. *Reproductive Sciences (Thousand Oaks, Calif.)*, *21*(4), 538–542. https://doi.org/10.1177/1933719113503416.

Pandey, N., Malik, R. K., Kaushik, J. K., & Singroha, G. (2013). Gassericin A: A circular bacteriocin produced by lactic acid bacteria Lactobacillus gasseri. *World Journal of Microbiology and Biotechnology*, *29*(11), 1977–1987. https://doi.org/10.1007/s11274-013-1368-3.

Pandya, S., Ravi, K., Srinivas, V., Jadhav, S., Khan, A., Arun, A., et al. (2017). Comparison of culture-dependent and culture-independent molecular methods for characterization of vaginal microflora. *Journal of Medical Microbiology*, *66*(2), 149–153. https://doi.org/10.1099/jmm.0.000407.

Parolin, C., Marangoni, A., Laghi, L., Foschi, C., Ñahui Palomino, R. A., Calonghi, N., et al. (2015). Isolation of vaginal lactobacilli and characterization of anti-Candida activity. *PLoS One, 10*(6), e0131220. https://doi.org/10.1371/journal.pone.0131220.

Pascual, L. M., Daniele, M. B., Giordano, W., Pájaro, M. C., & Barberis, I. L. (2008). Purification and partial characterization of novel bacteriocin L23 produced by Lactobacillus fermentum L23. *Current Microbiology*, *56*(4), 397–402. https://doi.org/10.1007/s00284-007-9094-4.

Pascual, L. M., Daniele, M. B., Pájaro, C., & Barberis, L. (2006). Lactobacillus species isolated from the vagina: Identification, hydrogen peroxide production and nonoxynol-9 resistance. *Contraception*, *73*(1), 78–81. https://doi.org/10.1016/j.contraception.2005.06.066.

Patton, D. L., Thwin, S. S., Meier, A., Hooton, T. M., Stapleton, A. E., & Eschenbach, D. A. (2000). Epithelial cell layer thickness and immune cell populations in the normal human

vagina at different stages of the menstrual cycle. *American Journal of Obstetrics and Gynecology*, *183*(4), 967–973.

Pavlova, S. I., Kilic, A. O., Kilic, S. S., So, J.-S., Nader-Macias, M. E., Simoes, J. A., et al. (2002). Genetic diversity of vaginal lactobacilli from women in different countries based on 16S rRNA gene sequences. *Journal of Applied Microbiology*, *92*(3), 451–459.

Peeters, M., & Piot, P. (1985). Adhesion of Gardnerella vaginalis to vaginal epithelial cells: Variables affecting adhesion and inhibition by metronidazole. *Genitourinary Medicine*, *61*(6), 391–395.

Pegg, A. E. (2009). Mammalian polyamine metabolism and function. *IUBMB Life*, *61*(9), 880–894. https://doi.org/10.1002/iub.230.

Pericone, C. D., Overweg, K., Hermans, P. W., & Weiser, J. N. (2000). Inhibitory and bactericidal effects of hydrogen peroxide production by Streptococcus pneumoniae on other inhabitants of the upper respiratory tract. *Infection and Immunity*, *68*(7), 3990–3997.

Petricevic, L., Domig, K. J., Nierscher, F. J., Sandhofer, M. J., Fidesser, M., Krondorfer, I., et al. (2014). Characterisation of the vaginal Lactobacillus microbiota associated with preterm delivery. *Scientific Reports*, *4*, 1–6. https://doi.org/10.1038/srep05136.

Phukan, N., Parsamand, T., Brooks, A. E. S., Nguyen, T. N. M., & Simoes-Barbosa, A. (2013). The adherence of Trichomonas vaginalis to host ectocervical cells is influenced by lactobacilli. *Sexually Transmitted Infections*, *89*(6), 455–459. https://doi.org/10.1136/sextrans-2013-051039.

Piot, P., Van Dyck, E., Peeters, M., Hale, J., Totten, P. A., & Holmes, K. K. (1984). Biotypes of Gardnerella vaginalis. *Journal of Clinical Microbiology*, *20*(4), 677–679.

Platz Christensen, J. J., Mattsby-Baltzer, I., Thomsen, P., & Wiqvist, N. (1993). Endotoxin and interleukin-1 alpha in the cervical mucus and vaginal fluid of pregnant women with bacterial vaginosis. *American Journal of Obstetrics and Gynecology*, *169*(5), 1161–1166.

Pridmore, R. D., Pittet, A.-C., Praplan, F., & Cavadini, C. (2008). Hydrogen peroxide production by Lactobacillus johnsonii NCC 533 and its role in anti-Salmonella activity. *FEMS Microbiology Letters*, *283*(2), 210–215. https://doi.org/10.1111/j.1574-6968.2008.01176.x.

Prince, A. L., Ma, J., Kannan, P. S., Alvarez, M., Gisslen, T., Harris, R. A., et al. (2016). The placental membrane microbiome is altered among subjects with spontaneous preterm birth with and without chorioamnionitis. *American Journal of Obstetrics and Gynecology, 214*(5), 627.e1–627.e16. https://doi.org/10.1016/j.ajog.2016.01.193.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., et al. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, *490*(7418), 55–60. https://doi.org/10.1038/nature11450.

Rath, D., Amlinger, L., Rath, A., & Lundgren, M. (2015). The CRISPR-Cas immune system: Biology, mechanisms and applications. *Biochimie*, *117*, 119–128. https://doi.org/10.1016/j.biochi.2015.03.025.

Ravel, J., Gajer, P., Abdo, Z., Schneider, G. M., Koenig, S. S. K., McCulle, S. L., et al. (2011). Vaginal microbiome of reproductive-age women. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(Suppl. 1), 4680–4687. https://doi.org/10.1073/pnas.1002611107.

Rea, M. C., Sit, C. S., Clayton, E., O'Connor, P. M., Whittal, R. M., Zheng, J., et al. (2010). Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against Clostridium difficile. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(20), 9352–9357. https://doi.org/10.1073/pnas.0913554107.

Reid, G., Heinemann, C., Velraeds, M., van der Mei, H. C., & Busscher, H. J. (1999). Biosurfactants produced by Lactobacillus. *Methods in Enzymology*, *310*, 426–433.

Rizzo, A., Fiorentino, M., Buommino, E., Donnarumma, G., Losacco, A., & Bevilacqua, N. (2015). Lactobacillus crispatus mediates anti-inflammatory cytokine interleukin-10 induction in response to Chlamydia trachomatis infection in vitro. *International Journal of Medical Microbiology*, *305*(8), 815–827.

Roberton, A. M., Wiggins, R., Horner, P. J., Greenwood, R., Crowley, T., Fernandes, A., et al. (2005). A novel bacterial mucinase, glycosulfatase, is associated with bacterial vaginosis. *Journal of Clinical Microbiology*, *43*(11), 5504–5508. https://doi.org/10.1128/JCM.43.11.5504-5508.2005.

Rolfe, R. D., Hentges, D. J., Campbell, B. J., & Barrett, J. T. (1978). Factors related to the oxygen tolerance of anaerobic bacteria. *Applied and Environmental Microbiology*, *36*(2), 306–313.

Romero, R., Hassan, S. S., Gajer, P., Tarca, A. L., Fadrosh, D. W., Bieda, J., et al. (2014a). The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term. *Microbiome*, *2*(1), 18. https://doi.org/10.1186/2049-2618-2-18.

Romero, R., Hassan, S. S., Gajer, P., Tarca, A. L., Fadrosh, D. W., Nikita, L., et al. (2014b). The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome*, *2*(1), 4. https://doi.org/10.1186/2049-2618-2-4.

Romero, R., Sirtori, M., Oyarzun, E., Avila, C., Mazor, M., Callahan, R., et al. (1989). Infection and labor V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. *American Journal of Obstetrics and Gynecology*, *161*(3), 817–824.

Rose, W. A., McGowin, C. L., Spagnuolo, R. A., Eaves-Pyles, T. D., Popov, V. L., & Pyles, R. B. (2012). Commensal bacteria modulate innate immune responses of vaginal epithelial cell multilayer cultures. *PLoS One, 7*(3), e32728. https://doi.org/10.1371/journal.pone.0032728.

Rosenstein, I. J., Fontaine, E. A., Morgan, D. J., Sheehan, M., Lamont, R. F., & Taylor-Robinson, D. (1997). Relationship between hydrogen peroxide-producing strains of lactobacilli and vaginosis-associated bacterial species in pregnant women. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology*, *16*(7), 517–522. https://doi.org/10.1007/BF01708235.

Ross, S. A., Novak, Z., Ashrith, G., Rivera, L. B., Britt, W. J., Hedges, S., et al. (2005). Association between genital tract cytomegalovirus infection and bacterial vaginosis. *Journal of Infectious Diseases*, *192*(10), 1727–1730. https://doi.org/10.1086/497150.

Ruíz, F. O., Pascual, L., Giordano, W., & Barberis, L. (2015). Bacteriocins and other bioactive substances of probiotic lactobacilli as biological weapons against *Neisseria gonorrhoeae*. *Pathogens and Disease*, *73*(3), 1–10, ftv013, Pages 1–10. https://doi.org/10.1093/femspd/ftv013.

Sallenave, J. M. (2002). Antimicrobial activity of antiproteinases. *Biochemical Society Transactions*, *30*(2), 111–115.

Santiago, G. L. D. S., Cools, P., Verstraelen, H., Trog, M., Missine, G., El Aila, N., et al. (2011). Longitudinal study of the dynamics of vaginal microflora during two consecutive menstrual cycles. *PLoS One*, *6*(11), e28180. https://doi.org/10.1371/journal.pone.0028180.

Santiago, G. L. D. S., Tency, I., Verstraelen, H., Verhelst, R., Trog, M., Temmerman, M., et al. (2012). Longitudinal qPCR study of the dynamics of L. crispatus, L. iners, A. vaginae, (sialidase positive) G. vaginalis, and P. bivia in the vagina. *PLoS One, 7*(9), e45281. https://doi.org/10.1371/journal.pone.0045281.

Sautter, R. L., & Brown, W. J. (1980). Sequential vaginal cultures from normal young women. *Journal of Clinical Microbiology*, *11*(5), 479–484.

Schellenberg, J. J., Links, M. G., Hill, J. E., Dumonceaux, T. J., Kimani, J., Jaoko, W., et al. (2011). Molecular definition of vaginal microbiota in East African commercial sex workers. *Applied and Environmental Microbiology*, *77*(12), 4066–4074. https://doi.org/10.1128/AEM.02943-10.

Shah, P., & Swiatlo, E. (2008). A multifaceted role for polyamines in bacterial pathogens. *Molecular Microbiology*, *68*(1), 4–16. https://doi.org/10.1111/j.1365-2958.2008.06126.x.

Shedlovsky, L., & Belcher, D. (1942). Titrations of human seminal fluid with acids and alkalis and their effects on the survival of sperm motility. *American Journal of Physiology*, *136*, 535–541.

Shi, Y., Chen, L., Tong, J., & Xu, C. (2009). Preliminary characterization of vaginal microbiota in healthy Chinese women using cultivation-independent methods. *The Journal of Obstetrics and Gynaecology Research*, *35*(3), 525–532. https://doi.org/10.1111/j.1447-0756.2008.00971.x.

Shipitsyna, E., Roos, A., Datcu, R., Hallén, A., Fredlund, H., Jensen, J. S., et al. (2013). Composition of the vaginal microbiota in women of reproductive age—Sensitive and specific molecular diagnosis of bacterial vaginosis is possible? *PLoS One, 8*(4), e60670. https://doi.org/10.1371/journal.pone.0060670.

Skarin, A., & Sylwan, J. (1986). Vaginal lactobacilli inhibiting growth of Gardnerella vaginalis, Mobiluncus and other bacterial species cultured from vaginal content of women with bacterial vaginosis. *APMIS: Acta Pathologica, Microbiologica, Et Immunologica Scandinavica*, *94B*(1–6), 399–403. https://doi.org/10.1111/j.1699-0463.1986.tb03074.x.

Smith, B. C., McAndrew, T., Chen, Z., Harari, A., Barris, D. M., Viswanathan, S., et al. (2012). The cervical microbiome over 7 years and a comparison of methodologies for its characterization. *PLoS One, 7*(7), e40425. https://doi.org/10.1371/journal.pone.0040425.

Sobel, J. D., Karpas, Z., & Lorber, A. (2012). Diagnosing vaginal infections through measurement of biogenic amines by ion mobility spectrometry. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *163*(1), 81–84. https://doi.org/10.1016/j.ejogrb.2012.03.022.

Sochacki, K. A., Barns, K. J., Bucki, R., & Weisshaar, J. C. (2011). Real-time attack on single Escherichia coli cells by the human antimicrobial peptide LL-37. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(16), E77–81. https://doi.org/10.1073/pnas.1101130108.

Song, Y. L., Kato, N., Matsumiya, Y., Liu, C. X., Kato, H., & Watanabe, K. (1999). Identification of and hydrogen peroxide production by fecal and vaginal lactobacilli isolated from Japanese women and newborn infants. *Journal of Clinical Microbiology*, *37*(9), 3062–3064.

Spandorfer, S. D., Neuer, A., Giraldo, P. C., Rosenwaks, Z., & Witkin, S. S. (2001). Relationship of abnormal vaginal flora, proinflammatory cytokines and idiopathic infertility in women undergoing IVF. *The Journal of Reproductive Medicine*, *46*(9), 806–810.

Spear, G. T., French, A. L., Gilbert, D., Zariffard, M. R., Mirmonsef, P., Sullivan, T. H., et al. (2014). Human α-amylase present in lower genital tract mucosal fluid processes glycogen to support vaginal colonization by Lactobacillus. *Journal of Infectious Diseases*, *210*(7), 1019–1028. https://doi.org/10.1093/infdis/jiu231.

Spear, G. T., Kersh, E., Guenthner, P., Vishwanathan, S. A., Gilbert, D., Zariffard, M. R., et al. (2012). Longitudinal assessment of pigtailed macaque lower genital tract microbiota by pyrosequencing reveals dissimilarity to the genital microbiota of healthy humans. *AIDS Research and Human Retroviruses*, *28*(10), 1244–1249. https://doi.org/10.1089/AID.2011.0382.

Spear, G. T., Sikaroodi, M., Zariffard, M. R., Landay, A. L., French, A. L., & Gillevet, P. M. (2008). Comparison of the diversity of the vaginal microbiota in HIV-infected and HIV-uninfected women with or without bacterial vaginosis. *198*(8), 1131–1140. https://doi.org/10.1086/591942.

Spear, G. T., Zariffard, M. R., Cohen, M. H., & Sha, B. E. (2008). Vaginal IL-8 levels are positively associated with Candida albicans and inversely with lactobacilli in HIV-infected women. *Journal of Reproductive Immunology*, *78*(1), 76–79. https://doi.org/10.1016/j.jri.2007.11.001.

Spurbeck, R. R., & Arvidson, C. G. (2008). Inhibition of Neisseria gonorrhoeae epithelial cell interactions by vaginal Lactobacillus species. *Infection and Immunity*, *76*(7), 3124–3130. https://doi.org/10.1128/IAI.00101-08.

Spurbeck, R. R., & Arvidson, C. G. (2010). Lactobacillus jensenii surface-associated proteins inhibit Neisseria gonorrhoeae adherence to epithelial cells. *Infection and Immunity*, *78*(7), 3103–3111. https://doi.org/10.1128/IAI.01200-09.

Srinivasan, S., & Fredricks, D. N. (2009). The human vaginal bacterial biota and bacterial vaginosis. *Interdisciplinary Perspectives on Infectious Diseases*, *2008*(1), 1–22. https://doi.org/10.1155/2008/750479.

Srinivasan, S., Hoffman, N. G., Morgan, M. T., Matsen, F. A., Fiedler, T. L., Hall, R. W., et al. (2012). Bacterial communities in women with bacterial vaginosis: High resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS One, 7*(6), e37818. https://doi.org/10.1371/journal.pone.0037818.

Srinivasan, S., Liu, C., Mitchell, C. M., Fiedler, T. L., Thomas, K. K., Agnew, K. J., et al. (2010). Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. *PLoS One, 5*(4), e10197. https://doi.org/10.1371/journal.pone.0010197.

Srinivasan, S., Morgan, M. T., Fiedler, T. L., Djukovic, D., Hoffman, N. G., Raftery, D., et al. (2015). Metabolic signatures of bacterial vaginosis. *mBio, 6*(2), e00204–15. https://doi.org/10.1128/MBio00204-15.

Stapleton, A. E., Au-Yeung, M., Hooton, T. M., Fredricks, D. N., Roberts, P. L., Czaja, C. A., et al. (2011). Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. *Clinical Infectious Diseases*, *52*(10), 1212–1217. https://doi.org/10.1093/cid/cir183.

Statnikov, A., Alekseyenko, A. V., Li, Z., Henaff, M., Perez-Perez, G. I., Blaser, M. J., et al. (2013). Microbiomic signatures of psoriasis: Feasibility and methodology comparison. *Scientific Reports*, *3*(1), 2620. https://doi.org/10.1038/srep02620.

Stoyancheva, G., Marzotto, M., Dellaglio, F., & Torriani, S. (2014). Bacteriocin production and gene sequencing analysis from vaginal Lactobacillus strains. *Archives of Microbiology*, *196*(9), 645–653. https://doi.org/10.1007/s00203-014-1003-1.

Strus, M., Brzychczy-Włoch, M., Gosiewski, T., Kochan, P., & Heczko, P. B. (2006). The in vitro effect of hydrogen peroxide on vaginal microbial communities. *FEMS Immunology & Medical Microbiology*, *48*(1), 56–63. https://doi.org/10.1111/j.1574-695X.2006.00120.x.

Subramaniam, A., Kumar, R., Cliver, S., Zhi, D., Szychowski, J., Abramovici, A., et al. (2016). Vaginal microbiota in pregnancy: Evaluation based on vaginal flora, birth outcome, and race. *American Journal of Perinatology*, *33*(04), 401–408.

Sumati, A. H., & Saritha, N. K. (2009). Association of urinary tract infection in women with bacterial vaginosis. *Journal of Global Infectious Diseases*, *1*(2), 151–152. https://doi.org/10.4103/0974-777X.56254.

Swartz, J. D., Lachman, M., Westveer, K., O'Neill, T., Geary, T., Kott, R. W., et al. (2014). Characterization of the vaginal microbiota of ewes and cows reveals a unique microbiota with low levels of lactobacilli and near-neutral pH. *Frontiers in Veterinary Science*, *1*(Suppl. 1), 19. https://doi.org/10.3389/fvets.2014.00019.

Swidsinski, A., Loening-Baucke, V., Mendling, W., Dörffel, Y., Schilling, J., Halwani, Z., et al. (2014). Infection through structured polymicrobial Gardnerella biofilms (StPM-GB). *Histology and Histopathology*, *29*(5), 567–587. http://doi.org/10.14670/HH-29.10.567.

Tärnberg, M., Jakobsson, T., Jonasson, J., & Forsum, U. (2002). Identification of randomly selected colonies of lactobacilli from normal vaginal fluid by pyrosequencing of the 16S rDNA variable V1 and V3 regions. *APMIS: Acta Pathologica, Microbiologica, Et Immunologica Scandinavica*, *110*(11), 802–810.

Tevi-Bénissan, C., Belec, L., Levy, M., Schneider-Fauveau, V., Si Mohamed, A., Hallouin, M. C., et al. (1997). In vivo semen-associated pH neutralization of cervicovaginal secretions. *Clinical and Diagnostic Laboratory Immunology*, *4*(3), 367–374.

The Human Microbiome Project Consortium (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, *486*(7402), 207–214. https://doi.org/10.1038/nature11234.

Thomas, S. (1928). Döderlein's bacillus: Lactobacillus acidophilus. *Journal of Infectious Diseases*, *43*, 218–227.

Totten, P. A., Amsel, R., Hale, J., Piot, P., & Holmes, K. K. (1982). Selective differential human blood bilayer media for isolation of Gardnerella (Haemophilus) vaginalis. *Journal of Clinical Microbiology*, *15*(1), 141–147.

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., et al. (2009). A core gut microbiome in obese and lean twins. *Nature*, *457*(7228), 480–484. https://doi.org/10.1038/nature07540.

Valore, E. V., Wiley, D. J., & Ganz, T. (2006). Reversible deficiency of antimicrobial polypeptides in bacterial vaginosis. *Infection and Immunity*, *74*(10), 5693–5702. https://doi.org/10.1128/IAI.00524-06.

van de Wijgert, J. H. H. M., Borgdorff, H., Verhelst, R., Crucitti, T., Francis, S., Verstraelen, H., et al. (2014). The vaginal microbiota: What have we learned after a decade of molecular characterization? *PLoS One, 9*(8), e105998. https://doi.org/10.1371/journal.pone.0105998.

Vásquez, A., Jakobsson, T., Ahrné, S., Forsum, U., & Molin, G. (2002). Vaginal Lactobacillus flora of healthy Swedish women. *Journal of Clinical Microbiology*, *40*(8), 2746–2749. https://doi.org/10.1128/JCM.40.8.2746-2749.2002.

Vera Pingitore, E., Hébert, E. M., Nader-Macias, M. E., & Sesma, F. (2009). Characterization of salivaricin CRL 1328, a two-peptide bacteriocin produced by Lactobacillus salivarius CRL 1328 isolated from the human vagina. *Research in Microbiology*, *160*(6), 401–408. https://doi.org/10.1016/j.resmic.2009.06.009.

Verhelst, R., Verstraelen, H., Claeys, G., Verschraegen, G., Delanghe, J., Van Simaey, L., et al. (2004). Cloning of 16S rRNA genes amplified from normal and disturbed vaginal microflora suggests a strong association between Atopobium vaginae, Gardnerella vaginalis and bacterial vaginosis. *BMC Microbiology*, *4*(1), 16. https://doi.org/10.1186/1471-2180-4-16.

Verstraelen, H., & Swidsinski, A. (2013). The biofilm in bacterial vaginosis: Implications for epidemiology, diagnosis and treatment. *Current Opinion in Infectious Diseases*, *26*(1), 86–89. https://doi.org/10.1097/QCO.0b013e32835c20cd.

Verstraelen, H., Verhelst, R., Claeys, G., De Backer, E., Temmerman, M., & Vaneechoutte, M. (2009). Longitudinal analysis of the vaginal microflora in pregnancy suggests that L. crispatus promotes the stability of the normal vaginal microflora and that L. gasseri and/or L. iners are more conducive to the occurrence of abnormal vaginal microflora. *BMC Microbiology*, *9*(1), 116. https://doi.org/10.1186/1471-2180-9-116.

Vodstrcil, L. A., Twin, J., Garland, S. M., Fairley, C. K., Hocking, J. S., Law, M. G., et al. (2017). The influence of sexual activity on the vaginal microbiota and Gardnerella vaginalis clade diversity in young women. *PLoS One, 12*(2), e0171856. https://doi.org/10.1371/journal.pone.0171856.

Walther-António, M. R. S., Jeraldo, P., Miller, M. E. B., Yeoman, C. J., Nelson, K. E., Wilson, B. A., et al. (2014). Pregnancy's stronghold on the vaginal microbiome. *PLoS One, 9*(6), e98514. https://doi.org/10.1371/journal.pone.0098514.

Wang, S., Wang, Q., Yang, E., Yan, L., Li, T., & Zhuang, H. (2017). Antimicrobial compounds produced by vaginal Lactobacillus crispatus are able to strongly inhibit Candida albicans growth, hyphal formation and regulate virulence-related gene expressions. *Frontiers in Microbiology*, *8*(1722), 564. https://doi.org/10.3389/fmicb.2017.00564.

Wassenaar, T. M., & Panigrahi, P. (2014). Is a foetus developing in a sterile environment? *Letters in Applied Microbiology*, *59*(6), 572–579. https://doi.org/10.1111/lam.12334.

Waters, T. P., & Mercer, B. (2011). Preterm PROM: Prediction, prevention, principles. *Clinical Obstetrics and Gynecology*, *54*(2), 307–312. https://doi.org/10.1097/GRF.0b013e318217d4d3.

Wennerholm, U. B., Holm, B., Mattsby-Baltzer, I., Nielsen, T., Platz Christensen, J. J., Sundell, G., et al. (1998). Interleukin-1alpha, interleukin-6 and interleukin-8 in cervico/vaginal secretion for screening of preterm birth in twin gestation. *Acta Obstetricia et Gynecologica Scandinavica*, *77*(5), 508–514.

Wertz, J., Isaacs-Cosgrove, N., Holzman, C., & Marsh, T. L. (2008). Temporal shifts in microbial communities in nonpregnant African-American women with and without bacterial vaginosis. *Interdisciplinary Perspectives on Infectious Diseases*, *2008*(8), 181253–181259. https://doi.org/10.1155/2008/181253.

Wiesenfeld, H. C., Hillier, S. L., Krohn, M. A., Landers, D. V., & Sweet, R. L. (2003). Bacterial vaginosis is a strong predictor of Neisseria gonorrhoeae and Chlamydia trachomatis infection. *Clinical Infectious Diseases*, *36*(5), 663–668. https://doi.org/10.1086/367658.

Wilks, M., Wiggins, R., Whiley, A., Hennessy, E., Warwick, S., Porter, H., et al. (2004). Identification and H(2)O(2) production of vaginal lactobacilli from pregnant women at high risk of preterm birth and relation with outcome. *Journal of Clinical Microbiology*, *42*(2), 713–717. https://doi.org/10.1128/JCM.42.2.713-717.2004.

Willson, J., & Goforth, M. L. (1942). Effects of an excess of ingested carbohydrate upon the glycogen content of vaginal epithelium. *The Journal of Clinical Endocrinology and Metabolism*, *2*, 223–225.

Witkin, S. S., Mendes-Soares, H., Linhares, I. M., Jayaram, A., Ledger, W. J., & Forney, L. J. (2013). Influence of vaginal bacteria and D- and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase inducer: Implications for protection against upper genital tract infections. *mBio, 4*(4), e00460–13. https://doi.org/10.1128/mBio.00460-13.

Wolrath, H., Boren, H., Hallen, A., & Forsum, U. (2002). Trimethylamine content in vaginal secretion and its relation to bacterial vaginosis. *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, *110*(11), 819–824. https://doi.org/10.1034/j.1600-0463.2002.1101108.x.

Yamamoto, T., Zhou, X., Williams, C. J., Hochwalt, A., & Forney, L. J. (2009). Bacterial populations in the vaginas of healthy adolescent women. *Journal of Pediatric and Adolescent Gynecology*, *22*(1), 11–18. https://doi.org/10.1016/j.jpag.2008.01.073.

Yarlett, N., Goldberg, B., Moharrami, M. A., & Bacchi, C. J. (1993). Trichomonas vaginalis: Characterization of ornithine decarboxylase. *Biochemical Journal*, *293*(2), 487–493. https://doi.org/10.1042/bj2930487.

Yeoman, C. J., Chia, N., Yildirim, S., Miller, M. E. B., Kent, A., Stumpf, R., et al. (2011). Towards an evolutionary model of animal-associated microbiomes. *Entropy*, *13*(12), 570–594. https://doi.org/10.3390/e13030570.

Yeoman, C. J., Thomas, S. M., Miller, M. E. B., Ulanov, A. V., Torralba, M., Lucas, S., et al. (2013). A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. *PLoS One, 8*(2), e56111. https://doi.org/10.1371/journal.pone.0056111.

Yeoman, C. J., Yildirim, S., Thomas, S. M., Durkin, A. S., Torralba, M., Sutton, G., et al. (2010). Comparative genomics of Gardnerella vaginalis strains reveals substantial differences in metabolic and virulence potential. *PLoS One, 5*(8), e12411. https://doi.org/10.1371/journal.pone.0012411.

Yildirim, S., Yeoman, C. J., Janga, S. C., Thomas, S. M., Ho, M., Leigh, S. R., et al. (2014). Primate vaginal microbiomes exhibit species specificity without universal Lactobacillus dominance. *The ISME Journal*, *8*(12), 2431–2444. https://doi.org/10.1038/ismej.2014.90.

Yoshimura, K., Morotomi, N., Fukuda, K., Nakano, M., Kashimura, M., Hachisuga, T., et al. (2011). Intravaginal microbial flora by the 16S rRNA gene sequencing. *American Journal of Obstetrics and Gynecology, 205*(3), 235.e1–9. https://doi.org/10.1016/j.ajog.2011.04.018.

Zevin, A. S., Xie, I. Y., Birse, K., Arnold, K., Romas, L., Westmacott, G., et al. (2016). Microbiome composition and function drives wound-healing impairment in the female genital tract. *PLoS Pathogens, 12*(9), e1005889. https://doi.org/10.1371/journal.ppat.1005889.

Zhang, R., Daroczy, K., Xiao, B., Yu, L., Chen, R., & Liao, Q. (2012). Qualitative and semi-quantitative analysis of Lactobacillus species in the vaginas of healthy fertile and postmenopausal Chinese women. *Journal of Medical Microbiology*, *61*(Pt. 5), 729–739. https://doi.org/10.1099/jmm.0.038687-0.

Zhou, X., Bent, S. J., Schneider, M. G., Davis, C. C., Islam, M. R., & Forney, L. J. (2004). Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. *Microbiology (Reading, England)*, *150*(Pt. 8), 2565–2573. https://doi.org/10.1099/mic.0.26905-0.

Zhou, X., Brown, C. J., Abdo, Z., Davis, C. C., Hansmann, M. A., Joyce, P., et al. (2007). Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. *The ISME Journal*, *1*(2), 121–133. https://doi.org/10.1038/ismej.2007.12.

Zhou, X., Westman, R., Hickey, R., Hansmann, M. A., Kennedy, C., Osborn, T. W., et al. (2009). Vaginal microbiota of women with frequent vulvovaginal candidiasis. *Infection and Immunity*, *77*(9), 4130–4135. https://doi.org/10.1128/IAI.00436-09.

Zozaya-Hinchliffe, M., Lillis, R., Martin, D. H., & Ferris, M. J. (2010). Quantitative PCR assessments of bacterial species in women with and without bacterial vaginosis. *Journal of Clinical Microbiology*, *48*(5), 1812–1819. https://doi.org/10.1128/JCM.00851-09.

FURTHER READING

Koumans, E. H., Sternberg, M., Bruce, C., McQuillan, G., Kendrick, J., Sutton, M., et al. (2007). The prevalence of bacterial vaginosis in the United States, 2001–2004; associations with symptoms, sexual behaviors, and reproductive health. *Sexually Transmitted Diseases*, *34*(11), 864–869. https://doi.org/10.1097/OLQ.0b013e318074e565.

CHAPTER 3

THE VAGINAL METABOLOME AND MICROBIOTA OF CERVICAL HPV-POSITIVE AND HPV-NEGATIVE WOMEN: A CROSS-SECTIONAL ANALYSIS

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Contributions: Designed and implemented the Parent Study, advised on statistical analysis, interpretation, and manuscript preparation.

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The vaginal metabolome and microbiota of cervical HPV-positive and HPV-negative women: a cross-sectional analysis

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Contribution to authorship:
RMB, JR, JMR and EG designed and implemented the Parent study. JB and MS performed statistical analysis. TN, JB, EK, RMB, CJY, PG and MS advised on statistical analyses as well as provided interpretation of the data. JB, CJY, MS and RMB wrote the main manuscript text. All authors contributed to the final version of the manuscript.

Disclosures of interest:
JR is the co-founder of LUCA Biologics, a biotechnology company focusing on translating microbiome research into live biotherapeutics drugs for women health. All remaining authors have no disclosures to declare. Completed disclosure of interest forms are available to view online as supporting information.

Details of ethics approval:
All participants provided written informed consent, and ethical approval was obtained from the Institutional Review Boards of the University of Maryland Baltimore (UMB) [HP-00045732; 09/12/2011], Montana State University [CY010818-EX; 1/8/18], and the University of Maryland School of Public Health [10–0301; 07/28/2010]. This secondary analysis was not assessed by an external peer review for scientific quality, and there was not a patient or public panel that participated in review. A core outcome set for reporting data was not available on the topic of sexually transmitted infection research, however we did follow STROBE guidelines in the report of this case control study[43–45]. All samples were collected and analyzed in accordance with the relevant guidelines and regulations.

Data Availability:
The questionnaire and metabolome data are available at the National Center for Biotechnology Information (NCBI) Database of Genotypes and Phenotypes (dbGaP) under Accession number phs001386.v1.p1. Metagenomic sequence data were submitted to the public NCBI Sequence Read Archive (SRA) with the accession number PRJNA391039.

Code Availability:
The code used to analyze these data has been provided as supplemental material (see Appendix S1).

Abstract

Objective: Characterize the vaginal metabolome of cervical HPV infected and uninfected women.

Design: Cross-sectional

Setting: The Center for Health Behavior Research at the University of Maryland School of Public Health.

Sample: Thirty-nine participants; 13 categorized as HPV-negative and 26 as HPV-positive (any genotype; HPV+), 14 of which were positive with at least one high-risk HPV strain (hrHPV).

Method: Self-collected mid-vaginal swabs were profiled for bacterial composition by 16S rRNA gene amplicon sequencing, metabolites by both gas- and liquid-chromatography mass spectrometry, and 37 types of HPV DNA.

Main Outcome Measures: Metabolite abundances

Results: Vaginal microbiota clustered into Community State Type (CST) I (*L. crispatus*-dominated), CST III (*L. iners*-dominated), and CST IV (low-*Lactobacillus*, "molecular-BV"). HPV+ women had higher biogenic amine and phospholipid concentrations than HPV-negative women after adjustment for CST and cigarette smoking. Metabolomic profiles of HPV+ and HPV − women differed in stratums of CST. Among women in CST III, HPV+ there were higher concentrations of biogenic amines and glycogen-related metabolites compared to CST III HPV-. In CST IV, HPV+ participants there were lower concentrations of glutathione, glycogen, and phospholipid-related metabolites than CST IV, HPV-. Across all CSTs, women with hrHPV strains had lower concentrations of amino acids, lipids and peptides compared to women who had only low-risk HPV (lrHPV).

Conclusions: The vaginal metabolome of HPV+ women differed from HPV− women in terms of several metabolites, including biogenic amines, glutathione, and lipid-related metabolites. If the temporal relationship between increased levels of reduced glutathione and oxidized glutathione and HPV incidence/persistence is confirmed in future studies, anti-oxidant therapies may be considered as a non-surgical HPV control intervention.

Tweetable abstract:

Metabolomics study: Vaginal microenvironment of HPV+ women may be informative for non-surgical interventions

Keywords

vaginal metabolome; vaginal microbiota; 16S rRNA gene amplicon sequencing; human papillomavirus

Introduction:

Cervical cancer is the third most prevalent cancer and the second most common cause of cancer-related deaths in women worldwide.[1] Human papillomavirus (HPV) is causatively linked to over 90% of cervical cancer cases.[2, 3] Persistent detection of cervical HPV is

associated with increased risk for dysplasia[4, 5] and cervical malignancy[6] but the factors affecting HPV-persistence are not fully understood.

Emerging data suggests an association between cervical HPV infections and the vaginal microenvironment[7–19]. The vaginal microbiota can be categorized into one of five major community state types (CSTs), four of which are dominated by *Lactobacillus* spp. (CST I, II, III and V)[20]. Vaginal *Lactobacillus* spp. provide broad-spectrum protection by producing lactic acid[21], bacteriocins[22, 23], and biosurfactants[24], and by adherence to the mucosa which forms barriers against pathogenic infection[25, 26]. CST IV is characterized by a paucity in *Lactobacillus*, high vaginal pH (>4.5), and high relative abundances of various anaerobic bacteria, including *Gardnerella, Prevotella* and *Atopobium* spp.[20] These microbial features are also characteristic of bacterial vaginosis (BV), which can be associated with a myriad of symptoms and adverse health outcomes[27] leading to the proposal of CST IV being termed "molecular-BV" [28].

Molecular studies have found HPV-positive participants are more likely to present with *L. iners*-dominated (CST III) or low-*Lactobacillus* molecular-BV states than HPV-negative women[14,17, 29–32]. Brotman *et al*. reported in a small cohort study that *L. gasseri*-dominated CST II was associated with increased clearance of detectable HPV[30]. With national surveys estimating that 29% of U.S. women have Nugent BV[33], the vaginal microbiota may be a population-level risk factor for HPV that has largely been overlooked.

The vaginal microenvironment also comprises both microbially- and host-produced metabolites that may influence clinical endpoints. While the vaginal metabolome is largely influenced by resident microbiota[17, 34–38], prior research has identified metabolites associated with important clinical conditions including BV[39, 40], pre-eclampsia[41], preterm labor[42] and more recently, Ilhan *et al*. observed an association in the vaginal metabolome between lipids and cervical cancer[17]. However, the impact of HPV infection remains relatively unexplored. We sought to compare the vaginal metabolome by HPV detection status and vaginal CSTs. These data may provide insight into HPV susceptibility, mechanisms for HPV persistence and lead to the discovery of new targets for intervention.

Methods:

Ethics statement:

All participants provided written informed consent, and ethical approval was obtained from the Institutional Review Boards of the University of Maryland Baltimore (UMB), Montana State University and the University of Maryland School of Public Health. This secondary analysis was not assessed by an external peer review for scientific quality, and there was no patient or public panel that participated in review. A core outcome set for reporting data was not available on the topic of sexually transmitted infection research; however, we did follow STROBE guidelines in the report of this case control study[43–45]. All samples were collected and analyzed in accordance with the relevant guidelines and regulations. This project was funded by two University of Maryland seed grants ((1) a University of Maryland Cancer Epidemiology Alliance Joint Research Pilot Grant sponsored by the University of Maryland Greenebaum Cancer Center (Brotman), and (2) a University of Maryland College Park/

University of Maryland Baltimore Seed Grant (Brotman and Glover)), and in part by the National Institute of Allergy and Infectious Diseases grants R01-AI116799 (Brotman). The funder did not play a role in the conduct of the study or in manuscript preparation.

Study population and sample collection:

This is a secondary analysis and the primary study has previously been described[29, 34]. Forty women were enrolled in a cross-sectional study at the Center for Health Behavior Research at the University of Maryland School of Public Health (UMSPH). Briefly, participants were excluded if they were pregnant, lactating, had the presence of an acute or chronic illness, if they reported use of an antibiotic or antimycotic in the prior 30 days and/or alcohol or drug dependency within the prior 12 months. The final sample size was 39 women. All samples were self-collected, mid-vaginal swabs.

Taxonomic assignment, community state type profiling and HPV detection:

DNA extraction, PCR amplification, and Roche 454 pyrosequencing of 16S rRNA gene amplicons (V1–V3) using primers 27F-YM+3 and 543R from the vaginal swabs has been previously described[29]. Taxonomic assignments were performed using the Ribosomal Database Project Naïve Bayesian Classifier. *Lactobacillus* species assignments were performed using higher order Markov Chain models and the software speciateIT (speciateIT.sourceforge.net). For each sample, CST classifications were assigned as previously described, using Jensen-Shannon divergence and Ward linkage hierarchical clustering[29, 34]. The CSTs reflected several *Lactobacillus*-dominated states I (*L. crispatus*), CST III (L. *iners*), and CST IV (low-*Lactobacillus*, molecular BV) as described by Ravel et al in 2011[20]. DNA extracted from the swabs were tested for 37 types of HPV DNA using the Roche Linear Array HPV Genotyping Test according to manufacturer's instructions. Samples with any HPV genotype detected were defined as HPV+. Samples with a high-risk HPV genotype (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 or 68) were defined as hrHPV+, and samples that were negative for all 37 types of HPV were defined as HPV-. HPV+ samples that were not listed as hrHPV were categorized as low-risk HPV (lrHPV) types.

Sample preparation for metabolomics:

Vaginal samples were eluted from swabs using phosphate buffered saline (PBS) and subjected to non-targeted Gas Chromatography (GC) and Liquid Chromatography (LC) mass spectrometry (MS)-based metabolomics conducted by Metabolon Inc., as previously described[34]. Briefly, LC-MS was performed using a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a ThermoFisher Scientific Orbitrap Elite high resolution/accurate mass spectrometer and samples were run in positive- and negative-ion sense. GC-MS was performed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization, after samples had been dried under vacuum desiccation, and derivatized under dried nitrogen using bistrimethyl-silyl-triflouroacetamide (BSTFA). Raw data was extracted, peaks identified, QC processed, and compounds identified using Metabolon's proprietary database of reference spectrum. Results were manually curated to ensure that data were accurate and to remove any system

artifacts, miss-assignments, and background noise. The provided dataset reflected the relative concentration of 619 metabolites.

Metabolite pre-processing:

Metabolites were excluded from the analysis if they were undetected in ≥ 90% of each biological group (HPV+ vs. HPV-). Remaining "missing" values were assumed to be below the level of detection and were imputed using one-half the minimum value obtained for a given metabolite[46–49]. The final dataset consisted of the relative concentration of 599 metabolites that were subsequently log-transformed to meet assumptions of linearity, normality, and heterogeneity for linear regression.

Statistical analysis:

CST, demographic and behavioral factors associated with HPV detection status were assessed using Fisher's exact test (Table S1).

An exploratory non-metric multidimensional scaling (nMDS) was applied to a Bray-Curtis dissimilarity matrix comprised of the 599 metabolites and 39 individuals. Analysis of similarities (ANOSIM) was used to evaluate the separation of groups and a permutational multivariate analysis of variance (PERMANOVA) was performed to assess the proportion of variation that CST, HPV, hrHPV, and smoking status contributed to the entire vaginal metabolome. The Bray-Curtis dissimilarity matrix, nMDS, ANOSIM, and PERMANOVA were conducted using the *vegan* package in R Statistical Software with 10,000 permutations.

Next, we performed linear regression of log-transformed metabolites on HPV and CST after adjustment for a binary variable on current smoking status (Model 1). Smoking was the only covariate as previous work linked it to both HPV and the vaginal metabolome[34] and to avoid overfitting the small sample size. We fit another model that included HPV-by-CST interaction terms to determine whether the associations of HPV with metabolites differed by CST (Model 2). Lastly, we fit a simple linear regression model of log-transformed metabolites to assess differences in women with hrHPV and lrHPV (Model 3). Additional covariates were excluded due to the small sample size. Analyses were performed using the *lm()* function in R Statistical Software and p-values were adjusted using the Storey false discovery rate in the package *qvalue*.[50] Metabolic super- and sub-pathways were identified using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Partial-least squares discriminant analysis (PLS-DA) was performed to identify discriminatory metabolites between HPV+ and HPV− status within each stratum of CST. In contrast to a linear model which examines the effect of HPV upon each singular metabolite, this method explores the vaginal metabolome as a whole system. Data were subset by CST and score plots were generated wherein each point represented an individual, and similar groups exhibited clustering, while different groups separated farther apart. PLS-DA models were validated based upon accuracy, variance (R^2), and cross-validated using the quality assessment Q^2 where values close to 1 indicate reliable models and negative Q^2 values indicate possible model over-fitting. Variable importance in projection (VIP) score plots were used to evaluate the discriminatory metabolites. PLS-DA, score plots, and VIP score

plots were conducted in the open source software MetaboAnalyst 4.0 (www.metaboanalyst.ca).

We then evaluated the association between microbial phylotypes and HPV detection using linear discriminant analyses effect size (LEfSe) (http://huttenhower.sph.harvard.edu/lefse/)[51]. LEfSe used the non-parametric Kruskal-Wallis rank-sum test to detect significantly differentially abundant phylotypes associated with HPV detection, then performed pairwise Wilcoxon rank-sum tests between each level of CST. Finally, linear discriminant analyses (LDA) were used to estimate the effect size where a larger LDA score indicates a larger effect. The alpha-level for both the Kruskal-Wallis rank sum and the Wilcoxon rank-sum tests was set to 0.01.

Finally, we examined how HPV-associated metabolites related to HPV-associated bacterial species using Spearman's correlation. P-values were adjusted using the *qvalue* package. Only correlations with q-values <0.05 were used for cluster analyses. Correlation and cluster analyses was performed and visualized using the functions *rcorr, corplot()* and Ward's D2 method function in R Statistical Software.

Results:

Participant characteristics:

Participant characteristics are detailed in Table S1. The average age of participants was 29 years (SD: 9.1; range:19–45). Thirteen participants were HPV-, 12 had lrHPV detected, and 14 were hrHPV+. There was a range of hygiene, lifestyle and sexual practices reported. Compared to HPV− women, HPV+ women were more likely to self-report having had vaginal discharge in the past 24 hours, smoking between 11–20 cigarettes a day, and >7 lifetime sexual partners. HPV− women were primarily classified (77%) into *Lactobacillus*-spp.-dominated CSTs (CST I and III), with just 23% categorized into the low-*Lactobacillus* CST IV. By comparison, 50% of lrHPV+ women and 29% of hrHPV+ women were categorized into CST IV.

The vaginal metabolome clusters broadly by CST:

We did not observe clear separation of the vaginal metabolome between HPV+ or HPV− women (ANOSIM R= −0.07, p-value =0.92). Instead, the vaginal metabolomes of women with CST IV microbiota clustered distinctly from those with *Lactobacillus*-dominated microbiota (CST I and CST III) (ANOSIM R = 0.54, p-value = 0.001; Figure S1A, B). Approximately 31% of the conditional variation and 23% of the marginal variation within the vaginal metabolome was explained by CST (p-value <0.0001; Table S2).

HPV detection is associated with specific metabolites (higher biogenic amines and lower glutathione and lipid concentrations):

Regression analysis identified eight metabolites that were significantly different between HPV+ and HPV− women (q-value <0.05; Figure 1; Table S3; raw data, Figure S2) after adjustment for CST and smoking status (Model 1). Notably, with the exception of 2-hydroxyhippurate, a xenobiotic, the relative concentration of the remaining metabolites,

including putrescine (197%) and ethanolamine (107%) were higher in HPV+ women when compared to HPV− women.

To evaluate whether the relationship between HPV detection status and a given metabolite differed by CSTs, we additionally included an interaction term between CST and HPV detection (Model 2). Within CST I women, no metabolites were significantly associated with HPV detection after adjustment for multiple comparisons. Within CST III women, seven metabolites including n-acetyl-cadaverine (9438%), n-acetyl-putrescine (641%) and putrescine (1210%), all biogenic amines or their derivatives, were found to be significantly higher in HPV+ women than in HPV− women (Table S3). Within CST IV women, HPV+ women had lower concentrations of glutathione, glycogen, and phospholipid-related metabolites than HPV− women (Table S3). The metabolites, reduced-glutathione (GSH) and oxidized-glutathione (GSSG) were 6535% and 2321% lower, respectively, in HPV+ women than in HPV− women. Additionally, the lipids glycerophosphorylcholine (336%), 3-hydroxydecanoate (339%) and choline phosphate (737%) were lower in HPV+ women.

High-risk HPV detection vs Low-risk HPV detection: differences in lyso- and monacylglycero- lipids:

We contrasted the metabolites between lrHPV (n=12) vs. hrHPV (n=14) women (Model 3, Table S3). Thirty-two metabolites, primarily lyso- and monacylglycero- lipids, were significantly different between hrHPV+ vs lrHPV+ women after adjustment for multiple comparisons (q-value < 0.05).

HPV detection is associated with CST-specific metabolite differences in a Partial Least-Squares Discriminant Analysis:

To interrogate potential biomarkers that might discriminate the vaginal metabolomes of HPV+ and HPV− women within each stratum of CST, data were subset into their respective CSTs and the multivariate technique, PLS-DA was applied. While Q^2 suggested that the model was overfit, R^2 values ranged from 0.6 to 0.98 and PLS-DA appropriately discriminated between HPV+ and HPV− women within each stratum of CST with no overlap at the 95% confidence level (Figure 2). Within CST I, higher concentrations of histamine, 3-n-acetyl-L-cysteine-S-yl acetaminophen, and gamma-aminobutyrate (GABA), a byproduct of bacterial acid stress resistance, were associated with HPV detection. Within CST III, high concentrations of 3-n-acetyl-L-cysteine-S-yl acetaminophen were important in discriminating HPV+ and HPV− women along with higher concentrations of the biogenic amines, n-acetyl-cadaverine, cadaverine, and tyramine. Within CST IV, low concentrations of heme, glycerophosphorylcholine and oxidized-glutathione were indicative of HPV detection (Figure 2).

HPV detection is associated with over-representation of several non-*Lactobacillus* spp.:

LEfSe analysis identified differences in the composition of the vaginal microbiota that may relate to HPV detection status even after stratifying participants into CSTs. Notably, in CST IV, HPV+ women significant over-representation of *Gardnerella vaginalis, Eggerthella*, *Atopobium* (other than *A. vaginae*) and *Dialister spp.*, among others, were observed (Figure

S3). In contrast, CST IV, HPV− individuals were found to have significant over-representation of *Bifidobacteriaceae* and *Atopobium vaginae* Figure S3).

Vaginal microbiota correlate with biogenic amines, lysine, and histidine metabolome differences:

Using Spearman's correlation analysis, two clusters of metabolites were observed, suggesting similarities in their metabolic capacities and/or requirements with regard to this subset of detectable metabolites (Figure 3). The first cluster consisted of *L. crispatus, L. jensenii* and *L. vaginalis.* These species were strongly negatively correlated with biogenic amine-, lysine- and histidine- metabolism and moderately to strongly- positively correlated with lysolipid-, phospholipid-, glutathione-, and glycogen- metabolism. The second cluster consisted of anaerobic species such as *Eggerthella*, *Gardnerella*, and *Gemella.* This cluster was strongly positively correlated with biogenic amine, lysine, and histidine metabolism and moderately to strongly negatively correlated with lipid-, glutathione- and glycogen-metabolism.

Discussion:

Main findings:

Overall, the vaginal metabolome did not cluster by HPV detection status and instead grouped broadly by bacterial composition. Women with CST IV (molecular-BV) microbiota presented a vaginal metabolome that clustered distinctly from those with other CSTs. However, in statistical models that controlled for the driving effects of smoking and CST, higher biogenic amine and phospholipid concentrations were observed in HPV+ women compared with HPV− women, and significant differences in metabolomic profiles of HPV+ and HPV− women were evident within each stratum of CST. Within CST III, HPV+ women had higher concentrations of biogenic amines compared to HPV− women. Within CST IV, HPV+ women had lower concentrations of glutathione, glycogen, and phospholipid-related metabolites than HPV− women. In a secondary analysis, hrHPV women had higher concentrations of multiple lysolipids and monoacylglycerol-lipids compared to lrHPV women. From the microbial perspective, HPV+ women with CST-IV microbiota had an over-representation of *Atopobium spp.* (other than *A. vaginae*), *Dialister, Eggerthella, Gemella*, and *Gardnerella* spp. These taxa were strongly positively correlated with the biogenic amines putrescine, n-acetyl-cadaverine and n-acetylputrescine.

Strengths and limitations:

We applied an untargeted metabolomics approach to examine HPV+ and HPV− vaginal samples. Furthermore, we were able to assess this association while addressing the vaginal microbiome and smoking behaviors as sources of participant heterogeneity. A previous study has assessed the metabolome of urinary samples based on HPV infection status[52]. Our study dually applied both GC- and LC-MS metabolomics to characterize the vaginal metabolome, enabling the separation of both soluble, non-volatile and volatile compounds. While the number of participants examined herein is comparable to the Godoy-Vitorino study[52], the sample size remains small relative to the number of metabolites measured, and this reduced our ability to identify and adjust for other potentially confounding variables

beyond smoking; a variable previously established as being associated with differences in the vaginal metabolome[29]. It may also have limited our power to detect significant differences in metabolites and microbiota with greater natural variation.

Interpretation:

The association between molecular BV with increased risk for gynecological and reproductive outcomes, including acquisition of HPV and other STIs, is well documented[53, 54]. Our group and others have previously documented microbial community to be a major driver of the vaginal metabolome[17, 34, 35, 38, 55], it is therefore not surprising that in this study, vaginal metabolomes clustered largely according to CST and not by HPV-detection status. However, when controlling for CST and in analyses within each stratum of CST, statistically significant metabolite differences by HPV detection status were observed.

Consistent with the recent study of the urinary metabolome of women varying in HPV status published by Godoy-Vitorino *et al.* [52], we also observed higher levels of the central nervous system neuromodulator 4-hydoxybutric acid (aka gamma-hydroxybutryic acid or GHB), a precursor to GABA, in HPV+ women. Consistent with the recent study by Ilhan *et al.*[17], we also observed higher levels of several lyso- and monoacylglycerol-lipids in HPV+ women, including choline phosphate, and glycerophosphorylcholine. Within CST IV, HPV+ women had lower levels of glutathione-related metabolites including, oxidized-glutathione and reduced-glutathione. These metabolites may represent oxidative stress and total glutathione depletion. Byproducts of oxidative stress may cause irreversible damage to membrane lipids, proteins and DNA, and have been implicated as potential co-factors in HPV carcinogenesis and persistence[56, 57]. Future longitudinal studies designed to establish the association of these compounds with disease progression, in combination with specific hrHPV strains, are merited.

Another primary result was that the biogenic amines putrescine and ethanolamine, were significantly higher in HPV+ women after adjustment for CST and smoking status. Cadaverine, tyramine, and two biogenic amine derivatives, n-acetyl-cadaverine and n-acetyl-putrescine, had higher concentrations in HPV+ versus HPV− women in the *L. iners*-dominated CST. Biogenic amines have been shown to vary by CST, having the greatest relative concentration within the *Lactobacillus* spp.-depauperate CST IV[59] and are elevated among BV-sufferers[37, 39, 40, 60–64], with cadaverine, putrescine, and trimethylamine specifically associated with the malodor reported in BV[39, 40, 61, 63]. Their role in STIs may be multifaceted, with those identified in this study all being formed by the decarboxylation of specific amino acids, a metabolic process utilized by various bacteria to overcome acidic environmental barriers[59], such as those documented in the vagina. Biogenic amines have also been reported to alter pathogen virulence, including protecting pathogens such as *Neisseria gonorrhea* from both the anti-microbial effects of lactic acid and host innate immune defenses[65, 66]. The relationship between biogenic amines and HPV deserves further exploration.

Additionally, HPV+ *L. iners* and *L. crispatus*-dominated women exhibited higher concentrations of the acetaminophen derivative, 3-n-acetyl-L-cysteine-S-yl acetaminophen, suggesting HPV-detection was associated with higher use of the common pain reliever. We

also observed over-representation of *Gardnerella, Gemella*, and *Atopobium* spp. in HPV+ women with CST IV microbiota which is consistent with a study conducted by Di Paola *et al.*[67]. *Eggerthella* and *Gemella* possess genes for the production of the biogenic amines agmatine and putrescine, which have been associated with BV[39, 40, 59] and, together, should be studied further to evaluate if they reflect a microbial microenvironment with increased risk of HPV acquisition, persistence, or reactivation[67]. Lastly, our analyses between lrHPV and hrHPV women also pointed to behavioral differences within our population that segregated with detection status. While we observed higher concentrations of the tobacco constituent, cotinine, in hrHPV+ women, this may reflect greater levels of tobacco use among hrHPV+ women or be an artifact of our inability to control for smoking status due to the small sample size.

Conclusion:

Significant differences in the vaginal metabolome were identified by HPV detection status, independent of bacterial community state or smoking status, both known influencers of the vaginal metabolome. This cross-sectional study cannot inform a causal relationship between HPV, the vaginal metabolome, the CST or other host/behavioral characteristics. However, the identified metabolite differences may provide important insights into the biochemical changes in the vaginal microenvironment that increase the risk of HPV infection and how the infection persists. Reduced-glutathione and oxidized-glutathione have known associations with HPV and may represent increased oxidative stress and total glutathione depletion. Chronic oxidative stress environments may compromise normal host responses to infection or increase risk of disease progression. The increased levels of reduced-glutathione and oxidized-glutathione observed in women with HPV detection supports further investigation. These data provide information on possible physiological pathways that may either induce HPV reactivation or clearance that could be targets for therapeutic non-surgical interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES:

1. Schoell WM, Janicek MF, Mirhashemi R. Epidemiology and biology of cervical cancer. SeminSurgOncol. 1999;16(3):203–11.
2. Pfister H. Human papillomaviruses and genital cancer. AdvCancer Res. 1987;48:113–47.
3. Salzman NP, Howley PM. The Papovaviridae. New York: Plenum Press; 1986.
4. Plummer M, Herrero R, Franceschi S, Meijer CJ, Snijders P, Bosch FX, et al. Smoking and cervical cancer: pooled analysis of the IARC multi-centric case--control study. Cancer Causes Control. 2003;14(9):805–14. [PubMed: 14682438]
5. International Collaboration of Epidemiological Studies of Cervical C, Appleby P, Beral V, Berrington de Gonzalez A, Colin D, Franceschi S, et al. Carcinoma of the cervix and tobacco smoking: collaborative reanalysis of individual data on 13,541 women with carcinoma of the cervix and 23,017 women without carcinoma of the cervix from 23 epidemiological studies. Int J Cancer. 2006;118(6):1481–95. [PubMed: 16206285]
6. Schlecht NF, Kulaga S, Robitaille J, Ferreira S, Santos M, Miyamura RA, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA : the journal of the American Medical Association. 2001;286(24):3106–14. [PubMed: 11754676]
7. Rodriguez-Cerdeira C, Sanchez-Blanco E, Alba A. Evaluation of Association between Vaginal Infections and High-Risk Human Papillomavirus Types in Female Sex Workers in Spain. ISRN obstetrics and gynecology. 2012;2012:240190. [PubMed: 22900198]
8. McNicol P, Paraskevas M, Guijon F. Variability of polymerase chain reaction-based detection of human papillomavirus DNA is associated with the composition of vaginal microbial flora. Journal of medical virology. 1994;43(2):194–200. [PubMed: 8083669]
9. Mao C, Hughes JP, Kiviat N, Kuypers J, Lee SK, Adam DE, et al. Clinical findings among young women with genital human papillomavirus infection. Am J Obs Gyn. 2003;188(3):677–84.
10. Klomp JM, Boon ME, Van Haaften M, Heintz APM. Cytologically diagnosed Gardnerella vaginalis infection and cervical (pre)neoplasia as established in population-based cervical screening. American journal of obstetrics and gynecology. 2008;199(5):480.e1–5. [PubMed: 18565490]
11. Dols JA, Reid G, Kort R, Schuren FH, Tempelman H, Bontekoe TR, et al. PCR-based identification of eight Lactobacillus species and 18 hr-HPV genotypes in fixed cervical samples of South African women at risk of HIV and BV. Diagnostic cytopathology. 2012;40(6):472–7. [PubMed: 22021225]
12. Wiley DJ, Masongsong EV, Lu S, Heather LS, Salem B, Giuliano AR, et al. Behavioral and sociodemographic risk factors for serological and DNA evidence of HPV6, 11, 16, 18 infections. Cancer Epidemiol. 2012;36(3):e183–9. [PubMed: 22277329]
13. Nam KH, Kim YT, Kim SR, Kim SW, Kim JW, Lee MK, et al. Association between bacterial vaginosis and cervical intraepithelial neoplasia. Journal of gynecologic oncology. 2009;20(1):39–43. [PubMed: 19471662]
14. Lee JE, Lee S, Lee H, Song Y-M, Lee K, Han MJ, et al. Association of the Vaginal Microbiota with Human Papillomavirus Infection in a Korean Twin Cohort. PloS one. 2013;8(5):e63514. [PubMed: 23717441]
15. Gillet E, Meys JF, Verstraelen H, Bosire C, De Sutter P, Temmerman M, et al. Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. BMC Infect Dis. 2011;11:10. [PubMed: 21223574]
16. Mitra A, MacIntyre DA, Marchesi JR, Lee YS, Bennett PR, Kyrgiou M. The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? Microbiome. 2016;4(1):58. [PubMed: 27802830]
17. Ilhan ZE, Laniewski P, Thomas N, Roe DJ, Chase DM, Herbst-Kralovetz MM. Deciphering the complex interplay between microbiota, HPV, inflammation and cancer through cervicovaginal metabolic profiling. EBioMedicine. 2019.
18. Dareng EO, Ma B, Famooto AO, Adebamowo SN, Offiong RA, Olaniyan O, et al. Prevalent high-risk HPV infection and vaginal microbiota in Nigerian women. Epidemiol Infect. 2016;144(1): 123–37. [PubMed: 26062721]

19. Shannon B, Yi TJ, Perusini S, Gajer P, Ma B, Humphrys MS, et al. Association of HPV infection and clearance with cervicovaginal immunology and the vaginal microbiota. Mucosal Immunol. 2017;10(5):1310–9. [PubMed: 28120845]
20. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011;108 Suppl 1:4680–7. [PubMed: 20534435]
21. Boskey ER, Cone RA, Whaley KJ, Moench TR. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. Human reproduction (Oxford, England). 2001;16(9):1809–13.
22. Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, et al. Defense factors of vaginal lactobacilli. American journal of obstetrics and gynecology. 2001;185(2):375–9. [PubMed: 11518895]
23. Ocana VS, Pesce De Ruiz Holgado AA, Nader-Macias ME. Characterization of a bacteriocin-like substance produced by a vaginal Lactobacillus salivarius strain. Applied and Environmental Microbiology. 1999;65(12):5631–5. [PubMed: 10584033]
24. Reid G, Heinemann C, Velraeds M, van der Mei HC, Busscher HJ. Biosurfactants produced by Lactobacillus. Methods in enzymology. 1999;310:426–33. [PubMed: 10547809]
25. Kaewsrichan J, Peeyananjarassri K, Kongprasertkit J. Selection and identification of anaerobic lactobacilli producing inhibitory compounds against vaginal pathogens. FEMS ImmunolMedMicrobiol. 2006;48(1):75–83.
26. Boris S, Barbés C. Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect. 2000;2(5):543–6. [PubMed: 10865199]
27. Borgogna J-LC, Yeoman CJ. The Application of Molecular Methods Towards an Understanding of the Role of the Vaginal Microbiome in Health and Disease. The Human Microbiome. Methods in Microbiology2017. p. 37–91.
28. McKinnon LR, Achilles SL, Bradshaw CS, Burgener A, Crucitti T, Fredricks DN, et al. The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. AIDS Res Hum Retroviruses. 2019;35(3):219–28. [PubMed: 30638028]
29. Brotman RM, He X, Gajer P, Fadrosh D, Sharma E, Mongodin EF, et al. Association between cigarette smoking and the vaginal microbiota: a pilot study. BMC Infect Dis. 2014;14:471. [PubMed: 25169082]
30. Brotman RM, Shardell MD, Gajer P, Tracy JK, Zenilman JM, Ravel J, et al. Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. J Infect Dis. 2014;210(11):1723–33. [PubMed: 24943724]
31. Onywera H, Williamson AL, Mbulawa ZZA, Coetzee D, Meiring TL. The cervical microbiota in reproductive-age South African women with and without human papillomavirus infection. Papillomavirus Res. 2019;7:154–63. [PubMed: 30986570]
32. Laniewski P, Cui H, Roe DJ, Barnes D, Goulder A, Monk BJ, et al. Features of the cervicovaginal microenvironment drive cancer biomarker signatures in patients across cervical carcinogenesis. Sci Rep. 2019;9(1):7333. [PubMed: 31089160]
33. Koumans EH, Sternberg M, Bruce C, McQuillan G, Kendrick J, Sutton M, et al. The prevalence of bacterial vaginosis in the United States, 2001–2004; associations with symptoms, sexual behaviors, and reproductive health. Sex TransmDis. 2007;34(11):864–9.
34. Nelson TM, Borgogna JC, Michalek RD, Roberts DW, Rath JM, Glover ED, et al. Cigarette smoking is associated with an altered vaginal tract metabolomic profile. Sci Rep. 2018;8(1):852. [PubMed: 29339821]
35. Hillier SL. Diagnostic microbiology of bacterial vaginosis. American journal of obstetrics and gynecology. 1993;169(2 Pt 2):455–9. [PubMed: 8357044]
36. Wang L, Koppolu S, Chappell C, Moncla BJ, Hillier SL, Mahal LK. Studying the effects of reproductive hormones and bacterial vaginosis on the glycome of lavage samples from the cervicovaginal cavity. PLoS One. 2015;10(5):e0127021. [PubMed: 25993513]
37. Sobel JD, Karpas Z, Lorber A. Diagnosing vaginal infections through measurement of biogenic amines by ion mobility spectrometry. European journal of obstetrics, gynecology, and reproductive biology. 2012.

38. Witkin SS, Ledger WJ. Complexities of the uniquely human vagina. Sci Transl Med. 2012;4(132): 132fs11. [PubMed: 22553249]

39. Yeoman CJ, Thomas SM, Miller ME, Ulanov AV, Torralba M, Lucas S, et al. A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. PLoS One. 2013;8(2):e56111. [PubMed: 23405259]

40. Srinivasan S, Morgan MT, Fiedler TL, Djukovic D, Hoffman NG, Raftery D, et al. Metabolic signatures of bacterial vaginosis. mBio. 2015;6(2):10.1128/mBio.00204-15.

41. Dobierzewska A, Soman S, Illanes SE, Morris AJ. Plasma cross-gestational sphingolipidomic analyses reveal potential first trimester biomarkers of preeclampsia. PLoS One. 2017;12(4):e0175118. [PubMed: 28384202]

42. Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Gomez R, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med. 2010;23(12):1344–59. [PubMed: 20504069]

43. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Int J Surg. 2014;12(12):1500–24. [PubMed: 25046751]

44. Field N, Cohen T, Struelens MJ, Palm D, Cookson B, Glynn JR, et al. Strengthening the Reporting of Molecular Epidemiology for Infectious Diseases (STROME-ID): an extension of the STROBE statement. Lancet Infect Dis. 2014;14(4):341–52. [PubMed: 24631223]

45. Gallo V, Egger M, McCormack V, Farmer PB, Ioannidis JP, Kirsch-Volders M, et al. STrengthening the Reporting of OBservational studies in Epidemiology - Molecular Epidemiology (STROBE-ME): an extension of the STROBE statement. Eur J Clin Invest. 2012;42(1):1–16.

46. Yang J, Zhao X, Lu X, Lin X, Xu G. A data preprocessing strategy for metabolomics to reduce the mask effect in data analysis. Front Mol Biosci. 2015;2:4. [PubMed: 25988172]

47. Smilde AK, van der Werf MJ, Bijlsma S, van der Werff-van der Vat BJ, Jellema RH. Fusion of mass spectrometry-based metabolomics data. Anal Chem. 2005;77(20):6729–36. [PubMed: 16223263]

48. Wei R, Wang J, Su M, Jia E, Chen S, Chen T, et al. Missing Value Imputation Approach for Mass Spectrometry-based Metabolomics Data. Sci Rep. 2018;8(1):663. [PubMed: 29330539]

49. Xia J, Wishart DS. Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. Nat Protoc. 2011;6(6):743–60. [PubMed: 21637195]

50. Storey JD. A Direct Approach to False Discovery Rates. Journal of the Royal Statistical Society Series B (Statistical Methodology). 2002;64(3):479–98.

51. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60. [PubMed: 21702898]

52. Godoy-Vitorino F, Ortiz-Morales G, Romaguera J, Sanchez MM, Martinez-Ferrer M, Chorna N. Discriminating high-risk cervical Human Papilloma Virus infections with urinary biomarkers via non-targeted GC-MS-based metabolomics. PLoS One. 2018;13(12):e0209936. [PubMed: 30592768]

53. Martin DH. The Microbiota of the Vagina and Its Influence on Women's Health and Disease. AmJ MedSci. 2011.

54. Nardis C, Mosca L, Mastromarino P. Vaginal microbiota and viral sexually transmitted diseases. Ann Ig. 2013;25(5):443–56. [PubMed: 24048183]

55. Sobel JD. Bacterial vaginosis. Annu Rev Med. 2000;51:349–56. [PubMed: 10774469]

56. De Marco F. Oxidative stress and HPV carcinogenesis. Viruses. 2013;5(2):708–31. [PubMed: 23403708]

57. Borges BES, Brito EB, Fuzii HT, Baltazar CS, Sa AB, Silva C, et al. Human papillomavirus infection and cervical cancer precursor lesions in women living by Amazon rivers: investigation of relations with markers of oxidative stress. Einstein (Sao Paulo). 2018;16(3):eAO4190.

58. Ferrini MG, Nolazco G, Vernet D, Gonzalez-Cadavid NF, Berman J. Increased vaginal oxidative stress, apoptosis, and inducible nitric oxide synthase in a diabetic rat model: implications for vaginal fibrosis. Fertil Steril. 2006;86(4 Suppl):1152–63. [PubMed: 16978624]

59. Nelson TM, Borgogna JL, Brotman RM, Ravel J, Walk ST, Yeoman CJ. Vaginal biogenic amines: biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis? Frontiers in physiology. 2015;6:253. [PubMed: 26483694]

60. Vitali B, Cruciani F, Picone G, Parolin C, Donders G, Laghi L. Vaginal microbiome and metabolome highlight specific signatures of bacterial vaginosis. Eur J Clin Microbiol Infect Dis. 2015;34(12):2367–76. [PubMed: 26385347]

61. Wolrath H, Boren H, Hallen A, Forsum U. Trimethylamine content in vaginal secretion and its relation to bacterial vaginosis. APMIS. 2002;110(11):819–24. [PubMed: 12588422]

62. Hillier SL, Krohn MA, Rabe LK, Klebanoff SJ, Eschenbach DA. The normal vaginal flora, H2O2-producing lactobacilli, and bacterial vaginosis in pregnant women. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1993;16 Suppl 4:S273–S81.

63. Chen KC, Forsyth PS, Buchanan TM, Holmes KK. Amine content of vaginal fluid from untreated and treated patients with nonspecific vaginitis. J Clin Invest. 1979;63(5):828–35. [PubMed: 447831]

64. Chen KC, Amsel R, Eschenbach DA, Holmes KK. Biochemical diagnosis of vaginitis: determination of diamines in vaginal fluid. J Infect Dis. 1982;145(3):337–45. [PubMed: 7061879]

65. Goytia M, Shafer WM. Polyamines can increase resistance of Neisseria gonorrhoeae to mediators of the innate human host defense. Infect Immun. 2010;78(7):3187–95. [PubMed: 20439477]

66. Gong Z, Tang MM, Wu X, Phillips N, Galkowski D, Jarvis GA, et al. Arginine- and Polyamine-Induced Lactic Acid Resistance in Neisseria gonorrhoeae. PLoS One. 2016;11(1):e0147637. [PubMed: 26808268]

67. Di Paola M, Sani C, Clemente AM, Iossa A, Perissi E, Castronovo G, et al. Characterization of cervico-vaginal microbiota in women developing persistent high-risk Human Papillomavirus infection. Sci Rep. 2017;7(1):10200. [PubMed: 28860468]

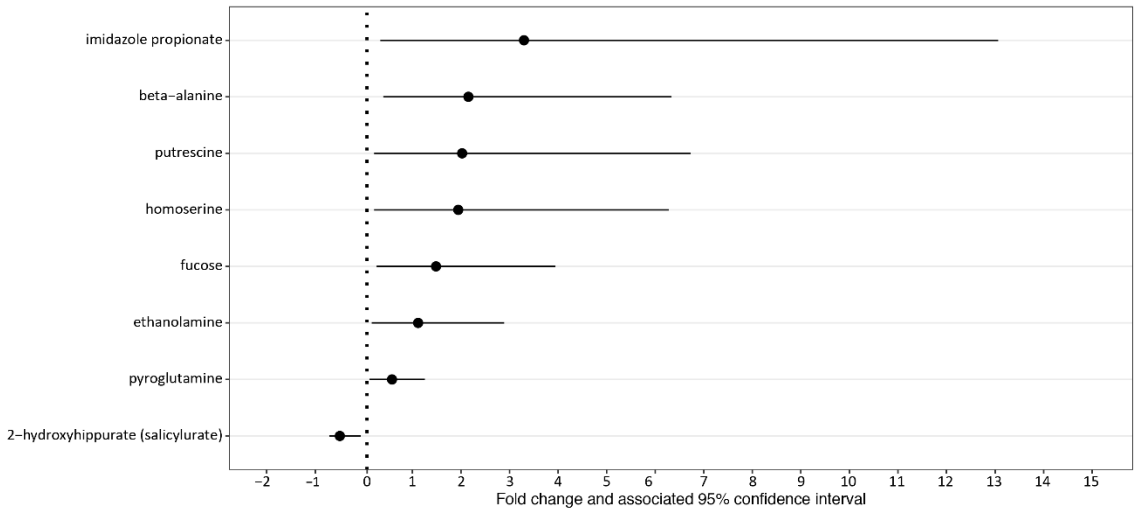

Figure 1| Fold changes and associated 95% confidence intervals of significantly different metabolites between HPV+ and HPV- women after adjustment for CST and smoking status. For each metabolite, the dot represents the fold change calculated from exponentiated model coefficients and the line represents the associated 95% confidence interval.

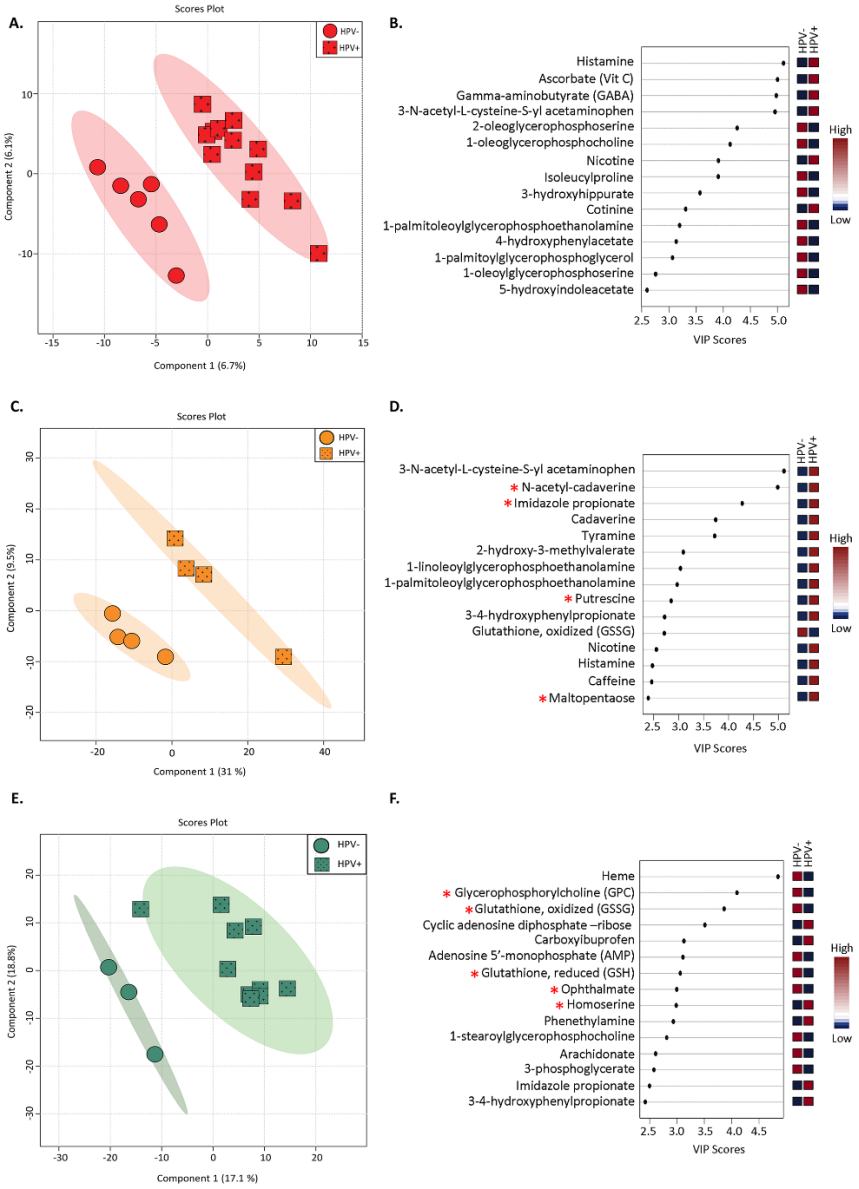

Figure 2| Discriminatory metabolites associated with HPV status stratified by CST. HPV- and HPV+ women within CST-I are compared in the first row (A,B). HPV- and HPV+ women within CST-III are compared in second row (C,D) and HPV- and HPV+ within CST-IV are compared in the third row (E,F). A,C,E show discrimination of groups using partial least squares discriminant analysis. B,D,F show the metabolites most strongly influencing discrimination by the PLS-DA. The variable importance in projection (VIP) score is the weighted sum of squares for the PLS-DA loading with the amount of variation explained by each component taken into account. Asterisks indicate metabolites which are also significant via regression analyses.

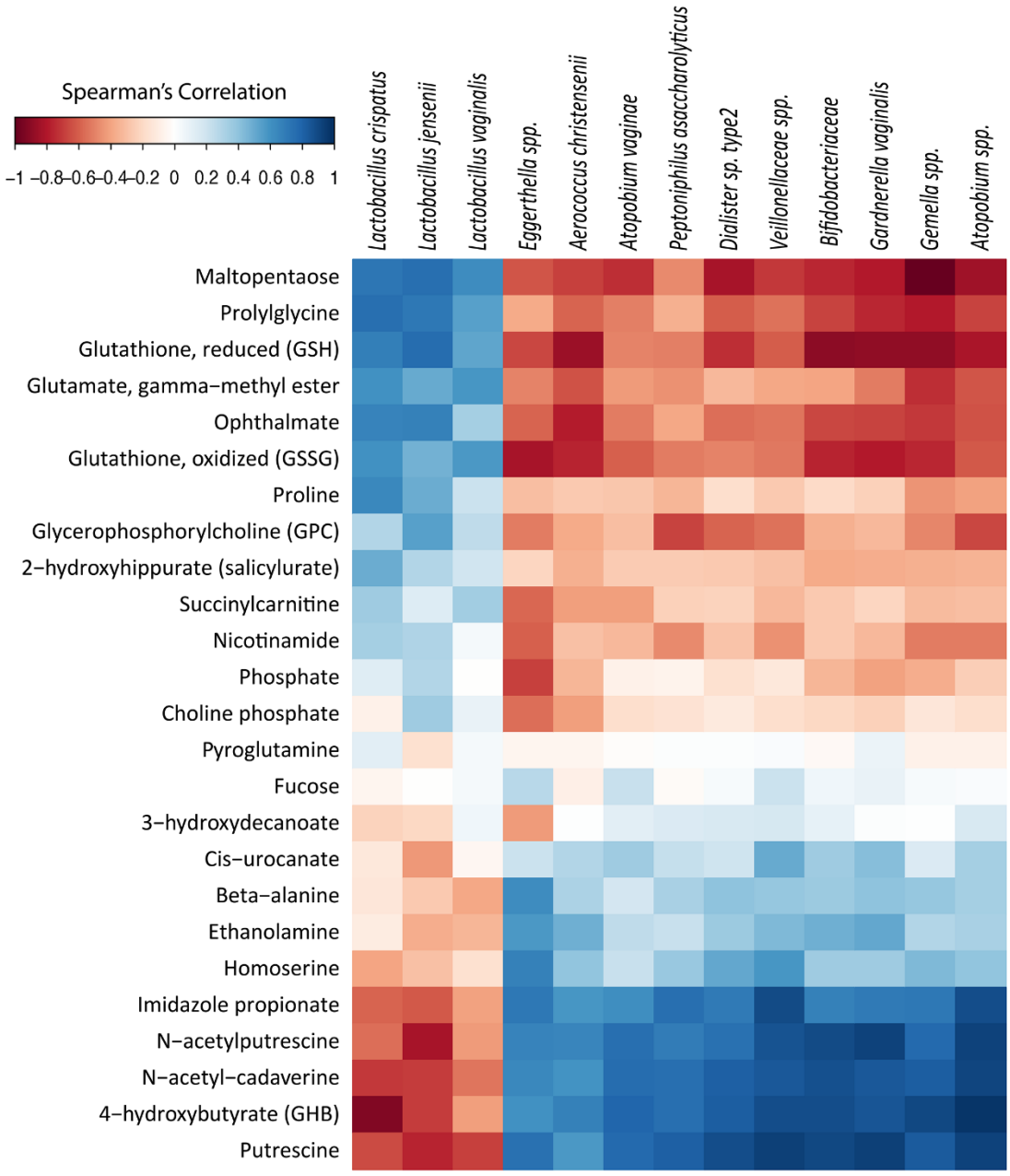

Figure 3| Correlation analysis of the microbiome and metabolome. Spearman's correlation analysis was conducted using potential microbiome biomarkers of HPV as identified by linear discriminant analysis effect size (LEfSe) and potential metabolite biomarkers identified through multiple linear regression.

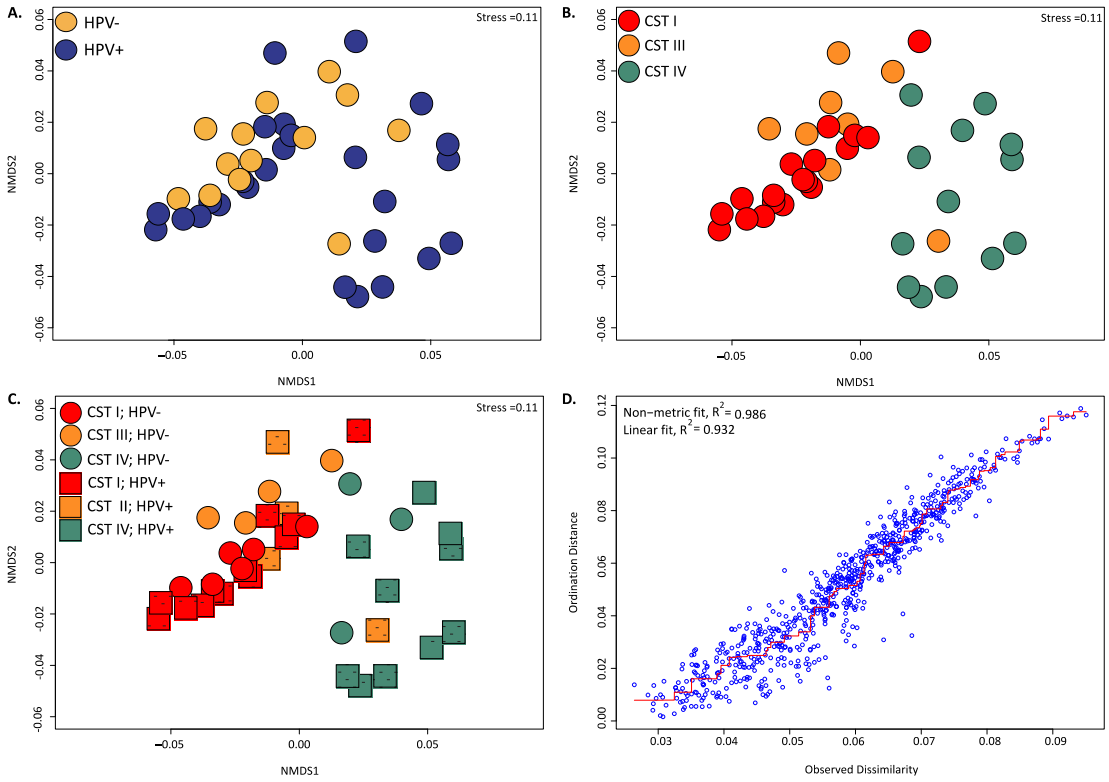

Figure S1. The vaginal metabolomes by CST. Shown are nMDS plots of the metabolomes clustered by A) HPD detection status B) Community State Type (CST) and C) CST and HPV. A Shepard's plot of dissimilarity (D) indicates that the observed dissimilarity and ordination distances were highly correlated (non-metric R=0.986).

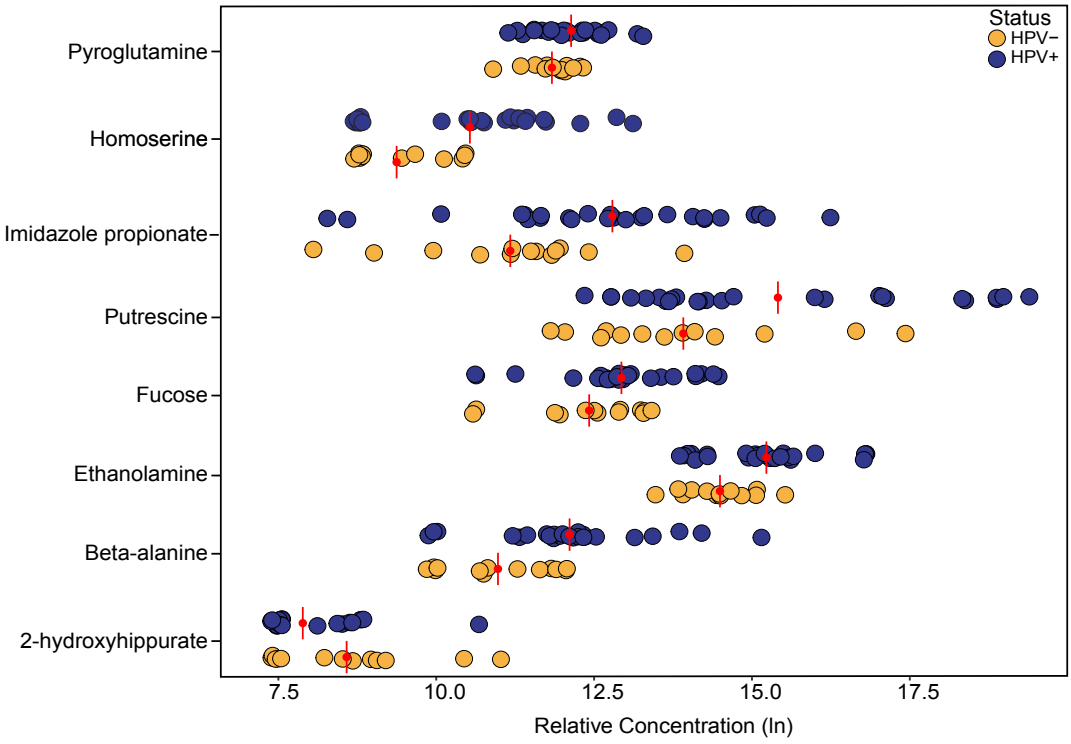

Figure S2| Relative (log-transformed) concentration of significantly different metabolites between HPV+ and HPV- women after adjustment for CST and smoking status. Red line indicates the average of a metabolite for a given group. Each dot represents a single individual (blue=HPV+; yellow=HPV-).

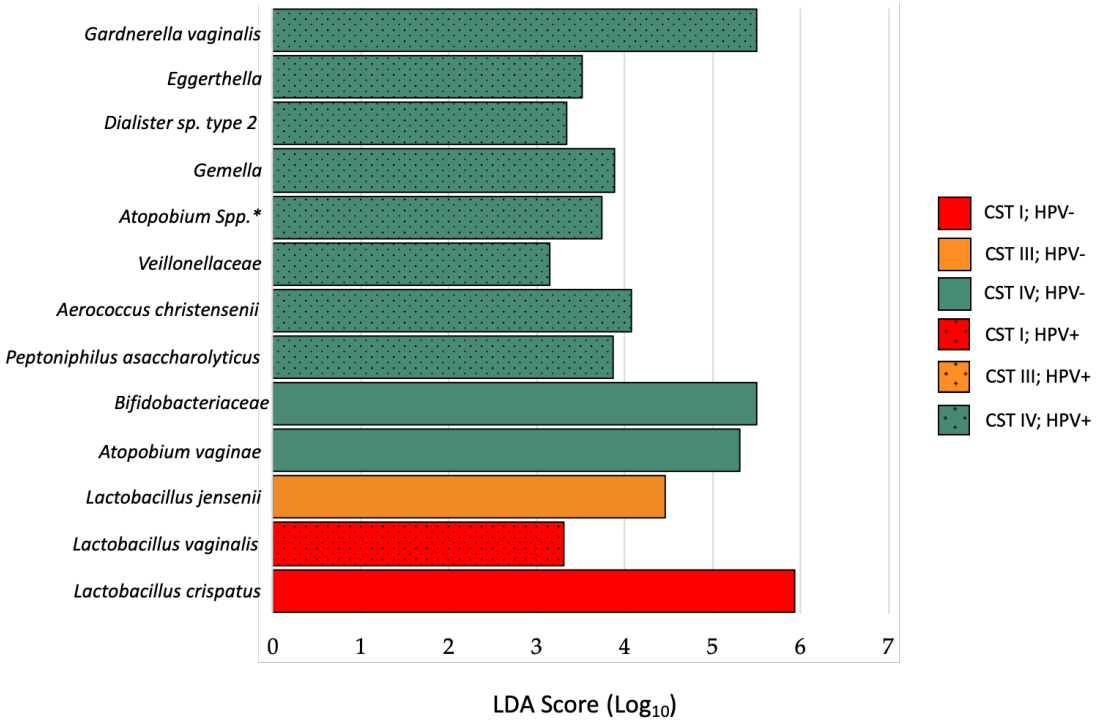

Figure S3| The association between vaginal taxa and detection status as identified by LEfSE. Over-represented taxa were considered significant if they had an LDA score >2 and a q-value <0.05. *Does not include Atopobium vaginae

Table S1. Participant characteristics and demographics

Participant Details	HPV-	HPV+		P-value*
	n (%)	**hrHPV-** (n%)	**hrHPV+** (n%)	
Age				0.078
18-28	10 (77.0)	3 (25.0)	9 (64.3)	
29-39	2 (15.0)	3 (25.0)	2 (14.3)	
40-45	1 (7.7)	6 (50.0)	3 (21.4)	
Race				0.051
African American	2 (15.0)	7 (58.0)	6 (43.0)	
Asian	3 (23.0)	0 (0.0)	1 (7.1)	
Hispanic	3 (23.0)	0 (0.0)	0 (0.0)	
Hispanic/White	0 (0.0)	0 (0.0)	1 (7.1)	
Multi-racial	0 (0.0)	1 (8.3)	0 (0.0)	
White	0 (0.0)	1 (8.3)	0 (0.0)	
Other	5 (39.0)	3 (25.0)	6 (43.0)	
Community State Type				0.601
CST-I	6 (46.0)	5 (41.7)	1 (7.1)	
CST-III	4 (31.0)	1 (8.3)	3 (21.0)	
CST-IV	3 (23.0)	6 (50.0)	4 (29.0)	
Marital status				0.085
Currently married	1 (7.7)	2 (16.7)	3 (21.0)	
Never married	12 (91.3)	5 (41.7)	9 (64.0)	
Separated, divorced, widowed	0 (0.0)	1 (8.3)	0 (0.0)	
Other	0 (0.0)	4 (33.3)	2 (14.0)	
Birth Control				0.039
Injection	0 (0.0)	2 (16.7)	1 (7.1)	
OCP	3 (23.1)	2 (16.7)	2 (14.0)	
Non-hormonal - other	1 (7.7)	5 (41.7)	8 (51.7)	
No answer	9 (69.2)	3 (25.0)	3 (21.0)	
Lifetime Sexual Partners, no (%)				0.015
0-4	7 (54.0)	2 (16.7)	1 (7.1)	
5-7	4 (31.0)	1 (8.3)	5(35.7)	
8+	2 (15.0)	8 (66.7)	7 (50.0)	
No answer	0 (0.0)	1 (8.3)	1 (7.1)	
Male Sex partner, no (%)				0.087
0 in last 2 months	6 (46.0)	1 (8.3)	1 (7.1)	
1 in last 2 months	7 (54.0)	8 (66.7)	10 (71.4)	
2-3 in last 2 months	0 (0.0)	2 (16.7)	2 (14.0)	
No answer	0 (0.0)	1 (8.3)	1 (7.1)	

Female Sex partner, no (%)				0.071
0 in last 2 months	11 (84.6)	7 (58.0)	11 (78.6)	
1 in last 2 months	1 (7.7)	1 (8.3)	0 (0.0)	
No answer	1 (7.7)	4 (33.3)	3 (21.3)	
Nugent Score				0.016
0-3	10 (77.0)	6 (50.0)	10 (71.0)	
4-6	0 (0.0)	6 (50.0)	3 (21.0)	
7-10	3 (23.0)	0 (0.0)	1 (7.0)	
Vaginal pH				0.443
≤ 4	5 (38.5)	4 (33.3)	5 (35.7)	
4.1-4.5	0 (0.0)	0 (0.0)	3 (21.0)	
4.6-5.0	3 (23.0)	1 (8.3)	2 (14.0)	
≥ 5.1	5 (38.5)	7 (58.0)	4 (29.0)	
Vaginal odor				0.154
Yes	3 (23.0)	0 (0.0)	4 (29.0)	
No	10 (77.0)	12 (100.0)	10 (71.0)	
Vaginal irritation, 24 hours prior				1.000
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
No	13 (100.0)	12 (100.0)	14 (100.0)	
Vaginal itching, 24 hours prior				1.000
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
No	13 (100.0)	12 (100.0)	14 (100.0)	
Vaginal discharge, 24 hours prior				0.073
Yes	0 (0.0)	4 (33.3)	4 (29.0)	
No	13 (100.0)	8	10 (71.0)	
Vaginal burning, 24 hours prior				1.000
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
No	13 (100.0)	12 (100.0)	14 (100.0)	
Pain urinating, 24 hours prior				1.000
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
No	13 (100.0)	12 (100.0)	14 (100.0)	
Douching, 2 months prior				0.429
1 time per week	0 (0.0)	0 (0.0)	1 (7.1)	
More than once per week	1 (7.7)	2 (16.7)	3 (21.0)	
1-2 times per month	0 (0.0)	1 (8.3)	0 (0.0)	
Every now and then	0 (0.0)	1 (8.3)	0 (0.0)	
Never	12 (91.3)	7 (58.0)	10 (71.0)	
No answer	0 (0.0)	1 (8.3)	0 (0.0)	
Smoking Status				0.009
Smoker	3 (23.0)	10 (83.3)	6 (42.9)	
Non-smoker	10 (77.0)	2 (16.7)	8 (57.1)	
Cigs. smoked per day, no.				0.006
1-10	2 (15.4)	7 (58.3)	1 (7.1)	
11-20	1 (7.7)	3 (25.0)	5 (35.7)	
non-smoker	10 (76.9)	2 (16.7)	8 (57.1)	

* p-values obtained from Fisher's exact test

Table S2. PERMANOVA results

PERMANOVA; Conditional Means:					
	DF	**Sum of Squares**	**R2**	**F**	**P-value**
Community State Type	2	0.020622	0.30788	7.9587	<0.0001
HPV	1	0.001254	0.01872	0.9677	0.3976
hrHPV	1	0.001285	0.01918	0.9916	0.3923
Smoking Status	1	0.001068	0.01594	0.824	0.5266
Residual	33	0.042754	0.63829		
Total	38	0.066982	1		
PERMANOVA; Marginal Means:					
	DF	**Sum of Squares**	**R2**	**F**	**P-value**
Community State Type	2	0.015621	0.23321	6.0286	<0.0001
HPV	1	0.001367	0.0204	1.0549	0.3473
hrHPV	1	0.001136	0.01696	0.8766	0.4696
Smoking Status	1	0.001068	0.01594	0.824	<0.0001
Residual	33	0.042754	0.63829		
Total	38	0.066982	1		

Table S3. Statistically significant metabolites as determined by regression analysis

HPV+ vs. HPV- accounting for smoking and CST							
KEGG ID	SUPER PATHWAY	SUB PATHWAY	METABOLITE	P-VALUE	Q-VALUE	EXP (COEF)	PERCENT(%) DIFFERENCE
	Amino Acid	Glutamate Metabolism	pyroglutamine	0.026	0.045	1.52	52.5
C00263	Amino Acid	Glycine, Serine and Threonine Metabolism	homoserine	0.025	0.044	2.89	189.0
	Amino Acid	Histidine Metabolism	imidazole propionate	0.019	0.035	4.25	324.9
C00134	Amino Acid	Polyamine Metabolism	putrescine	0.026	0.046	2.97	197.0
C01018	Carbohydrate	Pentose Metabolism	fucose	0.015	0.027	2.44	143.6
C00189	Lipid	Phospholipid Metabolism	ethanolamine	0.023	0.041	2.07	106.8
C00099	Nucleotide	Pyrimidine Metabolism, Uracil containing	beta-alanine	0.010	0.019	3.13	213.0
C07588	Xenobiotics	Benzoate Metabolism	2-hydroxyhippurate (salicylurate)	0.020	0.035	0.45	-55.5
	CST III HPV+ compared to CST III HPV- accounting for smoking						
	Amino Acid	Histidine Metabolism	cis-urocanate	0.009	0.041	4.01	301.1
	Amino Acid	Histidine Metabolism	imidazole propionate	0.001	0.004	43.53	4253.2
	Amino Acid	Lysine Metabolism	N-acetyl-cadaverine	0.003	0.013	95.39	9438.6
C02714	Amino Acid	Polyamine Metabolism	N-acetylputrescine	0.006	0.027	7.41	641.2
C00134	Amino Acid	Polyamine Metabolism	putrescine	0.006	0.029	13.10	1210.4
C06218	Carbohydrate	Glycogen Metabolism	maltopentaose	0.002	0.011	8.77	777.5
	Peptide	Dipeptide	prolylglycine	0.005	0.021	5.26	426.2
	CST IV HPV+ compared to CST IV HPV- accounting for smoking						
	Amino Acid	Glutamate Metabolism	glutamate, gamma-methyl ester	0.001	0.003	0.16	-630.7
C00127	Amino Acid	Glutathione Metabolism	glutathione, oxidized (GSSG)	0.009	0.046	0.04	-2321.2
C00051	Amino Acid	Glutathione Metabolism	glutathione, reduced (GSH)	0.005	0.028	0.09	-6535.7
	Amino Acid	Glutathione Metabolism	ophthalmate	0.000	0.001	0.08	-1554.3

C00263	Amino Acid	Glycine, Serine and Threonine Metabolism	homoserine	0.002	0.011	12.64	2115.4
C00148	Amino Acid	Urea cycle; Arginine and Proline Metabolism	proline	0.000	0.001	0.17	-608.9
C06218	Carbohydrate	Glycogen Metabolism	maltopentaose	0.004	0.021	0.16	-1305.8
C00153	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	nicotinamide	0.010	0.049	0.23	-464.4
C00009	Energy	Oxidative Phosphorylation	phosphate	0.003	0.015	0.84	-219.0
	Energy	TCA Cycle	succinylcarnitine	0.007	0.036	0.21	-680.1
	Lipid	Fatty Acid, Monohydroxy	3-hydroxydecanoate	0.007	0.034	0.38	-339.8
C00989	Lipid	Fatty Acid, Monohydroxy	4-hydroxybutyrate (GHB)	0.002	0.010	4.89	545.3
C00588	Lipid	Phospholipid Metabolism	choline phosphate	0.001	0.003	0.14	-737.5
C00670	Lipid	Phospholipid Metabolism	glycerophosphorylcholine (GPC)	0.005	0.029	0.03	-336.8
			hrHPV+ compared to lrHPV+				
	Amino Acid	Leucine, Isoleucine and Valine Metabolism	3-methylglutarylcarnitine (C6)	0.033	0.037	3.81	281.2
	Amino Acid	Leucine, Isoleucine and Valine Metabolism	tiglyl carnitine	0.041	0.045	3.46	246.4
C00216	Carbohydrate	Pentose Metabolism	arabinose	0.013	0.016	2.35	135.0
	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	1-methylnicotinamide	0.001	0.002	8.16	716.2
C00026	Energy	TCA Cycle	alpha-ketoglutarate	0.019	0.022	2.97	196.7
C00042	Energy	TCA Cycle	succinate	0.022	0.025	0.29	-71.5
C01181	Lipid	Carnitine Metabolism	deoxycarnitine	0.032	0.036	0.20	-79.7
	Lipid	Lysolipid	1-linoleoylglycerophosphoserine*	0.015	0.017	4.90	389.8
	Lipid	Lysolipid	1-oleoylglycerophosphoserine	0.011	0.013	6.37	536.8

	Lipid	Lysolipid	1-palmitoylglycerophosphoethanolamine	0.046	0.049	2.26	126.3
	Lipid	Lysolipid	1-stearoylglycerophosphoethanolamine	0.044	0.047	2.45	145.4
	Lipid	Lysolipid	2-oleoylglycerophosphocholine*	0.004	0.005	4.61	361.1
	Lipid	Lysolipid	2-oleoylglycerophosphoserine*	0.030	0.034	4.35	334.6
	Lipid	Lysolipid	2-palmitoleoylglycerophosphoethanolamine*	0.024	0.027	2.56	155.7
	Lipid	Monoacylglycerol	1-linoleoylglycerol (1-monolinolein)	0.024	0.027	2.30	129.9
	Lipid	Monoacylglycerol	1-oleoylglycerol (1-monoolein)	0.021	0.024	1.72	72.0
	Lipid	Monoacylglycerol	2-linoleoylglycerol (2-monolinolein)	0.021	0.025	2.92	192.3
	Lipid	Monoacylglycerol	2-oleoylglycerol (2-monoolein)	0.029	0.033	2.21	121.5
C00114	Lipid	Phospholipid Metabolism	choline	0.025	0.029	0.60	-40.3
C00294	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	inosine	0.006	0.007	4.79	378.9
C00212	Nucleotide	Purine Metabolism, Adenine containing	adenosine	0.005	0.006	2.82	181.6
	Nucleotide	Purine Metabolism, Adenine containing	N6-carbamoylthreonyladenosine	0.029	0.032	3.31	231.0
C00387	Nucleotide	Purine Metabolism, Guanine containing	guanosine	0.011	0.014	4.19	318.6
	Nucleotide	Purine Metabolism, Guanine containing	N2,N2-dimethylguanosine	0.041	0.045	4.50	349.7
	Peptide	Dipeptide	prolyltryptophan	0.040	0.043	4.86	386.4

	Xenobiotics	Tobacco Metabolite	cotinine	0.036	0.039	0.34	-66.0
	Xenobiotics	Tobacco Metabolite	hydroxycotinine	0.037	0.041	0.35	-64.6
C16359	Xenobiotics	Xanthine Metabolism	1-methylurate	0.046	0.049	2.97	197.4
	Xenobiotics	Xanthine Metabolism	1,3-dimethylurate	0.031	0.035	2.48	148.2
C16357	Xenobiotics	Xanthine Metabolism	3-methylxanthine	0.037	0.040	3.12	211.8
	Xenobiotics	Xanthine Metabolism	7-methylurate	0.034	0.038	3.12	211.7
C16353	Xenobiotics	Xanthine Metabolism	7-methylxanthine	0.036	0.040	2.95	195.4

Appendix S1. HPV code.

```
#this code was written by Joanna-Lynn Borgogna for the purpose of
analyzing the metabolomic data for the manuscript
#The vaginal metabolome and microbiota of cervical HPV-positive and HPV-
negative women: a cross-sectional analysis
HPV.raw<-read.csv("HPV_rawdata.csv",header=TRUE,
na.strings='',sep=',',fill=TRUE) #working file
#################
#impute function#
################################################################
################################################################
################################################################
num.v1<-function(x) as.numeric(x) #converting data into numeric form
new<-lapply(HPV.raw[37:635],num.v1) #applying this to entire dataset this
is the beginning of the metabolomic dataset

NA2_halfmin <- function(x) replace(x, is.na(x), (0.5*min(x, na.rm =
TRUE))) #replacing NAs with 1/2 the minimum value for given metabolite
new.2<-lapply(new, NA2_halfmin) #this applies the function

0.5*min(new$X1.3.7.trimethylurate,na.rm=TRUE) ##this is a sanity check to
verify that it is working:
min(new.2$X1.3.7.trimethylurate) ##this is a sanity check to verify that
the function is returning what it should

HPV.imp<-cbind(HPV.raw[,c(1:36)],new.2) #binding the new columns together
lndat<-log(HPV.imp[37:635]) #log transform values
HPV.ln<-cbind(HPV.imp[,c(1:36)],lndat) #this is binding/grabbing the
metadata columns so now we are workign with logged data


################################################################
################################################################
################################################################
# count table + fisher table
#############################################################
#HPV by age
hpv.cstXage<-as.matrix(table(HPV.ln$agecat,HPV.ln$newHPV))
fisher.test(hpv.cstXage,workspace=2e8)

#HPV by race
Hpv.diseaseXrace<-as.matrix(table(HPV.ln$race,HPV.ln$newHPV))
fisher.test(Hpv.diseaseXrace,workspace=2e8)

#HPV by cst
hpv.cstXdisease<-as.matrix(table(HPV.ln$CST,HPV.ln$newHPV))
fisher.test(hpv.cstXdisease,workspace=2e8)

#HPV by marital status
HPV.diseaseXmarital<-as.matrix(table(HPV.ln$marital_cat,HPV.ln$newHPV))
fisher.test(HPV.diseaseXmarital,workspace=2e8)

#HPV by Hormonal contraceptive
HPV.diseaseXHC<-as.matrix(table(HPV.ln$hc_type,HPV.ln$newHPV))
fisher.test(HPV.diseaseXHC,workspace=2e8)

#HPV by num. sex partner
```

```
HPVdiseaseXnumsex<-as.matrix(table(HPV.ln$lifetimecat,HPV.ln$newHPV))
fisher.test(HPVdiseaseXnumsex,workspace=2e8)

#HPV by sex with men
HPV.diseaseXnumsexMale<-
as.matrix(table(HPV.ln$sexpartmale2mo,HPV.ln$newHPV))
fisher.test(HPV.diseaseXnumsexMale,workspace=2e8)

#HPV by sex with women
HPV.diseaseXnumsexwom<-
as.matrix(table(HPV.ln$sexpartfemale2mo,HPV.ln$newHPV))
fisher.test(HPV.diseaseXnumsexwom,workspace=2e8)

#HPV by nugent
HPVdiseaseXnugent<-as.matrix(table(HPV.ln$nugent_cat,HPV.ln$newHPV))
fisher.test(HPVdiseaseXnugent,workspace=2e8)

#HPV by vaginal ph
HPVdiseaseXvagph<-as.matrix(table(HPV.ln$ph_cat,HPV.ln$newHPV))
fisher.test(HPVdiseaseXvagph,workspace=2e8)

#HPV by vaginal odor (24 hours)
HPVdiseaseXvagod<-as.matrix(table(HPV.ln$vag_odor,HPV.ln$newHPV))
fisher.test(HPVdiseaseXvagod,workspace=2e8)

#HPV by vaginal irritation (24 hours)
HPVdiseaseXvag_ir<-as.matrix(table(HPV.ln$vag_irr,HPV.ln$newHPV))
fisher.test(HPVdiseaseXvag_ir,workspace=2e8)

#HPV by vaginal itching (24 hours)
HPVdiseaseXvag_it<-as.matrix(table(HPV.ln$vag_itch,HPV.ln$newHPV))
fisher.test(HPVdiseaseXvag_it,workspace=2e8)

#HPV by vaginal burn
HPVdiseaseXvag_burn<-as.matrix(table(HPV.ln$vag_burn,HPV.ln$newHPV))
fisher.test(HPVdiseaseXvag_burn,workspace=2e8)

###HPV by pain urinating
HPVdiseaseXpain_urin<-as.matrix(table(HPV.ln$pain_uri,HPV.ln$newHPV))
fisher.test(HPVdiseaseXpain_urin,workspace=2e8)

###HPV by vaginal discharge
HPVdiseaseXvag_dis<-as.matrix(table(HPV.ln$vag_dis,HPV.ln$newHPV))
fisher.test(HPVdiseaseXvag_dis,workspace=2e8)

#HPV by douching
HPVdiseaseXdouch<-as.matrix(table(HPV.ln$douchefreq,HPV.ln$newHPV))
fisher.test(HPVdiseaseXdouch,workspace=2e8)

#HPV by smoking status
HPVdiseaseXsmo<-as.matrix(table(HPV.ln$smoking_status,HPV.ln$newHPV))
fisher.test(HPVdiseaseXsmo,workspace=2e8)

#HPV by cigg. day
HPVdiseaseXcigg<-as.matrix(table(HPV.ln$ciggavg30d_cat,HPV.ln$newHPV))
fisher.test(HPVdiseaseXcigg,workspace=2e8)

############################
```

```
#NMDS
############################
require(fpc)
require(cluster)
library(vegan)
set.seed(1)

example_NMDS=metaMDS(HPV.ln[(37:635)],distance="bray",k=2)
stressplot(example_NMDS) #Shepherd's dissimilarity plot
example_NMDS$stress #Stress

ordiplot(example_NMDS,type="n",ylim=c(-0.06,0.06),xlim=c(-0.06,0.06))
orditorp(example_NMDS,display="sites",label="n",cex =
4,pch=15,col="red")#"black")
orditorp(example_NMDS,display="sites",cex=1,air=0.01)


#anosim#
met.dist<-vegdist(HPV.ln[37:635],method="bray")
met.ano.cst<-anosim(met.dist,HPV.ln$CST)#anosim by CSt
summary(met.ano.cst)

met.ano.hpv<-anosim(met.dist,HPV.ln$anyHPV) #anosim by anyHPV
summary(met.ano.hpv)

##########
#Permanova#
set.seed(14)
HPV.raw.bc<-vegdist(decostand(HPV.ln[,c(37:635)],"norm"),method="bray")

p1.conditional<-
adonis2(HPV.raw.bc~CST+anyHPV+hrHPV+smoking_status,data=HPV.ln,permutatio
ns=10000)
p1.conditional

p1.marginal<-
adonis2(HPV.raw.bc~CST+anyHPV+hrHPV+smoking_status,data=HPV.ln,by="margin
",permutations=10000)
p1.marginal
##########################################################################
##############


#MLR
# accounting for CST and smoking status but not stratifiying by CST:
#create dummy variables for smoking status:
smoker<-ifelse(HPV.ln$Smoking.Status=="smoker",1,0)
nonsmoker<-ifelse(HPV.ln$Smoking.Status=="nonsmoker",1,0)

#create dummy variables for HPV detection status:
HPVpos<-ifelse(HPV.ln$anyHPV=="HPV+",1,0)
HPVneg<-ifelse(HPV.ln$anyHPV=="HPV-",1,0)
hrHPVpos<-ifelse(HPV.ln$hrHPV=="hrHPV+",1,0)

#for CST
CST1<-ifelse(HPV.ln$CST=="CST-I",1,0)
CST3<-ifelse(HPV.ln$CST=="CST-III",1,0)
CST4<-ifelse(HPV.ln$CST=="CST-IV",1,0)
```

```
#source("https://bioconductor.org/biocLite.R")
#biocLite("qvalue")
require(qvalue)


#model: yi=B0+B1HPV+B2CST+B3SmokingStatus+ei
HPV_constant_CST_SS<-
(lapply(HPV.ln[37:635],function(x)summary(lm(x~HPVpos+smoker+factor(CST),
data=HPV.ln))$coef[,4]))
HPV_constant_CST_SS.coef<-
(lapply(HPV.ln[37:635],function(x)summary(lm(x~HPVpos+smoker+factor(CST),
data=HPV.ln))$coef[,1])) #performs test

#qvalues:
HPV_unlisto_SS_CST<-unlist(HPV_constant_CST_SS)
HPV_unlisto_SS_CST.qval<-qvalue(HPV_unlisto_SS_CST,fdr.level=0.05)

#obtaining coefficients and 95% CIs of significant (q-value <0.05)
metabolites from above
#pyro
pyroglutamine.lm<-
(lm(pyroglutamine.~HPVpos+smoker+factor(CST),data=HPV.ln))
homoserine.lm<-(lm(homoserine~HPVpos+smoker+factor(CST),data=HPV.ln))
imidazole.lm<-
(lm(imidazole.propionate~HPVpos+smoker+factor(CST),data=HPV.ln))
putrescine.lm<-(lm(putrescine~HPVpos+smoker+factor(CST),data=HPV.ln))
fucose.lm<-(lm(fucose~HPVpos+smoker+factor(CST),data=HPV.ln))
ethanolamine.lm<-(lm(ethanolamine~HPVpos+smoker+factor(CST),data=HPV.ln))
beta.lm<-(lm(beta.alanine~HPVpos+smoker+factor(CST),data=HPV.ln))
hdyro.lm<-
(lm(X2.hydroxyhippurate..salicylurate.~HPVpos+smoker+factor(CST),data=HPV
.ln))


#obtaining CIs:
HPV.CIS<-rbind(c(exp(confint(pyroglutamine.lm,"HPVpos",level=0.95))),
           exp(confint(homoserine.lm,"HPVpos",level=0.95)),
           exp(confint(imidazole.lm,"HPVpos",level=0.95)),
           exp(confint(putrescine.lm,"HPVpos",level=0.95)),
           exp(confint(fucose.lm,"HPVpos",level=0.95)),
           exp(confint(ethanolamine.lm,"HPVpos",level=0.95)),
           exp(confint(beta.lm,"HPVpos",level=0.95)),
           exp(confint(hdyro.lm,"HPVpos",level=0.95)))



#create figure #1:
HPV.coefs<- as.data.frame(matrix(nrow = 8))
HPV.coefs$Metabolite <- c("pyroglutamine", "homoserine","imidazole
propionate","putrescine","fucose","ethanolamine","beta-alanine","2-
hydroxyhippurate (salicylurate)")
HPV.coefs$OR<-cbind(c(exp(pyroglutamine.lm$coefficients['HPVpos'])-1,
                   exp(homoserine.lm$coefficients['HPVpos'])-1,
                   exp(imidazole.lm$coefficients['HPVpos'])-1,
                   exp(putrescine.lm$coefficients['HPVpos'])-1,
                   exp(fucose.lm$coefficients['HPVpos'])-1,
                   exp(ethanolamine.lm$coefficients['HPVpos'])-1,
```

```
                    exp(beta.lm$coefficients['HPVpos'])-1,
                    exp(hdyro.lm$coefficients['HPVpos'])-1))

HPV.coefs$lower<-CIS[,1]-1
HPV.coefs$upper<-CIS[,2]-1


HPV.coefs$Met.fac <- factor(HPV.coefs$Metabolite, c("2-hydroxyhippurate
(salicylurate)", "pyroglutamine", "ethanolamine",
"fucose","homoserine","putrescine","beta-alanine","imidazole
propionate"))

require(ggplot2)
dodger = position_dodge(width = 0.3)
image1<-ggplot(data=HPV.coefs, aes(y=OR,x=Met.fac))+
  geom_pointrange(aes(ymin=lower,ymax=upper),
                  position=dodger,size=.5)+
  geom_hline(yintercept=0.0,linetype="dotted",size=1)+
  coord_flip(ylim=c(-2,15,breaks=1))+
  labs( x = "Metabolite") +
  scale_y_continuous(name="Odds ratios and associated 95% Confidence
Intervals, \n obtained from a model comparing HPV+ vs HPV- women and
accounting for smoking status and CST", limits=c(-2, 15),breaks=c(-2,-
1,0,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15))+
  theme_bw()+
  ggExtra::removeGridX()
image1



#####################################################
###################within CST ####################
############################
#model: yij=B0+B1HPV+B2CST+B3SmokingStatus+B4HPV*CST
#baseline: CST I:
mod.CST1<-
(lapply(HPV.ln[37:635],function(x)summary(lm(x~HPVpos+CST3+CST4+smoker+(H
PVpos*CST3)+(HPVpos*CST4),data=HPV.ln))$coef[,4])) #performs test
mod.CST1.coef<-
(lapply(HPV.ln[37:635],function(x)summary(lm(x~HPVpos+CST3+CST4+smoker+(H
PVpos*CST3)+(HPVpos*CST4),data=HPV.ln))$coef[,1])) #performs test

mod.CST1_unlist<-unlist(mod.CST1)
mod.CST1.qval<-qvalue(mod.CST1_unlist,fdr.level=0.05)


#baseline: CST III:
mod.CST3<-
(lapply(HPV.ln[37:635],function(x)summary(lm(x~HPVpos+CST1+CST4+smoker+(H
PVpos*CST1)+(HPVpos*CST4),data=HPV.ln))$coef[,4])) #performs test
mod.CST3.coef<-
(lapply(HPV.ln[37:635],function(x)summary(lm(x~HPVpos+CST1+CST4+smoker+(H
PVpos*CST1)+(HPVpos*CST4),data=HPV.ln))$coef[,1])) #performs test

mod.CST3_unlist<-unlist(mod.CST3)
mod.CST3.qval<-qvalue(mod.CST3_unlist,fdr.level=0.05)

#baseline: CST IV:
```

```
mod.CST4<-
(lapply(HPV.ln[37:635],function(x)summary(lm(x~HPVpos+CST1+CST3+smoker+(H
PVpos*CST1)+(HPVpos*CST3),data=HPV.ln))$coef[,4])) #performs test
mod.CST4.coef<-
(lapply(HPV.ln[37:635],function(x)summary(lm(x~HPVpos+CST1+CST3+smoker+(H
PVpos*CST1)+(HPVpos*CST3),data=HPV.ln))$coef[,1])) #performs test

mod.CST4_unlist<-unlist(mod.CST4)
mod.CST4.qval<-qvalue(mod.CST4_unlist,fdr.level=0.05)


########################
#evaluating hrHPV and lrHPV
########################
########################
########################

#to evaluate this coefficient, we use the baseline of anyHPV+, hrHPV-
#and will look at the coefficient of anyhPV+, hrHPV+
anyHPVneg_hrHPVneg<-ifelse(HPV.ln$newHPV=="HPV-_hrHPV-",1,0)
anyHPVpos_hrHPVneg<-ifelse(HPV.ln$newHPV=="HPV+_hrHPV-",1,0)
anyHPVpos_hrHPVpos<-ifelse(HPV.ln$newHPV=="HPV+_hrHPV+",1,0)


hrHPV_constant<-
(lapply(HPV.ln[37:635],function(x)summary(lm(x~anyHPVneg_hrHPVneg+anyHPVp
os_hrHPVpos,data=HPV.ln))$coef[,4])) #performs test
hrHPV_constant.coef<-
(lapply(HPV.ln[37:635],function(x)summary(lm(x~anyHPVneg_hrHPVneg+anyHPVp
os_hrHPVpos,data=HPV.ln))$coef[,1])) #performs test
hrHPV_constant.unlist<-unlist(hrHPV_constant)
hrHPV_constant.unlist.qval<-qvalue(hrHPV_constant.unlist,fdr.level=0.05)

#install.packages("corrplot")
library(corrplot)
#correlation chart
###################################
#spearman's correlation on significant metabolites (determined via
regression and model 1 and model 2) as well as taxa (LEFSE)
MetandTaxa.dat<-read.csv("MetandTaxa.cst",header=TRUE,
na.strings='',sep=',',fill=TRUE) #working file

sp.analysis<-cor(MetandTaxa.dat[7:48],method="spearman")
sp.analysis[lower.tri(sp.analysis)]<-NA


sp.dat<-read.csv("MetandTaxa_spearmanresults.csv",header=TRUE,
na.strings='',sep=',',fill=TRUE) #working file
rnames <- sp.dat[,1]
mat_data <- data.matrix(sp.dat[,2:ncol(sp.dat)]) #transforming into a
data matrix
corrplot(mat_data,
method="color",is.corr=TRUE,tl.cex=1,tl.col="black",cl.pos="b",hclust.met
hod = "ward.D2")
```

CHAPTER 4

THE ASSOCIATION OF *CHLAMYDIA TRACHOMATIS* AND MYCOPLASMA GENITALIUM INFECTION WITH THE VAGINAL METABOLOME

Contribution of Authors and Co-Authors

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Contributions: Conducted statistical analysis, interpretation of data, prepared manuscript.

Co-Author: Michelle D. Shardell

Contributions:

Co-Author: Carl J. Yeoman

Contributions: Conducted statistical analysis, interpretation of data, prepared manuscript.

Co-Author: Khalil G. Ghanem

Contributions: Conceived study, aided in interpretation of the data.

Co-Author: Herlin Kadriu

Contributions: Aided in statistical analysis and preparing the methods and results.

Co-Author: Alexander V. Ulanov

Contributions: Conducted untargeted liquid chromatography.

Co-Author: Charlotte A. Gaydos

Contributions: Conducted *Mycoplasma genitalium* testing.

Co-Author: Justin Hardick

Contributions: Conducted *Mycoplasma genitalium* testing.

Co-Author: Courtney K. Robinson

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The association of *Chlamydia trachomatis* and *Mycoplasma genitalium* infection with the vaginal metabolome

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Chlamydia trachomatis (CT) and Mycoplasma genitalium (MG) are two highly prevalent bacterial sexually transmitted infections (STIs) with a significant rate of co-infection in some populations. Vaginal metabolites are influenced by resident vaginal microbiota, affect susceptibility to sexually transmitted infections (STIs), and may impact local inflammation and patient symptoms. Examining the vaginal metabolome in the context of CT mono (CT+) and CT/MG co-infection (CT+/MG+) may identify biomarkers for infection or provide new insights into disease etiology and pathogenesis. Yet, the vaginal metabolome in the setting of CT infection is understudied and the composition of the vaginal metabolome in CT/MG co-infected women is unknown. Therefore, in this analysis, we used an untargeted metabolomic approach combined with 16S rRNA gene amplicon sequencing to characterize the vaginal microbiota and metabolomes of CT+, CT+/MG+, and uninfected women. We found that CT+ and CT+/MG+ women had distinct vaginal metabolomic profiles as compared to uninfected women both before and after adjustment for the vaginal microbiota. This study provides important foundational data documenting differences in the vaginal metabolome between CT+, CT+/MG+ and uninfected women. These data may guide future mechanistic studies that seek to provide insight into the pathogenesis of CT and CT/MG infections.

INTRODUCTION

Vaginal metabolites are influenced by resident vaginal microbiota, affect susceptibility to sexually transmitted infections (STIs), and impact local inflammation and symptoms [1,2]. A healthy, "optimal" vaginal microbiota is dominated by *Lactobacillus* species, which produce the metabolite lactic acid. Lactic acid lowers the pH of the vaginal microenvironment [1-4], and, through immunomodulatory and direct inhibitory effects, may protect against acquisition of STIs, including *Chlamydia trachomatis* (CT) and HIV [2,5-7]. In contrast, women with non-optimal microbiota, as epitomized by the clinical condition of bacterial vaginosis (BV), have vaginal microbial communities that are low in *Lactobacillus* spp.; and are instead colonized by a variety of anaerobes. Some of these bacteria produce metabolites such as biogenic amines and short chain fatty acids that may be pro-inflammatory and have been linked with symptoms such as vaginal malodor and discomfort [2,8-12]. These metabolites may also increase susceptibility to STIs, including bolstering chlamydial persistence [8,13-15]. Women with low-*Lactobacillus* non-optimal vaginal microbiota have an increased risk for acquisition of STIs and ascending infection (including pelvic inflammatory disease [PID]) [16-20].

CT is the most prevalent bacterial STI in the United States, and causes significant morbidity, including PID and infertility in young women [21]. *Mycoplasma genitalium* (MG) is an emerging, highly drug resistant STI, which appears to be more prevalent than gonorrhea, and has been linked to cervicitis and PID in women [22-24]. Interestingly, rates of co-infection with CT and MG are high in many female populations (36% of CT infected women had MG co-infection in one study done in Baltimore, MD, [25], nearly 38% of CT infected women were found to have MG co-infection in a second study [26] and 70.7% of women with MG in a high risk cohort were found to have CT co-infection [24]. Endometrial MG infection has been associated with endometritis and endometrial CT infection, suggesting that these infections may co-locate or perhaps affect PID risk in some women [24]; however the impact of co-infection on patient outcomes is understudied [1].

New molecular techniques have enabled a higher resolution understanding of BV, the vaginal microbiota, and associations with STI, including CT and MG infections. 16S rRNA gene amplicon sequencing techniques have identified several different kinds vaginal microbiota, or "community state types" (CSTs) [27] based on bacterial composition and relative abundance. Four are dominated by different *Lactobacillus* species (CST I, *Lactobacillus crispatus*; CST II, *L. gasseri*; CST III, *L. iners*; CST V, *L. jensenii*). A fifth community (CST IV) is low in *Lactobacillus* spp. and has been termed "molecular-Bacterial Vaginosis" (molecular-BV). [3]. Molecular-BV and CST III have been associated with increased risk of CT acquisition [28]. Emerging evidence suggests that low-*Lactobacillus* vaginal microbiota may be associated with increased risk for MG infection as well [17,28].

Associations between the vaginal microbiota and enhanced risk of CT and MG infection are likely mediated, at least in part, through vaginal metabolites. CT and MG may also independently influence the vaginal metabolome through direct, host-mediated or microbiota-mediated mechanisms, perhaps impacting clinical outcomes. Therefore, examining the vaginal metabolome in the context of these infections may identify biomarkers for infection or provide new insights into disease etiology and pathogenesis. Yet, to date, only one study has examined the vaginal metabolome in the setting of CT infection [29], and the composition of the vaginal metabolome in CT/MG co-infected women is unknown. Therefore, in this study, we utilized untargeted gas chromatography-mass spectrometry (GC-MS) to characterize the vaginal metabolome of CT mono-, CT/MG co-infected, and uninfected women. As STI-infected women are known to have a higher prevalence of BV [28,30,31], we additionally integrated analysis of the vaginal metabolome with 16S rRNA gene amplicon

sequencing in order to understand whether the identified differences in vaginal metabolites remained statistically significant after controlling for the composition of the vaginal microbiota.

RESULTS:

Participant characteristics and vaginal microbiota composition (CST) by infection state

Vaginal samples from 145 race-matched women (77 uninfected, 54 CT+ (mono-infected), and 14 CT+/MG+ (co-infected)) were analyzed. CT+ and CT+/MG+ women were generally younger, had more sexual partners, and a lower proportion reported "always" using condoms as compared to uninfected women (**Supplementary Table S1**). Seven (50%) of the CT+/MG+ women had evidence of macrolide resistance based on the SpeeDx MG testing. A much larger proportion of CT+ (64.8%) and CT+/MG+ (78.6%) women had molecular-BV (low-*Lactobacillus* species vaginal microbiota, CST IV) as compared with uninfected women (26.0%, $p<0.01$), and **Supplementary Figure S1**).

Vaginal metabolomic profiles separate women based on infection status and CST

An exploratory PCA plot revealed that metabolomes clustered by infection status with infected women clustering separately from uninfected women, and CT+ and CT+/MG+ women largely clustering together (**Fig. 1A**). A second PCA showed that women in CST IV (low-*Lactobacillus* vaginal microbiota) clustered separately from those with *L. crispatus*-, *L. gasseri*-, or *L. jensenii*-dominated vaginal microbiota (CST I, II, and V, respectively). Women with *L. iners* dominated vaginal microbiota (CST III) were interspersed between the two clusters (**Fig. 1B**). Both multiple linear regression and PLS-DA identified several metabolites that significantly distinguished: 1) CT+ vs. uninfected women; 2) CT+/MG+ vs. uninfected women; and 3) CT+/MG+ vs. CT+ women (**Supplementary Tables S2-S3**).

Multiple vaginal metabolites distinguish CT mono-, CT/MG co-infected and uninfected women before and after adjustment for CST

Multiple linear regression demonstrated that there were significant differences in individual vaginal metabolites in CT+ and CT+/MG+ compared with uninfected women both before and after adjustment for CST. **Supplementary Tables S2** and **S3** summarize all significant metabolites identified by multiple linear regression, grouped by metabolic pathway based on the Kyoto Encyclopedia of Genes and Genomes (KEGG). All results reported below have a q-value <0.05 unless otherwise stated.

In unadjusted analyses, the relative concentrations of 99 and 64 metabolites were significantly different when comparing CT+ and CT+/MG+ women to uninfected women, respectively (**Supplementary Table S2**). After adjustment for CST, 64 and 36 metabolites significantly differed when comparing CT+ and CT+/MG+ co-infected women, respectively, to uninfected women (**Supplementary Table S3**). 93% of metabolites CT+ vs. uninfected patients (60/64) and 94% of metabolites in CT+/MG+ co-infected women vs. uninfected women (34/36)) which were significant before adjustment for CST were also significant after adjustment for CST. Comparatively fewer metabolites differentiated CT+/MG+ and CT+ women both before (7 metabolites) and after adjustment for CST (3 metabolites). All of the metabolites that distinguished these two groups after adjustment for CST were significant prior to adjustment for CST.

Amino acid metabolism:

Women with CST IV (molecular-BV), irrespective of infection status, had greater vaginal concentrations of the biogenic amines cadaverine, putrescine, tyramine and ethanolamine (part of the lipid super pathway but is also considered a biogenic amine) based on linear regression (one-sided p-value <0.05) compared with women with *Lactobacillus*-dominated CSTs. Consistent with this result, (given the higher proportion of women with molecular-BV in the infected groups), before adjustment for CST, the biogenic amines cadaverine, putrescine, tyramine and ethanolamine were increased in the CT+, and cadaverine, putrescine and ethanolamine were increased in the CT+/MG+ as compared with the uninfected women. After adjustment for CST, only ethanolamine was increased in infected as compared with uninfected patients.

Cinnamic acid or its associated derivatives were higher in CT+ and CT+/MG+ women compared to uninfected women with and without adjustment for CST. Hydrocinnamic acid was increased in CT+/MG+ as compared to CT+ women, but there were no differences in biogenic amines either before or after adjustment for CST between these two groups.

Carbohydrate metabolism:

Several sugars and derivatives, including lactose and sorbitol, were significantly lower in both CT+ and CT+/MG+ women compared to uninfected women, irrespective of adjustment for CST. Metabolites involved in pentose metabolism, such as xylose, were higher in CT+ and CT+/MG+ relative to uninfected women both before and after adjustment for CST. Glyceric acid, which is involved in glycolysis, was decreased in CT+ and CT+/MG+ relative to uninfected women before and after adjustment for CST.

Lipid metabolism:

Multiple long chain fatty acids (LCFAs), including decanoic, pentadecanoic, heptadecanoic, oleic, arachidic, behenic, cerotic, and myristic acid were higher in CT+ as compared to uninfected women without adjustment for CST; the majority of these remained significantly higher after adjustment for CST. Fewer LCFAs, including pentadecanoic, heptadecanoic, and oleic acids were elevated in CT+/MG+ as compared to uninfected women, all of which remained significantly higher after adjustment for CST. The LCFA palmitoleic acid was decreased in CT+/MG+ as compared to CT+ women, but only before adjustment for CST. The sterol, 5α-cholestan-3-ol, was higher in CT mono-infected women compared to uninfected women without adjustment for CST. Multiple long chain fatty alcohols including octadecanol, eicosanol, heptadecanol and hexadecanol, were higher with and without CST adjustment in CT+ and CT+/MG+ as compared with uninfected women. Octadecanol was lower in CT+/MG+ co-infected as compared with CT+ women both before and after adjustment for CST.

Energy metabolism:

α-ketoglutaric acid, citric acid and glutaric acid, all metabolites involved in the TCA cycle, were lower in CT+ compared to uninfected women both before and after adjustment for CST. These metabolites were also significantly decreased in CT+/MG+ women relative to uninfected women after adjustment for CST.

Nucleic acid metabolism:

Several metabolites involved in purine and pyrimidine metabolism, including adenine, guanine and cytosine, were increased in CT+ relative to uninfected women both before and after adjustment for

CST. These were also higher in CT/MG co-infected women before adjustment for CST; guanine and cytosine were higher after adjustment for CST. When comparing CT+/MG+ to CT+ women, we did not observe any significant differences in nucleotide abundance with or without adjustment for CST.

Partial least squares discriminate analysis (PLS-DA)

We utilized PLS-DA to identify the most discriminatory metabolites between each infection state (**Fig. 2**). PLS-DA was able to effectively separate CT+ and CT+/MG+ from uninfected women in two components with accuracies ranging from 94-98%. Permutation tests provided evidence that the differences between these clusters were significant (CT+ vs. uninfected women, p-value <0.0001, 0/2000 permutations; CT+/MG+ vs. uninfected women, p-value =0.0025, 5/2000 permutations). Variable importance plots (VIP) indicated that octadecanol, eicosanol, and lactose (VIP scores > 2.5) contributed the most to the class separation between CT+ and uninfected women, with octadecanol and eicosanol being higher in CT+ women and lactose being lower in CT+ than uninfected women. (**Fig. 2B**). The most important discriminatory metabolites between CT+/MG+ and uninfected women were octadecanol, erythronic acid, sophorose, and isomaltose with octadecanol, sophorose and isomaltose being higher in CT+/MG+ than uninfected women. (**Fig. 2D**). PLS-DA was not able to effectively separate CT+/MG+ co-infected women from CT+ women (**Fig. 2E**). The lack of separation was cross-validated with R^2 coefficients of 0.39 and 0.79 and Q^2 values of -0.35 and 0.22. Permutation tests further provided evidence against the separation of these two groups (p-value 0.167, 334/2000 permutations)

Finally, we subset the data into CST III (*L. iners* dominated) and CST-IV (low-*Lactobacillus*, molecular-BV) and performed separate PLS-DAs to evaluate differences between infection status within strata of CST (**Fig. 3**). CT+ and CT+/MG+ women clustered together and were discriminated from uninfected women, irrespective of CST (CST III, p-value = <0.0001, 0/2000 permutations; CST IV p-value <0.0001, 0/2000 permutations). Octadecanol, eicosanol and lactose were significant in discriminating between the CT+ and uninfected subjects for women in both CST III and CST IV.

DISCUSSION

We found that numerous vaginal metabolites distinguished CT+ and CT+/MG+ from uninfected women, with and without adjustment for the composition of the vaginal microbiota. When comparing CT+/MG+ to CT+ women, we observed significant overlap of vaginal metabolome profiles.

Previous literature suggests that women with BV are at increased risk for acquisition of CT and MG [1], and women with CT infection are most likely be in CST IV [28]. Consistent with this, a larger proportion of the CT+ and CT+/MG+ as compared with the uninfected women in our study cohort had molecular-BV (CST IV). Since biogenic amines (thought to be produced by BV-associated bacteria) are known to be higher in women with molecular-BV, it is not surprising, therefore, that the biogenic amines putrescine and cadaverine were higher in CT mono- and CT/MG co-infected as compared to uninfected women prior to, but not after adjustment for CST. Biogenic amines are produced via specific amino acid decarboxylation reactions which involve the consumption of hydrogen ions and the reduction of intracellular and extracellular acidity, a bacterial acid resistance and mitigation mechanism [8,32,33]. This reduction of vaginal acidity may promote colonization by BV-associated bacteria. Additionally, the production of biogenic amines may increase the virulence of various pathogens, and may protect pathogens from innate immune defenses [34,35]. Biogenic amines may reduce the host's immune defenses against STIs, including CT or MG [8].

While some of the differences in vaginal metabolites between infection groups (such as putrescine and cadaverine, above) may relate to the higher proportion of infected women with molecular-BV, we cannot discount the possibility that these CST-related metabolites are important to the infection process. Differences in other metabolites remained consistent even after adjustment for CST, suggesting a role for the infecting pathogen(s) (or perhaps an interaction between the host, pathogen and underlying microbiota) in impacting the metabolome. For example, we observed significantly higher concentrations of most LCFAs and fatty alcohols when comparing the vaginal metabolites of CT+ and CT+/MG+ to uninfected women both before and after adjustment for CST. This effect, however, appeared to be attenuated by MG co-infection: CT+/MG+ women had several LCFAs and fatty alcohols that were lower compared to CT+ women.

It is possible that the higher levels of LCFAs are related to CT survival, growth, and/or pathogenesis. Chlamydiae are obligate intracellular organisms that are auxotrophic for a variety of essential metabolites, including many lipids that they acquire from the host cell. Lipids are trafficked from various sources within the host cell into the inclusion and to the envelope of the reticulate bodies. The acquisition of lipids from host stores is thought to be critical for chlamydial replication and development [36]. In eukaryotic cells, cholesterol esters, which are formed by the addition of a LCFA to the hydroxyl group of cholesterol, are the main transport and storage form of cholesterol [37]. The enzyme acyl-CoA:cholesterol acyltransferase (ACAT) catalyzes the production of these esters [38]. This process is important because in the un-esterified form, cholesterol can be toxic to the cell. A recent report [38] showed that chlamydial growth can be inhibited by blocking ACAT with a specific inhibitor, 4-hydroxycinnamic acid amide. Addition of a cholesterol ester (cholesterol esterified with a LCFA) rescued ACAT dependent inhibition [38].

These data raise the question of whether CT may be upregulating host production of LCFAs and cholesterol esters as part of a survival or growth mechanism. The relatively lower levels of lipid and lipid derivatives in CT/MG co-infected women versus uninfected women may reflect a direct or indirect competition between CT and MG for host lipids. Alternatively, the higher fatty alcohols and LCFAs may reflect greater wax ester production. Wax esters, a type of neutral lipid, are formed by the esterification of a fatty alcohol with a fatty acid derived from either acyl-CoA or acyl-ACP [39]. Neutral lipids are commonly found in vaginal fluid, along with secretions from the cervix, squamous epithelial cells and material from the sebaceous, apocrine and Bartholin's glands [40]. Thus, it is possible that the higher abundance of fatty acids, LCFAs and fatty alcohols reflects greater vaginal discharge associated with chlamydial infections. These questions are intriguing, but speculative. Larger, mechanistic studies will be required to further explore them.

No studies have examined the vaginal metabolome in the context of MG mono- or CT/MG co-infection, and only one other report [29] has examined the vaginal metabolome in the context of CT infection. That study found fewer distinguishing metabolites but had a number of important differences from ours, including a smaller sample size, the use of 1H-NMR rather than GC-MS to detect a total of only 54 metabolites and a lack of testing for MG. Furthermore, the study design was quite different: CT infected women in the Parolin study were specifically selected not to have BV. Importantly, by controlling for CST rather than eliminating subjects with molecular-BV, our results may capture important changes that occur as a result of complex interactions between the host, CT and dysbiotic vaginal microbiota which would be missed by an approach that excludes CT mono-infected women with BV.

There are several limitations to our study. First, while we have the largest group of CT+ women studied to date in a metabolomic analysis, the CT+/MG+ co-infected group was small, limiting our

ability to control for other potential confounders [8]. Secondly, these data are cross-sectional, with samples taken at the time of CT and CT/MG infection. Therefore, causal inferences (e.g. regarding the impact of the vaginal metabolome on susceptibility to CT or CT/MG co-infection) are not possible; nor is it possible to account for short term fluctuations in the vaginal microbiota. Batch effects could have introduced bias. Although we took a broad, untargeted GC-MS based approach, the library of identified metabolites does not reflect all the small metabolites present within the vaginal environment, and we may have missed crucial metabolites in our analysis. Importantly, the use of untargeted GC-based mass spectrometry excluded the detection of volatile fatty acids such as trimethylamine, acetate, or butyrate. The vaginal samples were stored in Amies transport medium. A number of metabolites, including lactic acid, were found in the transport medium and therefore had to be removed from analysis. Finally, given the limitations of study design, we are only able to identify broad associations - it is not possible to untangle from this data what specific factors, or interaction between factors (i.e. host, vaginal microbiota and pathogen) are impacting specific metabolites and ultimately how this may affect susceptibility to, clearance of, or morbidity from CT and MG infections.

Despite these limitations, our study shows that a distinct vaginal metabolic profile distinguishes CT+ and CT+/MG+ from uninfected women, with significant overlap between CT+ and CT+/MG+ women. This study provides important foundational data documenting cross-sectional differences in the vaginal metabolome. Larger, mechanistic studies will be needed to explore the complex interactions between host, vaginal microbiota and pathogens, in order to better understand the pathogenesis of CT and CT/MG co-infection.

METHODS:

Study population and sample collection:

Patient samples were drawn from three separate cohorts. CT positive cases (CT+) were drawn from the first (CT infected) visit of the Chlamydia Adolescent/Adult Reproductive Management (CHARM) study [41]. CHARM recruited predominantly African-American CT+ young women of reproductive age in Baltimore, MD and followed them over time after treatment for CT. Women were also tested for gonorrhea (GC) via NAAT and trichomoniasis via wet mount microscopy. Race-matched uninfected controls were drawn from enrollment visits of the Hormonal Contraception Longitudinal (HCL) study, (a cohort of low-risk women of reproductive age starting and stopping hormonal contraception recruited in Baltimore, MD) and the Human Microbiome Project Prospective Women's Health study at UMB (UMB-HMP), (a cohort of women of reproductive age recruited from clinics in Alabama and followed daily for 10 weeks)[42]. Women in HCL and UMB-HMP were tested for CT, GC (via NAAT). Trichomoniasis was tested for via microscopy in HCL and inPouch in UMB-HMP. In UMB-HMP, if diagnosis of an STI or symptomatic condition was made at baseline, it was treated, and subjects were offered enrollment 30 days after treatment completion. Subjects were tested again for gonorrhea, chlamydia and trichomoniasis at their enrollment visit. All samples were self-collected mid-vaginal Copan Eswab specimens placed in Amies transport media and frozen at -80°C until use.

Ethics statement: All participants provided written informed consent. Ethical approval was obtained from the Institutional Review Boards of Johns Hopkins University, the University of Maryland

Baltimore (UMB), and Montana State University, and all research was conducted in compliance with relevant guidelines and regulations.

Taxonomic assignment, community state type profiling and *M. genitalium* detection

Vaginal Copan Eswabs were extracted with either the QS DSP Virus/Pathogen Midi Kit (Qiagen) on the QiaSymphony platform or with the MagAttract Microbial DNA Kit (Qiagen) automated on a Hamilton Microlab Star robot. For the QS DSP Midi kit extraction, samples were thawed on ice and a 500µl aliquot from the vaginal swab was used as input [43]. For the MagAttract kit, samples were thawed on ice and a 200µl aliquot from the vaginal swab was used as input following the manufacturer protocol. Cells were lysed via shaking on the TissueLyser (Qiagen) at 20Hz for 20 minutes and the final elution volume was 110µl. Negative controls of water were extracted in the same manner as samples.

The vaginal microbiota was characterized by sequencing the V3-V4 regions of the 16S rRNA gene. Library construction was performed using either a 1-step protocol with sequencing carried out on the Illumina MiSeq platform for 600 cycles, resulting in 2 x 300 bp read lengths, or a 2-step PCR protocol, sequencing was carried out on the Illumina HiSeq 2500 platform using Rapid Run Chemistry, and sequence data were processed according to Holm *et al.* [43]. Amplicon sequence variants (ASVs) generated by DADA2 were classified taxonomically using the RDP Naïve Bayesian Classifier [44] trained with the SILVA v128 16S rRNA gene database [43,45]. ASVs of major vaginal taxa were assigned species-level annotations using speciateIT (http://ravel-lab.org/speciateit/). Taxonomic data from a pool of over 12,000 vaginal samples were used to assign community state types (CSTs). Low-abundant taxa present at less than $10^{-5.5}$ abundance across the project were removed and samples with fewer than 5,000 reads were removed from analysis. Hierarchical clustering based on Jensen-Shannon distances between samples and Ward linkage was used to determine clusters for CST assignment. Four CSTs dominated by *Lactobacillus* spp. were identified: CST I (*L. crispatus*), CST II (*L. gasseri*), CST III (*L. iners*), and CST V (*L. jensenii*). CST IV contained more diverse organisms with low levels of *Lactobacillus* sp., (i.e. molecular-BV See **Supplementary Fig. S1** for heatmap of 25 most abundant taxa with CST groupings).

Finally, DNA extracted from vaginal swabs was tested for MG using SpeeDx, a multiplex qPCR DNA based assay for detection of MG and resistance to macrolides by detecting one or more of 5 mutations in the 23S rRNA gene [46,47].

Sample selection and preparation for metabolomics:

289 samples were tested for MG: 157 CT+ cases from CHARM and 132 race matched CT- controls from HCL and HMP. Two control (i.e. CT-) samples tested positive for MG and were excluded. After exclusion of additional samples due to insufficient sample for metabolomic testing, unavailable 16S rRNA gene sequencing data, or co-infection with GC or *Trichomonas*, samples from 145 women were available for analysis (77 uninfected, 54 CT+ and 14 CT+/MG+ samples).

For quality control, an aliquot of 10µl was taken from each sample and mixed in 10 replicates. Additionally, two samples of sterile Amies transport medium (controls) were run in duplicate. Samples, Amies controls, and QC supernatants were dried in a vacuum and derivatized with 50µl methoxyamine hydrochloride (Sigma-Aldrich, MO, USA) (40 mg/mL^{-1} in pyridine) for 60 min at 50°C, then with 50 µl MSTFA+1%TMCS (Thermo, MA, USA) at 70°C for 120 min, followed by a 2-hour incubation at room temperature. 30µl of 1mg/ml hentriacontanoic acid was added to each sample prior to derivatization for use as an internal standard for normalization.

Metabolite profiles were acquired using a gas-chromatography mass-spectrometry (GC-MS) system (Agilent Inc, CA, USA) consisting of an Agilent 7890 gas chromatograph, an Agilent 5975 MSD and 7683B autosampler. Gas chromatography was performed on a ZB-5MS (60m×0.32mm I.D. and 0.25mm film thickness) capillary column (Phenomenex, CA, USA). The inlet and MS interface temperatures were 250°C, and the ion source temperature was adjusted to 230°C. An aliquot of 1ml was injected with the split ratio of 10:1. The helium carrier gas was kept at a constant flow rate of 2.4 ml/min. The temperature program was: 5-min isothermal heating at 70°C, followed by an oven temperature increase of 5°C/min to °C after which a final 10min incubation at 310°C was performed. The mass spectrometer was operated in positive electron impact mode (EI) at 69.9 eV ionization energy at m/z 30-800 scan range. The spectra of all chromatogram peaks were evaluated using the AMDIS 2.71 (NIST, MD, USA) using a custom-built database (460 unique metabolites) of the University of Illinois Carver Metabolomics Center. All known artificial peaks were identified and removed prior to data mining. To allow comparison between samples, all data were normalized to the internal standard in each chromatogram and sample volume. The instrument variability was within the standard acceptance limit (5%).

Metabolite pre-processing and statistical analyses:

Metabolites detected in Amies (See **Supplementary Table S4**) were removed from analysis. Metabolites were excluded from analysis if they were undetected in ≥ 90% of samples in all biological groups (CT+, CT+/MG+ and uninfected). Remaining missing values were assumed to be below the level of detection and were imputed (across all samples, irrespective of biological groups) using one half the minimum value obtained for a given metabolite [48-51]. The final dataset available for analysis consisted of 163 metabolites. Following imputation of missing values, data were mean-centered, and a generalized log-transformation was applied to meet assumptions of linearity and normality required for regression models. Mean centering and generalized log-transformations were performed using MetaboAnalyst 4.0 [51]. Metabolic super and sub pathways were identified using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Unadjusted associations between infection status and demographic and behavioral factors were assessed using Fisher's exact test (**Supplementary Table S1**). Samples were visualized using a principal component analysis (PCA) constructed in MetaboAnalyst 4.0 (**Fig. 1**)[51].

We fit a linear regression model of mean-centered, generalized log-transformed metabolites on infection status, with and without controlling for CST. For each model, a significance level $\alpha = 0.05$ was used. To correct for multiple comparisons, p-values were adjusted using the Storey false discovery rate in the package *qvalue* [52]. Linear regression and false discovery rate were conducted using R Statistical Software. Fold changes (FC) were calculated using $FC_i = \exp(mean(\log(X_i) - mean(\log(Y_i))$ where *i* is the metabolite of interest for the mean value of group of interest (X) and mean value of reference group (Y). Fold change values <1 indicate lower metabolite concentration group X than in group Y (e.g, CT infected versus uninfected). Metabolite fold changes were calculated for 1) CT mono-infected vs. uninfected women, 2) CT/MG co-infected vs. uninfected women, and 3) CT/MG co-infected vs. CT mono-infected women. We additionally subset the data and calculated the metabolite fold change between infection states within CST III and CST IV. CSTs I, II, and V were not included as only two CT mono-infected, and no CT/MG co-infected women had vaginal microbiota corresponding to these CSTs, limiting our ability to make comparisons to uninfected women.

We performed partial least squares discriminant analysis (PLS-DA) on the mean centered, generalized log-transformed metabolite data. PLS-DA models were assessed based upon variance

(R^2) and 10-fold cross-validation, which provides a quality assessment Q^2 with values close to 1 indicate reliable models and negative values indicate model overfitting. To assess whether differences between the groups were significant, a permutation test using the sum of squares between/sum of squares within (B/W) ratio was calculated. Variable importance in projection (VIP) score plots were used to display the top ten discriminatory metabolites. VIP scores ≥ 2.5 were considered important within a given model. PLS-DA, and VIP score plots were computed using MetaboAnalyst 4.0.

Competing Interests

ST is a consultant for Biofire Diagnostics. All other authors declare no competing interests.

Author Contributions

ST, RB and KG conceived of the study. JH and CG conducted the MG testing. CR organized and collected the samples as well as the microbiome data, AU conducted the metabolomics testing. JB, ST, CY, MS and HK conducted the analysis. JB, ST and CY drafted the paper. All authors, including JR, PB, KG and RB contributed significantly to the methods and to revising the manuscript.

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Data availability

We are currently obtaining approvals in order to submit the data to a database such as dbGAP. It will be available for download to interested parties by the time of publication, should this paper be accepted.

References:

1 Molenaar, M. C., Singer, M. & Ouburg, S. The two-sided role of the vaginal microbiome in Chlamydia trachomatis and Mycoplasma genitalium pathogenesis. *J Reprod Immunol* 130, 11-17, doi:10.1016/j.jri.2018.08.006 (2018).

2 Aldunate, M. *et al.* Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. *Front Physiol* 6, 164, doi:10.3389/fphys.2015.00164 (2015).

3 McKinnon, L. R. *et al.* The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. *AIDS Res Hum Retroviruses* 35, 219-228, doi:10.1089/AID.2018.0304 (2019).

4 Boskey, E. R., Cone, R. A., Whaley, K. J. & Moench, T. R. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Human reproduction (Oxford, England)* 16, 1809-1813, doi: file://C:\Documents and Settings\rbrotman\My Documents\articles\boskey origins of vaginal acidity 2001 Human Repro.pdf (2001).

5 Gong, Z., Luna, Y., Yu, P. & Fan, H. Lactobacilli inactivate Chlamydia trachomatis through lactic acid but not H2O2. *PLoS One* 9, e107758, doi:10.1371/journal.pone.0107758 (2014).

6 Tuddenham, S. & Ghanem, K. G. A microbiome variable in the HIV-prevention equation. *Science* 356, 907-908, doi:10.1126/science.aan6103 (2017).

7 Martin, D. H. & Marrazzo, J. M. The Vaginal Microbiome: Current Understanding and Future Directions. *J Infect Dis* 214 Suppl 1, S36-41, doi:10.1093/infdis/jiw184 (2016).

8 Nelson, T. *et al.* Vaginal Biogenic Amines: Biomarkers of Bacterial Vaginosis or Precursors to Vaginal Dysbiosis? *Frontiers in physiology* 6 (2015).

9 Srinivasan, S. *et al.* Metabolic signatures of bacterial vaginosis. *MBio* 6, doi:10.1128/mBio.00204-15 (2015).

10 Yeoman, C. J. *et al.* A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. *PLoS One* 8, e56111, doi:10.1371/journal.pone.0056111 (2013).

11 Vitali, B. *et al.* Vaginal microbiome and metabolome highlight specific signatures of bacterial vaginosis. *Eur J Clin Microbiol Infect Dis* 34, 2367-2376, doi:10.1007/s10096-015-2490-y (2015).

12 Laghi, L. *et al.* Rifaximin modulates the vaginal microbiome and metabolome in women affected by bacterial vaginosis. *Antimicrob Agents Chemother* 58, 3411-3420, doi:10.1128/AAC.02469-14 (2014).

13 Ziklo, N., Huston, W. M., Taing, K., Katouli, M. & Timms, P. In vitro rescue of genital strains of Chlamydia trachomatis from interferon-gamma and tryptophan depletion with indole-positive, but not indole-negative Prevotella spp. *BMC Microbiol* 16, 286, doi:10.1186/s12866-016-0903-4 (2016).

14 Ziklo, N., Vidgen, M. E., Taing, K., Huston, W. M. & Timms, P. Dysbiosis of the Vaginal Microbiota and Higher Vaginal Kynurenine/Tryptophan Ratio Reveals an Association with Chlamydia trachomatis Genital Infections. *Front Cell Infect Microbiol* 8, 1, doi:10.3389/fcimb.2018.00001 (2018).

15 Jordan, S. J. *et al.* Lower Levels of Cervicovaginal Tryptophan Are Associated With Natural Clearance of Chlamydia in Women. *J Infect Dis* 215, 1888-1892, doi:10.1093/infdis/jix240 (2017).

16 Onderdonk, A. B., Delaney, M. L. & Fichorova, R. N. The Human Microbiome during Bacterial Vaginosis. *Clinical microbiology reviews* 29, 223-238, doi:10.1128/CMR.00075-15 (2016).

17 Lokken, E. M. *et al.* Association of Recent Bacterial Vaginosis With Acquisition of Mycoplasma genitalium. *Am J Epidemiol* 186, 194-201, doi:10.1093/aje/kwx043 (2017).

18 Brotman, R. M. *et al.* Bacterial vaginosis assessed by gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. *J Infect Dis* 202, 1907-1915 (2010).

19 Ness, R. B. *et al.* Bacterial vaginosis and risk of pelvic inflammatory disease. *Obstetrics and gynecology* 104, 761-769, doi:10.1097/01.AOG.0000139512.37582.17 (2004).

20 Ness, R. B. *et al.* A cluster analysis of bacterial vaginosis-associated microflora and pelvic inflammatory disease. *Am J Epidemiol* 162, 585-590, doi:10.1093/aje/kwi243 (2005).

21 Torrone, E., Papp, J. & Weinstock, H. Prevalence of Chlamydia trachomatis Gential Infection Among Persons Aged 14-39 years — United States, 2007-2012. *Morbidity and Mortality Weekly Report 2014*, 834-838 (2014).

22 Lis, R., Rowhani-Rahbar, A. & Manhart, L. E. Mycoplasma genitalium infection and female reproductive tract disease: a meta-analysis. *Clin Infect Dis* 61, 418-426, doi:10.1093/cid/civ312 (2015).

23 Wiesenfeld, H. C. & Manhart, L. E. Mycoplasma genitalium in Women: Current Knowledge and Research Priorities for This Recently Emerged Pathogen. *J Infect Dis* 216, S389-S395, doi:10.1093/infdis/jix198 (2017).

24 Taylor, B. D. *et al.* Risk factors for Mycoplasma genitalium endometritis and incident infection: a secondary data analysis of the T cell Response Against Chlamydia (TRAC) Study. *Sex Transm Infect* 94, 414-420, doi:10.1136/sextrans-2017-053376 (2018).

25 Gaydos, C., Maldeis, N. E., Hardick, A., Hardick, J. & Quinn, T. C. Mycoplasma genitalium as a contributor to the multiple etiologies of cervicitis in women attending sexually transmitted disease clinics. *Sexually transmitted diseases* 36, 598-606, doi:10.1097/OLQ.0b013e3181b01948 (2009).

26 Huppert, J. S. *et al.* Mycoplasma genitalium detected by transcription-mediated amplification is associated with Chlamydia trachomatis in adolescent women. *Sexually transmitted diseases* 35, 250-254, doi:10.1097/OLQ.0b013e31815abac6 (2008).

27 Ravel, J. *et al.* Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A* 108 Suppl 1, 4680-4687, doi:10.1073/pnas.1002611107 (2011).

28 Tamarelle, J. *et al.* The vaginal microbiota and its association with human papillomavirus, Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium infections: a systematic review and meta-analysis. *Clin Microbiol Infect* 25, 35-47, doi:10.1016/j.cmi.2018.04.019 (2019).

29 Parolin, C. *et al.* Insights Into Vaginal Bacterial Communities and Metabolic Profiles of Chlamydia trachomatis Infection: Positioning Between Eubiosis and Dysbiosis. *Front Microbiol* 9, 600, doi:10.3389/fmicb.2018.00600 (2018).

30 Borgogna, J.-L. C. & Yeoman, C. J. in *The Human Microbiome Methods in Microbiology* 37-91 (2017).

31 Bautista, C. T. *et al.* Bacterial vaginosis: a synthesis of the literature on etiology, prevalence, risk factors, and relationship with chlamydia and gonorrhea infections. *Mil Med Res* 3, 4, doi:10.1186/s40779-016-0074-5 (2016).

32 Kanjee, U. & Houry, W. A. Mechanisms of acid resistance in Escherichia coli. *Annu Rev Microbiol* 67, 65-81, doi:10.1146/annurev-micro-092412-155708 (2013).

33 Shah, P. & Swiatlo, E. A multifaceted role for polyamines in bacterial pathogens. *Mol Microbiol* 68, 4-16, doi:10.1111/j.1365-2958.2008.06126.x (2008).

34 Gong, Z. *et al.* Arginine- and Polyamine-Induced Lactic Acid Resistance in Neisseria gonorrhoeae. *PLoS One* 11, e0147637, doi:10.1371/journal.pone.0147637 (2016).

35 Goytia, M. & Shafer, W. M. Polyamines can increase resistance of Neisseria gonorrhoeae to mediators of the innate human host defense. *Infect Immun* 78, 3187-3195, doi:10.1128/IAI.01301-09 (2010).

36 Cocchiaro, J. L. & Valdivia, R. H. New insights into Chlamydia intracellular survival mechanisms. *Cell Microbiol* 11, 1571-1578, doi:10.1111/j.1462-5822.2009.01364.x (2009).

37 Cox, R. A. & Garcia-Palmieri, M. R. in *Clinical Methods: The History, Physical, and Laboratory Examinations* (eds rd, H. K. Walker, W. D. Hall, & J. W. Hurst) (1990).

38 Peters, J. & Byrne, G. I. Chlamydia trachomatis growth depends on eukaryotic cholesterol esterification and is affected by Acyl-CoA:cholesterol acyltransferase inhibition. *Pathog Dis* 73, ftv028, doi:10.1093/femspd/ftv028 (2015).

39 Hamilton, R. J. R. J. *Waxes : chemistry, molecular biology and functions*. (Oily Press, 1995).

40 Huggins, G. R. & Preti, G. Volatile constituents of human vaginal secretions. *Am J Obstet Gynecol* 126, 129-136 (1976).

41 Mark, K., Brotman RM, Martinez-Greiwe S, Terplan M, Bavoil P, Ravel J. Chlamydia in Adolescent/Adult Reproductive Management Trial Study (CHARM): Clinical Core Protocol. *Contemporary Clinical Trials Communications* (2019). *In Press*

42 Ravel, J. *et al.* Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. *Microbiome*, 1:29 (2013).

43 Holm JB, H. M., Robinson CK, Settles ML, Ott S, Fu L, Yang Hm, Gajer P, McComb E, Ghanem K, Brotman R, Ravel J. *Ultra-high throughput multiplexing and sequencing of >500 bp amplicon regions on the Illumina HiSeq 2500 platform* (bioRxiv, 2018).

44 Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73, 5261-5267 (2007).

45 Quast, C. *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41, D590-596, doi:10.1093/nar/gks1219 (2013).

46 Tabrizi, S. N. *et al.* Prospective Evaluation of ResistancePlus MG, a New Multiplex Quantitative PCR Assay for Detection of Mycoplasma genitalium and Macrolide Resistance. *J Clin Microbiol* 55, 1915-1919, doi:10.1128/JCM.02312-16 (2017).

47 Tabrizi, S. N. *et al.* Multiplex Assay for Simultaneous Detection of Mycoplasma genitalium and Macrolide Resistance Using PlexZyme and PlexPrime Technology. *PLoS One* 11, e0156740, doi:10.1371/journal.pone.0156740 (2016).

48 Yang, J., Zhao, X., Lu, X., Lin, X. & Xu, G. A data preprocessing strategy for metabolomics to reduce the mask effect in data analysis. *Front Mol Biosci* 2, 4, doi:10.3389/fmolb.2015.00004 (2015).

49 Smilde, A. K., van der Werf, M. J., Bijlsma, S., van der Werff-van der Vat, B. J. & Jellema, R. H. Fusion of mass spectrometry-based metabolomics data. *Anal Chem* 77, 6729-6736, doi:10.1021/ac051080y (2005).

50 Wei, R. *et al.* Missing Value Imputation Approach for Mass Spectrometry-based Metabolomics Data. *Sci Rep* 8, 663, doi:10.1038/s41598-017-19120-0 (2018).

51 Xia, J. & Wishart, D. S. Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. *Nat Protoc* 6, 743-760, doi:10.1038/nprot.2011.319 (2011).

52 Storey, J. D. A Direct Approach to False Discovery Rates. *Journal of the Royal Statistical Society. Series B (Statistical Methodology)* 64, 479-498 (2002).

Figure 1 | The vaginal metabolome clusters by infection status and by CST. Two-dimensional principal component analysis (PCA) score plots demonstrate statistical clustering of metabolites by A) infection status and B) by CST.

Figure 2 | Discriminatory metabolites associated with infection status. CT+ and uninfected women are compared in the first column (A, B), CT+/MG+ and uninfected women are compared in second column (C,D) and CT+/MG+ women compared to CT+ women are compared in the last row (E, F). A, C, and E show discrimination of groups using partial least squares discriminant analysis. B, D, and F show the metabolites most strongly influencing discrimination by the PLS-DA. The variable importance in projection (VIP) score is the weighted sum of squares for the PLS-DA loading with the amount of variation explained by each component taken into account. Asterisks indicate metabolites which are also significant via regression analyses.

Figure 3 | Discriminatory metabolites associated with infection status stratified by CST. Women within CST-III are compared in the first column (A, B) and women within CST-IV are compared in the second column (C, D). A and C show discrimination of groups using partial least squares discriminant analysis. B and D show the metabolites most strongly influencing discrimination by the PLS-DA. Asterisks indicate metabolites which are also significant via regression analyses.

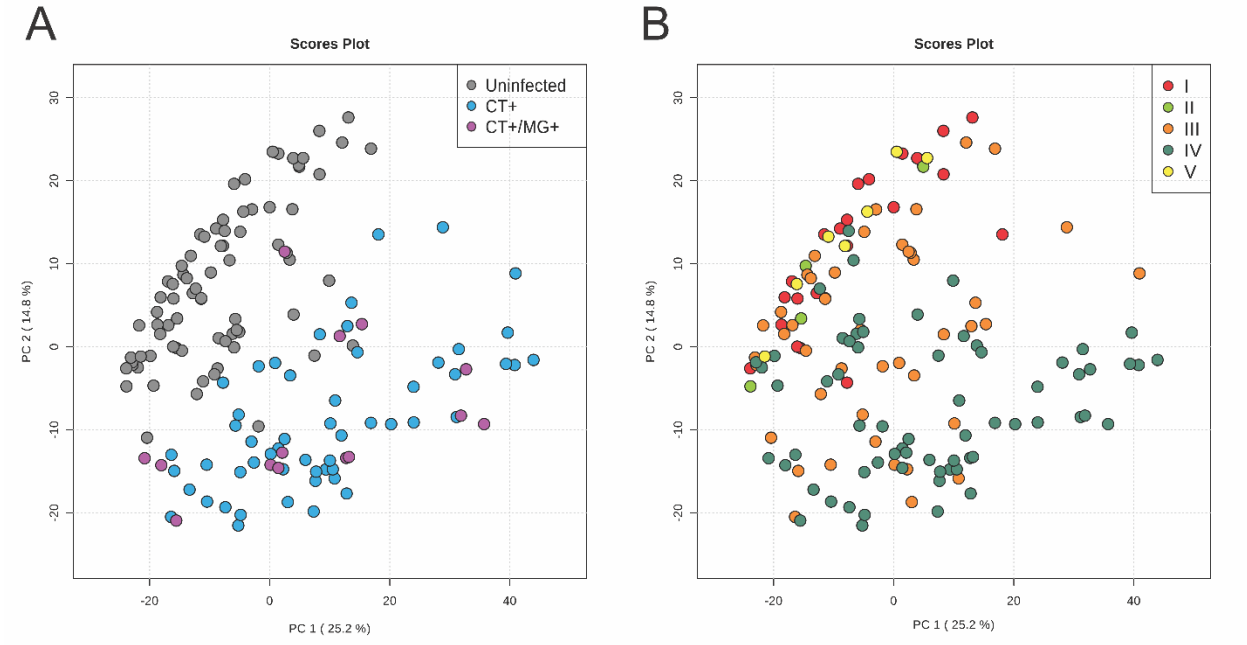

Figure 1 | The vaginal metabolome clusters by infection status and by CST. A) color-coded by infection status and B) color coded by CST.

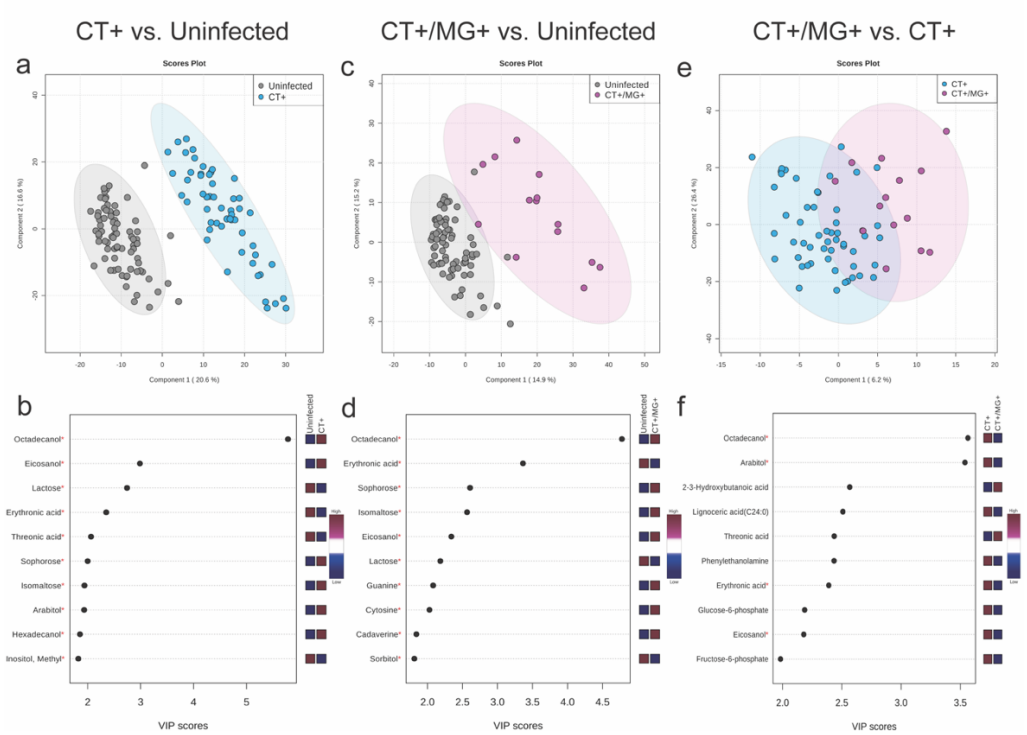

Figure 2 | Discriminatory metabolites associated with infection status. CT+ and uninfected women are compared in the first column (A, B), CT+/MG+ and uninfected women are compared in second column (C,D) and CT+/MG+ women compared to CT+ women are compared in the last row (E, F). A, C, and E show discrimination of groups using partial least squares discriminant analysis. B, D, and F show the metabolites most strongly influencing discrimination by the PLS-DA. Asterisks indicate metabolites which are also significant via regression analyses.

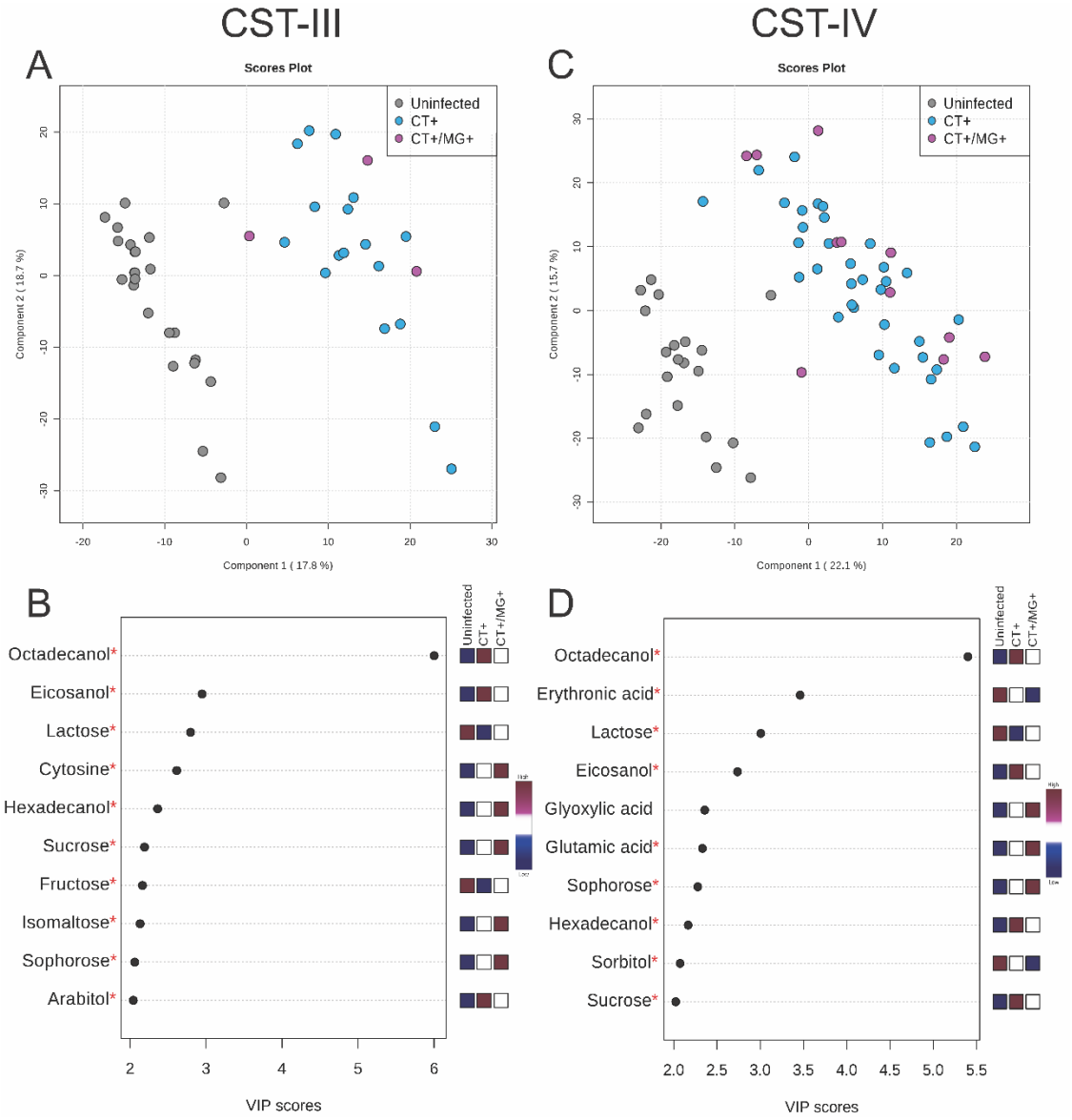

Figure 3 | Discriminatory metabolites associated with infection status stratified by CST. Women within CST-III are compared in the first column (A, B) and women within CST-IV are compared in the second column (C, D). A and C show discrimination of groups using partial least squares discriminant analysis. B and D show the metabolites most strongly influencing discrimination by the PLS-DA. Asterisks indicate metabolites which are also significant via regression analyses.

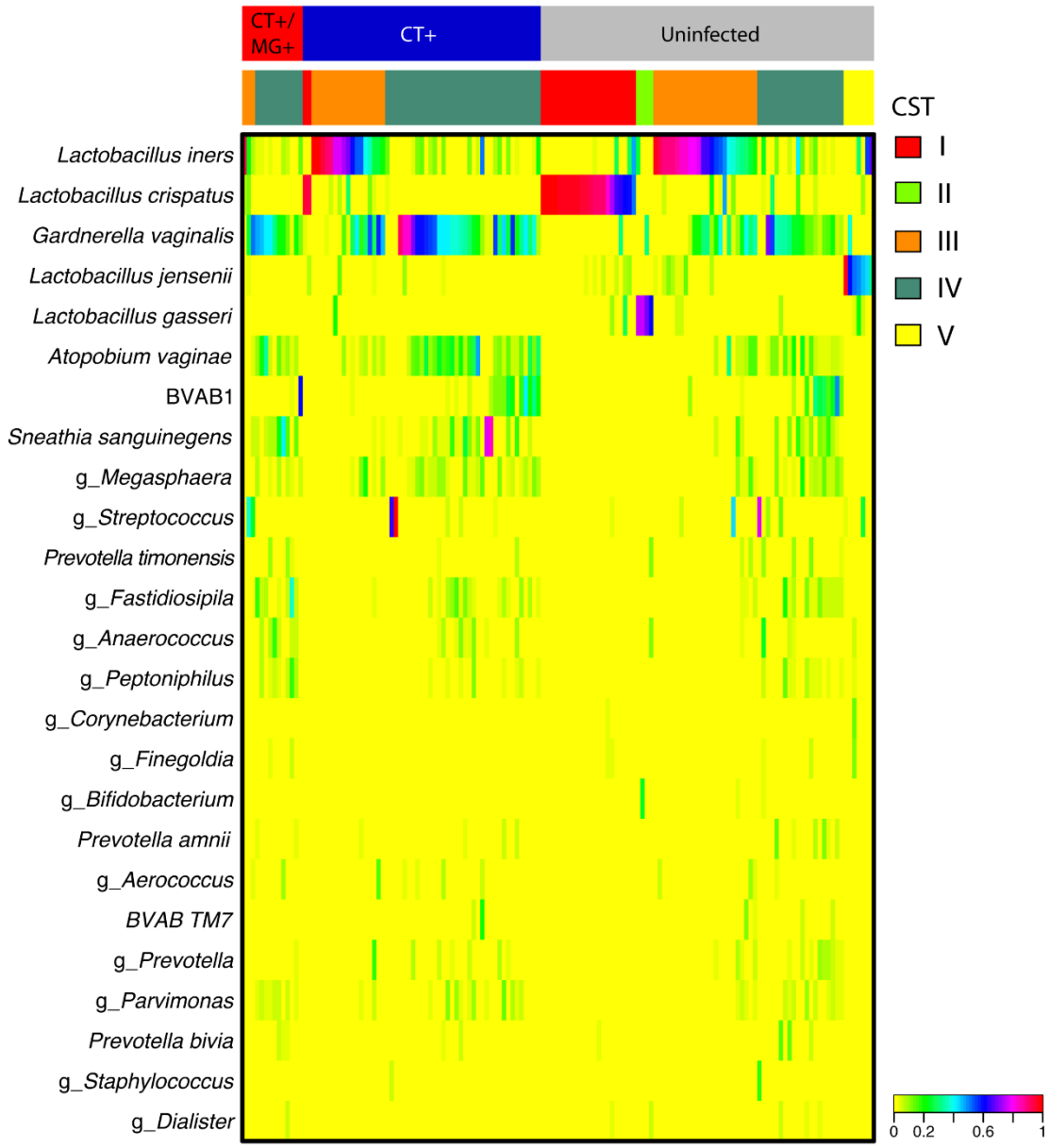

Figure S1 | Heatmap displaying the relative abundance of the 25 most abundant taxa in samples from MG+/CT+ co-infected, CT+ mono-infected, and uninfected women. Community state types (CSTs) are indicated in the second row from the top.

Table S1| Demographic, behavioral, and biological factors associated with infection status

Participant Details	Uninfected (77) n (%)	CT+ (54) n (%)	CT+/MG+ (14) n (%)	P-value*
Age				<0.0001
13-20	7 (9.1)	39 (72.2)	10 (71.4)	
21-25	31 (40.2)	11 (20.4)	4 (28.6)	
26-30	18 (23.4)	4 (7.4)	0 (0.0)	
31-35	16 (20.8)	0 (0)	0 (0.0)	
36-45	5 (6.5)	0 (0)	0 (0.0)	
Community State Type				<0.0001
I	22 (28.6)	2 (3.7)	0 (0.0)	
II	4 (5.2)	0 (0.0)	0 (0.0)	
III	24 (31.2)	17 (31.5)	3 (21.4)	
IV	20 (26.0)	35 (64.8)	11 (78.6)	
V	7 (9.0)	0 (0.0)	0 (0.0)	
Hormonal Contraceptive				0.243
Using	28 (36.4)	21 (38.9)	2 (14.3)	
Not using	49 (63.6)	33 (61.1)	12 (85.7)	
Sexual Partners, no (%)				<0.0001
0 in last 2-3 months	11 (14.3)	3 (5.6)	0 (0.0)	
1 in last 2-3 months	55 (71.4)	22 (40.7)	3 (21.4)	
2 in last 2-3 months	7 (9.1)	18 (33.3)	5 (35.7)	
More than 2 in the last 2-3 months	0 (0.0)	8 (14.8)	6 (42.9)	
Declined to answer	4 (5.2)	3 (5.6)	0 (0.0)	
Race				0.876
African American	59 (76.6)	47 (87.0)	14 (100.0)	
Asian	2 (2.6)	1 (1.9)	0 (0.0)	
Latino	5 (6.5)	2 (3.7)	0 (0.0)	
Mixed	1 (1.3)	0 (0.0)	0 (0.0)	
Native American	1 (1.3)	0 (0.0)	0 (0.0)	
White	9 (11.7)	4 (7.4)	0 (0.0)	
Condom Use				<0.0001
Always	31 (40.3)	4 (7.4)	1 (7.1)	
Sometimes	7 (9.1)	37 (68.5)	12 (85.7)	
Never	25 (32.5)	13 (24.1)	1 (7.1)	
Declined to answer	14 (18.2)	0 (0.0)	0 (0.0)	
History of Douching				0.139
Yes	10 (13.0)	13 (24.1)	4 (28.6)	
No	67 (87.0)	41 (75.9)	10 (71.4)	
Pelvic Inflamatory Disease				< 0.0001
Yes	0 (0.0)	13 (24.1)	2 (14.3)	
No	77 (100.0)	41 (75.9)	12 (85.7)	
HIV				0.0033
Positive	0 (0.0)	3 (5.5)	2 (14.3)	
Negative	77 (100.0)	48 (88.8)	12 (85.7)	
Unknown/no answer	0 (0.0)	3 (5.5)	0 (0.0)	

* p-value determined using Fisher's exact test

Table S2| Significant metabolites associated with infection status identified by multiple linear regression and unadjusted for CST

CT+ women vs. Uninfected women							
KEGG ID	**Super Pathway**	**Sub Pathway**	**Metabolite**	**P-value**	**Q-value**	**Coefficient**	**Fold Change**
	Amino Acid	Alanine and Aspartate Metabolism	beta-alanine	0.0138	0.0310	0.8	2.23
C00025	Amino Acid	Glutamate Metabolism	Glutamic acid	0.0091	0.0220	1.9	7.56
C01879	Amino Acid	Glutathione Metabolism	Pyroglutamic acid	0.0073	0.0179	-0.9	0.42
C00168	Amino Acid	Glycine, Serine and Threonine Metabolism	3-Hydroxypyruvic acid	<0.0001	<0.0001	-2.1	0.16
C00490	Amino Acid	Leucine, Isoleucine and Valine Metabolism	2-Methylsuccinic acid	0.0220	0.0464	-0.7	0.59
	Amino Acid	Leucine, Isoleucine and Valine Metabolism	3-Hydroxybutanoic acid	0.0004	0.0015	-1.8	0.16
C00141	Amino Acid	Leucine, Isoleucine and Valine Metabolism	3-methyl-2-oxobutanoic acid	0.0003	0.0014	1.3	3.17
C00123	Amino Acid	Leucine, Isoleucine and Valine Metabolism	Leucine	0.0017	0.0054	-1.8	0.16
C01672	Amino Acid	Lysine Metabolism	cadaverine	<0.0001	0.0003	2.5	12.69
C00047	Amino Acid	Lysine Metabolism	Lysine	<0.0001	<0.0001	2.4	9.78
C00245	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	Taurine	<0.0001	<0.0001	1.6	3.97
C01481	Amino Acid	Phenylalanine and Tyrosine Metabolism	Cinnamic acid, m-hydroxy	<0.0001	<0.0001	1.7	3.95

C00483	Amino Acid	Phenylalanine and Tyrosine Metabolism	Tyramine	0.0017	0.0054	1.9	7.58
C00296	Amino acid	Phenylalanine, tyrosine and tryptophan biosynthesis	Quinic acid	<0.0001	<0.0001	-1.6	0.17
C02735	Amino Acid	Polyamine	Phenylethanolamine	0.0008	0.0030	1.5	4.44
C00134	Amino Acid	Polyamine Metabolism	Putrescine	<0.0001	0.0003	2.4	9.48
C00366	Amino Acid	Purine metabolism	Uric acid	<0.0001	<0.0001	2.1	8.22
C00078	Amino Acid	Tryptophan Metabolism	Tryptophan	0.0044	0.0116	-0.7	0.49
C05942	Amino Acid	Urea cycle; Arginine and Proline Metabolism	Pyrrole-2-carboxylic acid	<0.0001	<0.0001	-1.7	0.21
C00086	Amino Acid	Urea cycle; Arginine and Proline Metabolism	Urea	0.0061	0.0154	-2.5	0.10
	Carbohydrate	Aminosugar metabolism	Erythronic acid	<0.0001	<0.0001	-4.6	0.01
C00140	Carbohydrate	Aminosugar metabolism	N-Acetyl-glucosamine	0.0022	0.0066	2.0	8.69
C00270	Carbohydrate	Aminosugar metabolism	N-Acetylneuraminic acid	<0.0001	<0.0001	2.1	10.17
C08250	Carbohydrate	Aminosugar metabolism	Sophorose	<0.0001	<0.0001	3.9	46.96
	Carbohydrate	Aminosugar metabolism	Threonic acid	<0.0001	<0.0001	-4.0	0.03
	Carbohydrate	Fructose, Mannose and Galactose Metabolism	2-O-Glycerol-b-D-galactopyranose	0.0011	0.0039	1.2	3.34

C00483	Amino Acid	Phenylalanine and Tyrosine Metabolism	Tyramine	0.0017	0.0054	1.9	7.58
C00296	Amino acid	Phenylalanine, tyrosine and tryptophan biosynthesis	Quinic acid	<0.0001	<0.0001	-1.6	0.17
C02735	Amino Acid	Polyamine	Phenylethanolamine	0.0008	0.0030	1.5	4.44
C00134	Amino Acid	Polyamine Metabolism	Putrescine	<0.0001	0.0003	2.4	9.48
C00366	Amino Acid	Purine metabolism	Uric acid	<0.0001	<0.0001	2.1	8.22
C00078	Amino Acid	Tryptophan Metabolism	Tryptophan	0.0044	0.0116	-0.7	0.49
C05942	Amino Acid	Urea cycle; Arginine and Proline Metabolism	Pyrrole-2-carboxylic acid	<0.0001	<0.0001	-1.7	0.21
C00086	Amino Acid	Urea cycle; Arginine and Proline Metabolism	Urea	0.0061	0.0154	-2.5	0.10
	Carbohydrate	Aminosugar metabolism	Erythronic acid	<0.0001	<0.0001	-4.6	0.01
C00140	Carbohydrate	Aminosugar metabolism	N-Acetyl-glucosamine	0.0022	0.0066	2.0	8.69
C00270	Carbohydrate	Aminosugar metabolism	N-Acetylneuraminic acid	<0.0001	<0.0001	2.1	10.17
C08250	Carbohydrate	Aminosugar metabolism	Sophorose	<0.0001	<0.0001	3.9	46.96
	Carbohydrate	Aminosugar metabolism	Threonic acid	<0.0001	<0.0001	-4.0	0.03
	Carbohydrate	Fructose, Mannose and Galactose Metabolism	2-O-Glycerol-b-D-galactopyranose	0.0011	0.0039	1.2	3.34

C00095	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Fructose	0.0036	0.0098	-1.6	0.32
C01582	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Galactose	0.0001	0.0006	1.8	8.01
	Carbohydrate	Fructose, Mannose and Galactose Metabolism	lactose	<0.0001	<0.0001	-5.4	0.01
C00392	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Mannitol	0.0006	0.0023	-1.6	0.19
C00507	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Rhamnose	0.0011	0.0039	1.7	7.04
C00794	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Sorbitol	<0.0001	<0.0001	-3.3	0.04
	Carbohydrate	Galactose Metabolism	Sucrose	<0.0001	<0.0001	3.5	41.83
	Carbohydrate	Glycogen Metabolism	Isomaltose	<0.0001	<0.0001	3.8	50.13
C00597	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	3-Phosphoglycerate	0.0032	0.0090	-1.0	0.38
C00258	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Glyceric acid	0.0002	0.0008	-1.3	0.30
	Carbohydrate	Pentose Metabolism	2-deoxy-D-ribose	0.0047	0.0124	0.7	2.08
C01904	Carbohydrate	Pentose Metabolism	Arabitol	<0.0001	<0.0001	3.8	56.01

C00257	Carbohydrate	Pentose Metabolism	Gluconic acid	<0.0001	<0.0001	-2.5	0.10
C00198	Carbohydrate	Pentose Metabolism	Gluconic acid, lactone	0.0008	0.0030	1.7	4.17
C00121	Carbohydrate	Pentose Metabolism	Ribose	0.0007	0.0026	1.3	4.79
	Carbohydrate	Pentose Metabolism	Ribose-5-P	0.0139	0.0311	0.5	1.50
	Carbohydrate	Pentose Metabolism	Sedoheptulose	<0.0001	0.0002	-1.5	0.21
C00181	Carbohydrate	Pentose Metabolism	Xylose	<0.0001	<0.0001	2.1	7.10
C00738	Carbohydrate		Hexose	<0.0001	<0.0001	2.7	15.01
C02225	Energy	TCA Cycle	2-Methylcitric acid	<0.0001	<0.0001	-1.1	0.36
C00147	Energy	TCA Cycle	alpha-Ketoglutaric acid	0.0040	0.0108	-0.9	0.52
	Energy	TCA Cycle	Citric acid	<0.0001	<0.0001	-2.8	0.08
C00489	Energy	TCA Cycle	Glutaric acid	<0.0001	<0.0001	-2.2	0.11
	Energy	TCA Cycle	Glutaric acid, 2-hydroxy	<0.0001	0.0001	2.8	15.03
	Lipid	Fatty acid	2-Oxoisocaproic acid	<0.0001	0.0005	2.1	10.12
C03044	Lipid	Fatty Acid	Butane,2-3-dihydroxy	0.0004	0.0015	-0.9	0.41
C05984	Lipid	Fatty Acid	Butanoic acid, 2-hydroxy	<0.0001	<0.0001	2.7	17.30
	Lipid	Fatty Acid	Butanoic acid, 2-methyl-2-hydroxy	<0.0001	<0.0001	2.3	11.20
C04181	Lipid	Fatty Acid	Butanoic acid, 3-methyl-3-hydroxy	<0.0001	<0.0001	1.8	4.33

C01585	Lipid	Fatty acid	Hexanoic acid, 2-hydroxy	0.0075	0.0182	0.4	1.53
	Lipid	Fatty acid	Pentanoic acid, 4-methyl-2-hydroxy	<0.0001	<0.0001	3.0	19.27
C00803	Lipid	Fatty acid	Pentanoic acid, 5-amino	0.0182	0.0388	1.1	3.12
	Lipid	Fatty Alcohol	1-hexadecanoylglycerol	0.0159	0.0347	0.9	2.16
	Lipid	Fatty Alcohol	1-O-Hexadecenylglycerol	0.0069	0.0172	0.5	1.61
	Lipid	Fatty Alcohol	1-Octadecanoylglycerol	<0.0001	<0.0001	3.1	17.55
	Lipid	Fatty Alcohol	2-Propylheptanol	<0.0001	<0.0001	-2.3	0.12
	Lipid	Fatty Alcohol	Eicosanol	<0.0001	<0.0001	5.8	252.72
	Lipid	Fatty Alcohol	Heptadecanol	<0.0001	<0.0001	2.9	14.96
C00823	Lipid	Fatty Alcohol	Hexadecanol	<0.0001	<0.0001	3.6	39.41
	Lipid	Fatty Alcohol	Octadecanol	<0.0001	<0.0001	11.3	40613.14
	Lipid	Glycerolipid Metabolism	alpha-Glycerophosphorylglycerol	0.0130	0.0294	-0.5	0.70
C00116	Lipid	Glycerolipid Metabolism	Glycerol	<0.0001	<0.0001	-2.8	0.07
C00093	Lipid	Glycerolipid Metabolism	Glycerol-3-p	0.0028	0.0079	-1.0	0.35
C01177	Lipid	Inositol Metabolism	Inositol-p	0.0180	0.0387	0.8	2.20
C03365	Lipid	Inositol Metabolism	Inositol, methyl	<0.0001	<0.0001	-3.6	0.04

	Lipid	Long Chain Fatty Acid	C10:0 (Decanoic acid)	<0.0001	<0.0001	1.2	3.11
	Lipid	Long Chain Fatty Acid	C15:0 (Pentadecanoic acid)	<0.0001	<0.0001	2.4	10.93
	Lipid	Long Chain Fatty Acid	C17:0 (Heptadecanoic acid)	<0.0001	<0.0001	1.8	6.23
	Lipid	Long Chain Fatty Acid	C18:1:1 (Oleic acid)	<0.0001	0.0002	1.6	4.60
	Lipid	Long Chain Fatty Acid	C20:0 (Arachidic acid)	<0.0001	<0.0001	0.8	2.28
	Lipid	Long Chain Fatty Acid	C22:0 (Behenic acid)	0.0041	0.0110	0.8	2.09
	Lipid	Long Chain Fatty Acid	C26:0 (Cerotic acid)	0.0061	0.0154	0.8	2.35
	Lipid	Long Chain Fatty Acid	C8:0 (Caprylic acid)	<0.0001	<0.0001	-1.6	0.34
	Lipid	Long Chain Fatty Acids	C14:0 (Myristic acid)	0.0002	0.0008	0.8	1.90
C00189	Lipid	Polyamine Metabolism	Ethanolamine	<0.0001	0.0001	1.6	4.12
C02483	Lipid	Prenol Lipids	Tocopherol-y	0.0016	0.0052	-1.2	0.38
C00041	Lipid	Quinone and hydroquinone lipids	alpha-Tocopherol	0.0150	0.0330	1.2	2.80
	Lipid	Sterol	5-a-Cholestan-3-ol	0.0052	0.0135	1.0	2.50
C01444	Nucleotide	Purine metabolism	Oxamic acid	0.0019	0.0058	-2.1	0.11
C00262	Nucleotide	Purine Metabolism, (Hypo)Xanthine /Inosine containing	Hypoxanthine	0.0023	0.0067	1.6	5.20

C00385	Nucleotide	Purine Metabolism, (Hypo)Xanthine /Inosine containing	Xanthine	<0.0001	<0.0001	2.1	7.42
	Nucleotide	Purine Metabolism, Adenine containing	Adenine	<0.0001	<0.0001	1.9	7.64
C00242	Nucleotide	Purine Metabolism, Guanine containing	Guanine	<0.0001	<0.0001	3.4	29.22
C00872	Nucleotide	Pyrimidine Metabolism	Aminomalonic acid	<0.0001	0.0003	1.8	5.76
C00383	Nucleotide	Pyrimidine Metabolism	Malonic acid	<0.0001	<0.0001	1.8	5.83
C00380	Nucleotide	Pyrimidine Metabolism, Cytidine containing	Cytosine	<0.0001	<0.0001	2.9	14.91
C00178	Nucleotide	Pyrimidine Metabolism, Thymine containing	Thymine	<0.0001	0.0003	2.5	12.94
C00106	Nucleotide	Pyrimidine Metabolism, Uracil containing	Uracil	0.0003	0.0012	1.9	8.87
	Xenobiotics	Additive agent	Adipic acid	<0.0001	<0.0001	-2.7	0.07
	Xenobiotics	Benzoate Metabolism	Benzenepropanoic acid, a-hydroxy	0.0001	0.0006	1.3	2.95
	Xenobiotics	Benzoate Metabolism	Benzoic acid	<0.0001	<0.0001	0.9	2.69
C00503	Xenobiotics	Food Component/Plant	Erythritol	0.0003	0.0012	-1.0	0.41
	Xenobiotics		Diethyleneglycol	0.0012	0.0042	0.7	1.77
CT+/MG+ women vs. Uninfected women							
KEGG ID	**Super Pathway**	**Sub Pathway**	**Metabolite**	**P-value**	**Q-value**	**Coefficient**	**Fold Change**

C01879	Amino Acid	Glutathione Metabolism	Pyroglutamic acid	0.0020	0.0060	-1.7	0.17
C00168	Amino Acid	Glycine, Serine and Threonine Metabolism	3-Hydroxypyruvic acid	0.0107	0.0253	-1.4	0.24
C00141	Amino Acid	Leucine, Isoleucine and Valine Metabolism	3-methyl-2-oxobutanoic acid	0.0177	0.0381	1.3	3.82
C01672	Amino Acid	Lysine Metabolism	Cadaverine	0.0008	0.0029	3.4	30.54
	Amino Acid	Phenylalanine and Tyrosine Metabolism	Hydrocinnamic acid	<0.0001	0.0003	1.2	3.17
	Amino Acid	Phenylalanine and Tyrosine Metabolism	p-hydroxyhydrocinnamic acid	0.0045	0.0119	2.4	55.20
C00296	Amino acid	Phenylalanine, tyrosine and tryptophan biosynthesis	Quinic acid	0.0002	0.0009	-1.7	0.18
C00134	Amino Acid	Polyamine Metabolism	Putrescine	0.0014	0.0048	3.1	21.69
C00366	Amino Acid	Purine metabolism	Uric acid	0.0042	0.0111	1.5	4.65
	Carbohydrate	Aminosugar metabolism	Erythronic acid	<0.0001	<0.0001	-6.2	0.00
C00645	Carbohydrate	Aminosugar metabolism	N-Acetyl mannosamine	0.0033	0.0091	0.5	0.23
C00270	Carbohydrate	Aminosugar metabolism	n-acetylneuraminic acid	0.0017	0.0055	2.4	11.11
C08250	Carbohydrate	Aminosugar metabolism	Sophorose	<0.0001	<0.0001	4.8	126.81
	Carbohydrate	Aminosugar metabolism	Threonic acid	0.0061	0.0154	-2.4	0.09
C01582	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Galactose	0.0029	0.0081	2.2	9.24

	Carbohydrate	Fructose, Mannose and Galactose Metabolism	lactose	<0.0001	<0.0001	-4.1	0.02
C00507	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Rhamnose	0.0125	0.0288	2.1	7.86
C00794	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Sorbitol	<0.0001	<0.0001	-3.4	0.03
	Carbohydrate	Galactose Metabolism	Sucrose	<0.0001	0.0003	3.3	28.19
	Carbohydrate	Glycogen Metabolism	Isomaltose	<0.0001	0.0002	4.8	117.07
C00258	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Glyceric acid	<0.0001	<0.0001	-2.7	0.07
	Carbohydrate	Pentose Metabolism	2-deoxy-D-ribose	0.0006	0.0025	1.4	2.32
C00257	Carbohydrate	Pentose Metabolism	Gluconic acid	<0.0001	0.0002	-2.6	0.08
	Carbohydrate	Pentose Metabolism	Sedoheptulose	0.0005	0.0020	-2.0	0.14
C00181	Carbohydrate	Pentose Metabolism	Xylose	0.0028	0.0079	1.8	5.80
C00738	Carbohydrate		Hexose	0.0016	0.0052	2.8	17.27
	Energy	TCA Cycle	Citric acid	0.0003	0.0014	-2.6	0.07
C00489	Energy	TCA Cycle	Glutaric acid	0.0015	0.0051	-2.1	0.13
	Energy	TCA Cycle	Glutaric acid, 2-hydroxy	0.0095	0.0226	2.8	15.87
	Lipid	Fatty acid	2-Oxoisocaproic acid	0.0119	0.0275	2.2	2.90
C06255	Lipid	Fatty acid	2-Oxoisovaleric acid	0.0015	0.0049	0.9	1.75

C03044	Lipid	Fatty Acid	Butane,2-3-dihydroxy	0.0050	0.0130	-1.2	0.30
C05984	Lipid	Fatty Acid	Butanoic acid, 2-hydroxy	0.0151	0.0330	2.4	11.01
	Lipid	Fatty Acid	Butanoic acid, 2-methyl-2-hydroxy	0.0119	0.0275	1.8	5.99
C04181	Lipid	Fatty Acid	Butanoic acid, 3-methyl-3-hydroxy	0.0042	0.0111	1.8	5.88
	Lipid	Fatty acid	Pentanoic acid, 4-methyl-2-hydroxy	0.0110	0.0258	2.3	14.81
C00803	Lipid	Fatty acid	Pentanoic acid, 5-amino	0.0023	0.0067	2.4	0.23
	Lipid	Fatty Alcohol	1-octadecanoylglycerol	<0.0001	<0.0001	2.7	5.66
	Lipid	Fatty Alcohol	2-Propylheptanol	0.0004	0.0017	-1.6	2.19
	Lipid	Fatty Alcohol	Eicosanol	<0.0001	<0.0001	4.3	77.23
	Lipid	Fatty Alcohol	Heptadecanol	<0.0001	<0.0001	2.3	10.21
C00823	Lipid	Fatty Alcohol	Hexadecanol	0.0010	0.0036	3.0	20.28
	Lipid	Fatty Alcohol	Octadecanol	<0.0001	<0.0001	8.9	13.60
C00093	Lipid	Glycerolipid Metabolism	Glycerol-3-p	0.0022	0.0066	-1.7	0.19
C00137	Lipid	Inositol Metabolism	Inositol	0.0059	0.0150	-1.3	0.28
C03365	Lipid	Inositol Metabolism	Inositol, methyl	<0.0001	<0.0001	-3.1	25.52
C00137	Lipid	Inositol Metabolism	Inositol, myo	0.0171	0.0371	-1.1	0.02

	Lipid	Long Chain Fatty Acid	C15:0 (Pentadecanoic acid)	<0.0001	<0.0001	2.2	9.39
	Lipid	Long Chain Fatty Acid	C17:0 (Heptadecanoic acid)	<0.0001	0.0003	1.3	3.76
	Lipid	Long Chain Fatty Acid	C18:1:1 (Oleic acid)	0.0127	0.0289	1.6	4.84
	Lipid	Long Chain Fatty Acid	C8:0 (Caprylic acid)	0.0021	0.0064	-1.8	0.17
	Lipid	Phospholipid Metabolism	O-Phosphoethanolamine	0.0011	0.0039	0.7	1099.67
C00189	Lipid	Polyamine Metabolism	Ethanolamine	0.0013	0.0043	2.0	7.39
C00041	Lipid	Quinone and hydroquinone lipids	alpha-Tocopherol	0.0173	0.0374	1.9	856.07
C00385	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Xanthine	<0.0001	0.0001	2.2	8.94
	Nucleotide	Purine Metabolism, Adenine containing	Adenine	0.0138	0.0310	1.5	6.32
	Nucleotide	Purine Metabolism, Guanine containing	2'-Deoxyguanosine	0.0012	0.0040	0.5	0.35
C00242	Nucleotide	Purine Metabolism, Guanine containing	Guanine	0.0002	0.0007	3.9	47.66
C00872	Nucleotide	Pyrimidine Metabolism	Aminomalonic acid	0.0074	0.0181	1.9	0.03
C00383	Nucleotide	Pyrimidine Metabolism	Malonic acid	0.0227	0.0471	1.2	1577148.12

C00380	Nucleotide	Pyrimidine Metabolism, Cytidine containing	Cytosine	0.0001	0.0005	3.8	43.26
C00178	Nucleotide	Pyrimidine Metabolism, Thymine containing	Thymine	0.0066	0.0165	2.7	15.18
C00106	Nucleotide	Pyrimidine Metabolism, Uracil containing	Uracil	0.0068	0.0169	2.3	10.26
	Xenobiotics	Additive agent	Adipic acid	<0.0001	0.0001	-2.0	0.95
CT+/MG+ women vs. CT+ women							
KEGG ID	**Super Pathway**	**Sub Pathway**	**Metabolite**	**P-value**	**Q-value**	**Coefficient**	**Fold Change**
	Amino Acid	Phenylalanine and Tyrosine Metabolism	Hydrocinnamic acid	0.0042	0.0150	0.8	2.33
	Amino Acid	Phenylalanine and Tyrosine Metabolism	p-Hydroxyphenylacetic acid	0.0116	0.0359	0.7	2.91
	Carbohydrate	Aminosugar metabolism	Erythronic acid	0.0164	0.0474	-1.6	0.19
C01904	Carbohydrate	Pentose Metabolism	Arabitol	0.0114	0.0355	-2.4	8.76
	Lipid	Fatty Alcohol	Eicosanol	0.0002	0.0009	-1.5	0.22
	Lipid	Fatty Alcohol	Octadecanol	<0.0001	<0.0001	-2.5	0.00
	Lipid	Long Chain Fatty Acid	C16:1 (Palmitoleic acid)	0.0147	0.0439	-1.2	0.30

Table S3 | Significant metabolites associated with infection status identified by multiple linear regression and adjusted for CST

CT+ women vs. Uninfected women								
KEGG ID	**Super Pathway**	**Sub Pathway**	**Metabolite**	**P-value**	**Q-value**	**Coefficient**	**Fold change within CST III women**	**Fold change within CST IV women**
C00025	Amino Acid	Glutamate Metabolism	Glutamic acid	0.0001	0.0013	3.2	19.98	34.32
C00624	Amino Acid	Glutamate Metabolism	N-Acetylglutamic acid	0.0005	0.0019	1.4	3.35	3.67
C00168	Amino Acid	Glycine, Serine and Threonine Metabolism	3-Hydroxypyruvic acid	<0.0001	0.0003	-1.7	0.52	0.12
C00047	Amino Acid	Lysine Metabolism	Lysine	<0.0001	<0.0001	2.9	12.68	22.33
C00245	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	Taurine	<0.0001	0.0001	1.9	4.38	4.51
C01481	Amino Acid	Phenylalanine and Tyrosine Metabolism	Cinnamic acid, m-hydroxy	<0.0001	0.0004	1.8	5.95	4.85
C00082	Amino Acid	Phenylalanine and Tyrosine Metabolism	Tyrosine	0.0028	0.0019	2.0	4.55	11.27

C00296	Amino acid	Phenylalanine, tyrosine and tryptophan biosynthesis	Quinic acid	<0.0001	<0.0001	-1.8	0.15	0.18
C00366	Amino Acid	Purine metabolism	Uric acid	<0.0001	<0.0001	2.3	19.06	6.45
C05942	Amino Acid	Urea cycle; Arginine and Proline Metabolism	Pyrrole-2-carboxylic acid	0.0004	0.0019	-1.6	0.51	0.12
	Carbohydrate	Aminosugar metabolism	Erythronic acid	<0.0001	<0.0001	-4.1	0.02	0.01
C08250	Carbohydrate	Aminosugar metabolism	Sophorose	<0.0001	<0.0001	3.4	11.01	55.62
	Carbohydrate	Aminosugar metabolism	Threonic acid	<0.0001	<0.0001	-4.0	0.01	0.02
C00095	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Fructose	<0.0001	0.0003	-2.6	0.01	0.36
	Carbohydrate	Fructose, Mannose and Galactose Metabolism	lactose	<0.0001	<0.0001	-5.8	0.00	0.00
C00794	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Sorbitol	<0.0001	<0.0001	-3.2	0.04	0.04

	Carbohydrate	Galactose Metabolism	Sucrose	<0.0001	<0.0001	3.7	31.79	53.64
	Carbohydrate	Glycogen Metabolism	Isomaltose	0.0001	0.0013	3.1	9.48	35.35
C00597	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	3-Phosphoglycerate	0.0018	0.0019	-1.2	0.25	0.27
C00258	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Glyceric acid	0.0025	0.0019	-1.2	0.27	0.29
C01904	Carbohydrate	Pentose Metabolism	Arabitol	<0.0001	<0.0001	3.7	80.39	30.26
C00257	Carbohydrate	Pentose Metabolism	Gluconic acid	<0.0001	0.0004	-1.7	0.18	0.11
C00181	Carbohydrate	Pentose Metabolism	Xylose	<0.0001	<0.0001	2.3	18.17	6.88
C00738	Carbohydrate		Hexose	0.0002	0.0019	2.3	11.66	8.72
C00253	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	Nicotinic acid	<0.0001	0.0006	-1.9	0.07	0.22
C02225	Energy	TCA Cycle	2-Methylcitric acid	<0.0001	<0.0001	-1.2	0.31	0.26
C00147	Energy	TCA Cycle	Alpha-Ketoglutaric acid	<0.0001	0.0009	-1.4	0.23	0.31
	Energy	TCA Cycle	Citric acid	<0.0001	<0.0001	-2.8	0.08	0.05
C00489	Energy	TCA Cycle	Glutaric acid	<0.0001	<0.0001	-2.5	0.05	0.13

C03044	Lipid	Fatty Acid	Butane,2-3-dihydroxy	<0.0001	<0.0001	-1.4	0.19	0.27
	Lipid	Fatty Acid	Butanoic acid, 2-methyl-2-hydroxy	0.0072	0.0019	1.2	1.02	9.91
C04181	Lipid	Fatty Acid	Butanoic acid, 3-methyl-3-hydroxy	0.0006	0.0019	1.5	10.52	2.17
	Lipid	Fatty acid	Pentanoic acid, 4-methyl-2-hydroxy	0.0039	0.0019	1.8	2.64	7.68
	Lipid	Fatty Alcohol	1-hexadecanoylglycerol	0.0017	0.0019	1.3	3.51	3.86
	Lipid	Fatty Alcohol	1-Octadecanoylglycerol	<0.0001	<0.0001	3.4	17.37	49.93
	Lipid	Fatty Alcohol	2-Propylheptanol	<0.0001	<0.0001	-2.1	0.17	0.08
	Lipid	Fatty Alcohol	Eicosanol	<0.0001	<0.0001	5.8	397.40	324.25
	Lipid	Fatty Alcohol	Heptadecanol	<0.0001	<0.0001	2.9	19.48	16.49
C00823	Lipid	Fatty Alcohol	Hexadecanol	<0.0001	<0.0001	4.1	59.36	92.59
	Lipid	Fatty Alcohol	Octadecanol	<0.0001	<0.0001	11.4	########	63764.28
C00116	Lipid	Glycerolipid Metabolism	Glycerol	<0.0001	0.0011	-2.5	0.03	0.15
C03365	Lipid	Inositol Metabolism	Inositol, methyl	<0.0001	<0.0001	-3.6	0.03	0.02
	Lipid	Long Chain Fatty Acid	C10:0 (Decanoic acid)	<0.0001	0.0002	1.1	3.67	3.27
	Lipid	Long Chain Fatty Acid	C15:0 (Pentadecanoic acid)	<0.0001	<0.0001	2.2	10.36	8.56

	Lipid	Long Chain Fatty Acid	C17:0 (Heptadecanoic acid)	<0.0001	<0.0001	1.8	5.92	6.76
	Lipid	Long Chain Fatty Acid	C18:1:1 (Oleic acid)	<0.0001	0.0005	1.9	2.77	15.49
	Lipid	Long Chain Fatty Acid	C20:0 (Arachidic acid)	<0.0001	0.0003	0.9	2.07	2.91
	Lipid	Long Chain Fatty Acid	C8:0 (Caprylic acid)	<0.0001	<0.0001	-2.0	0.16	0.10
C00189	Lipid	Polyamine Metabolism	Ethanolamine	0.0006	0.0019	1.5	4.24	4.48
C12078	Lipid	Prenol Lipids	Dehydroabietic acid	0.0055	0.0019	-0.5	0.43	0.87
C02483	Lipid	Prenol Lipids	Tocopherol-y	0.0008	0.0019	-1.4	0.26	0.17
C01444	Nucleotide	Purine metabolism	Oxamic acid	0.0069	0.0019	-2.1	0.10	0.20
C00262	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Hypoxanthine	0.0084	0.0019	1.6	7.90	3.74
C00385	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Xanthine	<0.0001	<0.0001	1.6	3.64	7.72
	Nucleotide	Purine Metabolism, Adenine containing	Adenine	0.0002	0.0019	1.6	4.10	6.46
C00242	Nucleotide	Purine Metabolism, Guanine containing	Guanine	<0.0001	0.0002	3.1	18.66	27.29

C00872	Nucleotide	Pyrimidine Metabolism	Aminomalonic acid	<0.0001	0.0011	2.0	8.45	7.36
C00383	Nucleotide	Pyrimidine Metabolism	Malonic acid	<0.0001	0.0001	1.7	6.58	5.80
C00380	Nucleotide	Pyrimidine Metabolism, Cytidine containing	Cytosine	<0.0001	0.0005	2.8	23.83	9.66
	Xenobiotics	Additive agent	Adipic acid	<0.0001	<0.0001	-2.3	0.07	0.13
	Xenobiotics	Benzoate Metabolism	Benzenepropanoic acid, a-hydroxy	0.0013	0.0019	1.2	1.60	5.10
	Xenobiotics	Benzoate Metabolism	Benzoic acid	<0.0001	0.0012	0.9	4.31	1.95
C00503	Xenobiotics	Food Component /Plant	Erythritol	0.0010	0.0019	-1.0	0.38	0.36
	Xenobiotics		Diethylenglycol	0.0020	0.0019	0.7	3.00	1.90

CT+/MG+ women vs. Uninfected women

KEGG ID	Super Pathway	Sub Pathway	Metabolite	P-value	Q-value	Coefficient	Fold change within CST III women	Fold change within CST IV women
C00025	Amino Acid	Glutamate Metabolism	Glutamic acid	0.0019	0.0133	3.9	26.23	77.28
	Amino Acid	Phenylalanine and Tyrosine Metabolism	Hydrocinnamic acid	0.0011	0.0089	1.0	1.00	4.20
C00296	Amino acid	Phenylalanine, tyrosine and tryptophan biosynthesis	Quinic acid	0.0002	0.0018	-1.9	0.15	0.16

C00366	Amino Acid	Purine metabolism	Uric acid	0.0011	0.0084	1.9	9.67	4.75
	Carbohydrate	Aminosugar metabolism	Erythronic acid	<0.0001	<0.0004	-5.6	0.06	0.00
C08250	Carbohydrate	Aminosugar metabolism	Sophorose	<0.0001	0.0009	4.3	391.99	57.63
	Carbohydrate	Fructose, Mannose and Galactose Metabolism	lactose	<0.0001	<0.0006	-4.6	0.03	0.01
C00794	Carbohydrate	Fructose, Mannose and Galactose Metabolism	Sorbitol	<0.0001	0.0005	-3.3	0.19	0.02
	Carbohydrate	Galactose Metabolism	Sucrose	<0.0001	0.0009	3.6	106.91	30.64
	Carbohydrate	Glycogen Metabolism	Isomaltose	0.0013	0.0099	4.0	733.37	28.00
C00258	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Glyceric acid	<0.0001	0.0010	-2.5	0.34	0.05
C00257	Carbohydrate	Pentose Metabolism	Gluconic acid	0.0095	0.0498	-1.6	0.22	0.15
C00181	Carbohydrate	Pentose Metabolism	Xylose	0.0015	0.0113	2.0	15.48	5.11
C00147	Energy	TCA Cycle	Alpha-Ketoglutaric acid	0.0089	0.0470	-1.4	0.52	0.23
	Energy	TCA Cycle	Citric acid	0.0008	0.0067	-2.7	0.20	0.04

C00489	Energy	TCA Cycle	Glutaric acid	0.0004	0.0037	-2.4	0.12	0.10
C03044	Lipid	Fatty Acid	Butane,2-3-dihydroxy	0.0001	0.0016	-1.7	0.19	0.18
	Lipid	Fatty Alcohol	1-octadecanoylglycerol	<0.0001	<0.0007	3.0	29.64	22.21
	Lipid	Fatty Alcohol	2-Propylheptanol	0.0038	0.0240	-1.4	0.37	0.17
	Lipid	Fatty Alcohol	Eicosanol	<0.0001	<0.0002	4.4	46.99	88.43
	Lipid	Fatty Alcohol	Heptadecanol	<0.0001	<0.0003	2.3	8.12	10.87
C00823	Lipid	Fatty Alcohol	Hexadecanol	0.0003	0.0034	3.6	81.44	35.82
	Lipid	Fatty Alcohol	Octadecanol	<0.0001	<0.0001	8.9	4203.70	7608.50
C03365	Lipid	Inositol Metabolism	Inositol, methyl	<0.0001	<0.0005	-3.1	0.11	0.03
	Lipid	Long Chain Fatty Acid	C15:0 (Pentadecanoic acid)	<0.0001	<0.0008	2.0	6.24	7.64
	Lipid	Long Chain Fatty Acid	C17:0 (Heptadecanoic acid)	<0.0001	0.0010	1.4	4.34	3.95
	Lipid	Long Chain Fatty Acid	C18:1:1 (Oleic acid)	0.0050	0.0304	1.9	4.99	11.71
	Lipid	Long Chain Fatty Acid	C8:0 (Caprylic acid)	0.0003	0.0031	-2.2	0.14	0.08
	Lipid	Phospholipid Metabolism	O-Phosphoethanolamine	0.0045	0.0274	0.7	1.40	2.35
C00189	Lipid	Polyamine Metabolism	Ethanolamine	0.0061	0.0354	1.8	10.87	5.25

C00385	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Xanthine	0.0028	0.0185	1.5	2.50	7.31
	Nucleotide	Purine Metabolism, Guanine containing	2'-Deoxyguanosine	0.0085	0.0453	0.5	1.00	2.01
C00242	Nucleotide	Purine Metabolism, Guanine containing	Guanine	0.0013	0.0099	3.5	87.39	27.95
C00872	Nucleotide	Pyrimidine Metabolism	Aminomalonic acid	0.0066	0.0376	2.1	2.49	12.09
C00380	Nucleotide	Pyrimidine Metabolism, Cytidine containing	Cytosine	0.0004	0.0035	3.8	1528.40	10.40
	Xenobiotics	Additive agent	Adipic acid	0.0009	0.0075	-1.6	0.28	0.21

CT+/MG+ women vs. CT+ women

KEGG ID	Super Pathway	Sub Pathway	Metabolite	P-value	Q-value	Coefficient	Fold change within CST III women	Fold change within CST IV women
	Amino Acid	Phenylalanine and Tyrosine Metabolism	Hydrocinnamic acid	0.0071	0.0498	0.80	1.00	2.69
	Lipid	Fatty Alcohol	Eicosanol	0.0002	0.0030	-1.50	0.12	0.27
	Lipid	Fatty Alcohol	Octadecanol	<0.0001	<0.0001	-2.47	0.03	0.12

Table S4 | Metabolites detected in Amies controls

Metabolite	Average Relative Concentration / 200uL	Standard Error of Mean (200uL)
2-Methylmalic acid	21.58	0.78
3,4-Dihydroxybutanoic acid	2.90	0.12
Alanine	145.99	6.46
Benzoic acid, 2-methyl	86.74	0.95
butanoic acid, 4-hydroxy	1.08	0.06
C16:0	149.35	16.05
C18:0	68.50	6.76
Fumaric acid	3.84	0.17
Glucose	1.79	0.13
Glycolic acid	524.22	11.18
glyoxylic acid	49.49	2.17
lactic acid	1945.43	85.72
malic acid	1.33	0.10
Malic acid	2009.40	34.17
Oxalic acid	17.24	1.27
Pentanoic acid, 3-methyl-2-hydroxy	4.27	0.49
phosphoric acid	31046.09	1097.94
pyrophosphate (4:1)	2.81	0.30
pyruvic acid	10117.92	518.51
Succinic acid	14.71	1.78

```
#mgen 061019#
setwd("~/Desktop/")
Mgen_raw_sinf<-read.csv("Mgen_raw_061019.csv",header=TRUE,
na.strings='',sep=',',fill=TRUE,stringsAsFactors = FALSE) #working file
head(Mgen_raw_sinf[17])#first metabolite
head(Mgen_raw_sinf[179])#last metabolite


#create a function that reads in metabolites, removes NAs and replaces w/ 1/2 minimum value:
NA2_halfmin <- function(x) replace(x, is.na(x), (0.5*min(x, na.rm = TRUE)))
new.dat<-lapply(Mgen_raw_sinf[,c(17:179)], NA2_halfmin) #calling the function
0.5*min(Mgen_raw_sinf$X_1_5_anhydro_D_sorbitol,na.rm=TRUE) #min of raw data = 2.11239
min(new.dat$X_1_5_anhydro_D_sorbitol,na.rm=TRUE) #double check that we are getting
correct values out.. min = 2.11239 so we are good

#write this to a csv file so I can scale and glog transform in metabolanalyst
met<-cbind(Mgen_raw_sinf[,c(1:16)],new.dat) #this is binding/grabbing the metadata columns
write.csv(met,"Mgen_impute_sinf_061019.csv")


#read in Med centered glog data
Mgen.dat<-read.csv("Mgen_mglog_061019.csv",header=TRUE, na.strings='',sep=',',fill=TRUE)
#working file
ncol(Mgen.dat)#178
head(Mgen.dat[16])

##########################################################
ncol(Mgen.dat)
#linear regression
#create dummy varibles

uninfec<-ifelse(Mgen.dat$ctmgallg=="neg CT&MG",1,0)
CTMG<-ifelse(Mgen.dat$ctmgallg=="PosMG/CT",1,0)
posCT<-ifelse(Mgen.dat$ctmgallg=="PosCT",1,0)

#grabbing p-values for baseline of uninfected
b_uninfected_mgen<-
(lapply(Mgen.dat[16:178],function(x)summary(lm(x~posCT+CTMG+factor(CST_hl),data=Mgen.d
at))$coef[,4])) #performs test
write.csv(b_uninfected_mgen,file="Mgen_bUninfected_sinf_CST_061019.csv")

#grab coefficients
```

```
b_uninfected_mgen_coef<-
(lapply(Mgen.dat[16:178],function(x)summary(lm(x~posCT+CTMG+factor(CST_hl),data=Mgen.d
at))$coef[,1])) #performs test
write.csv(b_uninfected_mgen_coef,"Mgen_bUninfected_sinf_coef_CST_061019.csv")

#####adjust p-value
uninfected.unlist<-unlist(b_uninfected_mgen)
uninfected.qval<-p.adjust(uninfected.unlist,method="fdr")
write.csv(uninfected.qval,"Mgen_CTbUninfected_sinf_CST_qvalue_061019.csv")

#try to read in just p-values associated with CT+ and compare #s after this but for now do
general FDR


#### uninfected baseline w/out CST in model...:
b_uninfected_mgen_noCST<-
(lapply(Mgen.dat[16:178],function(x)summary(lm(x~posCT+CTMG,data=Mgen.dat))$coef[,4]))
#performs test
b_uninfected_mgen_noCST_coef<-
(lapply(Mgen.dat[16:178],function(x)summary(lm(x~posCT+CTMG,data=Mgen.dat))$coef[,1]))
#performs test

#writing to a file:
write.csv(b_uninfected_mgen_noCST,file="Mgen_bUninfected_sinf_noCST_061019.csv")
write.csv(b_uninfected_mgen_noCST_coef,"Mgen_bUninfected_sinf_noCST_coef_061019.csv")

#####adjust p-value
ctposvuninfected.unlist<-unlist(b_uninfected_mgen_noCST)
ctposvuninfected.qval<-p.adjust(ctposvuninfected.unlist,method="fdr")
write.csv(ctposvuninfected.qval,"Mgen_CTvUninfected_sinf_qvalue_nCST_061019.csv")

#grabbing p-values for  CTpos baseline... with CST
b_ctpos_mgen<-
(lapply(Mgen.dat[16:178],function(x)summary(lm(x~uninfec+CTMG+factor(CST_hl),data=Mgen.
dat))$coef[,4])) #performs test
b_ctpos_mgen_coef<-
(lapply(Mgen.dat[16:178],function(x)summary(lm(x~uninfec+CTMG+factor(CST_hl),data=Mgen.
dat))$coef[,1])) #performs test

#writing to a file:
write.csv(b_ctpos_mgen,file="Mgen_bCTpos_sinf_CST_pval_061019.csv")
write.csv(b_ctpos_mgen_coef,file="Mgen_bCTpos_CST_coef_061019.csv")

#####adjust p-value
```

```
ctpos.unlist<-unlist(b_ctpos_mgen)
ctpos.qval<-p.adjust(ctpos.unlist,method="fdr")
write.csv(ctpos.qval,"Mgen_ctpos_sinf_qvalue_CST_061019.csv")


#Adjusting code to drop CST for both coef and p-values
b_ctpos_mgen_noCST<-
(lapply(Mgen.dat[16:178],function(x)summary(lm(x~uninfec+CTMG,data=Mgen.dat))$coef[,4]))
#performs test
b_ctpos_mgen_noCST_coef<-
(lapply(Mgen.dat[16:178],function(x)summary(lm(x~uninfec+CTMG,data=Mgen.dat))$coef[,1]))
#performs test

#writing to a file:
write.csv(b_ctpos_mgen_noCST,file="Mgen_bCTpos_sinf_noCST_061019.csv")
write.csv(b_ctpos_mgen_noCST_coef,file="Mgen_bCTpos_sinf_noCST_coef_061019.csv")

#####adjust p-value
ctpos.noCST.unlist<-unlist(b_ctpos_mgen_noCST)
ctpos.qval<-p.adjust(ctpos.noCST.unlist,method="fdr")
write.csv(ctpos.qval,"Mgen_ctpos_sinf_nCST_qvalue_061019.csv")


#####################################################################
##### fishers####
##### fishers####
##### fishers####
# count table + fisher table for Sue:
#####################################################################
#disease by age
mgen.diseaseXage<-as.matrix(table(Mgen.dat$age_cat,Mgen.dat$ctmgallg))
fisher.test(mgen.diseaseXage,workspace=2e8) #pvalue=2.2e-16; <0.0001 two-sided


#disease by cst
mgen.cstXdisease<-table(Mgen.dat$CST_hl,Mgen.dat$ctmgallg)
mgen.cstXdisease.mat<-as.matrix(mgen.cstXdisease)
fisher.test(mgen.cstXdisease.mat,workspace=2e8) #pvalue=2.162e-07 so use <0.0001; two-
sided


#disease by HC
mgen.diseaseXhc<-as.matrix(table(Mgen.dat$HC_rec,Mgen.dat$ctmgallg))
fisher.test(mgen.diseaseXhc,workspace=2e8) #pvalue=  0.253; two-sided
```

```
#disease by num. sex partner
mgen.diseaseXnumsex<-as.matrix(table(Mgen.dat$partnersex.new,Mgen.dat$ctmgallg))
fisher.test(mgen.diseaseXnumsex,workspace=2e8) #pvalue= 0.01211; two-sided


#disease by race
mgen.diseaseXrace<-as.matrix(table(Mgen.dat$race1,Mgen.dat$ctmgallg))
fisher.test(mgen.diseaseXrace,workspace=2e8) #pvalue= 0.7269; two-sided

#disease by num. sex partner
mgen.diseaseXnumsex<-as.matrix(table(Mgen.dat$ctmgallg,Mgen.dat$partnersex.new))
fisher.test(mgen.diseaseXnumsex,workspace=2e8) #pvalue= 0.01211; two-sided


#disease by condom use
mgen.diseaseXcondom<-as.matrix(table(Mgen.dat$cond,Mgen.dat$ctmgallg))
fisher.test(mgen.diseaseXcondom,workspace=2e8)

mgen.diseaseXdouching<-as.matrix(table(Mgen.dat$douchestudy,Mgen.dat$ctmgallg))
fisher.test(mgen.diseaseXdouching,workspace=2e8)

#disease by PID
mgen.diseaseXPID<-as.matrix(table(Mgen.dat$pid1,Mgen.dat$ctmgallg))
fisher.test(mgen.diseaseXPID,workspace=2e8)


mgen.diseaseXHIV<-as.matrix(table(Mgen.dat$hivstatus,Mgen.dat$ctmgallg))
fisher.test(mgen.diseaseXHIV,workspace=2e8)

################################################################################
######################
################################################################################
######################
################################################################################
############################
#permanova
#### permanova + anosim on scaled
########### use this block###########
set.seed(14)
#you have to start with the raw data b/c bray curtis doesn't handle negative values... you must
scale w/in the distance matrix
Mgen.imp.sinf<-read.csv("Mgen_impute_sinf_061019.csv",header=TRUE,
na.strings='',sep=',',fill=TRUE) #working file
```

```
ncol(Mgen.imp.sinf)#180
head(Mgen.imp.sinf[18])



install.packages("vegan")
require(vegan)
Mgen.raw.bc<-vegdist(decostand(Mgen.imp.sinf[,c(19:181)],"norm"),method="bray") # no
warning

p1.raw.full.nm<-
adonis2(Mgen.raw.bc~CST_hl+ctmgallg+age_cat+partnersex.new+cond+pid1+hivstatus+cohort,
data=Mgen.imp.sinf,sqrt.dist=FALSE,permutations=10000)
p1.raw.full.nm

p1.raw.full.norm<-
adonis2(Mgen.raw.bc~CST_hl+ctmgallg+age_cat+partnersex.new+cond+pid1+hivstatus+cohort,
data=Mgen.imp.sinf,by="margin",sqrt.dist=FALSE,permutations=10000)
p1.raw.full.norm
```

CHAPTER 5

BIOGENIC AMINES AFFECT THE GROWTH AND LACTIC ACID PRODUCTIN OF VAGINAL *LACTOBACILLUS* SPP.

Contribution of Authors and Co-Authors

Manuscript in Chapter 5

Author: Joanna-Lynn C Borgogna

Contributions: Designed, implemented, analyzed and interpreted data; wrote manuscript.

Co-Author: Savannah Gray

Contributions: Performed lactic acid assays; aided in growth curves.

Co-Author: Elisa K Santori

Contributions: Optimized growth media, performed lactic acid assays, aided in growth curves.

Co-Author: Ben Americus

Contributions: Aided in growth curves and performed 16S rRNA sequencing on *L. crispatus* isolates.

Co-Author: Zhong Li

Contributions: Performed mass spectrometry-based metabolomics.

Co-Author: Alexander Ulanov

Contributions: Performed mass spectrometry-based metabolomics.

Co-Author: Jacques Ravel

Contributions: Designed and implemented parent study.

Co-Author: Rebecca M Brotman

Contributions: Designed and implemented parent study.

Co-Author: Carl J Yeoman

Contributions: Designed, implemented, and aided in interpretation of data and manuscript development.

Manuscript Information

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Title: Biogenic Amines Affect the Growth and Lactic Acid Production of Vaginal *Lactobacillus* spp.
Running title: Biogenic Amines Alter Lactobacillus Growth

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Key words Biogenic amines; Amino acids; Metabolomics; Lactobacillus; Growth Curves

Abstract:

Vaginal *Lactobacillus* spp. provide the primary defenses against infection associated with sexual and reproductive morbidities. This includes bacterial vaginosis (BV), the most common vaginal disorder amongst reproductive-aged women. One clinical symptom of BV is malodor, which has been linked to elevated concentrations of the biogenic amines putrescine and cadaverine. The biosynthesis of biogenic amines is well-described as a mechanism for decreasing acidity, and thereby may play a role in overcoming the lactic acid-induced acidic barrier of the vagina. Additionally, biogenic amines may directly impact the growth phenotypes of some bacteria and have thereby been hypothesized as possible precursors of vaginal dysbiosis. Herein, we utilized targeted liquid chromatography mass-spectrometry to determine the effect of physiological concentrations of select biogenic amines upon the specific growth properties of vaginal *Lactobacillus* spp. With the exception of *L. gasseri*, putrescine was associated with delayed growth (slower doubling times and longer lag times). The doubling time of *L. crispatus* was adversely affected by all amines. The lag time (but not doubling time) of *L. iners* was significantly affected by all amines and was decreased in the presence of exogenous spermidine and spermine. Exposure to biogenic amines was generally associated with reduced production of D- and L-lactic acid, exceptions being higher levels of D-lactic acid by *L. crispatus* "big" and L-lactic acid by *L. crispatus "*small*"* when grown in the presence of spermine. These data may guide future mechanistic studies that seek to provide insight into the competitiveness of vaginal *Lactobacillus* spp.

INTRODUCTION:

The vaginal microenvironment comprises microbiota and metabolites that have significant relationships with gynecological health and disease[1-5]. The typical vaginal microbiota of apparently healthy, reproductive-age women are dominated by one of four *Lactobacillus* species, specifically *L. crispatus*, *L. gasseri*, *L. iners* or *L. jensenii*[6]. This being a characteristic feature of the vaginal microbiomes classification into 4 of five recognized community state types (CSTs). *Lactobacillus* spp. prevent the colonization of potential pathogens through the production of lactic acid[7], bacteriocins[8, 9], and biosurfactants[10], and through competitive exclusion by adherence to the vaginal mucosa[11, 12]. In approximately 30% of women, *Lactobacillus* spp. are depauperate being replaced by an increased diversity of anaerobic and microaerophilic bacteria, including *Gardnerella, Prevotella,* and *Atopobium* spp[6]. These women are classified as CST-IV and, because their microbiota are consistent with that observed as part of the most common vaginal disorder, bacterial vaginosis (BV), this CST has recently been proposed as "molecular BV"[13].

Bacterial vaginosis is the most common vaginal disorder in reproductive-aged women, affecting 33% of all U.S. women[14]. BV is associated with adverse health outcomes, including increased risks of acquiring sexually transmitted infections (STIs)[11-13] such as HIV, urinary tract infections (UTIs)[14], the development of pelvic inflammatory disease[15] and infertility[16]. Furthermore, in pregnant women, BV is associated with increases in the risk of pre-term birth[17,18] and spontaneous abortions[15,18].

The vaginal microbiota has a large influence on the vaginal metabolome [4, 15-18], with ~31% of the variation being explained by CST[4]. In particular, compared to *Lactobacillus*

spp.-dominated CSTs, CST IV has been associated with higher concentrations of the biogenic amines (BAs) cadaverine and putrescine[3]. Similarly, the vaginal concentrations of cadaverine and putrescine have often been shown to be higher in BV-afflicted women[2, 17, 19-24], with both having been associated with the clinical sign malodor associated with BV.

Biogenic amines are primarily produced via specific amino acid decarboxylation reactions – a well-described acid stress-resistance mechanism involving the consumption of intracellular hydrogen and a concomitant increase in extracellular pH[25, 26]. BAs have been shown to increase pathogen virulence by mediating resistance to multiple antibiotics[27, 28], increased protection against oxygen, superoxide, and hydrogen peroxide toxicity[29], and altering susceptibility to host innate defenses[26, 30]. These characteristics may, at least partially explain why both CST-IV, and the relative abundances of several specific vaginal taxa associated with BV, including *Dialister*, *Mobiluncus*, *Atopobium*, *Prevotella*, *Mycoplasma,* and Gardnerella spp. have been correlated with the presence of these biogenic amines[2, 3, 5, 24, 31]. As such, we have previously hypothesized that BAs may play an essential role in the transition from a *Lactobacillus* spp. dominant microbiota to that of the low-*Lactobacillus* spp., CST IV[4, 32].

Beyond reducing the barriers to microbial colonization of the vaginal microenvironment, biogenic amines may have more direct influences on bacterial growth and competitiveness. A few studies have assessed the direct effect of biogenic amines upon the specific growth rate of various bacteria. Cunningham-Rundles and Maas demonstrated that low concentrations of putrescine led to a reduction in the growth rate of *Escherichia*

coli[33], while Tabor et al. reported an 87% reduction in the growth rate of *E. coli* in the absence of spermidine[34]. Only one study has examined the effect of BAs upon the growth properties of *Lactobacillus* species, although this was limited to several species involved in food production[35]. In 1964, Guirard and Snell reported that while spermidine and spermine, stimulated the growth rate of several lactobacilli, including *L. casei,* putrescine and cadaverine significantly reduced growth[35]. However, to date, there exists no known study evaluating the impact of biogenic amines upon the competitiveness of vaginal *Lactobacillus* spp. We sought to evaluate the direct effect of the most common biogenic amines found in the vagina, cadaverine, putrescine, spermine, spermidine, and tyramine upon the specific growth properties and lactic acid production of vaginal *Lactobacillus* spp.

RESULTS:

Community state type dynamics of participants:

Participant characteristics are reported in Table S1. Fourteen participants (43.7%) were characterized with CST IV vaginal microbiota and maintained this for the duration of the study; nine (64%) of these participants had Nugent scores ≥ 7 associated with each time point. Two participants (6.25%) were characterized with CST IV vaginal microbiota and transitioned to a *Lactobacillus*-dominant CST during the study. The vaginal microbiota of six participants (18.75%) were initially dominated by *Lactobacillus* spp. (CST I, II, or III) and transitioned to CST IV. Eight participants (25%) transitioned into and from multiple CSTs throughout the selected time points; six of these participants reported that they were menstruating for at least one day (Table S1). Finally, two participants (6.25%) were

characterized with, and maintained, a *Lactobacillus*-dominant CST (-I, II, or III) for the duration of the study. Twenty-five participants recorded at least one day of menstruation during the selected time points, representing 72 samples. While menstruating, women were predominantly typified by CST IV microbiota (79%) with CST III (16%) being the next most common.

Physiological concentrations of biogenic amines:

The physiological concentrations of cadaverine, putrescine, spermidine, spermine, and tyramine were measured by quantitative targeted liquid chromatography-based metabolomics and are reported in Tables 1 and S1 and visually displayed in Figure 1. Cadaverine, putrescine and tyramine were the most frequently detected amines (present within 97-98% of the samples). The average concentrations of these amines were highest in women with CST IV microbiota when compared to all others (p-values <0.001; Table 1; Figure 2). Spermidine and spermine were the least detected amines both among all samples (both present within 18% of all samples) and in CST IV women (present within 18% and 8% of the samples, respectively); further, their average concentrations were lowest in CST IV women when compared to all others. In marked contrast, the average concentrations of spermidine and spermine were higher in women dominated by *L. crispatus* (CST I) than those dominated by *L. iners* (p-values = 0.03 and 0.02, respectively) or those with CST IV microbiota (p-values=0.0007 and 0.006, respectively).

The effect of biogenic amines upon growth properties of vaginal Lactobacillus species:

We first assessed the minimal inhibitory concentrations (MICs) of select biogenic amines (Supplemental Table S2). As the physiological measurements were below the observed MICs, we then conducted growth assays using the maximum physiological concentration of each amine measured from vaginal samples (Table 1). The maximum concentration of cadaverine, putrescine, spermidine, and tyramine were observed in CST IV women, while the highest concentration of spermine was observed in CST I. Growth of pure cultures of several *Lactobacillus* spp. under *in vitro* conditions allowed us to assess the effect of the biogenic amines upon doubling- and lag-times (Figure 2, Figure S1, and Table 3). Cadaverine significantly increased the doubling time (slower growth rate) of *L. crispatus "big", L. gasseri,* and *L. jensenii* and was also associated with an increased lag time in *L. crispatus "small", L. iners* and *L. jensenii* (Table 2). Putrescine significantly increased the doubling time of *L. crispatus "big, L. crispatus "small"*, *L. gasseri,* and *L. iners* by an average of 26, 31, 37 and 41 minutes, respectively. The average doubling time for the positive controls of *L. crispatus "big",* L. *crispatus "small"*, *L. gasseri* and *L. iners* were 121, 115, 125 and 129 minutes, respectively. Conversely when exposed to putrescine, the doubling times pf *L. crispatus "big", L. crispatus "small", L. gasseri,* and *L. iners* increased to 148, 153, 156, and 171 minutes, respectively. Notably, spermidine was associated with increased doubling- and decreased lag-times with *L. crispatus "big"*. Spermine was also significantly associated with decreased lag times (but not doubling times) of *L. gasseri* and *L. iners.*

The effect of biogenic amines upon lactic acid production of vaginal Lactobacillus species:

We then assessed whether biogenic amines affected the production of D- and L-lactic acid isomers by vaginal lactobacilli when controlling for cell density (Table 3). In general, when grown in the presence of biogenic amines, vaginal lactobacilli produced less D- and L-lactic acid isomers. The notable exceptions were higher levels of D-lactic acid by *L. crispatus* "big" and L-lactic acid by *L. crispatus* "small" when grown in the presence of spermine. Cadaverine negatively affected the production of D-lactic acid by *L. crispatus "big"* and L-lactic acid by *L. iners.* Both *L. crispatus* morphotypes and *L. jensenii* were associated with decreased production of D-lactic acid when grown in the presence of putrescine, although these relationships were no longer significant after correction for multiple comparisons. Growth in tyramine was associated with decreased production of D-lactic acid in all vaginal *Lactobacillus* spp., except for *L. iners* which does not have a known genetic capacity to produce D-lactic acid. However, tyramine did significantly reduce the concentration of L-lactic acid that *L. iners* produced. After correction for multiple comparisons, only the associations between tyramine and *L. iners* and tyramine and *L. gasseri* remained significant. It is also worth noting that both D- and L-lactic acid production varied, by species, even among the positive controls.

DISCUSSION:

Biogenic amines are small cationic molecules that have been previously been observed to increase with CST-IV and with the prevalent vaginal disorder, bacterial vaginosis[13, 3, 36, 37]

[2-4, 24, 36, 38]. Consistently, we also observed higher concentrations of cadaverine, putrescine, and tyramine in specimens collected from women when they were in CST IV compared to other CSTs. However, the higher-order amines, spermine and spermidine, were minimally detected in CST IV women and higher in *Lactobacillus* spp. dominated CST as has also been previously reported[3]. Based on several characteristics we have previously hypothesized that they may be more than biomarkers of dysbiosis and disease and may be important precursors of each[3, 36, 37].

One way that we have hypothesized BAs may be involved in dysbiosis is by directly affecting the growth of vaginal lactobacilli[32]. In general, vaginal microbiota dominated by *Lactobacillus* species are thought to be protective against infection-related gynecological and reproductive diseases and this is largely related to their production of lactic acid which acidifies the vaginal microenvironment and limits the ability for outgrowth of potential pathogens. Consistent with our hypotheses, we found cadaverine, putrescine, and tyramine increased the doubling time of representative strains of each of the major vaginal *Lactobacillus* spp. To date, this is the first known study that has evaluated the effect of biogenic amines upon specific growth properties of vaginal *Lactobacillus* spp. One previous study reported that spermidine and spermine stimulated the growth of several *Lactobacillus* spp. of relevance to the food and beverage industry, while putrescine and cadaverine inhibited and severely reduced growth, respectively[35]. Accordingly, we observed that except for *L. jensenii*, putrescine increased the doubling time (decreased the growth rate) of all vaginal *Lactobacillus* spp. representing the major CSTs. Conversely, we

observed that spermidine and spermine had varying effects on the growth rates and lag times of vaginal lactobacilli, including decreasing the lag time of *L. iners*.

L. crispatus-dominated microbiota (CST I), in particular, are associated with positive health outcomes. Among *Lactobacillus* species-dominated CSTs, CST III (*L. iners*-dominated) may be more susceptible to vaginal disorders including BV and STI[39-42]. Women with *L. iners* (CST III) dominated microbiota have also been reported to have higher concentrations of metabolites associated with BV than women with *L. crispatus* dominated microbiota (CST I)[36]. Consistent with these observations, we observed higher concentrations of cadaverine, putrescine, and tyramine in CST III women compared to CST I women.

We also observed that the doubling time of *L. iners* was less affected by the BAs than *L. crispatus*. However, the lag time of *L. iners* was significantly longer in the presence of cadaverine and putrescine yet decreased in the presence of spermidine, spermine, and tyramine. Additionally, we observed that when *L. iners* was grown in the presence of cadaverine and tyramine, the production of L-lactic acid was markedly decreased. Taken together, these data may support the observation that *L. iners* acts as an intermediate between vaginal health and vaginal dysbiosis.

Another major finding was that BAs were generally associated with decreased production of D- and L-lactic acid. The production of lactic acid is dogmatically considered the primary method of pathogen exclusion by vaginal *Lactobacillus* spp. and the biosynthesis of BAs has been proposed as a method of bacterial acid stress resistance by which anerobic and BV-associated bacteria can colonize the vaginal environment[25, 26]. A

few studies have evaluated the production of D- and L- lactic acid by vaginal *Lactobacillus* spp[43-45]. Witkin et al. evaluated the production of lactic acid from axenic cultures of various vaginal *Lactobacillus* spp. and observed that *L. crispatus* had the highest concentration of D- and L-lactic acid production compared to *L. gasseri, L. iners,* and *L. jensenii*. While this was not the primary focus of our study, we did observe that *L. jensenii* produced very little amounts of L-lactic acid, which is consistent with values reported by Witkin et al[43]. Overall, with the exceptions of spermine (both *L. crispatus* morphotypes) and tyramine (*L. jensenii*), we observed decreased production of lactic acid when vaginal lactobacilli were grown in the presence of amines.

This is the first study to examine biogenic amine concentrations in a longitudinally-collected cohort of participants, however, it is possible the maximum physiological concentrations of each amine determined could differ in another cohort and thus our results provide important but potentially limited insight to the growth-impacts on vaginal *Lactobacillus* spp. Nevertheless, the detection and distribution of amines observed was consistent with previously reported literature[4, 24, 32, 36, 37] and the observed physiological concentrations fell well below the MICs. Therefore, while different concentrations of amines would likely influence the effect of the BAs upon *Lactobacillus* spp. growth, we anticipate that the overall trends would remain the same. This is also the first study evaluating the effect of biogenic amines upon specific growth properties of vaginal *Lactobacillus spp.*; however, another limitation is that our results are limited to the type strains of vaginal *Lactobacillus* species. It will be interesting to determine the generalizability of these data to additional clinical isolates of *Lactobacillus* spp. as well as

to anaerobic taxa associated with CST-IV- and BV. Finally, it is important to note that biogenic amines do not occur in the vaginal microenvironment in isolation and thus the synergistic and/or antagonistic effects of the biogenic amines upon one another needs to be explored before a full understanding of the true impact of biogenic amines on the growth of vaginal *Lactobacillus* species can be achieved.

METHODS:

Ethics statement:

All participants provided written informed consent. Ethical approval was obtained from the Institutional Review Boards of the University of Alabama at Birmingham (UAB), the University of Maryland Baltimore School of Medicine, and Montana State University (MSU), and all research was conducted in compliance with relevant guidelines and regulations.

Study population and sample collection:

This is a secondary analysis, and the primary study has previously been described[46]. Briefly, between September 2009 and July 2010, 135 nonpregnant, reproductive-aged women were enrolled in a longitudinal study at the University of Alabama at Birmingham and provided daily, self-collected mid-vaginal swabs for ten weeks. Genomic DNA was collected using a Copan ESwab (Copan Diagnostics, Murrieta, CA, USA), placed in Amies liquid transport medium, and frozen at -80°C until use. Samples for metabolomics were collected using a Starplex double-headed Dacron swab (Starplex Scientific, Cleveland, TN, USA) and stored dry in a tube at -80°C until later use.

Taxonomic assignment and community state type profiling

DNA extraction, PCR amplification, and Roche 454 pyrosequencing of 16S rRNA gene amplicons (V1-V3) using primers 27F and 533r from the vaginal swabs have been previously described[46]. Taxonomic assignments were performed as described by Ravel et al. in 2011 using a combination of pplacer and speciateIT (speciateIT.sourceforge.net)[46]. For each sample, CST classifications were assigned as previously described, using Jensen-Shannon divergence and Ward linkage hierarchical clustering[6, 46]. The CSTs reflected the major *Lactobacillus*-dominated states I (*L. crispatus*), II (*L. gasseri),* III (L. *iners*), and CST IV (low-*Lactobacillus*, molecular BV) as described by Ravel et al. in 2011[6].

Study population and metabolomics processing:

152 samples were selected from 32 participants prior to and following a transition from a *Lactobacillus* spp.-dominant CST to the *Lactobacillus* spp. depauperate, CST-IV (Table S1). Vaginal samples were eluted from swabs using 70% methanol and analyzed with the 5500 QTRAP LC/MS/MS system (Sciex, Framingham, MA) in Metabolomics Lab of Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign. Software Analyst 1.6.2 was used for data acquisition and analysis. The 1200 series HPLC system (Agilent Technologies, Santa Clara, CA) includes a degasser, an autosampler, and a binary pump. The LC separation was performed on an Agilent Eclipse XDB-C18 (4.6 x 150mm, 5μm) with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The flow rate was 0.4 mL/min. The linear gradient was as follows: 0-

3min, 95%A; 9-13min, 5%A; 13.5-18min, 95%A. The autosampler was set at 10°C. The injection volume was 5 μL. Mass spectra were acquired under positive electrospray ionization (ESI) with the ion spray voltage of +5000 V. The source temperature was 500 °C. The curtain gas, ion source gas 1, and ion source gas 2 were 33, 65, and 55 psi, respectively. Multiple reaction monitoring (MRM) was used for quantitation: cadaverine (m/z 103.0 → m/z 69.0); putrescine (m/z 189.0 → m/z 30.0); spermine (m/z 199.9 → m/z 91.1); spermidine (m/z 146.0 → m/z 30.0); and tyramine (m/z 138.1 → m/z 77.1). The levels of detection for each metabolite were as follows: cadaverine (100nM), putrescine (100nM), spermidine (20nM), spermine (20nM) and tyramine (5nM). Quantitative measurements are reported in Table S1. For the purpose of plotting, "missing values" were imputed with one-half the minimum value obtained for a given metabolite[47-50]. Boxplots of the range of amines were constructed in R Statistical Software.

Bacterial strains:

The following bacteria were obtained from ATCC (American Type Culture Collection) and used to evaluate the effect of biogenic amines upon bacterial growth in axenic cultures: *L. crispatus* VPI 3199 (ATCC 33820), *L. iners* AB107 (ATCC 55195), *L. gasseri* NCTC 2948 (ATCC 9857), and *L. jensenii* 62G (ATCC 25258). When grown in isolation upon agar, *L. crispatus* VPI 3199 had two distinct colony morphologies; one colony type was small, irregular undulate, opaque, buff in color, and flat; we termed this, *L. crispatus* "small". The other colony type was larger, opaque, with a distinct, "dot-like" center that became translucent closer to the perimeter and were irregular undulate; we termed this, *L.*

crispatus “big”. Colony PCR was conducted on both morphotypes using primers 42F and 1023R as described by Fredricks et al[51] and confirmed the identity of the two morphologies as *L. crispatus*. Both colony morphologies had distinct growth properties and as such we conducted all growth assays on both morphotypes.

Growth conditions, and biogenic amines:

Lactobacillus spp. were grown in MRS-NYC III (MNC; 10 g/liter proteose peptone, 10 g/liter beef extract, 5 g/liter yeast extract, 5 g/liter NaCl, 0.1 g/liter $MgSO_4$, 0.05 g/liter $MnSO_4$, 2 g/liter K_2HPO_4, 20 g/liter glucose, 100 ml/liter fetal bovine serum) as described by Witkin et al[43]. All cultures were incubated at 37°C in an atmosphere with 5% CO_2 and grown in sterile, 15mL, PYREX™ round-bottom, screwcap, glass test-tubes with no shaking. The approximate bacterial cell density was calculated using a modified most probable number enumeration wherein two-fold dilutions were carried out in 96-well plates beginning with OD_{600} ~ 0.5 for each bacterium until maximum dilution was apparent by no visible growth after 24 hours. Plates were incubated for 24 hours at 37°C and the cell density approximated and associated with corresponding OD (Table S3). Cadaverine, spermidine, spermine, and tyramine were obtained from ACROS Organics™. Putrescine was obtained from MPBiomedicals™. Biogenic amine treatments were prepared within 30 minutes of use by diluting pre-made 1M stocks (putrescine and cadaverine) and 0.1M stocks (spermidine and spermine) or powder-form (tyramine) into the appropriate concentration (Table 1) with MNC that had been pre-warmed in the incubator at 37°C.

Minimum inhibitory concentrations of biogenic amines:

The minimum inhibitory concentration (MIC) was defined as the minimal biogenic amine concentration needed to inhibit visible bacterial growth and was determined with a standard microdilution procedure. Biogenic amine concentrations ranged from 900mM to 0.78mM (Table S2) in a two-fold dilution series. To each dilution of the amine, bacteria were added 1:100, vortexed, and then plated in a 96-well microtiter plate. For each bacterium and amine, the test was carried out in triplicate in MNC and grown for 24 hours at 37 °C and evaluated in both anaerobic (grown in a vinyl anaerobic chamber (Coy Laboratories Products, Inc.)) and aerobic environments.

Growth curves:

As the physiological concentrations of the biogenic amines were less than the MICs, we then conducted growth curves to evaluate the direct effect of the biogenic amines upon the growth of the *Lactobacillus* spp. Bacteria were inoculated into 15mLs of MNC and grown overnight 37 °C, in an atmosphere of 5% CO2 with no shaking until they reached an OD_{600}=1.4. Saturated overnight cultures were diluted 1:100 into 15mLs of pre-warmed, fresh, MNC. Once the day cultures reached an $OD_{600} \cong 0.5$ (Table S3), they were inoculated with a 1:100 dilution into 15mLs of the respective treatment and control. For each bacterium, the amine treatment and positive controls were run in triplicate. Biogenic amines are volatile compounds; in order to ensure that the observed effects were due to the amine of interest, for each bacterium, treatments were completed in isolation over three separate days, yielding nine replicates per positive control and biogenic amine treatment.

200uL samples were obtained from each replicate, and the OD measured using the BioTek Epoch™ 2 every 30 minutes until stationary phase. Generation- and lag-time were calculated using the package *GrowthRates*() in R statistical software[52]. Differences in the generation and lag-time were calculated using *t.test()*, and p-values were adjusted using the false discovery rate method and *p.adjust()* in R statistical software.

Lactic acid assays:

We evaluated whether the biogenic amines influenced D- and L- lactic acid production by vaginal lactobacilli. Axenic cultures of each bacterium in pure MNC and in MNC supplemented with appropriate concentration of biogenic amines were grown in triplicate. As biogenic amines are volatile, the effect of each amine was tested on independent days. Once each sample had reached stationary, we spun down the cells at 4,500 x g for 10 minutes. For each sample, one milliliter of supernatant was collected, transferred to clean microcentrifuge tubes, and stored at −20°C. Samples were thawed in batches and D- and L-lactate measured colorimetrically by using EnzyChrom L-lactate and EnzyChrom D-lactate kits from BioAssay Systems (Hayward, CA). Values were converted to ng/ml or mM by reference to a standard curve that was generated in parallel to the test samples. Difference in concentrations of D- and L-lactic acid were analyzed using the Student's test as it has been shown this method is appropriate even with sample sizes of N=2, if the effect size is large[53]. P-values were adjusted for multiple comparisons using the false discovery rate method and the *p.adjust* function in R Statistical Software.

References:

1. Borgogna J-LC, Yeoman CJ. The Application of Molecular Methods Towards an Understanding of the Role of the Vaginal Microbiome in Health and Disease. The Human Microbiome. Methods in Microbiology2017. p. 37-91.

2. Yeoman CJ, Thomas SM, Miller ME, Ulanov AV, Torralba M, Lucas S, et al. A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. PLoS One. 2013;8(2):e56111.

3. Nelson T, Borgogna J, Brotman R, Ravel J, Walk S, Yeoman C. Vaginal Biogenic Amines: Biomarkers of Bacterial Vaginosis or Precursors to Vaginal Dysbiosis? Frontiers in physiology. 2015;6(253).

4. Nelson TM, Borgogna JC, Michalek RD, Roberts DW, Rath JM, Glover ED, et al. Cigarette smoking is associated with an altered vaginal tract metabolomic profile. Sci Rep. 2018;8(1):852.

5. Borgogna JC SM, Santori EK, Nelson TM, Rath JM, Glover ED, Ravel J, Gravitt P, Yeoman CJ, Brotman RM. . The vaginal metabolome and microbiota of cervical HPV-positive and HPV-negative women: a cross-sectional analysis. . British Journal of Obstetrics & Gynecology 2019(*In Press*).

6. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011;108 Suppl 1:4680-7.

7. Boskey ER, Cone RA, Whaley KJ, Moench TR. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. Human reproduction (Oxford, England). 2001;16(9):1809-13.

8. Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, et al. Defense factors of vaginal lactobacilli. American journal of obstetrics and gynecology. 2001;185(2):375-9.

9. Ocana VS, Pesce De Ruiz Holgado AA, Nader-Macias ME. Characterization of a bacteriocin-like substance produced by a vaginal Lactobacillus salivarius strain. Applied and Environmental Microbiology. 1999;65(12):5631-5.

10. Reid G, Heinemann C, Velraeds M, van der Mei HC, Busscher HJ. Biosurfactants produced by Lactobacillus. Methods in enzymology. 1999;310:426-33.

11. Kaewsrichan J, Peeyananjarassri K, Kongprasertkit J. Selection and identification of anaerobic lactobacilli producing inhibitory compounds against vaginal pathogens. FEMS ImmunolMedMicrobiol. 2006;48(1):75-83.

12. Boris S, Barbés C. Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect. 2000;2(5):543-6.

13. McKinnon LR, Achilles SL, Bradshaw CS, Burgener A, Crucitti T, Fredricks DN, et al. The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. AIDS Res Hum Retroviruses. 2019;35(3):219-28.

14. Kairys N, Garg M. Bacterial Vaginosis. StatPearls. Treasure Island (FL)2019.

15. Hillier SL. Diagnostic microbiology of bacterial vaginosis. American journal of obstetrics and gynecology. 1993;169(2):455-9.

16. Wang L, Koppolu S, Chappell C, Moncla BJ, Hillier SL, Mahal LK. Studying the effects of reproductive hormones and bacterial vaginosis on the glycome of lavage samples from the cervicovaginal cavity. PLoS One. 2015;10(5):e0127021.

17. Sobel JD, Karpas Z, Lorber A. Diagnosing vaginal infections through measurement of biogenic amines by ion mobility spectrometry. European journal of obstetrics, gynecology, and reproductive biology. 2012.

18. Witkin SS, Ledger WJ. Complexities of the uniquely human vagina. Sci Transl Med. 2012;4(132):132fs11.

19. Chen KC, Forsyth PS, Buchanan TM, Holmes KK. Amine content of vaginal fluid from untreated and treated patients with nonspecific vaginitis. J Clin Invest. 1979;63(5):828-35.

20. Chen KC, Amsel R, Eschenbach DA, Holmes KK. Biochemical diagnosis of vaginitis: determination of diamines in vaginal fluid. J Infect Dis. 1982;145(3):337-45.

21. Hillier SL, Krohn MA, Rabe LK, Klebanoff SJ, Eschenbach DA. The normal vaginal flora, H2O2-producing lactobacilli, and bacterial vaginosis in pregnant women. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1993;16 Suppl 4:S273-S81.

22. Wolrath H, Boren H, Hallen A, Forsum U. Trimethylamine content in vaginal secretion and its relation to bacterial vaginosis. APMIS. 2002;110(11):819-24.

23. Srinivasan S, Morgan MT, Fiedler TL, Djukovic D, Hoffman NG, Raftery D, et al. Metabolic signatures of bacterial vaginosis. mBio. 2015;6(2):10.1128/mBio.00204-15.

24. Vitali B, Cruciani F, Picone G, Parolin C, Donders G, Laghi L. Vaginal microbiome and metabolome highlight specific signatures of bacterial vaginosis. Eur J Clin Microbiol Infect Dis. 2015;34(12):2367-76.

25. Kanjee U, Houry WA. Mechanisms of acid resistance in Escherichia coli. Annu Rev Microbiol. 2013;67:65-81.

26. Shah P, Swiatlo E. A multifaceted role for polyamines in bacterial pathogens. Mol Microbiol. 2008;68(1):4-16.

27. Kwon DH, Lu CD. Polyamine effects on antibiotic susceptibility in bacteria. Antimicrob Agents Chemother. 2007;51(6):2070-7.

28. Kwon DH, Lu CD. Polyamines induce resistance to cationic peptide, aminoglycoside, and quinolone antibiotics in Pseudomonas aeruginosa PAO1. Antimicrob Agents Chemother. 2006;50(5):1615-22.

29. Chattopadhyay MK, Tabor CW, Tabor H. Polyamines protect Escherichia coli cells from the toxic effect of oxygen. Proc Natl Acad Sci U S A. 2003;100(5):2261-5.

30. Goytia M, Shafer WM. Polyamines can increase resistance of Neisseria gonorrhoeae to mediators of the innate human host defense. Infect Immun. 2010;78(7):3187-95.

31. King CC, Jamieson DJ, Wiener J, Cu-Uvin S, Klein RS, Rompalo AM, et al. Bacterial vaginosis and the natural history of human papillomavirus. Infect Dis Obstet Gynecol. 2011;2011:319460.

32. Nelson TM, Borgogna JL, Brotman RM, Ravel J, Walk ST, Yeoman CJ. Vaginal biogenic amines: biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis? Frontiers in physiology. 2015;6:253.

33. Cunningham-Rundles S, Maas WK. Isolation, characterization, and mapping of Escherichia coli mutants blocked in the synthesis of ornithine decarboxylase. J Bacteriol. 1975;124(2):791-9.

34. Tabor CW, Tabor H, Hafner EW. Escherichia coli mutants completely deficient in adenosylmethionine decarboxylase and in spermidine biosynthesis. J Biol Chem. 1978;253(10):3671-6.

35. Guirard BM, Snell EE. Effect of Polyamine Structure on Growth Stimulation and Spermine and Spermidine Content of Lactic Acid Bacteria. J Bacteriol. 1964;88:72-80.

36. Srinivasan S, Morgan MT, Fiedler TL, Djukovic D, Hoffman NG, Raftery D, et al. Metabolic signatures of bacterial vaginosis. MBio. 2015;6(2).

37. Yeoman CJ, Thomas SM, Miller MEB, Ulanov AV, Torralba M, Lucas S, et al. A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. PloS one. 2013;8(2):e56111.

38. McMillan A, Rulisa S, Sumarah M, Macklaim JM, Renaud J, Bisanz JE, et al. A multi-platform metabolomics approach identifies highly specific biomarkers of bacterial diversity in the vagina of pregnant and non-pregnant women. Sci Rep. 2015;5:14174.

39. van Houdt R, Ma B, Bruisten SM, Speksnijder AGCL, Ravel J, de Vries HJC. Lactobacillus iners-dominated vaginal microbiota is associated with increased susceptibility to Chlamydia trachomatis infection in Dutch women: a case–control study. Sexually Transmitted Infections. 2018;94(2):117.

40. Borgdorff H, Armstrong SD, Tytgat HL, Xia D, Ndayisaba GF, Wastling JM, et al. Unique Insights in the Cervicovaginal Lactobacillus iners and L. crispatus Proteomes and Their Associations with Microbiota Dysbiosis. PLoS One. 2016;11(3):e0150767.

41. Lee JE, Lee S, Lee H, Song Y-M, Lee K, Han MJ, et al. Association of the Vaginal Microbiota with Human Papillomavirus Infection in a Korean Twin Cohort. PloS one. 2013;8(5):e63514.

42. Brotman RM, Shardell MD, Gajer P, Tracy JK, Zenilman JM, Ravel J, et al. Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. J Infect Dis. 2014;210(11):1723-33.

43. Witkin SS, Mendes-Soares H, Linhares IM, Jayaram A, Ledger WJ, Forney LJ. Influence of vaginal bacteria and D- and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase inducer: implications for protection against upper genital tract infections. MBio. 2013;4(4).

44. O'Hanlon DE, Moench TR, Cone RA. Vaginal pH and microbicidal lactic acid when lactobacilli dominate the microbiota. PloS one. 2013;8(11):e80074-e.

45. Juarez Tomas MS, Ocana VS, Wiese B, Nader-Macias ME. Growth and lactic acid production by vaginal Lactobacillus acidophilus CRL 1259, and inhibition of uropathogenic Escherichia coli. J Med Microbiol. 2003;52(Pt 12):1117-24.

46. Ravel J, Brotman RM, Gajer P, Ma B, Nandy Melissa, Fadrosh D, et al. Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. Microbiome. 2013:1:29.

47. Yang J, Zhao X, Lu X, Lin X, Xu G. A data preprocessing strategy for metabolomics to reduce the mask effect in data analysis. Front Mol Biosci. 2015;2:4.

48. Wei R, Wang J, Su M, Jia E, Chen S, Chen T, et al. Missing Value Imputation Approach for Mass Spectrometry-based Metabolomics Data. Sci Rep. 2018;8(1):663.

49. Smilde AK, van der Werf MJ, Bijlsma S, van der Werff-van der Vat BJ, Jellema RH. Fusion of mass spectrometry-based metabolomics data. Anal Chem. 2005;77(20):6729-36.

50. Xia J, Wishart DS. Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. Nat Protoc. 2011;6(6):743-60.

51. Fredricks DN, Fiedler TL, Thomas KK, Oakley BB, Marrazzo JM. Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis. J Clin Microbiol. 2007;45(10):3270-6.

52. Hall BG, Acar H, Nandipati A, Barlow M. Growth Rates Made Easy. Molecular Biology and Evolution. 2013;31(1):232-8.

53. de Winter JCF. Using the Student's t-test with extremely small sample sizes. Pract Assess, Res Eval. 2013;18:1-12.

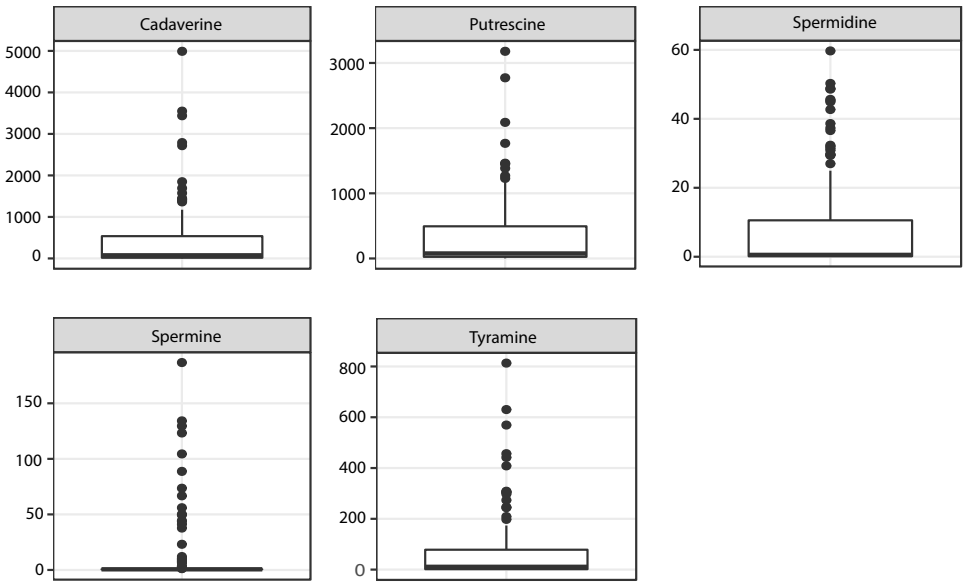

Figure 1 | Physiological concentrations of biogenic amines. Concentrations measured from 152 samples representing 32 women.

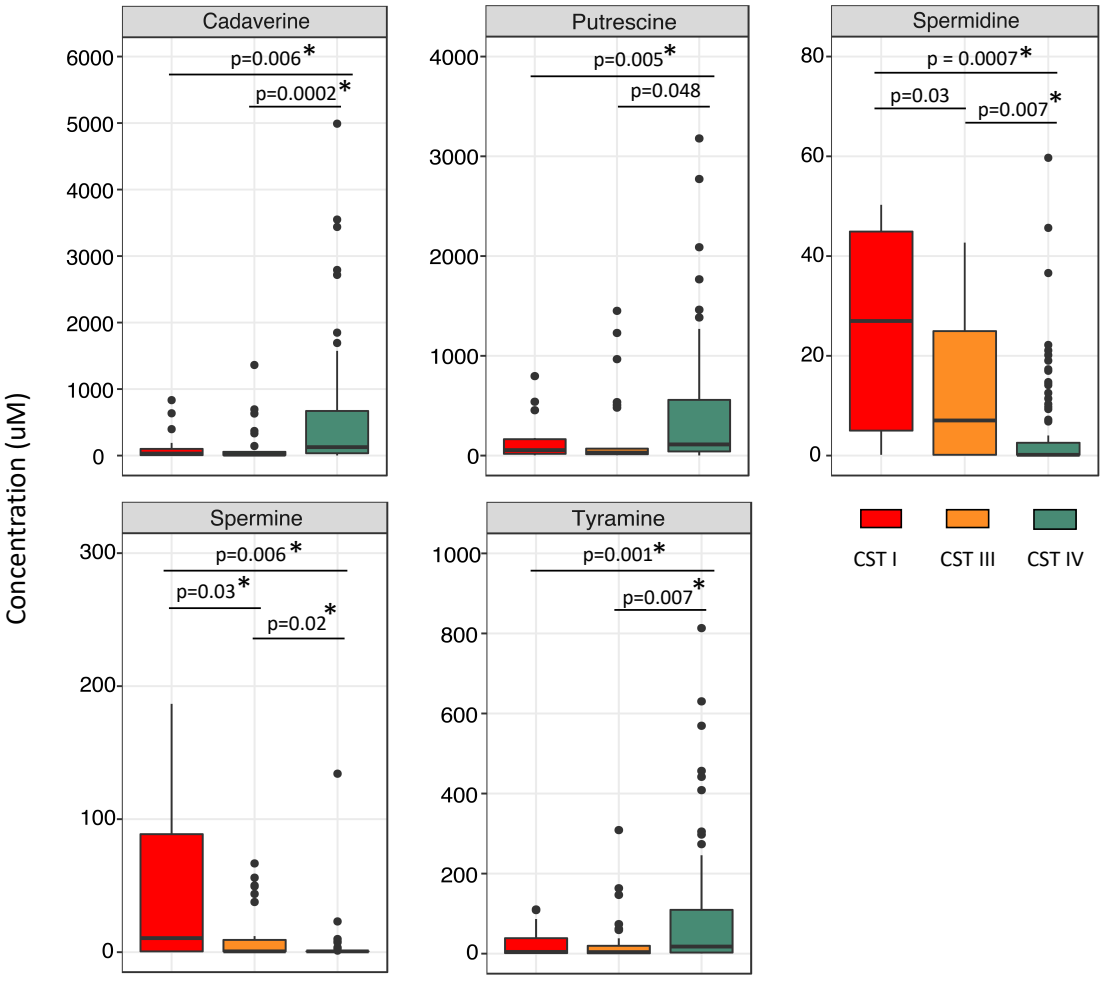

Figure 2 | Physiological concentrations of biogenic amines by CST. Concentrations measured from 152 samples representing 32 women. P-values obtained from pairwise comparisons; asterisks indicate significance (<0.05) after adjustment for multiple comparisons

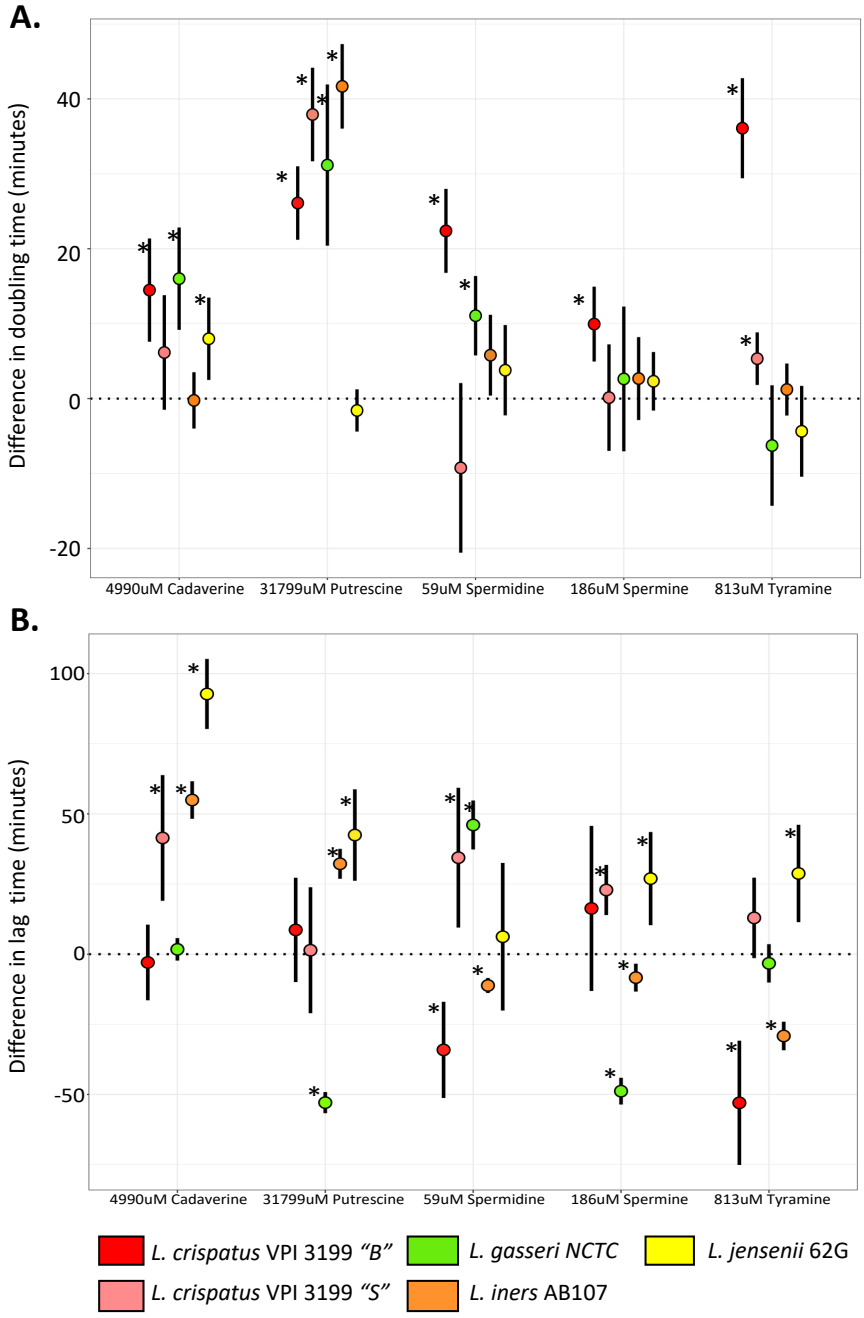

Figure 3| Effect of biogenic amines on the growth of vaginal *Lactobacillus* spp. The average difference in A) doubling time and B) lag time between treatments and controls for each biogenic amine and vaginal Lactobacillus spp. Dots represent mean and lines represent the associated 95% confidence interval.

Table 1 | Physiological concentrations of biogenic amines measured by LC-MS

		No. (%) detected in samples			
Amine	**All Samples (152)**	**CST I (17)**	**CST II (1)**	**CST III (29)**	**CST IV (105)**
Cadaverine	149 (98%)	16 (94%)	1 (100%)	29 (100%)	103 (98%)
Putrescine	148 (97%)	17 (100%)	1 (100%)	29 (100%)	101 (96%)
Spermidine	82 (18%)	14 (82%)	1 (100%)	19 (65%)	48 (47%)
Spermine	28 (18%)	9 (52%)	0 (0%)	10 (34%)	9 (8%)
Tyramine	149 (98%)	17 (100%)	1 (100%)	29 (100%)	102 (97%)
		Mean concentration (uM) (range)			
Cadaverine	396.57 (2.86 - 4990.55)	151.52 (3.11 - 833.61)	33.27	137.05 (3.07 - 1362.24)	511.24 (2.86 - 4990.55)[a]
Putrescine	328.22 (2.59 - 3179.17)	155.37 (3.01 - 796.64)	41.22	199.76 (2.59 - 1450.96)	397.04 (3.07 - 3179.17)[a]
Spermidine	14.91 (0.31 - 59.70)	29.26 (3.11 - 50.28)[c,d]	9.09	18.73 (0.31 - 42.69)	9.33 (0.45 - 59.70)[b]
Spermine	47.45 (1.18 - 186.68)	89.13 (10.55 - 186.68)[c,d]	ND	33.30 (1.45 - 66.73)	21.48 (1.18 - 134.19)[b]
Tyramine	67.98 (0.14 - 813.28)	27.41 (0.14 - 110.35)	2.03	32.13 (0.148 - 308.68)	85.58 (0.19 -813.28)[a]

a indicates p <0.001 versus concentration in all other combined groups
b indicates p <0.0009 versus concentration in all other groups combined
c indidates p <0.05 compared to *L. iners* dominated microbiota
d indicates p <0.006 compared to CST IV

Table 2A| Difference in doubling times of vaginal *Lactobacillus spp.* exposed to biogenic amines

Treatment	Bacteria	Average Difference in Doubling Time	Lower 95 CI	Upper 95 CI	Treatment mean (n=9)	Positive control mean (n=9)	p-value (t.test)	q-value
4990.5 uM Cadaverine	L. crispatus VPI 3199 morphotype "Big"	14.48	7.59	21.37	141.48	127.00	0.0004	0.0012
	L. crispatus VPI 3199 morphotype "Small"	6.16	-1.49	13.80	140.28	134.12	0.1069	0.1620
	L. gasseri NCTC	16.01	9.18	22.84	151.34	135.33	0.0002	0.0006
	L. iners AB107	-0.24	-4.00	3.52	118.67	118.91	0.8914	0.9141
	L. jensenii 62G	7.99	2.49	13.48	114.19	106.20	0.0078	0.0139
3179uM Putrescine	L. crispatus VPI 3199 morphotype "Big"	26.10	21.20	31.01	148.04	121.94	<0.0001	<0.0001
	L. crispatus VPI 3199 morphotype "Small"	37.92	31.68	44.16	153.15	115.23	<0.0001	<0.0001
	L. gasseri NCTC	31.17	20.41	41.93	156.24	125.07	0.0001	0.0002
	L. iners AB107	41.68	36.04	47.32	170.88	129.20	<0.0001	<0.0001
	L. jensenii 62G	-1.58	-4.40	1.24	88.17	89.75	0.2518	0.3297
59uM Spermidine	L. crispatus VPI 3199 morphotype "Big"	22.39	16.78	27.99	132.28	109.89	<0.0001	<0.0001
	L. crispatus VPI 3199 morphotype "Small"	-9.24	-20.56	2.08	121.14	130.38	0.1025	0.1602
	L. gasseri NCTC	11.07	5.76	16.37	160.53	149.46	0.0006	0.0014
	L. iners AB107	5.80	0.40	11.19	111.80	106.00	0.0369	0.0615
	L. jensenii 62G	3.79	-2.24	9.82	112.66	108.86	0.1991	0.2765
186uM Spermine	L. crispatus VPI 3199 morphotype "Big"	9.95	4.95	14.94	112.42	102.48	0.0008	0.0017
	L. crispatus VPI 3199 morphotype "Small"	0.13	-6.98	7.24	103.87	103.74	0.9691	0.9691
	L. gasseri NCTC	2.63	-7.04	12.29	124.11	121.48	0.5605	0.6228
	L. iners AB107	2.67	-2.86	8.21	125.39	122.72	0.3087	0.3859
	L. jensenii 62G	2.31	-1.59	6.22	75.46	73.15	0.2276	0.3076
813uM Tyramine	L. crispatus VPI 3199 morphotype "Big"	36.09	29.41	42.77	145.04	108.95	<0.0001	<0.0001
	L. crispatus VPI 3199 morphotype "Small"	5.33	1.82	8.84	117.82	112.49	0.0055	0.0101
	L. gasseri NCTC	-6.26	-14.30	1.78	131.21	137.47	0.1179	0.1734
	L. iners AB107	1.21	-2.26	4.68	114.03	112.82	0.4697	0.5338
	L. jensenii 62G	-4.36	-10.42	1.70	108.06	112.42	0.1458	0.2083

Table 2B| Difference in lag times of vaginal *Lactobacillus* spp. exposed to biogenic amines

Treatment	Bacteria	Average Difference in Lag Time	Lower 95 CI (lag)	Upper 95 CI (lag)	Treatment mean (n=9)	Positive control mean (n=9)	p-value (lag)	q-value (lag)
4990.5 uM Cadaverine	L. crispatus VPI 3199 morphotype "Big"	-2.95	-16.43	10.53	154.05	157.00	0.6490	0.6904
	L. crispatus VPI 3199 morphotype "Small"	41.43	19.03	63.83	228.26	186.83	0.0014	0.0030
	L. gasseri NCTC	1.72	-2.28	5.73	106.91	105.19	0.3695	0.4297
	L. iners AB107	54.96	48.27	61.65	178.95	123.99	<0.0001	<0.0001
	L. jensenii 62G	92.76	80.27	105.24	210.81	118.06	<0.0001	<0.0001
3179uM Putrescine	L. crispatus VPI 3199 morphotype "Big"	8.65	-9.95	27.24	199.53	190.88	0.3380	0.4024
	L. crispatus VPI 3199 morphotype "Small"	1.41	-21.04	23.86	281.55	280.14	0.8958	0.9141
	L. gasseri NCTC	-52.92	-56.68	-49.15	153.10	206.01	<0.0001	<0.0001
	L. iners AB107	32.21	26.88	37.54	189.43	157.22	<0.0001	<0.0001
	L. jensenii 62G	42.47	26.18	58.77	185.25	142.77	<0.0001	0.0002
59uM Spermidine	L. crispatus VPI 3199 morphotype "Big"	-34.12	-51.26	-16.98	197.52	231.64	0.0007	0.0016
	L. crispatus VPI 3199 morphotype "Small"	34.40	9.49	59.31	289.86	255.46	0.0099	0.0171
	L. gasseri NCTC	46.06	37.33	54.78	239.52	193.47	<0.0001	<0.0001
	L. iners AB107	-11.16	-13.84	-8.48	126.40	137.55	<0.0001	<0.0001
	L. jensenii 62G	6.22	-20.08	32.53	146.70	140.48	0.6221	0.6739
186uM Spermine	L. crispatus VPI 3199 morphotype "Big"	16.30	-13.12	45.72	142.09	125.79	0.2572	0.3297
	L. crispatus VPI 3199 morphotype "Small"	22.89	13.94	31.83	142.26	119.37	0.0001	0.0002
	L. gasseri NCTC	-48.83	-53.59	-44.07	126.86	175.69	<0.0001	<0.0001
	L. iners AB107	-8.36	-13.33	-3.40	154.62	162.99	0.0028	0.0059
	L. jensenii 62G	26.94	10.33	43.55	147.83	120.89	0.0034	0.0065
813uM Tyramine	L. crispatus VPI 3199 morphotype "Big"	-52.99	-75.14	-30.84	115.28	168.27	0.0003	0.0007
	L. crispatus VPI 3199 morphotype "Small"	12.94	-1.40	27.27	188.99	176.05	0.0730	0.1177
	L. gasseri NCTC	-3.27	-10.11	3.57	109.13	112.41	0.3255	0.3970
	L. iners AB107	-29.16	-34.25	-24.07	117.15	146.31	<0.0001	<0.0001
	L. jensenii 62G	28.77	11.41	46.13	182.49	153.72	0.0031	0.0063

Table 3 | Effect of BAs upon vaginal *Lactobacillus spp.* D- and L-lactic acid production (mM)

		L. crispatus VPI 3199 morphotype "Big"		L. crispatus VPI 3199 morphotype "Small"		L. gasseri NCTC		L. iners AB107		L. jensenii 62G	
Biogenic Amine	**Lactic Acid**	Positive Control (n=3)	Treatment (n=3)	Postive Control (n=3)	Treatment (n=3)	Positive Control (n=3)	Treatment (n=3)	Positive Control (n=3)	Treatment (n=3)	Positive Control (n=3)	Treatment (n=3)
	D-Lactic Acid (avg)	70.09	55.77	70.9	65.25	22.88	21.67	NA	NA	126.29	123.56
990uM Cadaverin	D-Lactic Acid (SD)	0.29	1.14	0.85	1.43	1.43	1.43	NA	NA	0	1.14
	L-Lactic Acid (avg)	55.12*	45.94*	51.94	50.88	16.65	13.3	52.65*	39.24*	4.13	3.78
	L-Lactic Acid (SD)	1.75	0.25	0.25	0.25	0.25	1.5	0.75	1.24	0	0
	D-Lactic Acid (avg)	58.19*	50.92*	63.23*	52.56*	67.88	56.98	NA	NA	60.05*	58.99*
3179uM Putrescin	D-Lactic Acid (SD)	0.56	1.14	1.42	0.6	0	2.28	NA	NA	0.57	0.57
	L-Lactic Acid (avg)	45.41	44	56	46.82	48.94	47.705	47.88	46.46	3.77	3.77
	L-Lactic Acid (SD)	0.5	2.5	1.99	1.0	0.5	0.25	0.5	0.25	1	0.5
	D-Lactic Acid (avg)	61.02	50.52	32.23	27.12	13.26	14.2	NA	NA	97.47	81.06
59uM Spermidine	D-Lactic Acid (SD)	4.58	0.4	1.42	4.55	0.46	0.81	NA	NA	11.66	7.49
	L-Lactic Acid (avg)	40.43	37.77	30.47	25.65	8.95	11.19	40.59	39.06	4.48	4.01
	L-Lactic Acid (SD)	8.82	1.78	2.04	3.58	0.41	1.41	1.24	2.2	0.35	0.2
	D-Lactic Acid (avg)	47.7*	51.57*	49.99	49.31	53.52	51.48	NA	NA	65.72*	54.69*
186uM Spermine	D-Lactic Acid (SD)	1.06	0.93	3.24	0.4	2.94	1.53	NA	NA	3.75	3.43
	L-Lactic Acid (avg)	42	38.83	44.94*	50.12*	33.53	33.89	42	39.41	2.25	2.36
	L-Lactic Acid (SD)	1.08	4.48	0.89	1.13	1.74	2.07	4.79	0	0.2	0.35
	D-Lactic Acid (avg)	103.79*	91.01*	91.95*	29.54*	29.81*	16.09*	NA	NA	139.03*	162.34*
813uM Tyramine	D-Lactic Acid (SD)	4.96	2.22	1.82	12.94	2.22	2.03	NA	NA	10.84	6.69
	L-Lactic Acid (avg)	85.17	78.82	70.11	38.33	23.3**	17.65**	59.06**	43.53**	7.89	10.6
	L-Lactic Acid (SD)	5.47	2.7	4.07	11.8	1.81	1.02	1.94	1.81	0.2	1.81

* indicates that the treatment and control were significantly different p-value <0.05
** indicates that the treatment and control were significantly different; q-value <0.05

Supplemental Materials

Table S1 | Quantitative biogenic amine measurements obtained from 153 samples (representing 34 women) using LC-MS

ID	W/D	Barcode	CST	Nugent Score	pH glove	Menstruating	Cadaverine	Putrescine	Spermidine	Spermine	Tyramine
AYAC03	W7D6	183704	IV	10	5.3	3	42.51	30.31	0.15	0.59	5.43
AYAC03	W8D1	199075	I	0	5.3	NA	635.83	796.64	5.01	0.59	108.87
AYAC03	W8D3	199091	I	0	5.3	NA	79.48	51.57	44.91	104.43	16.62
AYAC03	W8D5	199083	I	0	5.3	NA	54.53	54.90	29.57	44.36	11.85
AYAC09	W6D7	261179	I	1	4.4	NA	3.11	5.75	0.15	0.59	0.22
AYAC09	W7D2	261187	IV	5	4.4	2	1.43	1.29	0.15	0.59	0.07
AYAC09	W7D4	261195	IV	5	4.4	1	4.64	11.85	0.15	0.59	0.19
AYAC09	W7D6	261203	I	1	4.4	NA	41.03	82.99	5.90	0.59	2.03
EM01	W3D7	112061	III	3	4	NA	11.92	28.28	24.77	50.09	0.69
EM01	W4D3	112073	III	5	4.7	NA	334.55	536.02	29.76	49.54	59.15
EM01	W4D6	112085	III	8	5.3	NA	143.80	506.45	7.04	0.59	61.74
EM01	W5D2	112101	IV	8	5.5	NA	670.95	983.32	1.11	0.59	153.60
EM02	W7D3	198571	IV	9	4.7	NA	754.13	972.23	0.73	0.59	297.58
EM02	W7D5	198579	IV	10	4.7	1	9.35	7.37	0.15	0.59	1.41
EM02	W7D7	198591	IV	10	4.4	1	35.12	43.07	0.15	0.59	9.30
EM02	W8D2	198663	IV	10	5	NA	97.22	168.75	0.15	0.59	18.67
EM09	W2D1	113281	I	0	4	NA	3.57	17.76	48.80	129.57	0.20
EM09	W2D3	113289	IV	5	4.4	3	3.57	11.18	0.15	0.59	0.24
EM09	W2D5	113297	I	2	4.4	2	30.31	113.67	11.85	0.59	3.47
EM09	W2D7	113309	I	0	4	NA	10.26	90.38	48.61	88.72	3.79
EM09	W7D5	235339	I	0	4	NA	3.12	37.89	26.99	41.03	0.33
EM09	W8D7	235371	IV	9	5.5	NA	1848.35	2088.64	6.84	0.59	630.29
EM13	W3D4	261387	IV	8	4	NA	271.71	292.04	0.15	0.59	164.13
EM13	W3D6	261395	IV	6	5.3	2	121.81	129.94	0.15	0.59	63.40
EM13	W4D1	261407	IV	8	5	3	26.25	37.15	0.15	0.59	23.47
EM13	W4D3	261415	IV	7	5.3	1	1426.93	1767.03	4.03	0.59	161.92
EM13	W7D4	261516	IV	7	4	NA	2791.01	2772.53	17.06	0.59	813.28
EM13	W7D6	261524	IV	7	5	2	31.24	49.72	0.15	0.59	7.25
EM13	W8D1	261536	IV	8	5	2	1417.69	1269.82	1.63	0.59	148.61
EM13	W8D3	261544	IV	8	5	1	70.61	108.87	0.15	0.59	22.37
UAB003	W4D1	174808	III	1	4	1	7.21	13.86	32.35	43.81	9.54
UAB003	W4D4	174820	III	1	4	2	7.36	17.91	42.70	66.73	19.59
UAB003	W4D7	174836	III	NA	4	2	9.26	18.22	0.15	0.59	4.23
UAB003	W5D2	174844	IV	6	4.7	1	11.13	40.66	0.15	0.59	2.00
UAB003	W7D2	181207	IV	4	4	NA	741.19	1016.59	59.70	134.19	173.56
UAB003	W7D4	181215	IV	4	4	NA	977.78	1120.10	45.65	8.11	245.83
UAB003	W7D6	181223	IV	5	4	NA	510.15	558.20	21.07	3.07	103.14
UAB003	W8D1	181235	IV	6	4	NA	828.06	1007.35	36.60	9.87	154.89
UAB005	W4D5	175144	III	2	4	NA	13.96	33.09	31.98	56.01	2.99
UAB005	W4D7	175156	III	2	4	NA	13.29	37.52	9.09	6.41	4.51
UAB005	W5D2	175164	III	4	4.4	NA	632.14	1450.96	37.34	37.71	146.76
UAB005	W5D4	175172	IV	6	5	NA	168.39	741.19	2.46	0.59	78.74
UAB005	W6D6	181511	III	0	NA	NA	5.88	34.19	17.32	9.20	0.56
UAB005	W7D1	181523	IV	6	NA	3	608.11	883.51	12.55	0.59	109.42
UAB005	W7D3	181531	IV	5	4.7	1	159.51	175.78	19.04	23.10	66.73
UAB005	W7D5	181539	IV	7	5.3	NA	31.61	17.47	0.15	0.59	12.68
UAB006	W3D7	175284	III	5	5.3	NA	369.67	480.57	29.39	0.59	73.75
UAB006	W4D3	175296	IV	6	5	NA	41.59	74.67	11.44	0.59	9.11

UAB006	W4D6	175308	IV	7	5.5	NA	150.46	521.24	1.24	0.59	21.26
UAB008	W5D7	175668	IV	9	5.5	NA	1173.70	1016.59	17.30	7.45	441.76
UAB008	W6D2	181975	IV	9	5.5	2	1693.09	870.57	14.14	0.59	157.29
UAB008	W6D4	181983	IV	10	5.8	3	818.82	615.50	3.18	0.59	34.19
UAB008	W6D6	181991	IV	9	NA	2	3437.94	1462.05	17.00	0.59	56.56
UAB010	W1D7	175860	III	8	4.4	NA	1362.24	1229.16	30.87	0.59	308.68
UAB010	W2D2	175868	I	4	4	NA	190.38	175.78	31.42	10.55	70.61
UAB010	W2D4	175876	I	1	4	NA	23.10	25.69	3.11	0.59	6.73
UAB010	W2D6	175884	I	1	4	NA	833.61	539.72	45.28	73.56	110.35
UAB013	W7D5	187791	IV	9	5.8	2	49.72	61.00	0.15	0.59	16.97
UAB013	W8D1	187807	IV	8	5.8	2	43.99	47.13	0.15	0.59	10.31
UAB013	W8D4	187819	IV	7	5.8	1	61.55	69.68	0.15	0.59	4.58
UAB013	W8D7	187835	IV	3	5.8	NA	4.71	4.47	0.15	0.59	0.58
UAB015	W8D1	188201	IV	3	5.3	1	20.52	8.72	0.15	0.59	0.48
UAB015	W8D4	188213	III	4	4.4	2	31.24	41.40	0.53	0.59	10.35
UAB015	W8D7	188225	III	3	4.4	1	3.07	2.59	0.15	0.59	0.72
UAB015	W9D1	188233	III	3	5.3	1	11.15	6.76	0.15	0.59	0.95
UAB015	W9D3	188241	IV	7	5.5	3	17.12	15.75	0.15	0.59	1.27
UAB015	W9D7	188257	IV	NA	5.5	3	43.44	48.24	0.15	0.59	2.44
UAB017	W6D6	188559	IV	8	5.3	NA	133.08	358.58	0.15	0.59	85.76
UAB017	W6D7	188567	IV	8	5.3	NA	391.85	767.07	1.28	0.59	112.75
UAB017	W7D2	188575	IV	7	5.3	NA	22.73	20.70	0.15	0.59	4.64
UAB017	W7D3	188579	III	4	4	NA	41.59	69.68	0.15	0.59	38.26
UAB017	W7D5	188587	III	NA	NA	NA	34.19	29.76	0.15	0.59	4.47
UAB017	W7D6	188591	III	3	4	NA	66.54	73.19	24.95	1.47	10.13
UAB017	W8D1	188603	III	3	4	NA	13.59	13.73	0.15	0.59	0.43
UAB017	W8D2	188607	III	1	4	NA	9.20	27.73	10.81	0.59	1.42
UAB021	W4D1	177787	IV	3	4.7	3	8.63	4.21	0.46	0.59	1.11
UAB021	W4D4	177799	IV	8	5	1	621.05	277.25	0.45	0.59	117.37
UAB021	W4D7	177815	IV	8	5.3	NA	32.16	32.53	0.73	0.59	11.15
UAB021	W5D2	177823	IV	9	5.3	NA	2717.08	855.79	2.33	0.59	18.67
UAB022	W1D4	177863	IV	8	4.7	NA	119.96	125.32	0.15	0.59	50.46
UAB022	W1D6	177871	IV	7	5.5	2	3.31	1.29	0.15	0.59	0.90
UAB022	W2D1	177883	IV	8	5	3	41.03	24.58	0.15	0.59	3.36
UAB022	W2D3	177891	IV	8	4.7	1	13.05	3.07	0.15	0.59	0.73
UAB040	W7D7	187206	I	0	4.4	NA	6.62	3.01	0.15	0.59	1.39
UAB040	W8D2	187214	I	7	4.7	1	397.40	454.69	0.15	0.59	86.50
UAB040	W8D4	187222	IV	7	4.7	NA	767.07	809.58	9.76	0.59	95.19
UAB040	W8D6	187230	IV	7	5	1	73.75	65.43	0.15	0.59	21.81
UAB041	W3D4	191401	III	6	7	2	34.75	23.47	0.31	0.59	1.77
UAB041	W3D6	191409	IV	6	7	3	16.01	14.90	1.84	0.59	0.56
UAB041	W4D1	191421	III	3	4.7	1	56.56	51.38	0.45	0.59	3.84
UAB041	W4D3	191429	III	2	4.7	NA	29.57	47.50	3.16	0.59	0.97
UAB055	W3D2	196418	IV	10	5	NA	887.21	109.61	0.15	0.59	10.87
UAB055	W3D4	196426	IV	10	5.3	2	61.74	51.20	2.81	0.59	1.69
UAB055	W3D6	196434	IV	8	5.3	2	2.86	1.29	0.15	0.59	0.07
UAB055	W4D1	196446	IV	9	5	NA	1574.80	1171.86	22.18	2.92	408.49
UAB055	W7D2	196546	IV	10	5.3	NA	192.23	41.40	0.15	0.59	48.24
UAB055	W7D4	196554	IV	10	5.3	1	229.20	53.79	0.15	0.59	42.51
UAB055	W7D6	196562	IV	10	5.3	1	1449.11	125.69	0.15	0.59	9.22
UAB055	W8D1	196574	IV	10	5	NA	234.74	56.37	0.15	0.59	4.08
UAB059	W5D6	197934	III	2	4.4	1	5.12	13.60	0.15	0.59	0.87
UAB059	W6D2	197946	IV	6	4.4	2	11.24	17.67	0.15	0.59	0.40

UAB059	W6D2	197946	IV	6	4.4	2	11.24	17.67	0.15	0.59	0.40
UAB059	W6D3	197954	IV	7	4.4	2	134.01	96.30	0.15	0.59	0.97
UAB059	W6D6	197962	IV	6	4.4	1	524.93	127.17	0.15	0.59	1.18
UAB062	W1D1	199661	II	1	4.7	NA	33.27	41.22	9.09	0.59	2.03
UAB062	W1D3	199669	III	1	4.7	1	3.31	3.31	0.15	0.59	0.16
UAB062	W1D5	199677	IV	3	7	3	1.43	1.29	0.15	0.59	0.07
UAB062	W2D1	199693	IV	6	5.5	1	19.96	9.81	0.15	0.59	2.05
UAB065	W3D3	200744	IV	3	5	NA	11.81	16.10	7.19	0.59	3.16
UAB065	W3D5	200752	I	3	4	NA	100.74	164.13	19.22	0.59	38.63
UAB065	W3D7	200764	IV	1	4	NA	33.82	52.86	0.15	0.59	9.83
UAB065	W4D2	200772	IV	0	4	NA	15.84	31.98	20.15	3.44	6.36
UAB066	W4D1	201105	IV	8	5.3	NA	674.65	541.57	0.15	0.59	147.13
UAB066	W4D3	201113	IV	6	7	3	59.33	20.70	0.15	0.59	2.79
UAB066	W4D5	201121	IV	5	7	2	439.91	104.99	0.15	0.59	0.63
UAB066	W4D7	201133	IV	9	5.8	NA	4990.55	111.83	9.33	0.59	12.37
UAB076	W6D7	204904	IV	8	5	NA	75.60	74.30	0.15	0.59	39.37
UAB076	W7D2	204912	IV	6	5.8	3	28.46	7.89	0.15	1.18	0.82
UAB076	W7D4	204920	IV	5	4.7	1	122.36	76.34	0.15	0.59	0.82
UAB076	W7D6	204928	IV	9	4.7	NA	28.28	60.81	0.15	0.59	1.47
UAB077	W2D4	205097	IV	9	4	NA	1388.11	885.36	1.55	0.59	569.29
UAB077	W2D6	205105	IV	9	7	2	707.92	421.42	14.73	0.59	12.81
UAB077	W3D1	205117	IV	7	7	3	221.80	177.26	10.31	0.59	3.33
UAB077	W3D3	205125	IV	7	4	1	1144.13	545.26	2.57	0.59	12.03
UAB077	W7D5	205261	IV	9	4.7	NA	208.86	167.83	0.15	0.59	197.77
UAB077	W7D7	205273	IV	8	7	3	92.79	109.98	2.57	0.59	2.72
UAB090	W4D3	209538	I	2	4	NA	11.26	16.03	38.63	186.68	4.23
UAB090	W4D5	209546	I	1	4.7	3	1.43	10.74	50.28	123.29	0.14
UAB090	W4D7	209558	III	4	4.7	2	7.80	13.86	13.70	12.07	0.57
UAB112	W6D2	221176	IV	NA	NA	NA	75.04	78.19	0.15	0.59	40.66
UAB112	W6D5	221100	III	NA	NA	NA	694.98	966.69	9.45	0.59	163.39
UAB112	W7D1	221204	IV	NA	NA	NA	67.46	89.65	0.15	0.59	43.25
UAB112	W8D3	221244	IV	9	5.5	NA	1162.61	1384.42	1.15	0.59	456.54
UAB116	W5D1	222488	IV	9	5.3	1	548.96	360.43	1.60	0.59	208.86
UAB116	W9D1	222616	IV	9	5	NA	467.63	353.04	7.12	0.59	121.07
UAB116	W9D3	222624	IV	9	5.3	1	240.29	178.74	0.15	0.59	131.42
UAB116	W9D5	222632	IV	8	7	3	48.80	42.14	0.15	0.59	21.07
UAB120	W4D2	223808	IV	7	4.7	NA	126.98	168.94	6.99	0.59	17.73
UAB120	W4D4	223816	IV	8	5.8	2	103.51	150.27	0.15	0.59	34.56
UAB120	W4D6	223824	IV	8	5.3	2	113.30	125.50	0.15	0.59	1.92
UAB120	W5D1	223836	IV	8	5.3	1	78.00	77.63	0.15	0.59	5.40
UAB121	W1D7	224073	IV	8	5.3	NA	619.20	609.96	0.15	0.59	273.56
UAB121	W2D1	224077	IV	9	5.5	1	3548.84	3179.17	14.49	0.59	26.62
UAB121	W2D3	224085	IV	9	5.5	1	615.50	515.69	0.15	0.59	4.92
UAB121	W2D5	224093	IV	9	5.3	1	119.59	120.51	0.64	0.59	2.50
UAB127	W4D2	226167	IV	6	5	NA	746.73	598.87	0.82	0.59	304.98
UAB127	W4D3	226171	IV	6	5	NA	155.08	151.93	0.87	0.59	77.82
UAB127	W4D5	226179	IV	6	5	NA	558.20	646.92	1.52	0.59	243.98
UAB127	W4D6	226183	IV	4	4.7	NA	966.69	988.87	7.15	0.59	304.98
UAB128	W5D2	226536	III	2	5	3	13.60	17.41	0.15	0.59	0.94
UAB128	W5D4	226544	IV	7	5	NA	491.66	480.57	2.01	0.59	87.98
UAB128	W5D7	226560	III	2	4	NA	6.93	4.69	0.15	0.59	0.48
UAB128	W6D3	226572	IV	6	NA	NA	60.63	52.68	0.51	0.59	17.41

Table S2 | Minimum inhibitory concentrations of select BAs upon vaginal *Lactobacillus* spp.

			Aerobic					Anaerobic		
Bacteria	**Cadaverine**	**Putrescine**	**Spermidine**	**Spermine**	**Tyramine**	**Cadaverine**	**Putrescine**	**Spermidine**	**Spermine**	**Tyramine**
L. crispatus VPI 3199 morphotype "Big"	12.5mM	12.5mM	3.125mM	1.5625mM	16.675 mM	25mM	12.5mM	6.25mM	1.5625mM	33.35 mM
L. crispatus VPI 3199 **morphotype "Small"**	12.5mM	12.5mM	3.125mM	1.5625mM	16.675 mM	25mM	12.5mM	3.125mM	1.5625mM	33.35 mM
L. gasseri NCTC	6.25mM	12.5mM	12.5mM	6.25mM	16.675 mM	25mM	25mM	6.25mM	3.125mM	33.35 mM
L. iners **AB107**	12.5mM	12.5mM	6.25mM	1.5625mM	16.675 mM	25mM	12.5mM	6.25mM	3.125mM	33.35 mM
L. jensenii **62G**	12.5mM	12.5mM	12.5mM	1.5625mM	16.675 mM	-	-	-	-	-

Values represent the MICs observed for each *Lactobacillus spp.* under aerobic and anaerobic conditions after 24 hours of incubation. Dashes indicate no growth after 24 hours

Table S3| Approximated bacterial cell density and associated OD600

Bacterial Strain	OD600	Approximate cell density
L. iners AB107 rep. 1	0.515	3.4E+07
L. iners AB107 rep. 2	0.519	3.4E+07
L. iners AB107 rep. 3	0.515	3.4E+07
L. crispatus VPI 3199 morphotype "Big" rep. 1	0.503	2.1E+06
L. crispatus VPI 3199 morphotype "Big" rep. 2	0.507	2.1E+06
L. crispatus VPI 3199 morphotype "Big" rep. 3	0.505	2.1E+06
L. jensenii 62G rep. 1	0.521	1.3E+08
L. jensenii 62G rep. 2	0.521	1.3E+08
L. jensenii 62G rep. 3	0.521	1.3E+08
L. crispatus VPI 3199 morphotype "Small" rep. 1	0.519	3.4E+07
L. crispatus VPI 3199 morphotype "Small" rep. 2	0.517	3.4E+07
L. crispatus VPI 3199 morphotype "Small" rep. 3	0.517	3.4E+07
L. gasseri NCTC rep. 1	0.520	3.4E+07
L. gasseri NCTC rep. 2	0.521	6.7E+07
L. gasseri NCTC rep. 3	0.520	6.7E+07

Bacterial cell density was estimated by conducting two-fold serial dilutions until maximally diluted as indicated by no visible growth after 24hrs incubation at 37C. The first well that displayed visible growth was then estimated to have "at least one cell", with each subsequent well estimated to have two times the number of cells of the previous well (i.e., at least one cell, at least two cells, at least four cells, etc.)

Table S4| Specific growth properties of vaginal *Lactobacillus* spp. under varying conditions

Major	Trial conditions	Treatment	Doubling Time	Lag Time	Growth Rate
L. crispatus VPI 3199 morphotype "Small"	Cad	4990uM Cad	132.2	191.6	0.0052
L. crispatus VPI 3199 morphotype "Small"	Cad	4990uM Cad	132.5	197.1	0.0052
L. crispatus VPI 3199 morphotype "Small"	Cad	4990uM Cad	133.5	209.8	0.0052
L. crispatus VPI 3199 morphotype "Small"	Cad	4990uM Cad	138.5	214.2	0.0050
L. crispatus VPI 3199 morphotype "Small"	Cad	4990uM Cad	139.5	235.7	0.0050
L. crispatus VPI 3199 morphotype "Small"	Cad	4990uM Cad	142.3	242.1	0.0049
L. crispatus VPI 3199 morphotype "Small"	Cad	4990uM Cad	146.3	244.7	0.0047
L. crispatus VPI 3199 morphotype "Small"	Cad	4990uM Cad	147.4	253.9	0.0047
L. crispatus VPI 3199 morphotype "Small"	Cad	4990uM Cad	150.4	265.3	0.0046
L. crispatus VPI 3199 morphotype "Small"	Cad	LCS PC	124.1	166.4	0.0056
L. crispatus VPI 3199 morphotype "Small"	Cad	LCS PC	124.1	175.4	0.0056
L. crispatus VPI 3199 morphotype "Small"	Cad	LCS PC	125.5	175.5	0.0055
L. crispatus VPI 3199 morphotype "Small"	Cad	LCS PC	128.6	177.3	0.0054
L. crispatus VPI 3199 morphotype "Small"	Cad	LCS PC	137.9	180.5	0.0050
L. crispatus VPI 3199 morphotype "Small"	Cad	LCS PC	139.8	184.3	0.0050
L. crispatus VPI 3199 morphotype "Small"	Cad	LCS PC	141.0	191.4	0.0049
L. crispatus VPI 3199 morphotype "Small"	Cad	LCS PC	141.7	215.0	0.0049
L. crispatus VPI 3199 morphotype "Small"	Cad	LCS PC	144.4	215.7	0.0048
L. crispatus VPI 3199 morphotype "Small"	Put	3179uM Put	146.2	243.0	0.0047
L. crispatus VPI 3199 morphotype "Small"	Put	3179uM Put	148.1	257.9	0.0047
L. crispatus VPI 3199 morphotype "Small"	Put	3179uM Put	149.8	269.4	0.0046
L. crispatus VPI 3199 morphotype "Small"	Put	3179uM Put	150.0	274.9	0.0046
L. crispatus VPI 3199 morphotype "Small"	Put	3179uM Put	150.2	287.2	0.0046
L. crispatus VPI 3199 morphotype "Small"	Put	3179uM Put	154.7	289.2	0.0045
L. crispatus VPI 3199 morphotype "Small"	Put	3179uM Put	158.1	293.1	0.0044
L. crispatus VPI 3199 morphotype "Small"	Put	3179uM Put	159.0	304.2	0.0044
L. crispatus VPI 3199 morphotype "Small"	Put	3179uM Put	162.4	315.1	0.0043
L. crispatus VPI 3199 morphotype "Small"	Put	LCS PC	108.6	250.0	0.0064
L. crispatus VPI 3199 morphotype "Small"	Put	LCS PC	109.4	267.0	0.0063
L. crispatus VPI 3199 morphotype "Small"	Put	LCS PC	110.1	268.9	0.0063
L. crispatus VPI 3199 morphotype "Small"	Put	LCS PC	110.8	269.0	0.0063
L. crispatus VPI 3199 morphotype "Small"	Put	LCS PC	111.3	274.0	0.0062
L. crispatus VPI 3199 morphotype "Small"	Put	LCS PC	115.2	277.1	0.0060
L. crispatus VPI 3199 morphotype "Small"	Put	LCS PC	121.6	283.4	0.0057
L. crispatus VPI 3199 morphotype "Small"	Put	LCS PC	124.4	313.1	0.0056
L. crispatus VPI 3199 morphotype "Small"	Put	LCS PC	125.5	318.8	0.0055
L. crispatus VPI 3199 morphotype "Small"	SPD	59uM SPD	110.5	236.5	0.0063
L. crispatus VPI 3199 morphotype "Small"	SPD	59uM SPD	113.8	263.4	0.0061
L. crispatus VPI 3199 morphotype "Small"	SPD	59uM SPD	114.9	272.6	0.0060
L. crispatus VPI 3199 morphotype "Small"	SPD	59uM SPD	115.2	300.4	0.0060
L. crispatus VPI 3199 morphotype "Small"	SPD	59uM SPD	119.2	303.4	0.0058
L. crispatus VPI 3199 morphotype "Small"	SPD	59uM SPD	119.5	304.1	0.0058

L. crispatus VPI 3199 morphotype "Small"	SPD	59uM SPD	124.5	305.4	0.0056
L. crispatus VPI 3199 morphotype "Small"	SPD	59uM SPD	128.9	309.6	0.0054
L. crispatus VPI 3199 morphotype "Small"	SPD	59uM SPD	143.7	313.3	0.0048
L. crispatus VPI 3199 morphotype "Small"	SPD	LCS PC	111.4	217.3	0.0062
L. crispatus VPI 3199 morphotype "Small"	SPD	LCS PC	111.7	221.5	0.0062
L. crispatus VPI 3199 morphotype "Small"	SPD	LCS PC	126.2	241.9	0.0055
L. crispatus VPI 3199 morphotype "Small"	SPD	LCS PC	126.5	261.5	0.0055
L. crispatus VPI 3199 morphotype "Small"	SPD	LCS PC	133.9	265.2	0.0052
L. crispatus VPI 3199 morphotype "Small"	SPD	LCS PC	139.2	265.2	0.0050
L. crispatus VPI 3199 morphotype "Small"	SPD	LCS PC	140.4	265.9	0.0049
L. crispatus VPI 3199 morphotype "Small"	SPD	LCS PC	141.3	276.9	0.0049
L. crispatus VPI 3199 morphotype "Small"	SPD	LCS PC	142.9	283.9	0.0049
L. crispatus VPI 3199 morphotype "Small"	Tyr	813uM Tyr	111.8	174.6	0.0062
L. crispatus VPI 3199 morphotype "Small"	Tyr	813uM Tyr	116.5	178.3	0.0059
L. crispatus VPI 3199 morphotype "Small"	Tyr	813uM Tyr	116.8	178.6	0.0059
L. crispatus VPI 3199 morphotype "Small"	Tyr	813uM Tyr	117.3	188.3	0.0059
L. crispatus VPI 3199 morphotype "Small"	Tyr	813uM Tyr	118.2	191.8	0.0059
L. crispatus VPI 3199 morphotype "Small"	Tyr	813uM Tyr	118.4	195.0	0.0059
L. crispatus VPI 3199 morphotype "Small"	Tyr	813uM Tyr	118.4	196.1	0.0059
L. crispatus VPI 3199 morphotype "Small"	Tyr	813uM Tyr	119.5	196.9	0.0058
L. crispatus VPI 3199 morphotype "Small"	Tyr	813uM Tyr	123.6	201.3	0.0056
L. crispatus VPI 3199 morphotype "Small"	Tyr	LCS PC	104.5	153.6	0.0066
L. crispatus VPI 3199 morphotype "Small"	Tyr	LCS PC	110.0	153.6	0.0063
L. crispatus VPI 3199 morphotype "Small"	Tyr	LCS PC	111.5	155.2	0.0062
L. crispatus VPI 3199 morphotype "Small"	Tyr	LCS PC	111.7	180.7	0.0062
L. crispatus VPI 3199 morphotype "Small"	Tyr	LCS PC	112.0	181.6	0.0062
L. crispatus VPI 3199 morphotype "Small"	Tyr	LCS PC	113.5	184.4	0.0061
L. crispatus VPI 3199 morphotype "Small"	Tyr	LCS PC	115.3	184.4	0.0060
L. crispatus VPI 3199 morphotype "Small"	Tyr	LCS PC	116.3	192.1	0.0060
L. crispatus VPI 3199 morphotype "Small"	Tyr	LCS PC	117.5	198.8	0.0059
L. crispatus VPI 3199 morphotype "Small"	SP	LCS PC	98.4	109.4	0.0070
L. crispatus VPI 3199 morphotype "Small"	SP	LCS PC	100.5	109.4	0.0069
L. crispatus VPI 3199 morphotype "Small"	SP	LCS PC	102.2	109.9	0.0068
L. crispatus VPI 3199 morphotype "Small"	SP	LCS PC	102.2	109.9	0.0068
L. crispatus VPI 3199 morphotype "Small"	SP	LCS PC	102.4	119.3	0.0068
L. crispatus VPI 3199 morphotype "Small"	SP	LCS PC	103.0	126.2	0.0067
L. crispatus VPI 3199 morphotype "Small"	SP	LCS PC	103.0	128.2	0.0067
L. crispatus VPI 3199 morphotype "Small"	SP	LCS PC	103.3	128.8	0.0067
L. crispatus VPI 3199 morphotype "Small"	SP	LCS PC	118.8	133.4	0.0058
L. crispatus VPI 3199 morphotype "Small"	SP	186uM SP	98.3	132.7	0.0070
L. crispatus VPI 3199 morphotype "Small"	SP	186uM SP	98.7	134.5	0.0070
L. crispatus VPI 3199 morphotype "Small"	SP	186uM SP	100.0	134.8	0.0069
L. crispatus VPI 3199 morphotype "Small"	SP	186uM SP	100.0	136.7	0.0069
L. crispatus VPI 3199 morphotype "Small"	SP	186uM SP	100.3	143.8	0.0069
L. crispatus VPI 3199 morphotype "Small"	SP	186uM SP	102.7	144.7	0.0067

L. crispatus VPI 3199 morphotype "Small"	SP	186uM SP	103.1	150.2	0.0067
L. crispatus VPI 3199 morphotype "Small"	SP	186uM SP	107.8	151.3	0.0064
L. crispatus VPI 3199 morphotype "Small"	SP	186uM SP	124.0	151.6	0.0056
L. gasseri NCTC	Put	3179uM Put	139.1	145.8	0.0050
L. gasseri NCTC	Put	3179uM Put	139.9	150.0	0.0050
L. gasseri NCTC	Put	3179uM Put	141.7	150.1	0.0049
L. gasseri NCTC	Put	3179uM Put	156.0	150.1	0.0044
L. gasseri NCTC	Put	3179uM Put	158.3	153.6	0.0044
L. gasseri NCTC	Put	3179uM Put	162.4	155.1	0.0043
L. gasseri NCTC	Put	3179uM Put	163.6	155.1	0.0042
L. gasseri NCTC	Put	3179uM Put	168.3	157.7	0.0041
L. gasseri NCTC	Put	3179uM Put	176.9	160.4	0.0039
L. gasseri NCTC	Put	LG PC	117.9	202.7	0.0059
L. gasseri NCTC	Put	LG PC	118.3	203.0	0.0059
L. gasseri NCTC	Put	LG PC	119.8	204.0	0.0058
L. gasseri NCTC	Put	LG PC	123.8	204.2	0.0056
L. gasseri NCTC	Put	LG PC	125.4	206.5	0.0055
L. gasseri NCTC	Put	LG PC	127.4	207.5	0.0054
L. gasseri NCTC	Put	LG PC	127.7	208.5	0.0054
L. gasseri NCTC	Put	LG PC	128.8	208.5	0.0054
L. gasseri NCTC	Put	LG PC	136.5	209.4	0.0051
L. gasseri NCTC	SPD	59uM SPD	160.3	227.1	0.0043
L. gasseri NCTC	SPD	59uM SPD	154.3	231.5	0.0045
L. gasseri NCTC	SPD	59uM SPD	157.2	233.5	0.0044
L. gasseri NCTC	SPD	59uM SPD	160.0	239.1	0.0043
L. gasseri NCTC	SPD	59uM SPD	160.0	240.6	0.0043
L. gasseri NCTC	SPD	59uM SPD	160.5	240.6	0.0043
L. gasseri NCTC	SPD	59uM SPD	164.3	241.5	0.0042
L. gasseri NCTC	SPD	59uM SPD	167.7	242.7	0.0041
L. gasseri NCTC	SPD	59uM SPD	160.5	259.1	0.0043
L. gasseri NCTC	SPD	LG PC	140.2	181.3	0.0049
L. gasseri NCTC	SPD	LG PC	144.4	183.2	0.0048
L. gasseri NCTC	SPD	LG PC	146.2	188.6	0.0047
L. gasseri NCTC	SPD	LG PC	147.2	189.4	0.0047
L. gasseri NCTC	SPD	LG PC	150.3	193.6	0.0046
L. gasseri NCTC	SPD	LG PC	151.2	199.6	0.0046
L. gasseri NCTC	SPD	LG PC	159.0	199.9	0.0044
L. gasseri NCTC	SPD	LG PC	159.1	200.3	0.0044
L. gasseri NCTC	SPD	LG PC	147.6	205.4	0.0047
L. gasseri NCTC	Tyr	813uM Tyr	119.9	100.0	0.0058
L. gasseri NCTC	Tyr	813uM Tyr	120.4	103.9	0.0058
L. gasseri NCTC	Tyr	813uM Tyr	126.1	104.1	0.0055
L. gasseri NCTC	Tyr	813uM Tyr	128.1	106.3	0.0054
L. gasseri NCTC	Tyr	813uM Tyr	129.9	108.0	0.0053
L. gasseri NCTC	Tyr	813uM Tyr	131.6	111.1	0.0053

L. gasseri NCTC	Tyr	813uM Tyr	139.9	111.9	0.0050
L. gasseri NCTC	Tyr	813uM Tyr	141.8	114.6	0.0049
L. gasseri NCTC	Tyr	813uM Tyr	143.3	122.4	0.0048
L. gasseri NCTC	Tyr	LG PC	122.7	104.4	0.0056
L. gasseri NCTC	Tyr	LG PC	129.9	105.3	0.0053
L. gasseri NCTC	Tyr	LG PC	133.7	106.7	0.0052
L. gasseri NCTC	Tyr	LG PC	139.1	108.8	0.0050
L. gasseri NCTC	Tyr	LG PC	142.0	109.0	0.0049
L. gasseri NCTC	Tyr	LG PC	142.8	117.2	0.0049
L. gasseri NCTC	Tyr	LG PC	142.8	118.1	0.0049
L. gasseri NCTC	Tyr	LG PC	143.3	119.6	0.0048
L. gasseri NCTC	Tyr	LG PC	141.0	122.7	0.0049
L. gasseri NCTC	SP	LG PC	93.3	170.2	0.0074
L. gasseri NCTC	SP	LG PC	115.6	171.0	0.0060
L. gasseri NCTC	SP	LG PC	118.0	173.7	0.0059
L. gasseri NCTC	SP	LG PC	122.7	173.9	0.0056
L. gasseri NCTC	SP	LG PC	124.7	175.5	0.0056
L. gasseri NCTC	SP	LG PC	125.9	177.9	0.0055
L. gasseri NCTC	SP	LG PC	127.5	178.1	0.0054
L. gasseri NCTC	SP	LG PC	130.2	180.2	0.0053
L. gasseri NCTC	SP	LG PC	135.4	180.6	0.0051
L. gasseri NCTC	SP	186uM SP	116.4	120.1	0.0060
L. gasseri NCTC	SP	186uM SP	119.1	122.3	0.0058
L. gasseri NCTC	SP	186uM SP	121.8	123.7	0.0057
L. gasseri NCTC	SP	186uM SP	122.5	123.7	0.0057
L. gasseri NCTC	SP	186uM SP	122.5	125.7	0.0057
L. gasseri NCTC	SP	186uM SP	125.2	128.1	0.0055
L. gasseri NCTC	SP	186uM SP	129.7	128.3	0.0053
L. gasseri NCTC	SP	186uM SP	130.0	131.6	0.0053
L. gasseri NCTC	SP	186uM SP	130.0	138.1	0.0053
L. gasseri NCTC	Cad	PC	124.2	101.2	0.0056
L. gasseri NCTC	Cad	PC	129.0	102.1	0.0054
L. gasseri NCTC	Cad	PC	129.4	102.2	0.0054
L. gasseri NCTC	Cad	PC	129.8	105.0	0.0053
L. gasseri NCTC	Cad	PC	130.9	105.8	0.0053
L. gasseri NCTC	Cad	PC	142.3	106.5	0.0049
L. gasseri NCTC	Cad	PC	142.3	106.9	0.0049
L. gasseri NCTC	Cad	PC	144.1	108.4	0.0048
L. gasseri NCTC	Cad	PC	145.8	108.7	0.0048
L. gasseri NCTC	Cad	4990uM Cad	141.9	102.4	0.0049
L. gasseri NCTC	Cad	4990uM Cad	147.4	102.6	0.0047
L. gasseri NCTC	Cad	4990uM Cad	150.9	102.7	0.0046
L. gasseri NCTC	Cad	4990uM Cad	151.0	105.7	0.0046
L. gasseri NCTC	Cad	4990uM Cad	152.0	105.7	0.0046
L. gasseri NCTC	Cad	4990uM Cad	152.3	107.5	0.0046

L. gasseri NCTC	Cad	4990uM Cad	153.6	107.7	0.0045
L. gasseri NCTC	Cad	4990uM Cad	153.6	110.5	0.0045
L. gasseri NCTC	Cad	4990uM Cad	159.5	117.4	0.0043
L. crispatus VPI 3199 morphotype "Big"	Cad	4990uM Cad	128.1	142.4	0.0054
L. crispatus VPI 3199 morphotype "Big"	Cad	4990uM Cad	133.2	145.5	0.0052
L. crispatus VPI 3199 morphotype "Big"	Cad	4990uM Cad	141.2	146.7	0.0049
L. crispatus VPI 3199 morphotype "Big"	Cad	4990uM Cad	141.2	146.7	0.0049
L. crispatus VPI 3199 morphotype "Big"	Cad	4990uM Cad	142.1	146.7	0.0049
L. crispatus VPI 3199 morphotype "Big"	Cad	4990uM Cad	142.1	146.7	0.0049
L. crispatus VPI 3199 morphotype "Big"	Cad	4990uM Cad	144.1	164.9	0.0048
L. crispatus VPI 3199 morphotype "Big"	Cad	4990uM Cad	145.6	170.5	0.0048
L. crispatus VPI 3199 morphotype "Big"	Cad	4990uM Cad	155.7	176.4	0.0045
L. crispatus VPI 3199 morphotype "Big"	Cad	LCB PC	119.6	136.5	0.0058
L. crispatus VPI 3199 morphotype "Big"	Cad	LCB PC	120.9	137.8	0.0057
L. crispatus VPI 3199 morphotype "Big"	Cad	LCB PC	123.9	148.7	0.0056
L. crispatus VPI 3199 morphotype "Big"	Cad	LCB PC	124.0	154.5	0.0056
L. crispatus VPI 3199 morphotype "Big"	Cad	LCB PC	124.9	157.0	0.0055
L. crispatus VPI 3199 morphotype "Big"	Cad	LCB PC	129.2	164.8	0.0054
L. crispatus VPI 3199 morphotype "Big"	Cad	LCB PC	130.2	165.1	0.0053
L. crispatus VPI 3199 morphotype "Big"	Cad	LCB PC	132.7	173.6	0.0052
L. crispatus VPI 3199 morphotype "Big"	Cad	LCB PC	137.7	175.0	0.0050
L. crispatus VPI 3199 morphotype "Big"	Put	3179uM Put	141.3	175.3	0.0049
L. crispatus VPI 3199 morphotype "Big"	Put	3179uM Put	143.8	185.6	0.0048
L. crispatus VPI 3199 morphotype "Big"	Put	3179uM Put	145.9	193.3	0.0048
L. crispatus VPI 3199 morphotype "Big"	Put	3179uM Put	146.2	194.6	0.0047
L. crispatus VPI 3199 morphotype "Big"	Put	3179uM Put	149.1	197.3	0.0046
L. crispatus VPI 3199 morphotype "Big"	Put	3179uM Put	149.2	198.8	0.0046
L. crispatus VPI 3199 morphotype "Big"	Put	3179uM Put	149.9	199.0	0.0046
L. crispatus VPI 3199 morphotype "Big"	Put	3179uM Put	152.8	224.0	0.0045
L. crispatus VPI 3199 morphotype "Big"	Put	3179uM Put	154.2	227.9	0.0045
L. crispatus VPI 3199 morphotype "Big"	Put	LCB PC	111.7	159.2	0.0062
L. crispatus VPI 3199 morphotype "Big"	Put	LCB PC	116.5	167.6	0.0060
L. crispatus VPI 3199 morphotype "Big"	Put	LCB PC	118.5	178.6	0.0059
L. crispatus VPI 3199 morphotype "Big"	Put	LCB PC	121.9	186.6	0.0057
L. crispatus VPI 3199 morphotype "Big"	Put	LCB PC	123.0	188.8	0.0056
L. crispatus VPI 3199 morphotype "Big"	Put	LCB PC	124.6	200.6	0.0056
L. crispatus VPI 3199 morphotype "Big"	Put	LCB PC	125.3	205.9	0.0055
L. crispatus VPI 3199 morphotype "Big"	Put	LCB PC	127.2	212.9	0.0055
L. crispatus VPI 3199 morphotype "Big"	Put	LCB PC	128.9	217.9	0.0054
L. crispatus VPI 3199 morphotype "Big"	SPD	59uM SPD	122.5	162.5	0.0057
L. crispatus VPI 3199 morphotype "Big"	SPD	59uM SPD	127.7	195.8	0.0054
L. crispatus VPI 3199 morphotype "Big"	SPD	59uM SPD	129.6	196.0	0.0053
L. crispatus VPI 3199 morphotype "Big"	SPD	59uM SPD	130.0	197.0	0.0053
L. crispatus VPI 3199 morphotype "Big"	SPD	59uM SPD	130.7	197.5	0.0053
L. crispatus VPI 3199 morphotype "Big"	SPD	59uM SPD	136.9	200.1	0.0051

L. crispatus VPI 3199 morphotype "Big"	SPD	59uM SPD	136.9	204.2	0.0051
L. crispatus VPI 3199 morphotype "Big"	SPD	59uM SPD	137.7	204.9	0.0050
L. crispatus VPI 3199 morphotype "Big"	SPD	59uM SPD	138.6	219.7	0.0050
L. crispatus VPI 3199 morphotype "Big"	SPD	LCB PC	100.9	184.3	0.0069
L. crispatus VPI 3199 morphotype "Big"	SPD	LCB PC	106.6	229.3	0.0065
L. crispatus VPI 3199 morphotype "Big"	SPD	LCB PC	106.6	231.5	0.0065
L. crispatus VPI 3199 morphotype "Big"	SPD	LCB PC	106.8	232.1	0.0065
L. crispatus VPI 3199 morphotype "Big"	SPD	LCB PC	107.9	236.1	0.0064
L. crispatus VPI 3199 morphotype "Big"	SPD	LCB PC	112.1	237.6	0.0062
L. crispatus VPI 3199 morphotype "Big"	SPD	LCB PC	113.3	241.3	0.0061
L. crispatus VPI 3199 morphotype "Big"	SPD	LCB PC	115.1	244.0	0.0060
L. crispatus VPI 3199 morphotype "Big"	SPD	LCB PC	119.8	248.7	0.0058
L. crispatus VPI 3199 morphotype "Big"	Tyr	813uM Tyr	136.4	98.6	0.0051
L. crispatus VPI 3199 morphotype "Big"	Tyr	813uM Tyr	136.6	101.6	0.0051
L. crispatus VPI 3199 morphotype "Big"	Tyr	813uM Tyr	137.8	109.6	0.0050
L. crispatus VPI 3199 morphotype "Big"	Tyr	813uM Tyr	147.3	112.1	0.0047
L. crispatus VPI 3199 morphotype "Big"	Tyr	813uM Tyr	147.5	114.6	0.0047
L. crispatus VPI 3199 morphotype "Big"	Tyr	813uM Tyr	149.0	116.8	0.0047
L. crispatus VPI 3199 morphotype "Big"	Tyr	813uM Tyr	149.8	117.1	0.0046
L. crispatus VPI 3199 morphotype "Big"	Tyr	813uM Tyr	150.4	130.8	0.0046
L. crispatus VPI 3199 morphotype "Big"	Tyr	813uM Tyr	150.7	136.3	0.0046
L. crispatus VPI 3199 morphotype "Big"	Tyr	LCB PC	102.2	133.6	0.0068
L. crispatus VPI 3199 morphotype "Big"	Tyr	LCB PC	102.3	135.2	0.0068
L. crispatus VPI 3199 morphotype "Big"	Tyr	LCB PC	103.9	141.6	0.0067
L. crispatus VPI 3199 morphotype "Big"	Tyr	LCB PC	103.9	153.6	0.0067
L. crispatus VPI 3199 morphotype "Big"	Tyr	LCB PC	105.6	180.7	0.0066
L. crispatus VPI 3199 morphotype "Big"	Tyr	LCB PC	108.6	184.4	0.0064
L. crispatus VPI 3199 morphotype "Big"	Tyr	LCB PC	117.2	184.4	0.0059
L. crispatus VPI 3199 morphotype "Big"	Tyr	LCB PC	117.7	192.1	0.0059
L. crispatus VPI 3199 morphotype "Big"	Tyr	LCB PC	119.3	208.8	0.0058
L. crispatus VPI 3199 morphotype "Big"	SP	LCB PC	98.4	101.8	0.0070
L. crispatus VPI 3199 morphotype "Big"	SP	LCB PC	98.9	104.7	0.0070
L. crispatus VPI 3199 morphotype "Big"	SP	LCB PC	99.7	108.8	0.0070
L. crispatus VPI 3199 morphotype "Big"	SP	LCB PC	99.8	110.1	0.0069
L. crispatus VPI 3199 morphotype "Big"	SP	LCB PC	101.0	114.8	0.0069
L. crispatus VPI 3199 morphotype "Big"	SP	LCB PC	103.8	117.6	0.0067
L. crispatus VPI 3199 morphotype "Big"	SP	LCB PC	104.5	125.1	0.0066
L. crispatus VPI 3199 morphotype "Big"	SP	LCB PC	106.0	169.1	0.0065
L. crispatus VPI 3199 morphotype "Big"	SP	LCB PC	110.3	180.1	0.0063
L. crispatus VPI 3199 morphotype "Big"	SP	186uM SP	101.8	119.2	0.0068
L. crispatus VPI 3199 morphotype "Big"	SP	186uM SP	104.5	119.5	0.0066
L. crispatus VPI 3199 morphotype "Big"	SP	186uM SP	111.1	123.7	0.0062
L. crispatus VPI 3199 morphotype "Big"	SP	186uM SP	113.0	125.8	0.0061
L. crispatus VPI 3199 morphotype "Big"	SP	186uM SP	113.9	127.2	0.0061
L. crispatus VPI 3199 morphotype "Big"	SP	186uM SP	115.6	132.7	0.0060

L. crispatus VPI 3199 morphotype "Big"	SP	186uM SP	116.0	143.4	0.0060
L. crispatus VPI 3199 morphotype "Big"	SP	186uM SP	117.3	190.5	0.0059
L. crispatus VPI 3199 morphotype "Big"	SP	186uM SP	118.7	196.9	0.0058
L. jensenii 62G	Cad	4990uM Cad	107.7	185.4	0.0064
L. jensenii 62G	Cad	4990uM Cad	110.8	200.0	0.0063
L. jensenii 62G	Cad	4990uM Cad	110.9	202.9	0.0063
L. jensenii 62G	Cad	4990uM Cad	112.3	203.6	0.0062
L. jensenii 62G	Cad	4990uM Cad	116.0	204.9	0.0060
L. jensenii 62G	Cad	4990uM Cad	116.0	221.5	0.0060
L. jensenii 62G	Cad	4990uM Cad	116.4	221.5	0.0060
L. jensenii 62G	Cad	4990uM Cad	118.2	225.9	0.0059
L. jensenii 62G	Cad	4990uM Cad	119.3	231.6	0.0058
L. jensenii 62G	Cad	LJ PC	99.2	101.9	0.0070
L. jensenii 62G	Cad	LJ PC	99.5	112.1	0.0070
L. jensenii 62G	Cad	LJ PC	99.8	112.4	0.0069
L. jensenii 62G	Cad	LJ PC	102.6	116.6	0.0068
L. jensenii 62G	Cad	LJ PC	105.7	119.1	0.0066
L. jensenii 62G	Cad	LJ PC	108.6	119.8	0.0064
L. jensenii 62G	Cad	LJ PC	109.3	122.9	0.0063
L. jensenii 62G	Cad	LJ PC	114.7	128.8	0.0060
L. jensenii 62G	Cad	LJ PC	116.6	129.0	0.0059
L. jensenii 62G	Put	3179uM Put	84.4	163.9	0.0082
L. jensenii 62G	Put	3179uM Put	84.9	167.9	0.0082
L. jensenii 62G	Put	3179uM Put	86.0	173.6	0.0081
L. jensenii 62G	Put	3179uM Put	87.4	174.2	0.0079
L. jensenii 62G	Put	3179uM Put	87.5	180.6	0.0079
L. jensenii 62G	Put	3179uM Put	88.9	192.5	0.0078
L. jensenii 62G	Put	3179uM Put	89.5	201.3	0.0077
L. jensenii 62G	Put	3179uM Put	91.2	201.3	0.0076
L. jensenii 62G	Put	3179uM Put	93.7	212.0	0.0074
L. jensenii 62G	Put	LJ PC	84.6	124.6	0.0082
L. jensenii 62G	Put	LJ PC	88.0	125.2	0.0079
L. jensenii 62G	Put	LJ PC	88.0	125.3	0.0079
L. jensenii 62G	Put	LJ PC	90.1	141.6	0.0077
L. jensenii 62G	Put	LJ PC	90.3	141.8	0.0077
L. jensenii 62G	Put	LJ PC	90.4	145.7	0.0077
L. jensenii 62G	Put	LJ PC	90.9	155.1	0.0076
L. jensenii 62G	Put	LJ PC	92.0	159.6	0.0075
L. jensenii 62G	Put	LJ PC	93.6	166.1	0.0074
L. jensenii 62G	SPD	59uM SPD	102.1	148.0	0.0068
L. jensenii 62G	SPD	59uM SPD	102.1	108.3	0.0068
L. jensenii 62G	SPD	59uM SPD	109.6	121.9	0.0063
L. jensenii 62G	SPD	59uM SPD	111.6	122.0	0.0062
L. jensenii 62G	SPD	59uM SPD	115.6	133.4	0.0060
L. jensenii 62G	SPD	59uM SPD	116.0	148.2	0.0060

L. jensenii 62G	SPD	59uM SPD	116.3	163.0	0.0060
L. jensenii 62G	SPD	59uM SPD	119.9	181.7	0.0058
L. jensenii 62G	SPD	59uM SPD	120.8	193.7	0.0057
L. jensenii 62G	SPD	LJ PC	100.8	138.0	0.0069
L. jensenii 62G	SPD	LJ PC	104.1	102.7	0.0067
L. jensenii 62G	SPD	LJ PC	106.4	108.7	0.0065
L. jensenii 62G	SPD	LJ PC	107.3	130.8	0.0065
L. jensenii 62G	SPD	LJ PC	107.6	140.5	0.0064
L. jensenii 62G	SPD	LJ PC	112.0	148.2	0.0062
L. jensenii 62G	SPD	LJ PC	112.8	162.2	0.0061
L. jensenii 62G	SPD	LJ PC	113.1	163.7	0.0061
L. jensenii 62G	SPD	LJ PC	115.7	169.4	0.0060
L. jensenii 62G	Tyr	813uM Tyr	101.7	162.2	0.0068
L. jensenii 62G	Tyr	813uM Tyr	102.0	171.9	0.0068
L. jensenii 62G	Tyr	813uM Tyr	103.5	172.9	0.0067
L. jensenii 62G	Tyr	813uM Tyr	104.7	176.9	0.0066
L. jensenii 62G	Tyr	813uM Tyr	108.6	179.0	0.0064
L. jensenii 62G	Tyr	813uM Tyr	109.3	184.6	0.0063
L. jensenii 62G	Tyr	813uM Tyr	113.6	190.9	0.0061
L. jensenii 62G	Tyr	813uM Tyr	114.1	198.7	0.0061
L. jensenii 62G	Tyr	813uM Tyr	115.1	205.6	0.0060
L. jensenii 62G	Tyr	LJ PC	107.8	132.8	0.0064
L. jensenii 62G	Tyr	LJ PC	103.5	133.9	0.0067
L. jensenii 62G	Tyr	LJ PC	104.7	138.8	0.0066
L. jensenii 62G	Tyr	LJ PC	109.1	140.8	0.0064
L. jensenii 62G	Tyr	LJ PC	112.2	144.7	0.0062
L. jensenii 62G	Tyr	LJ PC	115.3	158.3	0.0060
L. jensenii 62G	Tyr	LJ PC	118.0	170.6	0.0059
L. jensenii 62G	Tyr	LJ PC	118.1	180.8	0.0059
L. jensenii 62G	Tyr	LJ PC	123.0	183.0	0.0056
L. jensenii 62G	SP	LJ PC	68.6	91.6	0.0101
L. jensenii 62G	SP	LJ PC	69.0	98.0	0.0100
L. jensenii 62G	SP	LJ PC	70.6	121.4	0.0098
L. jensenii 62G	SP	LJ PC	70.6	122.1	0.0098
L. jensenii 62G	SP	LJ PC	72.5	125.5	0.0096
L. jensenii 62G	SP	LJ PC	74.0	126.6	0.0094
L. jensenii 62G	SP	LJ PC	75.9	126.9	0.0091
L. jensenii 62G	SP	LJ PC	78.3	134.9	0.0089
L. jensenii 62G	SP	LJ PC	78.9	141.0	0.0088
L. jensenii 62G	SP	186uM SP	67.6	122.4	0.0103
L. jensenii 62G	SP	186uM SP	71.8	127.3	0.0097
L. jensenii 62G	SP	186uM SP	74.7	138.9	0.0093
L. jensenii 62G	SP	186uM SP	74.8	139.4	0.0093
L. jensenii 62G	SP	186uM SP	76.6	148.5	0.0090
L. jensenii 62G	SP	186uM SP	77.3	154.6	0.0090

L. jensenii 62G	SP	186uM SP	77.3	162.3	0.0090
L. jensenii 62G	SP	186uM SP	77.6	166.6	0.0089
L. jensenii 62G	SP	186uM SP	81.5	170.5	0.0085
L. iners AB107	Cad	4990uM Cad	115.1	171.1	0.0060
L. iners AB107	Cad	4990uM Cad	116.1	173.3	0.0060
L. iners AB107	Cad	4990uM Cad	118.0	173.5	0.0059
L. iners AB107	Cad	4990uM Cad	118.4	174.7	0.0059
L. iners AB107	Cad	4990uM Cad	118.9	177.7	0.0058
L. iners AB107	Cad	4990uM Cad	119.0	178.4	0.0058
L. iners AB107	Cad	4990uM Cad	119.3	182.7	0.0058
L. iners AB107	Cad	4990uM Cad	121.5	188.8	0.0057
L. iners AB107	Cad	4990uM Cad	121.7	190.4	0.0057
L. iners AB107	Cad	LI PC	112.4	114.0	0.0062
L. iners AB107	Cad	LI PC	112.7	114.7	0.0062
L. iners AB107	Cad	LI PC	114.5	122.2	0.0061
L. iners AB107	Cad	LI PC	119.6	123.7	0.0058
L. iners AB107	Cad	LI PC	120.6	124.2	0.0057
L. iners AB107	Cad	LI PC	121.2	125.9	0.0057
L. iners AB107	Cad	LI PC	121.5	128.5	0.0057
L. iners AB107	Cad	LI PC	121.7	129.6	0.0057
L. iners AB107	Cad	LI PC	125.9	133.3	0.0055
L. iners AB107	Put	3179uM Put	163.2	181.7	0.0042
L. iners AB107	Put	3179uM Put	164.7	184.8	0.0042
L. iners AB107	Put	3179uM Put	165.8	187.0	0.0042
L. iners AB107	Put	3179uM Put	167.1	187.1	0.0041
L. iners AB107	Put	3179uM Put	168.9	188.7	0.0041
L. iners AB107	Put	3179uM Put	172.9	188.9	0.0040
L. iners AB107	Put	3179uM Put	173.2	191.2	0.0040
L. iners AB107	Put	3179uM Put	176.4	196.6	0.0039
L. iners AB107	Put	3179uM Put	185.6	199.0	0.0037
L. iners AB107	Put	LI PC	122.8	150.9	0.0056
L. iners AB107	Put	LI PC	127.0	153.5	0.0055
L. iners AB107	Put	LI PC	128.3	153.5	0.0054
L. iners AB107	Put	LI PC	128.8	155.2	0.0054
L. iners AB107	Put	LI PC	130.3	156.8	0.0053
L. iners AB107	Put	LI PC	130.3	157.2	0.0053
L. iners AB107	Put	LI PC	130.8	159.5	0.0053
L. iners AB107	Put	LI PC	132.3	159.8	0.0052
L. iners AB107	Put	LI PC	132.3	168.7	0.0052
L. iners AB107	SPD	59uM SPD	102.6	120.8	0.0068
L. iners AB107	SPD	59uM SPD	106.5	125.3	0.0065
L. iners AB107	SPD	59uM SPD	107.3	125.5	0.0065
L. iners AB107	SPD	59uM SPD	109.2	125.5	0.0063
L. iners AB107	SPD	59uM SPD	113.2	126.3	0.0061
L. iners AB107	SPD	59uM SPD	113.5	126.7	0.0061

L. iners AB107	SPD	59uM SPD	117.1	127.8	0.0059
L. iners AB107	SPD	59uM SPD	118.3	132.5	0.0059
L. iners AB107	SPD	59uM SPD	118.5	127.1	0.0059
L. iners AB107	SPD	LI PC	95.2	135.0	0.0073
L. iners AB107	SPD	LI PC	101.5	135.1	0.0068
L. iners AB107	SPD	LI PC	105.7	135.9	0.0066
L. iners AB107	SPD	LI PC	105.7	136.0	0.0066
L. iners AB107	SPD	LI PC	106.4	137.3	0.0065
L. iners AB107	SPD	LI PC	107.5	138.5	0.0064
L. iners AB107	SPD	LI PC	110.0	139.5	0.0063
L. iners AB107	SPD	LI PC	110.4	140.2	0.0063
L. iners AB107	SPD	LI PC	111.6	140.6	0.0062
L. iners AB107	Tyr	813uM Tyr	112.2	111.9	0.0062
L. iners AB107	Tyr	813uM Tyr	112.7	112.7	0.0062
L. iners AB107	Tyr	813uM Tyr	112.9	112.8	0.0061
L. iners AB107	Tyr	813uM Tyr	113.3	113.2	0.0061
L. iners AB107	Tyr	813uM Tyr	113.5	115.7	0.0061
L. iners AB107	Tyr	813uM Tyr	113.9	115.9	0.0061
L. iners AB107	Tyr	813uM Tyr	119.5	118.6	0.0058
L. iners AB107	Tyr	813uM Tyr	119.5	125.5	0.0058
L. iners AB107	Tyr	813uM Tyr	108.9	128.0	0.0064
L. iners AB107	Tyr	LI PC	108.8	140.3	0.0064
L. iners AB107	Tyr	LI PC	109.3	142.2	0.0063
L. iners AB107	Tyr	LI PC	109.3	142.5	0.0063
L. iners AB107	Tyr	LI PC	110.0	144.8	0.0063
L. iners AB107	Tyr	LI PC	112.7	147.5	0.0062
L. iners AB107	Tyr	LI PC	116.1	147.9	0.0060
L. iners AB107	Tyr	LI PC	116.2	148.6	0.0060
L. iners AB107	Tyr	LI PC	116.4	151.0	0.0060
L. iners AB107	Tyr	LI PC	116.7	151.9	0.0059
L. iners AB107	SP	LI PC	118.1	154.9	0.0059
L. iners AB107	SP	LI PC	120.0	160.0	0.0058
L. iners AB107	SP	LI PC	120.9	160.4	0.0057
L. iners AB107	SP	LI PC	121.9	163.9	0.0057
L. iners AB107	SP	LI PC	122.6	164.4	0.0057
L. iners AB107	SP	LI PC	124.0	164.5	0.0056
L. iners AB107	SP	LI PC	125.3	164.6	0.0055
L. iners AB107	SP	LI PC	125.8	166.3	0.0055
L. iners AB107	SP	LI PC	125.9	167.8	0.0055
L. iners AB107	SP	186uM SP	113.1	144.9	0.0061
L. iners AB107	SP	186uM SP	122.7	145.6	0.0057
L. iners AB107	SP	186uM SP	122.9	153.5	0.0056
L. iners AB107	SP	186uM SP	122.9	155.0	0.0056
L. iners AB107	SP	186uM SP	124.4	155.8	0.0056
L. iners AB107	SP	186uM SP	124.9	158.9	0.0055

L. iners AB107	SP	186uM SP	127.0	159.1	0.0055
L. iners AB107	SP	186uM SP	132.6	159.4	0.0052
L. iners AB107	SP	186uM SP	138.1	159.4	0.0050

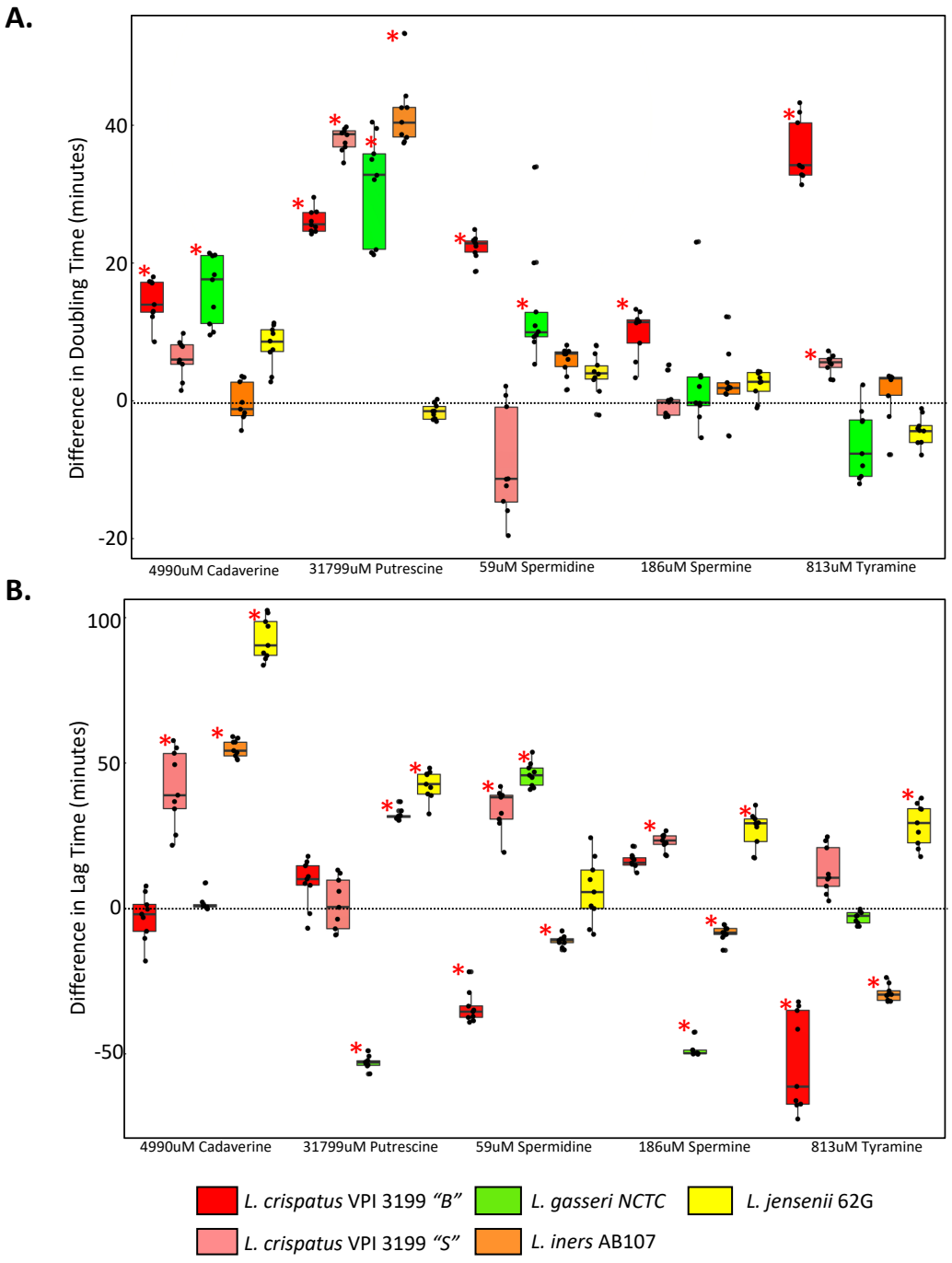

Figure S1| Effect of biogenic amines on the growth of vaginal *Lactobacillus* spp. Differences in A) doubling time and b) lag time between treatments and controls for each biogenic amine. Dots represent individual differences between each treatment and control.

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSION

Since 1892, the presence of *Lactobacillus* spp. within the vaginal microenvironment have been associated with reproductive and sexual health[1]. The application of molecular methods, such as 16S rRNA gene amplicon sequencing and metabolomics, to studies of the vaginal microenvironment has provided new and potentially important insights into the mechanistic underpinnings of the microbially-mediated preservation of health and dysbiosis and disease[2]. One such hypothesis from metabolomics is the potential role of a class of metabolite known as biogenic amines that may both disrupt the ability of vaginal *Lactobacillus* species to protect the vaginal microenvironment from infection and enhance the virulence of potential pathogens, including agents of sexually transmitted infections (STIs)[3]. The studies herein evaluate the association between biogenic amines and prevalent bacterial and viral STIs as well as the direct effect of exogenous BAs upon growth properties of vaginal *Lactobacillus* spp. to provide a more refined understanding of their potential role in vaginal sexual and reproductive health.

The studies reviewed in **Chapter 2**, which have employed a variety of molecular techniques, have shown that the vaginal microbiota of apparently healthy, asymptomatic, reproductive-aged women are dominated by vaginal *Lactobacillus* species while bacterial vaginosis and molecular-BV are characterized by an abundance of strict and facultative anaerobic bacteria. We now know that not all vaginal *Lactobacillus* spp. are created equal

– with some strains (*L. crispatus*) being more frequently associated with vaginal health while others (*L. iners*) are seemingly more susceptible to vaginal disorders. The competitive ability of each *Lactobacillus* spp. likely reflects different genomic capabilities with *L. crispatus* having the ability to produce bacteriocins, both D- and L-isomers of lactic acid and being able to ferment a wide variety of sugars. In contrast, *L. iners* has a limited ability to ferment sugars, does not have a known genetic capacity to produce D-lactic acid, and encodes for a variety of lysins and proteins associated with pro-inflammatory response. We also know that CST IV and BV are associated with a wide variety of reproductive disorders and molecular changes including a more diverse vaginal microbiota and an increased concentration of biogenic amines.

Utilizing both 16S rRNA gene amplicon sequencing and metabolomic based methods, we sought to assess the relationship between microbial composition, metabolites, and viral and bacterial STIs. In **Chapter 3**, we characterized the vaginal microbiota and profiled the vaginal metabolome of women who were negative for HPV-detection as well as those who were positive for both high-risk and low-risk HPV detection. Using both univariate and multivariate statistical techniques, we observed that women who were positive for HPV detection had higher concentrations of biogenic amines compared to women who were negative for HPV detection after adjusting for CST and for smoking status. We also observed increased concentrations of BAs in women with CST III (*L. iners*) microbiota who were positive for HPV detection.

In **Chapter 4**, the vaginal microbiota and metabolome of women who were positive for *Chlamydia trachomatis* (CT+) mono- and *C. trachomatis / Mycoplasma genitalium*

(CT+/MG+) co-infection as well as those who were negative for bacterial STIs were characterized using 16S rRNA gene amplicon sequencing and untargeted liquid chromatography mass spectrometry. Herein, we used a combination of linear regression and multivariate analysis and observed that CT+ and CT+/MG+ women predominantly had CST IV microbiota. Women who were positive for CT and CT+/MG+ had higher concentrations of biogenic amines including cadaverine, putrescine, and tyramine, than women who were uninfected when we did not control for CST. However, when we controlled for CST, only ethanolamine remained significant. Significant associations between infection status and age, CST, number of sexual partners, condom use, pelvic inflammatory disease, and HIV were also observed; however, CST and infection status were the only significant variables that contributed >3% of the variation to the vaginal metabolome.

In **Chapter 5**, we utilized both targeted liquid chromatography mass spectrometry and pure microbiology techniques to evaluate the effect of the most commonly detected biogenic amines in the vaginal microenvironment upon the growth properties of vaginal *Lactobacillus* spp. We observed that CST IV women had higher concentrations of cadaverine, putrescine, and tyramine than women with *Lactobacillus* spp.–dominated microbiota. We also demonstrated that each amine had varying effects upon the growth of vaginal *Lactobacillus* spp. In general, putrescine, cadaverine, and tyramine were associated with delayed growth (slower growth rates and/or longer lag times) while spermidine and spermine had more variable effects – both were associated with decreased lag time, but not growth rate, of *L. iners*. When *L. gasseri* was exposed to exogenous spermidine, it

displayed longer lag- and doubling-times. We also note that when grown in the presence of biogenic amines, vaginal lactobacilli generally produced less D- and L-lactic acid isomers. The notable exceptions were higher levels of D-lactic acid by *L. crispatus* "big" and L-lactic acid by *L. crispatus "small"* when grown in the presence of spermine.

Collectively, these data provide evidence that BAs directly affect specific growth properties, including the production of lactic acid, of vaginal *Lactobacillus* spp. Differences in growth properties (increased lag time, slower growth rates, and decreased production of lactic acid) may represent decreased competitiveness and fitness of vaginal *Lactobacillus* spp. when exposed to BAs, thus increasing ones susceptibility to colonization by anaerobic and/or potentially pathogenic bacteria. These microbiology experiments are augmented by the fact that we observe higher concentrations of amines in women with CST IV microbiota, in women with BV, and in women who are positive for viral and bacterial STIs.

These data provide the basis for a myriad of future studies. We have not evaluated the association between pertinent host behaviors such as sexual activity[4], type of sex[5], or sexual preferences[5], and biogenic amines. While we know that these host behaviors affect the vaginal microbiota, only a few studies have demonstrated the association between host behavioral factors such as smoking,[6] and douching[7] upon the biogenic amines. We also know that menses affects the vaginal microbiota[8], yet we have not evaluated the corresponding effect upon biogenic amines. Within a laboratory setting, our greatest limitation is our current inability to mimic the complex microenvironment of the vaginal tract, and observations of vaginal *Lactobacillus* spp., grown in isolation, certainly does not

imitate the composite interactions that undoubtedly occur therein. Nevertheless, these observations provide critical insight into the potential role that vaginal biogenic amines may have in disrupting vaginal colonization by *Lactobacillus* species, and in facilitating vaginal dysbiosis.

REFERENCES

1. Döderlein A. Das scheidensekret und seine bedeutung fur puerperalfieber. . Zentralblatt fur Bakteriology. 1892:11:699.

2. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011;108 Suppl 1:4680-7.

3. Nelson TM, Borgogna JL, Brotman RM, Ravel J, Walk ST, Yeoman CJ. Vaginal biogenic amines: biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis? Frontiers in physiology. 2015;6:253.

4. Vodstrcil LA, Twin J, Garland SM, Fairley CK, Hocking JS, Law MG, et al. The influence of sexual activity on the vaginal microbiota and Gardnerella vaginalis clade diversity in young women. PLoS One. 2017;12(2):e0171856.

5. Bradshaw CS, Walker SM, Vodstrcil LA, Bilardi JE, Law M, Hocking JS, et al. The Influence of Behaviors and Relationships on the Vaginal Microbiota of Women and Their Female Partners: The WOW Health Study. The Journal of Infectious Diseases. 2013;209(10):1562-72.

6. Nelson TM, Borgogna JC, Michalek RD, Roberts DW, Rath JM, Glover ED, et al. Cigarette smoking is associated with an altered vaginal tract metabolomic profile. Sci Rep. 2018;8(1):852.

7. Yeoman CJ, Thomas SM, Miller ME, Ulanov AV, Torralba M, Lucas S, et al. A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. PLoS One. 2013;8(2):e56111.

8. Hickey RJ, Abdo Z, Zhou X, Nemeth K, Hansmann M, Osborn TW, 3rd, et al. Effects of tampons and menses on the composition and diversity of vaginal microbial communities over time. BJOG. 2013;120(6):695-704; discussion -6.

REFERENCES CITED

REFERENCES CITED

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. *Sci Transl Med, 6*(237), 237ra265. doi:10.1126/scitranslmed.3008599

Aagaard, K., Riehle, K., Ma, J., Segata, N., Mistretta, T. A., Coarfa, C., . . . Versalovic, J. (2012). A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS One, 7*(6), e36466. doi:10.1371/journal.pone.0036466

Ahmed, A., Earl, J., Retchless, A., Hillier, S. L., Rabe, L. K., Cherpes, T. L., . . . Ehrlich, G. D. (2012). Comparative Genomic Analyses of 17 Clinical Isolates of Gardnerella vaginalis Provide Evidence of Multiple Genetically Isolated Clades Consistent with Subspeciation into Genovars. *Journal of Bacteriology, 194*(15), 3922. doi:10.1128/JB.00056-12

Al Kassaa, I., Hamze, M., Hober, D., Chihib, N. E., & Drider, D. (2014). Identification of vaginal lactobacilli with potential probiotic properties isolated from women in North Lebanon. *Microb Ecol, 67*(3), 722-734. doi:10.1007/s00248-014-0384-7

Albert, A. Y. K., Chaban, B., Wagner, E. C., Schellenberg, J. J., Links, M. G., van Schalkwyk, J., . . . Group, V. R. (2015). A Study of the Vaginal Microbiome in Healthy Canadian Women Utilizing cpn60-Based Molecular Profiling Reveals Distinct Gardnerella Subgroup Community State Types. *PLoS One, 10*(8), e0135620. doi:10.1371/journal.pone.0135620

Aldunate, M., Srbinovski, D., Hearps, A. C., Latham, C. F., Ramsland, P. A., Gugasyan, R., . . . Tachedjian, G. (2015). Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. *Front Physiol, 6*, 164. doi:10.3389/fphys.2015.00164

Aldunate, M., Srbinovski, D., Hearps, A. C., Latham, C. F., Ramsland, P. A., Gugasyan, R., . . . Tachedjian, G. (2015). Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. *Frontiers in Physiology, 6*(164). doi:10.3389/fphys.2015.00164

Aldunate, M., Tyssen, D., Johnson, A., Zakir, T., Sonza, S., Moench, T., . . . Tachedjian, G. (2013). Vaginal concentrations of lactic acid potently inactivate HIV. *The*

Journal of antimicrobial chemotherapy, 68(9), 2015-2025. doi:10.1093/jac/dkt156

Allsworth, J. E., & Peipert, J. F. (2007). Prevalence of Bacterial Vaginosis: 2001–2004 National Health and Nutrition Examination Survey Data. *Obstetrics & Gynecology, 109*(1), 114-120. doi:10.1097/01.Aog.0000247627.84791.91

Alnaif, B., & Drutz, H. P. (2000). Bacterial vaginosis increases in pessary users. *Int Urogynecol J Pelvic Floor Dysfunct, 11*(4), 219-222; discussion 222-213. doi:10.1007/pl00004026

Alves, P., Castro, J., Sousa, C., Cereija, T. B., & Cerca, N. (2014). Gardnerella vaginalis Outcompetes 29 Other Bacterial Species Isolated From Patients With Bacterial Vaginosis, Using in an In Vitro Biofilm Formation Model. *The Journal of Infectious Diseases, 210*(4), 593-596. doi:10.1093/infdis/jiu131

Amarasekara, R., Jayasekara, R. W., Senanayake, H., & Dissanayake, V. H. (2015). Microbiome of the placenta in pre-eclampsia supports the role of bacteria in the multifactorial cause of pre-eclampsia. *J Obstet Gynaecol Res, 41*(5), 662-669. doi:10.1111/jog.12619

The American Journal of Obstetrics and Diseases of Women and Children. (1896). *XXXIV*.

Amsel, R., Totten, P. A., Spiegel, C. A., Chen, K. C., Eschenbach, D., & Holmes, K. K. (1983). Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med, 74*(1), 14-22. doi:10.1016/0002-9343(83)91112-9

Anahtar, M. N., Byrne, E. H., Doherty, K. E., Bowman, B. A., Yamamoto, H. S., Soumillon, M., . . . Kwon, D. S. (2015). Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. *Immunity, 42*(5), 965-976. doi:10.1016/j.immuni.2015.04.019

Anderson, D. J., Marathe, J., & Pudney, J. (2014). The structure of the human vaginal stratum corneum and its role in immune defense. *Am J Reprod Immunol, 71*(6), 618-623. doi:10.1111/aji.12230

Andreu, A., Stapleton, A. E., Fennell, C. L., Hillier, S. L., & Stamm, W. E. (1995). Hemagglutination, adherence, and surface properties of vaginal Lactobacillus species. *J Infect Dis, 171*(5), 1237-1243. doi:10.1093/infdis/171.5.1237

Anton, G., Rid, J., Mylonas, I., Friese, K., & Weissenbacher, E. R. (2008). Evidence of a TH1-shift of local vaginal inflammatory response during bacterial vaginosis. *Infection, 36*(2), 147-152. doi:10.1007/s15010-007-7152-2

Antonio, M. A., Rabe, L. K., & Hillier, S. L. (2005). Colonization of the rectum by Lactobacillus species and decreased risk of bacterial vaginosis. *J Infect Dis, 192*(3), 394-398. doi:10.1086/430926

Antonio, M. A. D., Meyn, L. A., Murray, P. J., Busse, B., & Hillier, S. L. (2009). Vaginal Colonization by Probiotic Lactobacillus crispatus CTV-05 Is Decreased by Sexual Activity and Endogenous Lactobacilli. *The Journal of Infectious Diseases, 199*(10), 1506-1513. doi:10.1086/598686

Anukam, K. C., Macklaim, J. M., Gloor, G. B., Reid, G., Boekhorst, J., Renckens, B., . . . Siezen, R. J. (2013). Genome sequence of Lactobacillus pentosus KCA1: vaginal isolate from a healthy premenopausal woman. *PLoS One, 8*(3), e59239. doi:10.1371/journal.pone.0059239

Anukam, K. C., Osazuwa, E., Osemene, G. I., Ehigiagbe, F., Bruce, A. W., & Reid, G. (2006). Clinical study comparing probiotic Lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. *Microbes Infect, 8*(12-13), 2772-2776. doi:10.1016/j.micinf.2006.08.008

Arakawa, K., Kawai, Y., Iioka, H., Tanioka, M., Nishimura, J., Kitazawa, H., . . . Saito, T. (2009). Effects of gassericins A and T, bacteriocins produced by Lactobacillus gasseri, with glycine on custard cream preservation. *J Dairy Sci, 92*(6), 2365-2372. doi:10.3168/jds.2008-1240

Aroutcheva, A., Gariti, D., Simon, M., Shott, S., Faro, J., Simoes, J. A., . . . Faro, S. (2001). Defense factors of vaginal lactobacilli. *Am J Obstet Gynecol, 185*(2), 375-379. doi:10.1067/mob.2001.115867

Atassi, F., Brassart, D., Grob, P., Graf, F., & Servin, A. L. (2006a). Lactobacillus strains isolated from the vaginal microbiota of healthy women inhibit Prevotella bivia and Gardnerella vaginalis in coculture and cell culture. *FEMS Immunol Med Microbiol, 48*(3), 424-432. doi:10.1111/j.1574-695X.2006.00162.x

Atassi, F., Brassart, D., Grob, P., Graf, F., & Servin, A. L. (2006b). Vaginal Lactobacillus isolates inhibit uropathogenic Escherichia coli. *FEMS Microbiol Lett, 257*(1), 132-138. doi:10.1111/j.1574-6968.2006.00163.x

Atassi, F., & Servin, A. L. (2010). Individual and co-operative roles of lactic acid and hydrogen peroxide in the killing activity of enteric strain Lactobacillus johnsonii

NCC933 and vaginal strain Lactobacillus gasseri KS120.1 against enteric, uropathogenic and vaginosis-associated pathogens. *FEMS Microbiol Lett, 304*(1), 29-38. doi:10.1111/j.1574-6968.2009.01887.x

Averette, H. E., Weinstein, G. D., & Frost, P. (1970). Autoradiographic analysis of cell proliferation kinetics in human genital tissues. I. Normal cervix and vagina. *Am J Obstet Gynecol, 108*(1), 8-17. doi:10.1016/0002-9378(70)90195-x

Ayre, W. B. (1951). The glycogen-estrogen relationship in the vaginal tract. *J Clin Endocrinol Metab, 11*(1), 103-110. doi:10.1210/jcem-11-1-103

Barbieri, F., Montanari, C., Gardini, F., & Tabanelli, G. (2019). Biogenic Amine Production by Lactic Acid Bacteria: A Review. *Foods, 8*(1). doi:10.3390/foods8010017

Barfod, K. K., Roggenbuck, M., Hansen, L. H., Schjorring, S., Larsen, S. T., Sorensen, S. J., & Krogfelt, K. A. (2013). The murine lung microbiome in relation to the intestinal and vaginal bacterial communities. *BMC Microbiol, 13*, 303. doi:10.1186/1471-2180-13-303

Bautista, C. T., Wurapa, E. K., Sateren, W. B., Morris, S. M., Hollingsworth, B. P., & Sanchez, J. L. (2017). Association of Bacterial Vaginosis With Chlamydia and Gonorrhea Among Women in the U.S. Army. *American Journal of Preventive Medicine, 52*(5), 632-639. doi:https://doi.org/10.1016/j.amepre.2016.09.016

Benito, R., Vazquez, J. A., Berron, S., Fenoll, A., & Saez-Neito, J. A. (1986). A modified scheme for biotyping Gardnerella vaginalis. *J Med Microbiol, 21*(4), 357-359. doi:10.1099/00222615-21-4-357

Bergmann, S., Wild, D., Diekmann, O., Frank, R., Bracht, D., Chhatwal, G. S., & Hammerschmidt, S. (2003). Identification of a novel plasmin(ogen)-binding motif in surface displayed alpha-enolase of Streptococcus pneumoniae. *Mol Microbiol, 49*(2), 411-423. doi:10.1046/j.1365-2958.2003.03557.x

Bisanz, J. E., Enos, M. K., PrayGod, G., Seney, S., Macklaim, J. M., Chilton, S., . . . Reid, G. (2015). Microbiota at Multiple Body Sites during Pregnancy in a Rural Tanzanian Population and Effects of Moringa-Supplemented Probiotic Yogurt. *Applied and environmental microbiology, 81*(15), 4965-4975. doi:10.1128/AEM.00780-15

Blaskewicz, C. D., Pudney, J., & Anderson, D. J. (2011). Structure and function of intercellular junctions in human cervical and vaginal mucosal epithelia. *Biology of reproduction, 85*(1), 97-104. doi:10.1095/biolreprod.110.090423

Boakes, S., Ayala, T., Herman, M., Appleyard, A. N., Dawson, M. J., & Cortés, J. . (2012). Generation of an actagardine A variant library through saturation mutagenesis. *Applied Microbiology and Biotechnology, 95*, 1509-1517. doi:https://doi.org/10.1007/s00253-012-4041-0

Borgdorff, H., Armstrong, S. D., Tytgat, H. L., Xia, D., Ndayisaba, G. F., Wastling, J. M., & van de Wijgert, J. H. (2016). Unique Insights in the Cervicovaginal Lactobacillus iners and L. crispatus Proteomes and Their Associations with Microbiota Dysbiosis. *PLoS One, 11*(3), e0150767. doi:10.1371/journal.pone.0150767

Borgdorff, H., Tsivtsivadze, E., Verhelst, R., Marzorati, M., Jurriaans, S., Ndayisaba, G. F., . . . van de Wijgert, J. H. (2014). Lactobacillus-dominated cervicovaginal microbiota associated with reduced HIV/STI prevalence and genital HIV viral load in African women. *ISME J, 8*(9), 1781-1793. doi:10.1038/ismej.2014.26

Borges, B. E. S., Brito, E. B., Fuzii, H. T., Baltazar, C. S., Sa, A. B., Silva, C., . . . Pinheiro, M. (2018). Human papillomavirus infection and cervical cancer precursor lesions in women living by Amazon rivers: investigation of relations with markers of oxidative stress. *Einstein (Sao Paulo), 16*(3), eAO4190. doi:10.1590/s1679-45082018ao4190

Borgogna, J.-L. C., & Yeoman, C. J. (2017). The Application of Molecular Methods Towards an Understanding of the Role of the Vaginal Microbiome in Health and Disease. *Methods in microbiology., 44*, 37-91. doi:10.1016/bs.mim.2017.08.003

Borgogna JC, S. M., Santori EK, Nelson TM, Rath JM, Glover ED, Ravel J, Gravitt P, Yeoman CJ, Brotman RM. (2019). The vaginal metabolome and microbiota of cervical HPV-positive and HPV-negative women: a cross-sectional analysis. . *British Journal of Obstetrics & Gynecology.* (*In Press*).

Boris, S., & Barbés, C. (2000). Role played by lactobacilli in controlling the population of vaginal pathogens. *Microbes and infection / Institut Pasteur, 2*(5), 543-546. doi:papers2://publication/uuid/CA12AAA7-5393-4EC6-9AA0-9A4EEFDD4F8D

Boskey, E. R., Cone, R. A., Whaley, K. J., & Moench, T. R. (2001). Origins of vaginal acidity: high d/l lactate ratio is consistent with bacteria being the primary source. *Human Reproduction, 16*(9), 1809-1813. doi:10.1093/humrep/16.9.1809

Boskey, E. R., Telsch, K. M., Whaley, K. J., Moench, T. R., & Cone, R. A. (1999). Acid production by vaginal flora in vitro is consistent with the rate and extent of

vaginal acidification. *Infection and immunity, 67*(10), 5170-5175. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10496892

Bradshaw, C. S., Walker, S. M., Vodstrcil, L. A., Bilardi, J. E., Law, M., Hocking, J. S., . . . Fairley, C. K. (2013). The Influence of Behaviors and Relationships on the Vaginal Microbiota of Women and Their Female Partners: The WOW Health Study. *The Journal of Infectious Diseases, 209*(10), 1562-1572. doi:10.1093/infdis/jit664

Briselden, A. M., & Hillier, S. L. (1990). Longitudinal study of the biotypes of Gardnerella vaginalis. *J Clin Microbiol, 28*(12), 2761-2764. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2280007

Brotman, R. M., Bradford, L. L., Conrad, M., Gajer, P., Ault, K., Peralta, L., . . . Ravel, J. (2012). Association between Trichomonas vaginalis and vaginal bacterial community composition among reproductive-age women. *Sexually transmitted diseases, 39*(10), 807-812. doi:10.1097/OLQ.0b013e3182631c79

Brotman, R. M., Ghanem, K. G., Klebanoff, M. A., Taha, T. E., Scharfstein, D. O., & Zenilman, J. M. (2008). The effect of vaginal douching cessation on bacterial vaginosis: a pilot study. *American journal of obstetrics and gynecology, 198*(6), 628.e621-628.e6287. doi:10.1016/j.ajog.2007.11.043

Brotman, R. M., He, X., Gajer, P., Fadrosh, D., Sharma, E., Mongodin, E. F., . . . Rath, J. M. (2014). Association between cigarette smoking and the vaginal microbiota: a pilot study. *BMC infectious diseases, 14*, 471-471. doi:10.1186/1471-2334-14-471

Brotman, R. M., Klebanoff, M. A., Nansel, T. R., Yu, K. F., Andrews, W. W., Zhang, J., & Schwebke, J. R. (2010). Bacterial vaginosis assessed by gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. *The Journal of Infectious Diseases, 202*(12), 1907-1915. doi:10.1086/657320

Brotman, R. M., Ravel, J., Cone, R. A., & Zenilman, J. M. (2010). Rapid fluctuation of the vaginal microbiota measured by Gram stain analysis. *Sexually transmitted infections, 86*(4), 297-302. doi:10.1136/sti.2009.040592

Brotman, R. M., Shardell, M. D., Gajer, P., Fadrosh, D., Chang, K., Silver, M. I., . . . Gravitt, P. E. (2014). Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. *Menopause (New York, N.Y.), 21*(5), 450-458. doi:10.1097/GME.0b013e3182a4690b

Brotman, R. M., Shardell, M. D., Gajer, P., Tracy, J. K., Zenilman, J. M., Ravel, J., & Gravitt, P. E. (2014). Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *The Journal of Infectious Diseases, 210*(11), 1723-1733. doi:10.1093/infdis/jiu330

Burton, J. P., Cadieux, P. A., & Reid, G. (2003). Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. *Applied and environmental microbiology, 69*(1), 97-101. doi:10.1128/aem.69.1.97-101.2003

Burton, J. P., & Reid, G. (2002). Evaluation of the bacterial vaginal flora of 20 postmenopausal women by direct (Nugent score) and molecular (polymerase chain reaction and denaturing gradient gel electrophoresis) techniques. *J Infect Dis, 186*(12), 1770-1780. doi:10.1086/345761

Busarcevic, M., & Dalgalarrondo, M. (2012). Purification and genetic characterisation of the novel bacteriocin LS2 produced by the human oral strain Lactobacillus salivarius BGHO1. *Int J Antimicrob Agents, 40*(2), 127-134. doi:10.1016/j.ijantimicag.2012.04.011

Cadieux, P. A., Burton, J., Devillard, E., & Reid, G. (2009). Lactobacillus by-products inhibit the growth and virulence of uropathogenic Escherichia coli. *J Physiol Pharmacol, 60 Suppl 6*, 13-18. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20224146

Chaban, B., Links, M. G., Jayaprakash, T. P., Wagner, E. C., Bourque, D. K., Lohn, Z., . . . Money, D. M. (2014). Characterization of the vaginal microbiota of healthy Canadian women through the menstrual cycle. *Microbiome, 2*, 23-23. doi:10.1186/2049-2618-2-23

Chattopadhyay, M. K., Tabor, C. W., & Tabor, H. (2003). Polyamines protect Escherichia coli cells from the toxic effect of oxygen. *Proc Natl Acad Sci U S A, 100*(5), 2261-2265. doi:10.1073/pnas.2627990100

Chen, K. C., Amsel, R., Eschenbach, D. A., & Holmes, K. K. (1982). Biochemical diagnosis of vaginitis: determination of diamines in vaginal fluid. *J Infect Dis, 145*(3), 337-345. doi:10.1093/infdis/145.3.337

Chen, K. C., Forsyth, P. S., Buchanan, T. M., & Holmes, K. K. (1979). Amine content of vaginal fluid from untreated and treated patients with nonspecific vaginitis. *J Clin Invest, 63*(5), 828-835. doi:10.1172/JCI109382

Cherpes, T. L., Hillier, S. L., Meyn, L. A., Busch, J. L., & Krohn, M. A. (2008). A delicate balance: risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race, and

positive herpes simplex virus type 2 serology. *Sexually transmitted diseases, 35*(1), 78-83. doi:10.1097/OLQ.0b013e318156a5d0

Cherpes, T. L., Meyn, L. A., Krohn, M. A., Lurie, J. G., & Hillier, S. L. (2003). Association between Acquisition of Herpes Simplex Virus Type 2 in Women and Bacterial Vaginosis. *Clinical Infectious Diseases, 37*(3), 319-325. doi:10.1086/375819

Clish, C. B. (2015). Metabolomics: an emerging but powerful tool for precision medicine. *Cold Spring Harb Mol Case Stud, 1*(1), a000588. doi:10.1101/mcs.a000588

Cocchiaro, J. L., & Valdivia, R. H. (2009). New insights into Chlamydia intracellular survival mechanisms. *Cell Microbiol, 11*(11), 1571-1578. doi:10.1111/j.1462-5822.2009.01364.x

Cohen, C. R., Lingappa, J. R., Baeten, J. M., Ngayo, M. O., Spiegel, C. A., Hong, T., . . . Bukusi, E. A. (2012). Bacterial vaginosis associated with increased risk of female-to-male HIV-1 transmission: a prospective cohort analysis among African couples. *PLoS medicine, 9*(6), e1001251-e1001251. doi:10.1371/journal.pmed.1001251

Cohen, M. S., Black, J. R., Proctor, R. A., & Sparling, P. F. (1984). Host defences and the vaginal mucosa. A re-evaluation. *Scand J Urol Nephrol Suppl, 86*, 13-22. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6399405

Collado, M. C., Rautava, S., Aakko, J., Isolauri, E., & Salminen, S. (2016). Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Scientific Reports, 6*(1), 23129. doi:10.1038/srep23129

Collins, S., Beigi, R., Mellen, C., O'Sullivan, D., & Tulikangas, P. (2015). The effect of pessaries on the vaginal microenvironment. *Am J Obstet Gynecol, 212*(1), 60 e61-66. doi:10.1016/j.ajog.2014.07.024

Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol, 3*(10), 777-788. doi:10.1038/nrmicro1273

Cotter, P. D., Ross, R. P., & Hill, C. (2013). Bacteriocins - a viable alternative to antibiotics? *Nat Rev Microbiol, 11*(2), 95-105. doi:10.1038/nrmicro2937

Cox, R. A., & Garcia-Palmieri, M. R. (1990). Cholesterol, Triglycerides, and Associated Lipoproteins. In rd, H. K. Walker, W. D. Hall, & J. W. Hurst (Eds.), *Clinical Methods: The History, Physical, and Laboratory Examinations*. Boston.

Craciun, S., & Balskus, E. P. (2012). Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. *Proc Natl Acad Sci U S A, 109*(52), 21307-21312. doi:10.1073/pnas.1215689109

Cruden, D. L., & Galask, R. P. (1988). Reduction of Trimethylamine Oxide to Trimethylamine by Mobiluncus Strains Isolated from Patients with Bacterial Vaginosis. *Microbial Ecology in Health and Disease, 1*(2), 95-100. doi:10.3109/08910608809140187

Cunningham-Rundles, S., & Maas, W. K. (1975). Isolation, characterization, and mapping of Escherichia coli mutants blocked in the synthesis of ornithine decarboxylase. *J Bacteriol, 124*(2), 791-799. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1102531

Czaja, C. A., Stapleton, A. E., Yarova-Yarovaya, Y., & Stamm, W. E. (2007). Phase I trial of a Lactobacillus crispatus vaginal suppository for prevention of recurrent urinary tract infection in women. *Infect Dis Obstet Gynecol, 2007*, 35387. doi:10.1155/2007/35387

Damelin, L. H., Paximadis, M., Mavri-Damelin, D., Birkhead, M., Lewis, D. A., & Tiemessen, C. T. (2011). Identification of predominant culturable vaginal Lactobacillus species and associated bacteriophages from women with and without vaginal discharge syndrome in South Africa. *J Med Microbiol, 60*(Pt 2), 180-183. doi:10.1099/jmm.0.024463-0

Danielsson, D., Teigen, P. K., & Moi, H. (2011). The genital econiche: focus on microbiota and bacterial vaginosis. *Ann N Y Acad Sci, 1230*, 48-58. doi:10.1111/j.1749-6632.2011.06041.x

Dareng, E. O., Ma, B., Famooto, A. O., Adebamowo, S. N., Offiong, R. A., Olaniyan, O., . . . Adebamowo, C. A. (2016). Prevalent high-risk HPV infection and vaginal microbiota in Nigerian women. *Epidemiol Infect, 144*(1), 123-137. doi:10.1017/S0950268815000965

De Backer, E., Verhelst, R., Verstraelen, H., Alqumber, M. A., Burton, J. P., Tagg, J. R., . . . Vaneechoutte, M. (2007). Quantitative determination by real-time PCR of four vaginal Lactobacillus species, Gardnerella vaginalis and Atopobium vaginae indicates an inverse relationship between L. gasseri and L. iners. *BMC Microbiol, 7*, 115. doi:10.1186/1471-2180-7-115

De Marco, F. (2013). Oxidative stress and HPV carcinogenesis. *Viruses, 5*(2), 708-731. doi:10.3390/v5020708

de Winter, J. C. F. (2013). Using the Student's t-test with extremely small sample sizes. *Pract. Assess., Res. Eval., 18*, 1-12.

Deveau, H., Garneau, J. E., & Moineau, S. (2010). CRISPR/Cas system and its role in phage-bacteria interactions. *Annu Rev Microbiol, 64*, 475-493. doi:10.1146/annurev.micro.112408.134123

Di Paola, M., Sani, C., Clemente, A. M., Iossa, A., Perissi, E., Castronovo, G., . . . Torcia, M. G. (2017). Characterization of cervico-vaginal microbiota in women developing persistent high-risk Human Papillomavirus infection. *Sci Rep, 7*(1), 10200. doi:10.1038/s41598-017-09842-6

DiGiulio, D. B., Callahan, B. J., McMurdie, P. J., Costello, E. K., Lyell, D. J., Robaczewska, A., . . . Relman, D. A. (2015). Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci U S A, 112*(35), 11060-11065. doi:10.1073/pnas.1502875112

Ding, S. Z., O'Hara, A. M., Denning, T. L., Dirden-Kramer, B., Mifflin, R. C., Reyes, V. E., . . . Crowe, S. E. (2004). Helicobacter pylori and H2O2 increase AP endonuclease-1/redox factor-1 expression in human gastric epithelial cells. *Gastroenterology, 127*(3), 845-858. doi:10.1053/j.gastro.2004.06.017

Dingens, A. S., Fairfortune, T. S., Reed, S., & Mitchell, C. (2016). Bacterial vaginosis and adverse outcomes among full-term infants: a cohort study. *BMC Pregnancy Childbirth, 16*(1), 278. doi:10.1186/s12884-016-1073-y

Dobierzewska, A., Soman, S., Illanes, S. E., & Morris, A. J. (2017). Plasma cross-gestational sphingolipidomic analyses reveal potential first trimester biomarkers of preeclampsia. *PLoS One, 12*(4), e0175118. doi:10.1371/journal.pone.0175118

Döderlein, A. (1892). Das scheidensekret und seine bedeutung fur puerperalfieber. . *Zentralblatt fur Bakteriology*, 11:699.

Doerflinger, S. Y., Throop, A. L., & Herbst-Kralovetz, M. M. (2014). Bacteria in the vaginal microbiome alter the innate immune response and barrier properties of the human vaginal epithelia in a species-specific manner. *J Infect Dis, 209*(12), 1989-1999. doi:10.1093/infdis/jiu004

Dols, J. A., Reid, G., Kort, R., Schuren, F. H., Tempelman, H., Bontekoe, T. R., . . . Boon, M. E. (2012). PCR-based identification of eight Lactobacillus species and

18 hr-HPV genotypes in fixed cervical samples of South African women at risk of HIV and BV. *Diagn Cytopathol, 40*(6), 472-477. doi:10.1002/dc.21786

Dols, J. A. M., Reid, G., Brown, J. M., Tempelman, H., Bontekoe, T. R., Quint, W. G. V., & Boon, M. E. (2012). HPV Type Distribution and Cervical Cytology among HIV-Positive Tanzanian and South African Women. *ISRN obstetrics and gynecology, 2012*, 514146-514146. doi:10.5402/2012/514146

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A, 107*(26), 11971-11975. doi:10.1073/pnas.1002601107

Dong, X. D., Li, X. R., Luan, J. J., Liu, X. F., Peng, J., Luo, Y. Y., & Liu, C. J. (2015). Bacterial communities in neonatal feces are similar to mothers' placentae. *Can J Infect Dis Med Microbiol, 26*(2), 90-94. doi:10.1155/2015/737294

Donia, M. S., Cimermancic, P., Schulze, C. J., Wieland Brown, L. C., Martin, J., Mitreva, M., . . . Fischbach, M. A. (2014). A systematic analysis of biosynthetic gene clusters in the human microbiome reveals a common family of antibiotics. *Cell, 158*(6), 1402-1414. doi:10.1016/j.cell.2014.08.032

Doyle, R. M., Alber, D. G., Jones, H. E., Harris, K., Fitzgerald, F., Peebles, D., & Klein, N. (2014). Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery. *Placenta, 35*(12), 1099-1101. doi:10.1016/j.placenta.2014.10.007

Draper, D. L., Landers, D. V., Krohn, M. A., Hillier, S. L., Wiesenfeld, H. C., & Heine, R. P. (2000). Levels of vaginal secretory leukocyte protease inhibitor are decreased in women with lower reproductive tract infections. *Am J Obstet Gynecol, 183*(5), 1243-1248. doi:10.1067/mob.2000.107383

Drapkin, R., von Horsten, H. H., Lin, Y., Mok, S. C., Crum, C. P., Welch, W. R., & Hecht, J. L. (2005). Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Res, 65*(6), 2162-2169. doi:10.1158/0008-5472.CAN-04-3924

Drell, T., Lillsaar, T., Tummeleht, L., Simm, J., Aaspollu, A., Vain, E., . . . Metsis, M. (2013). Characterization of the vaginal micro- and mycobiome in asymptomatic reproductive-age Estonian women. *PLoS One, 8*(1), e54379. doi:10.1371/journal.pone.0054379

Dumonceaux, T. J., Schellenberg, J., Goleski, V., Hill, J. E., Jaoko, W., Kimani, J., . . . Severini, A. (2009). Multiplex detection of bacteria associated with normal microbiota and with bacterial vaginosis in vaginal swabs by use of oligonucleotide-coupled fluorescent microspheres. *J Clin Microbiol, 47*(12), 4067-4077. doi:10.1128/JCM.00112-09

El Aila, N. A., Tency, I., Claeys, G., Verstraelen, H., Saerens, B., Santiago, G. L. D. S., . . . Vaneechoutte, M. (2009). Identification and genotyping of bacteria from paired vaginal and rectal samples from pregnant women indicates similarity between vaginal and rectal microflora. *BMC infectious diseases, 9*, 167-167. doi:10.1186/1471-2334-9-167

Emmanuel, D. G., Madsen, K. L., Churchill, T. A., Dunn, S. M., & Ametaj, B. N. (2007). Acidosis and lipopolysaccharide from Escherichia coli B:055 cause hyperpermeability of rumen and colon tissues. *J Dairy Sci, 90*(12), 5552-5557. doi:10.3168/jds.2007-0257

Eren, A. M., Zozaya, M., Taylor, C. M., Dowd, S. E., Martin, D. H., & Ferris, M. J. (2011). Exploring the diversity of Gardnerella vaginalis in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation. *PLoS One, 6*(10), e26732. doi:10.1371/journal.pone.0026732

Eschenbach, D. A., Davick, P. R., Williams, B. L., Klebanoff, S. J., Young-Smith, K., Critchlow, C. M., & Holmes, K. K. (1989). Prevalence of hydrogen peroxide-producing Lactobacillus species in normal women and women with bacterial vaginosis. *J Clin Microbiol, 27*(2), 251-256. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2915019

Eschenbach, D. A., Patton, D. L., Hooton, T. M., Meier, A. S., Stapleton, A., Aura, J., & Agnew, K. (2001). Effects of vaginal intercourse with and without a condom on vaginal flora and vaginal epithelium. *J Infect Dis, 183*(6), 913-918. doi:10.1086/319251

Eschenbach, D. A., Patton, D. L., Meier, A., Thwin, S. S., Aura, J., Stapleton, A., & Hooton, T. M. (2000). Effects of oral contraceptive pill use on vaginal flora and vaginal epithelium. *Contraception, 62*(3), 107-112. doi:10.1016/s0010-7824(00)00155-4

Eschenbach, D. A., Thwin, S. S., Patton, D. L., Hooton, T. M., Stapleton, A. E., Agnew, K., . . . Stamm, W. E. (2000). Influence of the normal menstrual cycle on vaginal tissue, discharge, and microflora. *Clin Infect Dis, 30*(6), 901-907. doi:10.1086/313818

Falsen, E., Pascual, C., Sjoden, B., Ohlen, M., & Collins, M. D. (1999). Phenotypic and phylogenetic characterization of a novel Lactobacillus species from human sources: description of Lactobacillus iners sp. nov. *Int J Syst Bacteriol, 49 Pt 1*, 217-221. doi:10.1099/00207713-49-1-217

Farage, M., & Maibach, H. (2006). Lifetime changes in the vulva and vagina. *Arch Gynecol Obstet, 273*(4), 195-202. doi:10.1007/s00404-005-0079-x

Farage, M. A., & Maibach, H. I. (2011). Morphology and physiological changes of genital skin and mucosa. *Curr Probl Dermatol, 40*, 9-19. doi:10.1159/000321042

Farage, M. A., Miller, K. W., & Sobel, J. D. (2010). Dynamics of the Vaginal Ecosystem—Hormonal Influences. *Infectious Diseases: Research and Treatment, 3*, IDRT.S3903. doi:10.4137/IDRT.S3903

Ferris, M. J., Masztal, A., Aldridge, K. E., Fortenberry, J. D., Fidel, P. L., Jr., & Martin, D. H. (2004). Association of Atopobium vaginae, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. *BMC infectious diseases, 4*, 5. doi:10.1186/1471-2334-4-5

Ferris, M. J., Norori, J., Zozaya-Hinchliffe, M., & Martin, D. H. (2007). Cultivation-independent analysis of changes in bacterial vaginosis flora following metronidazole treatment. *J Clin Microbiol, 45*(3), 1016-1018. doi:10.1128/JCM.02085-06

Fettweis, J. M., Serrano, M. G., Sheth, N. U., Mayer, C. M., Glascock, A. L., Brooks, J. P., . . . Buck, G. A. (2012). Species-level classification of the vaginal microbiome. *BMC Genomics, 13 Suppl 8*, S17. doi:10.1186/1471-2164-13-S8-S17

Fichorova, R. N., Beatty, N., Sassi, R. R., Yamamoto, H. S., Allred, E. N., Leviton, A., & Investigators, E. (2015). Systemic inflammation in the extremely low gestational age newborn following maternal genitourinary infections. *Am J Reprod Immunol, 73*(2), 162-174. doi:10.1111/aji.12313

Fichorova, R. N., Onderdonk, A. B., Yamamoto, H., Delaney, M. L., DuBois, A. M., Allred, E., . . . Extremely Low Gestation Age Newborns Study, I. (2011). Maternal microbe-specific modulation of inflammatory response in extremely low-gestational-age newborns. *MBio, 2*(1), e00280-00210. doi:10.1128/mBio.00280-10

Field, N., Cohen, T., Struelens, M. J., Palm, D., Cookson, B., Glynn, J. R., . . . Abubakar, I. (2014). Strengthening the Reporting of Molecular Epidemiology for Infectious

Diseases (STROME-ID): an extension of the STROBE statement. *Lancet Infect Dis, 14*(4), 341-352. doi:10.1016/S1473-3099(13)70324-4

Fontaine, E. A., Claydon, E., & Taylor-Robinson, D. (1996). Lactobacilli from Women With or Without Bacterial Vaginosis and Observations on the Significance of Hydrogen Peroxide. *Microbial Ecology in Health and Disease, 9*(4), 135-141. doi:10.3109/08910609609166454

Forney, L. J., Gajer, P., Williams, C. J., Schneider, G. M., Koenig, S. S., McCulle, S. L., . . . Ravel, J. (2010). Comparison of self-collected and physician-collected vaginal swabs for microbiome analysis. *J Clin Microbiol, 48*(5), 1741-1748. doi:10.1128/JCM.01710-09

France, M. T., Mendes-Soares, H., & Forney, L. J. (2016). Genomic Comparisons of Lactobacillus crispatus and Lactobacillus iners Reveal Potential Ecological Drivers of Community Composition in the Vagina. *Applied and environmental microbiology, 82*(24), 7063-7073. doi:10.1128/AEM.02385-16

Frank, D. N., Manigart, O., Leroy, V., Meda, N., Valea, D., Zhang, W., . . . Janoff, E. N. (2012). Altered vaginal microbiota are associated with perinatal mother-to-child transmission of HIV in African women from Burkina Faso. *J Acquir Immune Defic Syndr, 60*(3), 299-306. doi:10.1097/QAI.0b013e31824e4bdb

Fredricks, D. N., Fiedler, T. L., & Marrazzo, J. M. (2005). Molecular identification of bacteria associated with bacterial vaginosis. *N Engl J Med, 353*(18), 1899-1911. doi:10.1056/NEJMoa043802

Fredricks, D. N., Fiedler, T. L., Thomas, K. K., Oakley, B. B., & Marrazzo, J. M. (2007). Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis. *J Clin Microbiol, 45*(10), 3270-3276. doi:10.1128/JCM.01272-07

Gajer, P., Brotman, R. M., Bai, G., Sakamoto, J., Schutte, U. M., Zhong, X., . . . Ravel, J. (2012). Temporal dynamics of the human vaginal microbiota. *Sci Transl Med, 4*(132), 132ra152. doi:10.1126/scitranslmed.3003605

Gallo, V., Egger, M., McCormack, V., Farmer, P. B., Ioannidis, J. P., Kirsch-Volders, M., . . . Vineis, P. (2012). STrengthening the Reporting of OBservational studies in Epidemiology - Molecular Epidemiology (STROBE-ME): an extension of the STROBE statement. *Eur J Clin Invest, 42*(1), 1-16. doi:10.1111/j.1365-2362.2011.02561.x

Gao, W., Weng, J., Gao, Y., & Chen, X. (2013). Comparison of the vaginal microbiota diversity of women with and without human papillomavirus infection: a cross-sectional study. *BMC infectious diseases, 13*, 271. doi:10.1186/1471-2334-13-271

Gardner, H. L., & Dukes, C. D. (1954). New etiologic agent in nonspecific bacterial vaginitis. *Science, 120*(3125), 853. doi:10.1126/science.120.3125.853

Gaydos, C., Maldeis, N. E., Hardick, A., Hardick, J., & Quinn, T. C. (2009). Mycoplasma genitalium as a contributor to the multiple etiologies of cervicitis in women attending sexually transmitted disease clinics. *Sexually transmitted diseases, 36*(10), 598-606. doi:10.1097/OLQ.0b013e3181b01948

Ghartey, J. P., Smith, B. C., Chen, Z., Buckley, N., Lo, Y., Ratner, A. J., . . . Burk, R. D. (2014). Lactobacillus crispatus dominant vaginal microbiome is associated with inhibitory activity of female genital tract secretions against Escherichia coli. *PLoS One, 9*(5), e96659. doi:10.1371/journal.pone.0096659

Gibbs, R. S. (2007). Asymptomatic bacterial vaginosis: is it time to treat? *Am J Obstet Gynecol, 196*(6), 495-496. doi:10.1016/j.ajog.2007.04.001

Gillet, E., Meys, J. F., Verstraelen, H., Bosire, C., De Sutter, P., Temmerman, M., & Broeck, D. V. (2011). Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. *BMC infectious diseases, 11*, 10. doi:10.1186/1471-2334-11-10

Gillissen, G., Schumacher, M., & Breuer-Werle, M. (1991). Modulation of antimicrobial effects of beta-lactams by amino acids in vitro. *Zentralbl Bakteriol, 275*(2), 223-232. doi:10.1016/s0934-8840(11)80069-1

Ginkel, P. D., Soper, D. E., Bump, R. C., & Dalton, H. P. (1993). Vaginal flora in postmenopausal women: the effect of estrogen replacement. *Infect Dis Obstet Gynecol, 1*(2), 94-97. doi:10.1155/S1064744993000225

Godoy-Vitorino, F., Ortiz-Morales, G., Romaguera, J., Sanchez, M. M., Martinez-Ferrer, M., & Chorna, N. (2018). Discriminating high-risk cervical Human Papilloma Virus infections with urinary biomarkers via non-targeted GC-MS-based metabolomics. *PLoS One, 13*(12), e0209936. doi:10.1371/journal.pone.0209936

Gong, Z., Luna, Y., Yu, P., & Fan, H. (2014). Lactobacilli inactivate Chlamydia trachomatis through lactic acid but not H2O2. *PLoS One, 9*(9), e107758. doi:10.1371/journal.pone.0107758

Gong, Z., Tang, M. M., Wu, X., Phillips, N., Galkowski, D., Jarvis, G. A., & Fan, H. (2016). Arginine- and Polyamine-Induced Lactic Acid Resistance in Neisseria gonorrhoeae. *PLoS One, 11*(1), e0147637. doi:10.1371/journal.pone.0147637

Goytia, M., Dhulipala, V. L., & Shafer, W. M. (2013). Spermine impairs biofilm formation by Neisseria gonorrhoeae. *FEMS Microbiol Lett, 343*(1), 64-69. doi:10.1111/1574-6968.12130

Gregoire, A. T., Kandil, O., & Ledger, W. J. (1971). The glycogen content of human vaginal epithelial tissue. *Fertil Steril, 22*(1), 64-68. doi:10.1016/s0015-0282(16)37989-4

Guirard, B. M., & Snell, E. E. (1964). Effect of Polyamine Structure on Growth Stimulation and Spermine and Spermidine Content of Lactic Acid Bacteria. *J Bacteriol, 88*, 72-80. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14197908

Gupta, S., Kumar, N., Singhal, N., Kaur, R., & Manektala, U. (2006). Vaginal microflora in postmenopausal women on hormone replacement therapy. *Indian J Pathol Microbiol, 49*(3), 457-461. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17001923

Hall, B. G., Acar, H., Nandipati, A., & Barlow, M. (2014). Growth rates made easy. *Mol Biol Evol, 31*(1), 232-238. doi:10.1093/molbev/mst187

Hamilton, R. J. (1995). *Waxes : chemistry, molecular biology and functions*: Oily Press.

Hammerschlag, M. R., Alpert, S., Onderdonk, A. B., Thurston, P., Drude, E., McCormack, W. M., & Bartlett, J. G. (1978). Anaerobic microflora of the vagina in children. *Am J Obstet Gynecol, 131*(8), 853-856. doi:10.1016/s0002-9378(16)33130-1

Hammerschlag, M. R., Alpert, S., Rosner, I., Thurston, P., Semine, D., McComb, D., & McCormack, W. M. (1978). Microbiology of the vagina in children: normal and potentially pathogenic organisms. *Pediatrics, 62*(1), 57-62. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/98750

Hammes, W. P., & Hertel, C. (2015). Lactobacillus. *Bergey's Manual of Systematics of Archaea and Bacteria*, 1-76. doi:doi:10.1002/9781118960608.gbm00604

10.1002/9781118960608.gbm00604

Harwich, M. D., Jr., Alves, J. M., Buck, G. A., Strauss, J. F., 3rd, Patterson, J. L., Oki, A. T., . . . Jefferson, K. K. (2010). Drawing the line between commensal and

pathogenic Gardnerella vaginalis through genome analysis and virulence studies. *BMC Genomics, 11*, 375. doi:10.1186/1471-2164-11-375

Hawes, S. E., Hillier, S. L., Benedetti, J., Stevens, C. E., Koutsky, L. A., Wolner-Hanssen, P., & Holmes, K. K. (1996). Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. *J Infect Dis, 174*(5), 1058-1063. doi:10.1093/infdis/174.5.1058

Hay, P. E., Lamont, R. F., Taylor-Robinson, D., Morgan, D. J., Ison, C., & Pearson, J. (1994). Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. *BMJ, 308*(6924), 295-298. doi:10.1136/bmj.308.6924.295

Hecht, J. L., Onderdonk, A., Delaney, M., Allred, E. N., Kliman, H. J., Zambrano, E., . . . Investigators, E. S. (2008). Characterization of chorioamnionitis in 2nd-trimester C-section placentas and correlation with microorganism recovery from subamniotic tissues. *Pediatr Dev Pathol, 11*(1), 15-22. doi:10.2350/07-06-0285.1

Hedges, S. R., Barrientes, F., Desmond, R. A., & Schwebke, J. R. (2006). Local and systemic cytokine levels in relation to changes in vaginal flora. *J Infect Dis, 193*(4), 556-562. doi:10.1086/499824

Hemmerling, A., Harrison, W., Schroeder, A., Park, J., Korn, A., Shiboski, S., & Cohen, C. R. (2009). Phase 1 dose-ranging safety trial of Lactobacillus crispatus CTV-05 for the prevention of bacterial vaginosis. *Sexually transmitted diseases, 36*(9), 564-569. doi:10.1097/OLQ.0b013e3181a74924

Hemmerling, A., Harrison, W., Schroeder, A., Park, J., Korn, A., Shiboski, S., . . . Cohen, C. R. (2010). Phase 2a study assessing colonization efficiency, safety, and acceptability of Lactobacillus crispatus CTV-05 in women with bacterial vaginosis. *Sexually transmitted diseases, 37*(12), 745-750. doi:10.1097/OLQ.0b013e3181e50026

Henderson, B., & Martin, A. (2011). Bacterial virulence in the moonlight: multitasking bacterial moonlighting proteins are virulence determinants in infectious disease. *Infection and immunity, 79*(9), 3476-3491. doi:10.1128/IAI.00179-11

Hernandez-Rodriguez, C., Romero-Gonzalez, R., Albani-Campanario, M., Figueroa-Damian, R., Meraz-Cruz, N., & Hernandez-Guerrero, C. (2011). Vaginal microbiota of healthy pregnant Mexican women is constituted by four Lactobacillus species and several vaginosis-associated bacteria. *Infect Dis Obstet Gynecol, 2011*, 851485. doi:10.1155/2011/851485

Hickey, R. J., Abdo, Z., Zhou, X., Nemeth, K., Hansmann, M., Osborn, T. W., 3rd, . . . Forney, L. J. (2013). Effects of tampons and menses on the composition and diversity of vaginal microbial communities over time. *BJOG, 120*(6), 695-704; discussion 704-696. doi:10.1111/1471-0528.12151

Hickey, R. J., Zhou, X., Settles, M. L., Erb, J., Malone, K., Hansmann, M. A., . . . Forney, L. J. (2015). Vaginal microbiota of adolescent girls prior to the onset of menarche resemble those of reproductive-age women. *MBio, 6*(2). doi:10.1128/mBio.00097-15

Hillier, S. L., Krohn, M. A., Klebanoff, S. J., & Eschenbach, D. A. (1992). The relationship of hydrogen peroxide-producing lactobacilli to bacterial vaginosis and genital microflora in pregnant women. *Obstet Gynecol, 79*(3), 369-373. doi:10.1097/00006250-199203000-00008

Hillier, S. L., Krohn, M. A., Nugent, R. P., & Gibbs, R. S. (1992). Characteristics of three vaginal flora patterns assessed by gram stain among pregnant women. Vaginal Infections and Prematurity Study Group. *Am J Obstet Gynecol, 166*(3), 938-944. doi:10.1016/0002-9378(92)91368-k

Hillier, S. L., Nugent, R. P., Eschenbach, D. A., Krohn, M. A., Gibbs, R. S., Martin, D. H., . . . et al. (1995). Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. *N Engl J Med, 333*(26), 1737-1742. doi:10.1056/NEJM199512283332604

Hillier, S. L. H., K. K. . (1999). *Bacterial Vaginosis* (3 ed.). New York: McGraw-Hill.

Hishinuma, F., Izaki, K., & Takahashi, H. (1969). Effects of Glycine and d-Amino Acids on Growth of Various Microorganisms. *Agricultural and Biological Chemistry, 33*(11), 1577-1586. doi:10.1080/00021369.1969.10859511

Hladik, F., Sakchalathorn, P., Ballweber, L., Lentz, G., Fialkow, M., Eschenbach, D., & McElrath, M. J. (2007). Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity, 26*(2), 257-270. doi:10.1016/j.immuni.2007.01.007

Holm JB, H. M., Robinson CK, Settles ML, Ott S, Fu L, Yang Hm, Gajer P, McComb E, Ghanem K, Brotman R, Ravel J. (2018). *Ultra-high throughput multiplexing and sequencing of >500 bp amplicon regions on the Illumina HiSeq 2500 platform*. bioRxiv. Retrieved from https://www.biorxiv.org/content/early/2018/09/14/417618

Huang, Y. E., Wang, Y., He, Y., Ji, Y., Wang, L. P., Sheng, H. F., . . . Zhou, H. W. (2015). Homogeneity of the vaginal microbiome at the cervix, posterior fornix, and vaginal canal in pregnant Chinese women. *Microb Ecol, 69*(2), 407-414. doi:10.1007/s00248-014-0487-1

Huggins, G. R., & Preti, G. (1976). Volatile constituents of human vaginal secretions. *Am J Obstet Gynecol, 126*(1), 129-136. doi:10.1016/0002-9378(76)90477-4

Human Microbiome Project, C. (2012). Structure, function and diversity of the healthy human microbiome. *Nature, 486*(7402), 207-214. doi:10.1038/nature11234

Hummelen, R., Fernandes, A. D., Macklaim, J. M., Dickson, R. J., Changalucha, J., Gloor, G. B., & Reid, G. (2010). Deep sequencing of the vaginal microbiota of women with HIV. *PLoS One, 5*(8), e12078. doi:10.1371/journal.pone.0012078

Hunter, C. A., Jr., Long, K. R., & Schumacher, R. R. (1959). A study of Doderlein's vaginal bacillus. *Ann N Y Acad Sci, 83*, 217-226. doi:10.1111/j.1749-6632.1960.tb40894.x

Huppert, J. S., Mortensen, J. E., Reed, J. L., Kahn, J. A., Rich, K. D., & Hobbs, M. M. (2008). Mycoplasma genitalium detected by transcription-mediated amplification is associated with Chlamydia trachomatis in adolescent women. *Sexually transmitted diseases, 35*(3), 250-254. doi:10.1097/OLQ.0b013e31815abac6

Hurmalainen, V., Edelman, S., Antikainen, J., Baumann, M., Lahteenmaki, K., & Korhonen, T. K. (2007). Extracellular proteins of Lactobacillus crispatus enhance activation of human plasminogen. *Microbiology, 153*(Pt 4), 1112-1122. doi:10.1099/mic.0.2006/000901-0

Husain, S. M., Wilks, M., Mupita, M., Reddy, S. P., Hennessy, E. M., Macfarlane, A. J., & Millar, M. R. (2014). Diversity and stability of cultured vaginal lactobacilli in pregnant women from a multi-ethnic urban UK population. *J Appl Microbiol, 117*(1), 258-265. doi:10.1111/jam.12506

Huttenhower, C., Kostic, A. D., & Xavier, R. J. (2014). Inflammatory bowel disease as a model for translating the microbiome. *Immunity, 40*(6), 843-854. doi:10.1016/j.immuni.2014.05.013

Huycke, M. M., Abrams, V., & Moore, D. R. (2002). Enterococcus faecalis produces extracellular superoxide and hydrogen peroxide that damages colonic epithelial cell DNA. *Carcinogenesis, 23*(3), 529-536. doi:10.1093/carcin/23.3.529

Hyman, R. W., Fukushima, M., Diamond, L., Kumm, J., Giudice, L. C., & Davis, R. W. (2005). Microbes on the human vaginal epithelium. *Proc Natl Acad Sci U S A, 102*(22), 7952-7957. doi:10.1073/pnas.0503236102

Hyman, R. W., Fukushima, M., Jiang, H., Fung, E., Rand, L., Johnson, B., . . . Giudice, L. C. (2014). Diversity of the vaginal microbiome correlates with preterm birth. *Reprod Sci, 21*(1), 32-40. doi:10.1177/1933719113488838

Ilhan, Z. E., Laniewski, P., Thomas, N., Roe, D. J., Chase, D. M., & Herbst-Kralovetz, M. M. (2019). Deciphering the complex interplay between microbiota, HPV, inflammation and cancer through cervicovaginal metabolic profiling. *EBioMedicine*. doi:10.1016/j.ebiom.2019.04.028

International Collaboration of Epidemiological Studies of Cervical, C. (2006). Carcinoma of the cervix and tobacco smoking: Collaborative reanalysis of individual data on 13,541 women with carcinoma of the cervix and 23,017 women without carcinoma of the cervix from 23 epidemiological studies. *International Journal of Cancer, 118*(6), 1481-1495. doi:10.1002/ijc.21493

Jain, A., Gupta, Y., Agrawal, R., Khare, P., & Jain, S. K. (2007). Biofilms--a microbial life perspective: a critical review. *Crit Rev Ther Drug Carrier Syst, 24*(5), 393-443. doi:10.1615/critrevtherdrugcarriersyst.v24.i5.10

Jelsbak, L., Thomsen, L. E., Wallrodt, I., Jensen, P. R., & Olsen, J. E. (2012). Polyamines are required for virulence in Salmonella enterica serovar Typhimurium. *PLoS One, 7*(4), e36149. doi:10.1371/journal.pone.0036149

Jespers, V., Menten, J., Smet, H., Poradosu, S., Abdellati, S., Verhelst, R., . . . Crucitti, T. (2012). Quantification of bacterial species of the vaginal microbiome in different groups of women, using nucleic acid amplification tests. *BMC Microbiol, 12*, 83. doi:10.1186/1471-2180-12-83

Jespers, V., van de Wijgert, J., Cools, P., Verhelst, R., Verstraelen, H., Delany-Moretlwe, S., . . . Vaginal Biomarkers Study, G. (2015). The significance of Lactobacillus crispatus and L. vaginalis for vaginal health and the negative effect of recent sex: a cross-sectional descriptive study across groups of African women. *BMC infectious diseases, 15*, 115. doi:10.1186/s12879-015-0825-z

Jin, L., Tao, L., Pavlova, S. I., So, J. S., Kiwanuka, N., Namukwaya, Z., . . . Wawer, M. (2007). Species diversity and relative abundance of vaginal lactic acid bacteria from women in Uganda and Korea. *J Appl Microbiol, 102*(4), 1107-1115. doi:10.1111/j.1365-2672.2006.03147.x

Jordan, S. J., Olson, K. M., Barnes, S., Wilson, L. S., Berryhill, T. F., Bakshi, R., . . . Geisler, W. M. (2017). Lower Levels of Cervicovaginal Tryptophan Are Associated With Natural Clearance of Chlamydia in Women. *J Infect Dis, 215*(12), 1888-1892. doi:10.1093/infdis/jix240

Juarez Tomas, M. S., Ocana, V. S., Wiese, B., & Nader-Macias, M. E. (2003). Growth and lactic acid production by vaginal Lactobacillus acidophilus CRL 1259, and inhibition of uropathogenic Escherichia coli. *J Med Microbiol, 52*(Pt 12), 1117-1124. doi:10.1099/jmm.0.05155-0

Kacerovsky, M., Vrbacky, F., Kutova, R., Pliskova, L., Andrys, C., Musilova, I., . . . Nekvindova, J. (2015). Cervical microbiota in women with preterm prelabor rupture of membranes. *PLoS One, 10*(5), e0126884. doi:10.1371/journal.pone.0126884

Kaewnopparat, S., Dangmanee, N., Kaewnopparat, N., Srichana, T., Chulasiri, M., & Settharaksa, S. (2013). In vitro probiotic properties of Lactobacillus fermentum SK5 isolated from vagina of a healthy woman. *Anaerobe, 22*, 6-13. doi:10.1016/j.anaerobe.2013.04.009

Kaewsrichan, J., Peeyananjarassri, K., & Kongprasertkit, J. (2006). Selection and identification of anaerobic lactobacilli producing inhibitory compounds against vaginal pathogens. *FEMS Immunol Med Microbiol, 48*(1), 75-83. doi:10.1111/j.1574-695X.2006.00124.x

Kainulainen, V., Loimaranta, V., Pekkala, A., Edelman, S., Antikainen, J., Kylvaja, R., . . . Korhonen, T. K. (2012). Glutamine synthetase and glucose-6-phosphate isomerase are adhesive moonlighting proteins of Lactobacillus crispatus released by epithelial
cathelicidin LL-37. *J Bacteriol, 194*(10), 2509-2519. doi:10.1128/JB.06704-11

Kairys, N., & Garg, M. (2019). Bacterial Vaginosis. In *StatPearls*. Treasure Island (FL).

Kalyoussef, S., Nieves, E., Dinerman, E., Carpenter, C., Shankar, V., Oh, J., . . . Herold, B. C. (2012). Lactobacillus proteins are associated with the bactericidal activity against E. coli of female genital tract secretions. *PLoS One, 7*(11), e49506. doi:10.1371/journal.pone.0049506

Kanjee, U., & Houry, W. A. (2013). Mechanisms of acid resistance in Escherichia coli. *Annu Rev Microbiol, 67*, 65-81. doi:10.1146/annurev-micro-092412-155708

Kaur, B., Balgir, P. P., Mittu, B., Kumar, B., & Garg, N. (2013). Biomedical applications of fermenticin HV6b isolated from Lactobacillus fermentum HV6b MTCC10770. *Biomed Res Int, 2013*, 168438. doi:10.1155/2013/168438

Kenyon, C., Colebunders, R., & Crucitti, T. (2013). The global epidemiology of bacterial vaginosis: a systematic review. *Am J Obstet Gynecol, 209*(6), 505-523. doi:10.1016/j.ajog.2013.05.006

Kilic, A. O., Pavlova, S. I., Alpay, S., Kilic, S. S., & Tao, L. (2001). Comparative study of vaginal Lactobacillus phages isolated from women in the United States and Turkey: prevalence, morphology, host range, and DNA homology. *Clin Diagn Lab Immunol, 8*(1), 31-39. doi:10.1128/CDLI.8.1.31-39.2001

Kindinger, L. M., MacIntyre, D. A., Lee, Y. S., Marchesi, J. R., Smith, A., McDonald, J. A., . . . Bennett, P. R. (2016). Relationship between vaginal microbial dysbiosis, inflammation, and pregnancy outcomes in cervical cerclage. *Sci Transl Med, 8*(350), 350ra102. doi:10.1126/scitranslmed.aag1026

King, C. C., Jamieson, D. J., Wiener, J., Cu-Uvin, S., Klein, R. S., Rompalo, A. M., . . . Sobel, J. D. (2011). Bacterial vaginosis and the natural history of human papillomavirus. *Infect Dis Obstet Gynecol, 2011*, 319460. doi:10.1155/2011/319460

Kiss, H., Kogler, B., Petricevic, L., Sauerzapf, I., Klayraung, S., Domig, K., . . . Kneifel, W. (2007). Vaginal Lactobacillus microbiota of healthy women in the late first trimester of pregnancy. *BJOG, 114*(11), 1402-1407. doi:10.1111/j.1471-0528.2007.01412.x

Klaenhammer, T. R. (1988). Bacteriocins of lactic acid bacteria. *Biochimie, 70*(3), 337-349. doi:10.1016/0300-9084(88)90206-4

Klebanoff, S. J., Hillier, S. L., Eschenbach, D. A., & Waltersdorph, A. M. (1991). Control of the microbial flora of the vagina by H2O2-generating lactobacilli. *J Infect Dis, 164*(1), 94-100. doi:10.1093/infdis/164.1.94

Klomp, J. M., Boon, M. E., Van Haaften, M., & Heintz, A. P. (2008). Cytologically diagnosed Gardnerella vaginalis infection and cervical (pre)neoplasia as established in population-based cervical screening. *Am J Obstet Gynecol, 199*(5), 480 e481-485. doi:10.1016/j.ajog.2008.04.036

Kong, H. H., Oh, J., Deming, C., Conlan, S., Grice, E. A., Beatson, M. A., . . . Segre, J. A. (2012). Temporal shifts in the skin microbiome associated with disease flares

and treatment in children with atopic dermatitis. *Genome Res, 22*(5), 850-859. doi:10.1101/gr.131029.111

Kostic, A. D., Gevers, D., Siljander, H., Vatanen, T., Hyotylainen, T., Hamalainen, A. M., . . . Xavier, R. J. (2015). The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe, 17*(2), 260-273. doi:10.1016/j.chom.2015.01.001

Koumans, E. H., Sternberg, M., Bruce, C., McQuillan, G., Kendrick, J., Sutton, M., & Markowitz, L. E. (2007). The prevalence of bacterial vaginosis in the United States, 2001-2004; associations with symptoms, sexual behaviors, and reproductive health. *Sexually transmitted diseases, 34*(11), 864-869. doi:10.1097/OLQ.0b013e318074e565

Kwon, D. H., & Lu, C. D. (2006). Polyamines induce resistance to cationic peptide, aminoglycoside, and quinolone antibiotics in Pseudomonas aeruginosa PAO1. *Antimicrob Agents Chemother, 50*(5), 1615-1622. doi:10.1128/AAC.50.5.1615-1622.2006

Kwon, D. H., & Lu, C. D. (2007). Polyamine effects on antibiotic susceptibility in bacteria. *Antimicrob Agents Chemother, 51*(6), 2070-2077. doi:10.1128/AAC.01472-06

Kyongo, J. K., Jespers, V., Goovaerts, O., Michiels, J., Menten, J., Fichorova, R. N., . . . Arien, K. K. (2012). Searching for lower female genital tract soluble and cellular biomarkers: defining levels and predictors in a cohort of healthy Caucasian women. *PLoS One, 7*(8), e43951. doi:10.1371/journal.pone.0043951

Laghi, L., Picone, G., Cruciani, F., Brigidi, P., Calanni, F., Donders, G., . . . Vitali, B. (2014). Rifaximin modulates the vaginal microbiome and metabolome in women affected by bacterial vaginosis. *Antimicrob Agents Chemother, 58*(6), 3411-3420. doi:10.1128/AAC.02469-14

Lambert, J. A., John, S., Sobel, J. D., & Akins, R. A. (2013). Longitudinal analysis of vaginal microbiome dynamics in women with recurrent bacterial vaginosis: recognition of the conversion process. *PLoS One, 8*(12), e82599. doi:10.1371/journal.pone.0082599

Lamont, R. F., Sobel, J. D., Akins, R. A., Hassan, S. S., Chaiworapongsa, T., Kusanovic, J. P., & Romero, R. (2011). The vaginal microbiome: new information about genital tract flora using molecular based techniques. *BJOG, 118*(5), 533-549. doi:10.1111/j.1471-0528.2010.02840.x

Laniewski, P., Cui, H., Roe, D. J., Barnes, D., Goulder, A., Monk, B. J., . . . Herbst-Kralovetz, M. M. (2019). Features of the cervicovaginal microenvironment drive cancer biomarker signatures in patients across cervical carcinogenesis. *Sci Rep, 9*(1), 7333. doi:10.1038/s41598-019-43849-5

Large, T. M., Walk, S. T., & Whittam, T. S. (2005). Variation in acid resistance among shiga toxin-producing clones of pathogenic Escherichia coli. *Applied and environmental microbiology, 71*(5), 2493-2500. doi:10.1128/AEM.71.5.2493-2500.2005

Larsen, B., Goplerud, C. P., Petzold, C. R., Ohm-Smith, M. J., & Galask, R. P. (1982). Effect of estrogen treatment on the genital tract flora of postmenopausal women. *Obstet Gynecol, 60*(1), 20-24. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6283447

Lash, A. F., & Kaplan, B. (1926). A Study of Döderlein's Vaginal Bacillus. *The Journal of Infectious Diseases, 38*(4), 333-340. doi:10.1093/infdis/38.4.333

Laxmi, U., Agrawal, S., Raghunandan, C., Randhawa, V. S., & Saili, A. (2012). Association of bacterial vaginosis with adverse fetomaternal outcome in women with spontaneous preterm labor: a prospective cohort study. *J Matern Fetal Neonatal Med, 25*(1), 64-67. doi:10.3109/14767058.2011.565390

Leccese Terraf, M. C., Mendoza, L. M., Juarez Tomas, M. S., Silva, C., & Nader-Macias, M. E. (2014). Phenotypic surface properties (aggregation, adhesion and biofilm formation) and presence of related genes in beneficial vaginal lactobacilli. *J Appl Microbiol, 117*(6), 1761-1772. doi:10.1111/jam.12642

Lee, J. E., Lee, S., Lee, H., Song, Y. M., Lee, K., Han, M. J., . . . Ko, G. (2013). Association of the vaginal microbiota with human papillomavirus infection in a Korean twin cohort. *PLoS One, 8*(5), e63514. doi:10.1371/journal.pone.0063514

Leitich, H., Bodner-Adler, B., Brunbauer, M., Kaider, A., Egarter, C., & Husslein, P. (2003). Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis. *Am J Obstet Gynecol, 189*(1), 139-147. doi:10.1067/mob.2003.339

Ling, Z., Kong, J., Liu, F., Zhu, H., Chen, X., Wang, Y., . . . Xiang, C. (2010). Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. *BMC Genomics, 11*, 488. doi:10.1186/1471-2164-11-488

Linhares, I. M., Summers, P. R., Larsen, B., Giraldo, P. C., & Witkin, S. S. (2011). Contemporary perspectives on vaginal pH and lactobacilli. *Am J Obstet Gynecol, 204*(2), 120 e121-125. doi:10.1016/j.ajog.2010.07.010

Lis, R., Rowhani-Rahbar, A., & Manhart, L. E. (2015). Mycoplasma genitalium infection and female reproductive tract disease: a meta-analysis. *Clin Infect Dis, 61*(3), 418-426. doi:10.1093/cid/civ312

Lokken, E. M., Balkus, J. E., Kiarie, J., Hughes, J. P., Jaoko, W., Totten, P. A., . . . Manhart, L. E. (2017). Association of Recent Bacterial Vaginosis With Acquisition of Mycoplasma genitalium. *Am J Epidemiol, 186*(2), 194-201. doi:10.1093/aje/kwx043

Machado, A., & Cerca, N. (2015). Influence of Biofilm Formation by Gardnerella vaginalis and Other Anaerobes on Bacterial Vaginosis. *J Infect Dis, 212*(12), 1856-1861. doi:10.1093/infdis/jiv338

MacIntyre, D. A., Chandiramani, M., Lee, Y. S., Kindinger, L., Smith, A., Angelopoulos, N., . . . Bennett, P. R. (2015). The vaginal microbiome during pregnancy and the postpartum period in a European population. *Sci Rep, 5*, 8988. doi:10.1038/srep08988

Macklaim, J. M., Fernandes, A. D., Di Bella, J. M., Hammond, J. A., Reid, G., & Gloor, G. B. (2013). Comparative meta-RNA-seq of the vaginal microbiota and differential expression by Lactobacillus iners in health and dysbiosis. *Microbiome, 1*(1), 12. doi:10.1186/2049-2618-1-12

Macklaim, J. M., Gloor, G. B., Anukam, K. C., Cribby, S., & Reid, G. (2011). At the crossroads of vaginal health and disease, the genome sequence of Lactobacillus iners AB-1. *Proc Natl Acad Sci U S A, 108 Suppl 1*, 4688-4695. doi:10.1073/pnas.1000086107

Malik, S., Siezen, R. J., Renckens, B., Vaneechoutte, M., Vanderleyden, J., & Lebeer, S. (2014). Draft Genome Sequence of Lactobacillus plantarum CMPG5300, a Human Vaginal Isolate. *Genome Announc, 2*(6). doi:10.1128/genomeA.01149-14

Mao, C., Hughes, J. P., Kiviat, N., Kuypers, J., Lee, S. K., Adam, D. E., & Koutsky, L. A. (2003). Clinical findings among young women with genital human papillomavirus infection. *Am J Obstet Gynecol, 188*(3), 677-684. doi:10.1067/mob.2003.164

Marcobal, A., De las Rivas, B., Landete, J. M., Tabera, L., & Munoz, R. (2012). Tyramine and phenylethylamine biosynthesis by food bacteria. *Crit Rev Food Sci Nutr, 52*(5), 448-467. doi:10.1080/10408398.2010.500545

Mark, K. S., Brotman, R. M., Martinez-Greiwe, S., Terplan, M., Bavoil, P., & Ravel, J. (2019). Chlamydia in adolescent/adult reproductive management trial study (CHARM): Clinical core protocol. *Contemp Clin Trials Commun, 16*, 100414. doi:10.1016/j.conctc.2019.100414

Marrazzo, J. M., Fiedler, T. L., Srinivasan, S., Thomas, K. K., Liu, C., Ko, D., . . . Fredricks, D. N. (2012). Extravaginal reservoirs of vaginal bacteria as risk factors for incident bacterial vaginosis. *J Infect Dis, 205*(10), 1580-1588. doi:10.1093/infdis/jis242

Marrazzo, J. M., Thomas, K. K., Fiedler, T. L., Ringwood, K., & Fredricks, D. N. (2010). Risks for acquisition of bacterial vaginosis among women who report sex with women: a cohort study. *PLoS One, 5*(6), e11139. doi:10.1371/journal.pone.0011139

Martin, D. H., Zozaya, M., Lillis, R., Miller, J., & Ferris, M. J. (2012). The microbiota of the human genitourinary tract: trying to see the forest through the trees. *Trans Am Clin Climatol Assoc, 123*, 242-256. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23303991

Martinez, R. C., Franceschini, S. A., Patta, M. C., Quintana, S. M., Nunes, A. C., Moreira, J. L., . . . De Martinis, E. C. (2008). Analysis of vaginal lactobacilli from healthy and infected Brazilian women. *Applied and environmental microbiology, 74*(14), 4539-4542. doi:10.1128/AEM.00284-08

Martinez-Pena, M. D., Castro-Escarpulli, G., & Aguilera-Arreola, M. G. (2013). Lactobacillus species isolated from vaginal secretions of healthy and bacterial vaginosis-intermediate Mexican women: a prospective study. *BMC infectious diseases, 13*, 189. doi:10.1186/1471-2334-13-189

Mastromarino, P., Brigidi, P., Macchia, S., Maggi, L., Pirovano, F., Trinchieri, V., . . . Matteuzzi, D. (2002). Characterization and selection of vaginal Lactobacillus strains for the preparation of vaginal tablets. *J Appl Microbiol, 93*(5), 884-893. doi:10.1046/j.1365-2672.2002.01759.x

Mastromarino, P., Cacciotti, F., Masci, A., & Mosca, L. (2011). Antiviral activity of Lactobacillus brevis towards herpes simplex virus type 2: role of cell wall associated components. *Anaerobe, 17*(6), 334-336. doi:10.1016/j.anaerobe.2011.04.022

Mastromarino, P., Di Pietro, M., Schiavoni, G., Nardis, C., Gentile, M., & Sessa, R. (2014). Effects of vaginal lactobacilli in Chlamydia trachomatis infection. *Int J Med Microbiol, 304*(5-6), 654-661. doi:10.1016/j.ijmm.2014.04.006

Mattsby-Baltzer, I., Platz-Christensen, J. J., Hosseini, N., & Rosen, P. (1998). IL-1beta, IL-6, TNFalpha, fetal fibronectin, and endotoxin in the lower genital tract of pregnant women with bacterial vaginosis. *Acta Obstet Gynecol Scand, 77*(7), 701-706. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9740515

McDonald, H. M., O'Loughlin, J. A., Jolley, P., Vigneswaran, R., & McDonald, P. J. (1992). Prenatal microbiological risk factors associated with preterm birth. *Br J Obstet Gynaecol, 99*(3), 190-196. doi:10.1111/j.1471-0528.1992.tb14497.x

McGregor, J. A., French, J. I., Jones, W., Milligan, K., McKinney, P. J., Patterson, E., & Parker, R. (1994). Bacterial vaginosis is associated with prematurity and vaginal fluid mucinase and sialidase: results of a controlled trial of topical clindamycin cream. *Am J Obstet Gynecol, 170*(4), 1048-1059; discussion 1059-1060. doi:10.1016/s0002-9378(94)70098-2

McGroarty, J. A., & Reid, G. (1988). Detection of a Lactobacillus substance that inhibits Escherichia coli. *Can J Microbiol, 34*(8), 974-978. doi:10.1139/m88-171

McGroarty, J. A., Tomeczek, L., Pond, D. G., Reid, G., & Bruce, A. W. (1992). Hydrogen peroxide production by Lactobacillus species: correlation with susceptibility to the spermicidal compound nonoxynol-9. *J Infect Dis, 165*(6), 1142-1144. doi:10.1093/infdis/165.6.1142

McKinnon, L. R., Achilles, S. L., Bradshaw, C. S., Burgener, A., Crucitti, T., Fredricks, D. N., . . . Tachedjian, G. (2019). The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. *AIDS Res Hum Retroviruses, 35*(3), 219-228. doi:10.1089/AID.2018.0304

McLean, N. W., & McGroarty, J. A. (1996). Growth inhibition of metronidazole-susceptible and metronidazole-resistant strains of Gardnerella vaginalis by Lactobacilli in vitro. *Applied and environmental microbiology, 62*(3), 1089-1092. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8975601

McMillan, A., Macklaim, J. M., Burton, J. P., & Reid, G. (2013). Adhesion of Lactobacillus iners AB-1 to human fibronectin: a key mediator for persistence in the vagina? *Reprod Sci, 20*(7), 791-796. doi:10.1177/1933719112466306

McNicol, P., Paraskevas, M., & Guijon, F. (1994). Variability of polymerase chain reaction-based detection of human papillomavirus DNA is associated with the composition of vaginal microbial flora. *J Med Virol, 43*(2), 194-200. doi:10.1002/jmv.1890430218

Mendes-Soares, H., Suzuki, H., Hickey, R. J., & Forney, L. J. (2014). Comparative functional genomics of Lactobacillus spp. reveals possible mechanisms for specialization of vaginal lactobacilli to their environment. *J Bacteriol, 196*(7), 1458-1470. doi:10.1128/JB.01439-13

Menge. (1894). Ueber ein bacterienfeindliches Verhalten der Seheidensekrete Nichtschwangerer. *Deutsche med. Wochenschrift*, 46-48.

Mercer, B. M. (2005). Preterm premature rupture of the membranes: current approaches to evaluation and management. *Obstet Gynecol Clin North Am, 32*(3), 411-428. doi:10.1016/j.ogc.2005.03.003

Miller, E. A., Beasley, D. E., Dunn, R. R., & Archie, E. A. (2016). Lactobacilli Dominance and Vaginal pH: Why Is the Human Vaginal Microbiome Unique? *Front Microbiol, 7*, 1936. doi:10.3389/fmicb.2016.01936

Mirmonsef, P., Hotton, A. L., Gilbert, D., Burgad, D., Landay, A., Weber, K. M., . . . Spear, G. T. (2014). Free glycogen in vaginal fluids is associated with Lactobacillus colonization and low vaginal pH. *PLoS One, 9*(7), e102467. doi:10.1371/journal.pone.0102467

Mirmonsef, P., Modur, S., Burgad, D., Gilbert, D., Golub, E. T., French, A. L., . . . Spear, G. T. (2015). Exploratory comparison of vaginal glycogen and Lactobacillus levels in premenopausal and postmenopausal women. *Menopause (New York, N.Y.), 22*(7), 702-709. doi:10.1097/GME.0000000000000397

Mitchell, C., Balkus, J. E., Fredricks, D., Liu, C., McKernan-Mullin, J., Frenkel, L. M., . . . Hitti, J. (2013). Interaction between lactobacilli, bacterial vaginosis-associated bacteria, and HIV Type 1 RNA and DNA Genital shedding in U.S. and Kenyan women. *AIDS Res Hum Retroviruses, 29*(1), 13-19. doi:10.1089/AID.2012.0187

Mitchell, C. M., Fredricks, D. N., Winer, R. L., & Koutsky, L. (2012). Effect of sexual debut on vaginal microbiota in a cohort of young women. *Obstet Gynecol, 120*(6), 1306-1313. doi:http://10.1097/AOG.0b013e31827075ac
10.1097/aog.0b013e31827075ac

Mitra, A., MacIntyre, D. A., Marchesi, J. R., Lee, Y. S., Bennett, P. R., & Kyrgiou, M. (2016). The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? *Microbiome, 4*(1), 58. doi:10.1186/s40168-016-0203-0

Molenaar, D., Bosscher, J. S., ten Brink, B., Driessen, A. J., & Konings, W. N. (1993). Generation of a proton motive force by histidine decarboxylation and electrogenic

histidine/histamine antiport in Lactobacillus buchneri. *J Bacteriol, 175*(10), 2864-2870. doi:10.1128/jb.175.10.2864-2870.1993

Moncla, B. J., Mietzner, T. A., & Hillier, S. L. (2012). In vitro activity of cationic peptides against Neisseria gonorrhoeae and vaginal Lactobacillus species: The effect of divalent cations. *Advances in Bioscience and Biotechnology*(3), 249–255. doi:http://doi.org/10.4236/abb.2012.33034

Nagaraja, T. G., & Titgemeyer, E. C. (2007). Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *J Dairy Sci, 90 Suppl 1*, E17-38. doi:10.3168/jds.2006-478

Nam, K. H., Kim, Y. T., Kim, S. R., Kim, S. W., Kim, J. W., Lee, M. K., . . . Jung, Y. W. (2009). Association between bacterial vaginosis and cervical intraepithelial neoplasia. *J Gynecol Oncol, 20*(1), 39-43. doi:10.3802/jgo.2009.20.1.39

Nardini, P., Nahui Palomino, R. A., Parolin, C., Laghi, L., Foschi, C., Cevenini, R., . . . Marangoni, A. (2016). Lactobacillus crispatus inhibits the infectivity of Chlamydia trachomatis elementary bodies, in vitro study. *Sci Rep, 6*, 29024. doi:10.1038/srep29024

Nardis, C., Mosca, L., & Mastromarino, P. (2013). Vaginal microbiota and viral sexually transmitted diseases. *Ann Ig, 25*(5), 443-456. doi:10.7416/ai.2013.1946

Nasioudis, D., Beghini, J., Bongiovanni, A. M., Giraldo, P. C., Linhares, I. M., & Witkin, S. S. (2015). alpha-Amylase in Vaginal Fluid: Association With Conditions Favorable to Dominance of Lactobacillus. *Reprod Sci, 22*(11), 1393-1398. doi:10.1177/1933719115581000

Nelson, T. M., Borgogna, J. C., Michalek, R. D., Roberts, D. W., Rath, J. M., Glover, E. D., . . . Brotman, R. M. (2018). Cigarette smoking is associated with an altered vaginal tract metabolomic profile. *Sci Rep, 8*(1), 852. doi:10.1038/s41598-017-14943-3

Nelson, T. M., Borgogna, J. L., Brotman, R. M., Ravel, J., Walk, S. T., & Yeoman, C. J. (2015). Vaginal biogenic amines: biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis? *Front Physiol, 6*, 253. doi:10.3389/fphys.2015.00253

Ness, R. B., Hillier, S. L., Kip, K. E., Soper, D. E., Stamm, C. A., McGregor, J. A., . . . Richter, H. E. (2004). Bacterial vaginosis and risk of pelvic inflammatory disease. *Obstet Gynecol, 104*(4), 761-769. doi:10.1097/01.AOG.0000139512.37582.17

Ness, R. B., Kip, K. E., Hillier, S. L., Soper, D. E., Stamm, C. A., Sweet, R. L., . . . Richter, H. E. (2005). A cluster analysis of bacterial vaginosis-associated microflora and pelvic inflammatory disease. *Am J Epidemiol, 162*(6), 585-590. doi:10.1093/aje/kwi243

Nieburgs, H. E. (1947). Gestational changes in the vaginal epithelium and their relation to the sex of the foetus. *J Obstet Gynaecol Br Emp, 54*(5), 653-655. doi:10.1111/j.1471-0528.1947.tb10749.x

Nikolaitchouk, N., Andersch, B., Falsen, E., Strombeck, L., & Mattsby-Baltzer, I. (2008). The lower genital tract microbiota in relation to cytokine-, SLPI- and endotoxin levels: application of checkerboard DNA-DNA hybridization (CDH). *APMIS, 116*(4), 263-277. doi:10.1111/j.1600-0463.2008.00808.x

Nugent, R. P., Krohn, M. A., & Hillier, S. L. (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol, 29*(2), 297-301. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1706728

O'Hanlon, D. E., Lanier, B. R., Moench, T. R., & Cone, R. A. (2010). Cervicovaginal fluid and semen block the microbicidal activity of hydrogen peroxide produced by vaginal lactobacilli. *BMC infectious diseases, 10*, 120. doi:10.1186/1471-2334-10-120

O'Hanlon, D. E., Moench, T. R., & Cone, R. A. (2011). In vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid but not hydrogen peroxide. *BMC infectious diseases, 11*, 200. doi:10.1186/1471-2334-11-200

O'Hanlon, D. E., Moench, T. R., & Cone, R. A. (2013). Vaginal pH and microbicidal lactic acid when lactobacilli dominate the microbiota. *PLoS One, 8*(11), e80074. doi:10.1371/journal.pone.0080074

Ocana, V. S., Pesce De Ruiz Holgado, A. A., & Nader-Macias, M. E. (1999a). Characterization of a bacteriocin-like substance produced by a vaginal Lactobacillus salivarius strain. *Applied and environmental microbiology, 65*(12), 5631-5635. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10584033

Ocana, V. S., Pesce de Ruiz Holgado, A. A., & Nader-Macias, M. E. (1999b). Selection of vaginal H2O2-generating Lactobacillus species for probiotic use. *Curr Microbiol, 38*(5), 279-284. doi:10.1007/pl00006802

Oh, H. Y., Kim, B. S., Seo, S. S., Kong, J. S., Lee, J. K., Park, S. Y., . . . Kim, M. K. (2015). The association of uterine cervical microbiota with an increased risk for

cervical intraepithelial neoplasia in Korea. *Clin Microbiol Infect, 21*(7), 674 e671-679. doi:10.1016/j.cmi.2015.02.026

Oh, H. Y., Seo, S. S., Kong, J. S., Lee, J. K., & Kim, M. K. (2015). Association between Obesity and Cervical Microflora Dominated by Lactobacillus iners in Korean Women. *J Clin Microbiol, 53*(10), 3304-3309. doi:10.1128/JCM.01387-15

Ojala, T., Kankainen, M., Castro, J., Cerca, N., Edelman, S., Westerlund-Wikstrom, B., . . . Auvinen, P. (2014). Comparative genomics of Lactobacillus crispatus suggests novel mechanisms for the competitive exclusion of Gardnerella vaginalis. *BMC Genomics, 15*, 1070. doi:10.1186/1471-2164-15-1070

Onderdonk, A. B., Delaney, M. L., & Fichorova, R. N. (2016). The Human Microbiome during Bacterial Vaginosis. *Clin Microbiol Rev, 29*(2), 223-238. doi:10.1128/CMR.00075-15

Onywera, H., Williamson, A. L., Mbulawa, Z. Z. A., Coetzee, D., & Meiring, T. L. (2019). The cervical microbiota in reproductive-age South African women with and without human papillomavirus infection. *Papillomavirus Res, 7*, 154-163. doi:10.1016/j.pvr.2019.04.006

Orfanelli, T., Jayaram, A., Doulaveris, G., Forney, L. J., Ledger, W. J., & Witkin, S. S. (2014). Human epididymis protein 4 and secretory leukocyte protease inhibitor in vaginal fluid: relation to vaginal components and bacterial composition. *Reprod Sci, 21*(4), 538-542. doi:10.1177/1933719113503416

Pandey, N., Malik, R. K., Kaushik, J. K., & Singroha, G. (2013). Gassericin A: a circular bacteriocin produced by lactic acid bacteria Lactobacillus gasseri. *World J Microbiol Biotechnol, 29*(11), 1977-1987. doi:10.1007/s11274-013-1368-3

Pandya, S., Ravi, K., Srinivas, V., Jadhav, S., Khan, A., Arun, A., . . . Madhivanan, P. (2017). Comparison of culture-dependent and culture-independent molecular methods for characterization of vaginal microflora. *J Med Microbiol, 66*(2), 149-153. doi:10.1099/jmm.0.000407

Parolin, C., Foschi, C., Laghi, L., Zhu, C., Banzola, N., Gaspari, V., . . . Marangoni, A. (2018). Insights Into Vaginal Bacterial Communities and Metabolic Profiles of Chlamydia trachomatis Infection: Positioning Between Eubiosis and Dysbiosis. *Front Microbiol, 9*, 600. doi:10.3389/fmicb.2018.00600

Parolin, C., Marangoni, A., Laghi, L., Foschi, C., Nahui Palomino, R. A., Calonghi, N., . . . Vitali, B. (2015). Isolation of Vaginal Lactobacilli and Characterization of

Anti-Candida Activity. *PLoS One, 10*(6), e0131220. doi:10.1371/journal.pone.0131220

Pascual, L. M., Daniele, M. B., Giordano, W., Pajaro, M. C., & Barberis, I. L. (2008). Purification and partial characterization of novel bacteriocin L23 produced by Lactobacillus fermentum L23. *Curr Microbiol, 56*(4), 397-402. doi:10.1007/s00284-007-9094-4

Pascual, L. M., Daniele, M. B., Pajaro, C., & Barberis, L. (2006). Lactobacillus species isolated from the vagina: identification, hydrogen peroxide production and nonoxynol-9 resistance. *Contraception, 73*(1), 78-81. doi:10.1016/j.contraception.2005.06.066

Patton, D. L., Thwin, S. S., Meier, A., Hooton, T. M., Stapleton, A. E., & Eschenbach, D. A. (2000). Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle. *Am J Obstet Gynecol, 183*(4), 967-973. doi:10.1067/mob.2000.108857

Pavlova, S. I., Kilic, A. O., Kilic, S. S., So, J. S., Nader-Macias, M. E., Simoes, J. A., & Tao, L. (2002). Genetic diversity of vaginal lactobacilli from women in different countries based on 16S rRNA gene sequences. *J Appl Microbiol, 92*(3), 451-459. doi:10.1046/j.1365-2672.2002.01547.x

Peebles, K., Velloza, J., Balkus, J. E., McClelland, R. S., & Barnabas, R. V. (2019). High Global Burden and Costs of Bacterial Vaginosis: A Systematic Review and Meta-Analysis. *Sexually transmitted diseases, 46*(5), 304-311. doi:10.1097/OLQ.0000000000000972

Peeters, M., & Piot, P. (1985). Adhesion of Gardnerella vaginalis to vaginal epithelial cells: variables affecting adhesion and inhibition by metronidazole. *Genitourin Med, 61*(6), 391-395. doi:10.1136/sti.61.6.391

Pegg, A. E. (2009). Mammalian polyamine metabolism and function. *IUBMB Life, 61*(9), 880-894. doi:10.1002/iub.230

Pereira, C. I., Matos, D., San Romao, M. V., & Crespo, M. T. (2009). Dual role for the tyrosine decarboxylation pathway in Enterococcus faecium E17: response to an acid challenge and generation of a proton motive force. *Applied and environmental microbiology, 75*(2), 345-352. doi:10.1128/AEM.01958-08

Pericone, C. D., Overweg, K., Hermans, P. W., & Weiser, J. N. (2000). Inhibitory and bactericidal effects of hydrogen peroxide production by Streptococcus

pneumoniae on other inhabitants of the upper respiratory tract. *Infection and immunity, 68*(7), 3990-3997. doi:10.1128/iai.68.7.3990-3997.2000

Peters, J., & Byrne, G. I. (2015). Chlamydia trachomatis growth depends on eukaryotic cholesterol esterification and is affected by Acyl-CoA:cholesterol acyltransferase inhibition. *Pathogens and disease, 73*(6), ftv028-ftv028. doi:10.1093/femspd/ftv028

Petricevic, L., Domig, K. J., Nierscher, F. J., Sandhofer, M. J., Fidesser, M., Krondorfer, I., . . . Kiss, H. (2014). Characterisation of the vaginal Lactobacillus microbiota associated with preterm delivery. *Sci Rep, 4*, 5136. doi:10.1038/srep05136

Pfister, H. (1987). Human papillomaviruses and genital cancer. *Adv Cancer Res, 48*, 113-147. doi:10.1016/s0065-230x(08)60691-0

Phukan, N., Parsamand, T., Brooks, A. E., Nguyen, T. N., & Simoes-Barbosa, A. (2013). The adherence of Trichomonas vaginalis to host ectocervical cells is influenced by lactobacilli. *Sexually transmitted infections, 89*(6), 455-459. doi:10.1136/sextrans-2013-051039

Piot, P., Van Dyck, E., Peeters, M., Hale, J., Totten, P. A., & Holmes, K. K. (1984). Biotypes of Gardnerella vaginalis. *J Clin Microbiol, 20*(4), 677-679. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6333436

Platz-Christensen, J. J., Mattsby-Baltzer, I., Thomsen, P., & Wiqvist, N. (1993). Endotoxin and interleukin-1 alpha in the cervical mucus and vaginal fluid of pregnant women with bacterial vaginosis. *Am J Obstet Gynecol, 169*(5), 1161-1166. doi:10.1016/0002-9378(93)90274-m

Plummer, M., Herrero, R., Franceschi, S., Meijer, C. J., Snijders, P., Bosch, F. X., . . . Group, I. M.-c. C. C. S. (2003). Smoking and cervical cancer: pooled analysis of the IARC multi-centric case--control study. *Cancer Causes Control, 14*(9), 805-814. doi:10.1023/b:caco.0000003811.98261.3e

Pridmore, R. D., Pittet, A. C., Praplan, F., & Cavadini, C. (2008). Hydrogen peroxide production by Lactobacillus johnsonii NCC 533 and its role in anti-Salmonella activity. *FEMS Microbiol Lett, 283*(2), 210-215. doi:10.1111/j.1574-6968.2008.01176.x

Prince, A. L., Ma, J., Kannan, P. S., Alvarez, M., Gisslen, T., Harris, R. A., . . . Aagaard, K. M. (2016). The placental membrane microbiome is altered among subjects with spontaneous preterm birth with and without chorioamnionitis. *Am J Obstet Gynecol, 214*(5), 627 e621-627 e616. doi:10.1016/j.ajog.2016.01.193

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., . . . Wang, J. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature, 490*(7418), 55-60. doi:10.1038/nature11450

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., . . . Glockner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res, 41*(Database issue), D590-596. doi:10.1093/nar/gks1219

Rath, D., Amlinger, L., Rath, A., & Lundgren, M. (2015). The CRISPR-Cas immune system: biology, mechanisms and applications. *Biochimie, 117*, 119-128. doi:10.1016/j.biochi.2015.03.025

Ravel, J., Gajer, P., Abdo, Z., Schneider, G. M., Koenig, S. S., McCulle, S. L., . . . Forney, L. J. (2011). Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A, 108 Suppl 1*, 4680-4687. doi:10.1073/pnas.1002611107

Rea, M. C., Sit, C. S., Clayton, E., O'Connor, P. M., Whittal, R. M., Zheng, J., . . . Hill, C. (2010). Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against Clostridium difficile. *Proc Natl Acad Sci U S A, 107*(20), 9352-9357. doi:10.1073/pnas.0913554107

Reid, G., Heinemann, C., Velraeds, M., van der Mei, H. C., & Busscher, H. J. (1999). Biosurfactants produced by Lactobacillus. *Methods Enzymol, 310*, 426-433. doi:10.1016/s0076-6879(99)10033-8

Rizzo, A., Fiorentino, M., Buommino, E., Donnarumma, G., Losacco, A., & Bevilacqua, N. (2015). Lactobacillus crispatus mediates anti-inflammatory cytokine interleukin-10 induction in response to Chlamydia trachomatis infection in vitro. *Int J Med Microbiol, 305*(8), 815-827. doi:10.1016/j.ijmm.2015.07.005

Roberton, A. M., Wiggins, R., Horner, P. J., Greenwood, R., Crowley, T., Fernandes, A., . . . Corfield, A. P. (2005). A novel bacterial mucinase, glycosulfatase, is associated with bacterial vaginosis. *J Clin Microbiol, 43*(11), 5504-5508. doi:10.1128/JCM.43.11.5504-5508.2005

Rodriguez-Cerdeira, C., Sanchez-Blanco, E., & Alba, A. (2012). Evaluation of Association between Vaginal Infections and High-Risk Human Papillomavirus Types in Female Sex Workers in Spain. *ISRN obstetrics and gynecology, 2012*, 240190. doi:10.5402/2012/240190

Rolfe, R. D., Hentges, D. J., Campbell, B. J., & Barrett, J. T. (1978). Factors related to the oxygen tolerance of anaerobic bacteria. *Applied and environmental microbiology, 36*(2), 306-313. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/697363

Romero, R., Hassan, S. S., Gajer, P., Tarca, A. L., Fadrosh, D. W., Bieda, J., . . . Ravel, J. (2014). The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term. *Microbiome, 2*, 18. doi:10.1186/2049-2618-2-18

Romero, R., Hassan, S. S., Gajer, P., Tarca, A. L., Fadrosh, D. W., Nikita, L., . . . Ravel, J. (2014). The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome, 2*(1), 4. doi:10.1186/2049-2618-2-4

Romero, R., Sirtori, M., Oyarzun, E., Avila, C., Mazor, M., Callahan, R., . . . Hobbins, J. C. (1989). Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. *Am J Obstet Gynecol, 161*(3), 817-824. doi:10.1016/0002-9378(89)90409-2

Rose, W. A., 2nd, McGowin, C. L., Spagnuolo, R. A., Eaves-Pyles, T. D., Popov, V. L., & Pyles, R. B. (2012). Commensal bacteria modulate innate immune responses of vaginal epithelial cell multilayer cultures. *PLoS One, 7*(3), e32728. doi:10.1371/journal.pone.0032728

Rosenstein, I. J., Fontaine, E. A., Morgan, D. J., Sheehan, M., Lamont, R. F., & Taylor-Robinson, D. (1997). Relationship between hydrogen peroxide-producing strains of lactobacilli and vaginosis-associated bacterial species in pregnant women. *Eur J Clin Microbiol Infect Dis, 16*(7), 517-522. doi:10.1007/bf01708235

Ross, S. A., Novak, Z., Ashrith, G., Rivera, L. B., Britt, W. J., Hedges, S., . . . Boppana, A. S. (2005). Association between genital tract cytomegalovirus infection and bacterial vaginosis. *J Infect Dis, 192*(10), 1727-1730. doi:10.1086/497150

Ruiz, F. O., Pascual, L., Giordano, W., & Barberis, L. (2015). Bacteriocins and other bioactive substances of probiotic lactobacilli as biological weapons against Neisseria gonorrhoeae. *Pathog Dis, 73*(3). doi:10.1093/femspd/ftv013

Sallenave, J. M. (2002). Antimicrobial activity of antiproteinases. *Biochem Soc Trans, 30*(2), 111-115. doi:10.1042/

Salzman, N. P., & Howley, P. M. . (1986). The Papovaviridae, the papillomaviruses. *1*.

Santiago, G. L., Cools, P., Verstraelen, H., Trog, M., Missine, G., El Aila, N., . . . Vaneechoutte, M. (2011). Longitudinal study of the dynamics of vaginal microflora during two consecutive menstrual cycles. *PLoS One, 6*(11), e28180. doi:10.1371/journal.pone.0028180

Santiago, G. L., Tency, I., Verstraelen, H., Verhelst, R., Trog, M., Temmerman, M., . . . Vaneechoutte, M. (2012). Longitudinal qPCR study of the dynamics of L. crispatus, L. iners, A. vaginae, (sialidase positive) G. vaginalis, and P. bivia in the vagina. *PLoS One, 7*(9), e45281. doi:10.1371/journal.pone.0045281

Sautter, R. L., & Brown, W. J. (1980). Sequential vaginal cultures from normal young women. *J Clin Microbiol, 11*(5), 479-484. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7381013

Schellenberg, J. J., Links, M. G., Hill, J. E., Dumonceaux, T. J., Kimani, J., Jaoko, W., . . . Plummer, F. A. (2011). Molecular definition of vaginal microbiota in East African commercial sex workers. *Applied and environmental microbiology, 77*(12), 4066-4074. doi:10.1128/AEM.02943-10

Schlecht, N. F., Kulaga, S., Robitaille, J., Ferreira, S., Santos, M., Miyamura, R. A., . . . Franco, E. L. (2001). Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. *JAMA, 286*(24), 3106-3114. doi:10.1001/jama.286.24.3106

Schoell, W. M., Janicek, M. F., & Mirhashemi, R. (1999). Epidemiology and biology of cervical cancer. *Semin Surg Oncol, 16*(3), 203-211. doi:10.1002/(sici)1098-2388(199904/05)16:3<203::aid-ssu2>3.0.co;2-c

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol, 12*(6), R60. doi:10.1186/gb-2011-12-6-r60

Shafer, W. M., Qu, X., Waring, A. J., & Lehrer, R. I. (1998). Modulation of Neisseria gonorrhoeae susceptibility to vertebrate antibacterial peptides due to a member of the resistance/nodulation/division efflux pump family. *Proc Natl Acad Sci U S A, 95*(4), 1829-1833. doi:10.1073/pnas.95.4.1829

Shah, P., & Swiatlo, E. (2008). A multifaceted role for polyamines in bacterial pathogens. *Mol Microbiol, 68*(1), 4-16. doi:10.1111/j.1365-2958.2008.06126.x

Shannon, B., Yi, T. J., Perusini, S., Gajer, P., Ma, B., Humphrys, M. S., . . . Kaul, R. (2017). Association of HPV infection and clearance with cervicovaginal immunology and the vaginal microbiota. *Mucosal Immunol, 10*(5), 1310-1319. doi:10.1038/mi.2016.129

Shedlovsky, L., Belcher, D., & Levenstein, I. (1942). Titrations of human seminal fluid with acids and alkalis and thier effects on the survival of sperm motility. *American Journal of Physiology-Legacy Content, 136*(3), 535-541. doi:10.1152/ajplegacy.1942.136.3.535

Shi, Y., Chen, L., Tong, J., & Xu, C. (2009). Preliminary characterization of vaginal microbiota in healthy Chinese women using cultivation-independent methods. *J Obstet Gynaecol Res, 35*(3), 525-532. doi:10.1111/j.1447-0756.2008.00971.x

Shipitsyna, E., Roos, A., Datcu, R., Hallen, A., Fredlund, H., Jensen, J. S., . . . Unemo, M. (2013). Composition of the vaginal microbiota in women of reproductive age--sensitive and specific molecular diagnosis of bacterial vaginosis is possible? *PLoS One, 8*(4), e60670. doi:10.1371/journal.pone.0060670

Skarin, A., & Sylwan, J. (1986). Vaginal lactobacilli inhibiting growth of Gardnerella vaginalis, Mobiluncus and other bacterial species cultured from vaginal content of women with bacterial vaginosis. *Acta Pathol Microbiol Immunol Scand B, 94*(6), 399-403. doi:10.1111/j.1699-0463.1986.tb03074.x

Smeianov, V., Scott, K., & Reid, G. (2000). Activity of cecropin P1 and FA-LL-37 against urogenital microflora. *Microbes Infect, 2*(7), 773-777. doi:10.1016/s1286-4579(00)90359-9

Smilde, A. K., van der Werf, M. J., Bijlsma, S., van der Werff-van der Vat, B. J., & Jellema, R. H. (2005). Fusion of mass spectrometry-based metabolomics data. *Anal Chem, 77*(20), 6729-6736. doi:10.1021/ac051080y

Smith, B. C., McAndrew, T., Chen, Z., Harari, A., Barris, D. M., Viswanathan, S., . . . Burk, R. D. (2012). The cervical microbiome over 7 years and a comparison of methodologies for its characterization. *PLoS One, 7*(7), e40425. doi:10.1371/journal.pone.0040425

Sobel, J. D. (2000). Bacterial vaginosis. *Annu Rev Med, 51*, 349-356. doi:10.1146/annurev.med.51.1.349

Sobel, J. D., Karpas, Z., & Lorber, A. (2012). Diagnosing vaginal infections through measurement of biogenic amines by ion mobility spectrometry. *Eur J Obstet Gynecol Reprod Biol, 163*(1), 81-84. doi:10.1016/j.ejogrb.2012.03.022

Sochacki, K. A., Barns, K. J., Bucki, R., & Weisshaar, J. C. (2011). Real-time attack on single Escherichia coli cells by the human antimicrobial peptide LL-37. *Proc Natl Acad Sci U S A, 108*(16), E77-81. doi:10.1073/pnas.1101130108

Song, Y. L., Kato, N., Matsumiya, Y., Liu, C. X., Kato, H., & Watanabe, K. (1999). Identification of and hydrogen peroxide production by fecal and vaginal lactobacilli isolated from Japanese women and newborn infants. *J Clin Microbiol, 37*(9), 3062-3064. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10449509

Spandorfer, S. D., Neuer, A., Giraldo, P. C., Rosenwaks, Z., & Witkin, S. S. (2001). Relationship of abnormal vaginal flora, proinflammatory cytokines and idiopathic infertility in women undergoing IVF. *J Reprod Med, 46*(9), 806-810. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11584481

Spear, G. T., French, A. L., Gilbert, D., Zariffard, M. R., Mirmonsef, P., Sullivan, T. H., . . . Hamaker, B. R. (2014). Human alpha-amylase present in lower-genital-tract mucosal fluid processes glycogen to support vaginal colonization by Lactobacillus. *J Infect Dis, 210*(7), 1019-1028. doi:10.1093/infdis/jiu231

Spear, G. T., Kersh, E., Guenthner, P., Vishwanathan, S. A., Gilbert, D., Zariffard, M. R., . . . Gillevet, P. (2012). Longitudinal assessment of pigtailed macaque lower genital tract microbiota by pyrosequencing reveals dissimilarity to the genital microbiota of healthy humans. *AIDS Res Hum Retroviruses, 28*(10), 1244-1249. doi:10.1089/AID.2011.0382

Spear, G. T., Sikaroodi, M., Zariffard, M. R., Landay, A. L., French, A. L., & Gillevet, P. M. (2008). Comparison of the diversity of the vaginal microbiota in HIV-infected and HIV-uninfected women with or without bacterial vaginosis. *J Infect Dis, 198*(8), 1131-1140. doi:10.1086/591942

Spear, G. T., Zariffard, M. R., Cohen, M. H., & Sha, B. E. (2008). Vaginal IL-8 levels are positively associated with Candida albicans and inversely with lactobacilli in HIV-infected women. *J Reprod Immunol, 78*(1), 76-79. doi:10.1016/j.jri.2007.11.001

Spiegel, C. A., Amsel, R., Eschenbach, D., Schoenknecht, F., & Holmes, K. K. (1980). Anaerobic bacteria in nonspecific vaginitis. *N Engl J Med, 303*(11), 601-607. doi:10.1056/NEJM198009113031102

Spurbeck, R. R., & Arvidson, C. G. (2008). Inhibition of Neisseria gonorrhoeae epithelial cell interactions by vaginal Lactobacillus species. *Infection and immunity, 76*(7), 3124-3130. doi:10.1128/IAI.00101-08

Spurbeck, R. R., & Arvidson, C. G. (2010). Lactobacillus jensenii surface-associated proteins inhibit Neisseria gonorrhoeae adherence to epithelial cells. *Infection and immunity, 78*(7), 3103-3111. doi:10.1128/IAI.01200-09

Srinivasan, S., & Fredricks, D. N. (2008). The human vaginal bacterial biota and bacterial vaginosis. *Interdisciplinary perspectives on infectious diseases, 2008*, 750479. doi:10.1155/2008/750479

Srinivasan, S., Hoffman, N. G., Morgan, M. T., Matsen, F. A., Fiedler, T. L., Hall, R. W., . . . Fredricks, D. N. (2012). Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS One, 7*(6), e37818. doi:10.1371/journal.pone.0037818

Srinivasan, S., Liu, C., Mitchell, C. M., Fiedler, T. L., Thomas, K. K., Agnew, K. J., . . . Fredricks, D. N. (2010). Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. *PLoS One, 5*(4), e10197. doi:10.1371/journal.pone.0010197

Srinivasan, S., Morgan, M. T., Fiedler, T. L., Djukovic, D., Hoffman, N. G., Raftery, D., . . . Fredricks, D. N. (2015). Metabolic signatures of bacterial vaginosis. *MBio, 6*(2). doi:10.1128/mBio.00204-15

Stapleton, A. E., Au-Yeung, M., Hooton, T. M., Fredricks, D. N., Roberts, P. L., Czaja, C. A., . . . Stamm, W. E. (2011). Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. *Clin Infect Dis, 52*(10), 1212-1217. doi:10.1093/cid/cir183

Statnikov, A., Alekseyenko, A. V., Li, Z., Henaff, M., Perez-Perez, G. I., Blaser, M. J., & Aliferis, C. F. (2013). Microbiomic signatures of psoriasis: feasibility and methodology comparison. *Sci Rep, 3*, 2620. doi:10.1038/srep02620

Storey, J. D. (2002). A Direct Approach to False Discovery Rates. *Journal of the Royal Statistical Society. Series B (Statistical Methodology), 64*(3), 479-498. Retrieved from http://www.jstor.org/stable/3088784

Stoyancheva, G., Marzotto, M., Dellaglio, F., & Torriani, S. (2014). Bacteriocin production and gene sequencing analysis from vaginal Lactobacillus strains. *Arch Microbiol, 196*(9), 645-653. doi:10.1007/s00203-014-1003-1

Strus, M., Brzychczy-Wloch, M., Gosiewski, T., Kochan, P., & Heczko, P. B. (2006). The in vitro effect of hydrogen peroxide on vaginal microbial communities. *FEMS Immunol Med Microbiol, 48*(1), 56-63. doi:10.1111/j.1574-695X.2006.00120.x

Subramaniam, A., Kumar, R., Cliver, S. P., Zhi, D., Szychowski, J. M., Abramovici, A., . . . Edwards, R. K. (2016). Vaginal Microbiota in Pregnancy: Evaluation Based on Vaginal Flora, Birth Outcome, and Race. *Am J Perinatol, 33*(4), 401-408. doi:10.1055/s-0035-1565919

Sumati, A. H., & Saritha, N. K. (2009). Association of urinary tract infection in women with bacterial vaginosis. *J Glob Infect Dis, 1*(2), 151-152. doi:10.4103/0974-777X.56254

Surgery, Gynecology, and Obstetrics. (1914). *XIX*.

Swartz, J. D., Lachman, M., Westveer, K., O'Neill, T., Geary, T., Kott, R. W., . . . Yeoman, C. J. (2014). Characterization of the Vaginal Microbiota of Ewes and Cows Reveals a Unique Microbiota with Low Levels of Lactobacilli and Near-Neutral pH. *Front Vet Sci, 1*, 19. doi:10.3389/fvets.2014.00019

Swidsinski, A., Mendling, W., Loening-Baucke, V., Swidsinski, S., Dorffel, Y., Scholze, J., . . . Verstraelen, H. (2008). An adherent Gardnerella vaginalis biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. *Am J Obstet Gynecol, 198*(1), 97 e91-96. doi:10.1016/j.ajog.2007.06.039

Tabor, C. W., Tabor, H., & Hafner, E. W. (1978). Escherichia coli mutants completely deficient in adenosylmethionine decarboxylase and in spermidine biosynthesis. *J Biol Chem, 253*(10), 3671-3676. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/348695

Tabrizi, S. N., Su, J., Bradshaw, C. S., Fairley, C. K., Walker, S., Tan, L. Y., . . . Garland, S. M. (2017). Prospective Evaluation of ResistancePlus MG, a New Multiplex Quantitative PCR Assay for Detection of Mycoplasma genitalium and Macrolide Resistance. *J Clin Microbiol, 55*(6), 1915-1919. doi:10.1128/JCM.02312-16

Tabrizi, S. N., Tan, L. Y., Walker, S., Twin, J., Poljak, M., Bradshaw, C. S., . . . Garland, S. M. (2016). Multiplex Assay for Simultaneous Detection of Mycoplasma genitalium and Macrolide Resistance Using PlexZyme and PlexPrime Technology. *PLoS One, 11*(6), e0156740. doi:10.1371/journal.pone.0156740

Tamarelle, J., Thiebaut, A. C. M., de Barbeyrac, B., Bebear, C., Ravel, J., & Delarocque-Astagneau, E. (2019). The vaginal microbiota and its association with human papillomavirus, Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium infections: a systematic review and meta-analysis. *Clin Microbiol Infect, 25*(1), 35-47. doi:10.1016/j.cmi.2018.04.019

Tarnberg, M., Jakobsson, T., Jonasson, J., & Forsum, U. (2002). Identification of randomly selected colonies of lactobacilli from normal vaginal fluid by pyrosequencing of the 16S rDNA variable V1 and V3 regions. *APMIS, 110*(11), 802-810. doi:10.1034/j.1600-0463.2002.1101106.x

Taylor, B. D., Zheng, X., O'Connell, C. M., Wiesenfeld, H. C., Hillier, S. L., & Darville, T. (2018). Risk factors for Mycoplasma genitalium endometritis and incident infection: a secondary data analysis of the T cell Response Against Chlamydia (TRAC) Study. *Sexually transmitted infections, 94*(6), 414-420. doi:10.1136/sextrans-2017-053376

Tevi-Benissan, C., Belec, L., Levy, M., Schneider-Fauveau, V., Si Mohamed, A., Hallouin, M. C., . . . Gresenguet, G. (1997). In vivo semen-associated pH neutralization of cervicovaginal secretions. *Clin Diagn Lab Immunol, 4*(3), 367-374. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9144379

Thomas, S. (1928). Döderlein's Bacillus: Lactobacillus Acidophilus. *The Journal of Infectious Diseases, 43*(3), 218–227.

Torrone, E., Papp, J., Weinstock, H., Centers for Disease, C., & Prevention. (2014). Prevalence of Chlamydia trachomatis genital infection among persons aged 14-39 years--United States, 2007-2012. *MMWR Morb Mortal Wkly Rep, 63*(38), 834-838. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25254560

Totten, P. A., Amsel, R., Hale, J., Piot, P., & Holmes, K. K. (1982). Selective differential human blood bilayer media for isolation of Gardnerella (Haemophilus) vaginalis. *J Clin Microbiol, 15*(1), 141-147. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6764766

Transactions of the American Gynecological Society. (1898). *23*.

Tuddenham, S., & Ghanem, K. G. (2017). A microbiome variable in the HIV-prevention equation. *Science, 356*(6341), 907-908. doi:10.1126/science.aan6103

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., . . . Gordon, J. I. (2009). A core gut microbiome in obese and lean twins. *Nature, 457*(7228), 480-484. doi:10.1038/nature07540

Vahle. (1896). Ueber das Vorkommen von Streptococcen in der Scheide Gebarender. *Zeit. f. Geb. u. Gyn., xxxv*, 92-214.

Valore, E. V., Wiley, D. J., & Ganz, T. (2006). Reversible deficiency of antimicrobial polypeptides in bacterial vaginosis. *Infection and immunity, 74*(10), 5693-5702. doi:10.1128/IAI.00524-06

van de Wijgert, J. H., Borgdorff, H., Verhelst, R., Crucitti, T., Francis, S., Verstraelen, H., & Jespers, V. (2014). The vaginal microbiota: what have we learned after a decade of molecular characterization? *PLoS One, 9*(8), e105998. doi:10.1371/journal.pone.0105998

van Houdt, R., Ma, B., Bruisten, S. M., Speksnijder, A., Ravel, J., & de Vries, H. J. C. (2018). Lactobacillus iners-dominated vaginal microbiota is associated with increased susceptibility to Chlamydia trachomatis infection in Dutch women: a case-control study. *Sexually transmitted infections, 94*(2), 117-123. doi:10.1136/sextrans-2017-053133

Vandenbroucke, J. P., von Elm, E., Altman, D. G., Gotzsche, P. C., Mulrow, C. D., Pocock, S. J., . . . Initiative, S. (2014). Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *Int J Surg, 12*(12), 1500-1524. doi:10.1016/j.ijsu.2014.07.014

Vasquez, A., Jakobsson, T., Ahrne, S., Forsum, U., & Molin, G. (2002). Vaginal lactobacillus flora of healthy Swedish women. *J Clin Microbiol, 40*(8), 2746-2749. doi:10.1128/jcm.40.8.2746-2749.2002

Vera Pingitore, E., Hebert, E. M., Nader-Macias, M. E., & Sesma, F. (2009). Characterization of salivaricin CRL 1328, a two-peptide bacteriocin produced by Lactobacillus salivarius CRL 1328 isolated from the human vagina. *Res Microbiol, 160*(6), 401-408. doi:10.1016/j.resmic.2009.06.009

Verhelst, R., Verstraelen, H., Claeys, G., Verschraegen, G., Delanghe, J., Van Simaey, L., . . . Vaneechoutte, M. (2004). Cloning of 16S rRNA genes amplified from normal and disturbed vaginal microflora suggests a strong association between Atopobium vaginae, Gardnerella vaginalis and bacterial vaginosis. *BMC Microbiol, 4*, 16. doi:10.1186/1471-2180-4-16

Verstraelen, H., & Swidsinski, A. (2013). The biofilm in bacterial vaginosis: implications for epidemiology, diagnosis and treatment. *Curr Opin Infect Dis, 26*(1), 86-89. doi:10.1097/QCO.0b013e32835c20cd

Verstraelen, H., Verhelst, R., Claeys, G., De Backer, E., Temmerman, M., & Vaneechoutte, M. (2009). Longitudinal analysis of the vaginal microflora in pregnancy suggests that L. crispatus promotes the stability of the normal vaginal microflora and that L. gasseri and/or L. iners are more conducive to the occurrence of abnormal vaginal microflora. *BMC microbiology, 9*, 116-116. doi:10.1186/1471-2180-9-116

Vitali, B., Cruciani, F., Picone, G., Parolin, C., Donders, G., & Laghi, L. (2015). Vaginal microbiome and metabolome highlight specific signatures of bacterial vaginosis. *Eur J Clin Microbiol Infect Dis, 34*(12), 2367-2376. doi:10.1007/s10096-015-2490-y

Vodstrcil, L. A., Twin, J., Garland, S. M., Fairley, C. K., Hocking, J. S., Law, M. G., . . . Bradshaw, C. S. (2017). The influence of sexual activity on the vaginal microbiota and Gardnerella vaginalis clade diversity in young women. *PLoS One, 12*(2), e0171856. doi:10.1371/journal.pone.0171856

Walther-António, M. R. S., Jeraldo, P., Berg Miller, M. E., Yeoman, C. J., Nelson, K. E., Wilson, B. A., . . . Creedon, D. J. (2014). Pregnancy's Stronghold on the Vaginal Microbiome. *PLoS One, 9*(6), e98514. doi:10.1371/journal.pone.0098514

Wang, L., Koppolu, S., Chappell, C., Moncla, B. J., Hillier, S. L., & Mahal, L. K. (2015). Studying the effects of reproductive hormones and bacterial vaginosis on the glycome of lavage samples from the cervicovaginal cavity. *PLoS One, 10*(5), e0127021-e0127021. doi:10.1371/journal.pone.0127021

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and environmental microbiology, 73*(16), 5261-5267. doi:10.1128/AEM.00062-07

Wassenaar, T. M., & Panigrahi, P. (2014). Is a foetus developing in a sterile environment? *Letters in Applied Microbiology, 59*(6), 572-579. doi:10.1111/lam.12334

Waters, T. P., & Mercer, B. (2011). Preterm PROM: prediction, prevention, principles. *Clin Obstet Gynecol, 54*(2), 307-312. doi:10.1097/GRF.0b013e318217d4d3

Wei, R., Wang, J., Su, M., Jia, E., Chen, S., Chen, T., & Ni, Y. (2018). Missing Value Imputation Approach for Mass Spectrometry-based Metabolomics Data. *Sci Rep, 8*(1), 663. doi:10.1038/s41598-017-19120-0

Wennerholm, U. B., Holm, B., Mattsby-Baltzer, I., Nielsen, T., Platz-Christensen, J. J., Sundell, G., & Hagberg, H. (1998). Interleukin-1alpha, interleukin-6 and interleukin-8 in cervico/vaginal secretion for screening of preterm birth in twin gestation. *Acta Obstet Gynecol Scand, 77*(5), 508-514. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9654172

Wertz, J., Isaacs-Cosgrove, N., Holzman, C., & Marsh, T. L. (2008). Temporal Shifts in Microbial Communities in Nonpregnant African-American Women with and without Bacterial Vaginosis. *Interdisciplinary perspectives on infectious diseases, 2008*, 181253-181253. doi:10.1155/2008/181253

Wiesenfeld, H. C., Hillier, S. L., Krohn, M. A., Landers, D. V., & Sweet, R. L. (2003). Bacterial Vaginosis Is a Strong Predictor of Neisseria gonorrhoeae and Chlamydia trachomatis Infection. *Clinical Infectious Diseases, 36*(5), 663-668. doi:10.1086/367658

Wiley, D. J., Masongsong, E. V., Lu, S., Heather, L. S., Salem, B., Giuliano, A. R., . . . Brown, D. R. (2012). Behavioral and sociodemographic risk factors for serological and DNA evidence of HPV6, 11, 16, 18 infections. *Cancer Epidemiol, 36*(3), e183-189. doi:10.1016/j.canep.2011.12.007

Wilks, M., Wiggins, R., Whiley, A., Hennessy, E., Warwick, S., Porter, H., . . . Millar, M. (2004). Identification and H(2)O(2) production of vaginal lactobacilli from pregnant women at high risk of preterm birth and relation with outcome. *Journal of clinical microbiology, 42*(2), 713-717. doi:10.1128/jcm.42.2.713-717.2004

Willson, J. R., & Goforth, M. L. (1942). Effect of an Excess of Ingested Carbohydrate upon the Glycogen Content of Vaginal Epithelium. *The Journal of Clinical Endocrinology & Metabolism, 2*(4), 223-225. doi:10.1210/jcem-2-4-223

Witkin, S. S., & Ledger, W. J. (2012). Complexities of the uniquely human vagina. *Sci Transl Med, 4*(132), 132fs111. doi:10.1126/scitranslmed.3003944

Witkin, S. S., Mendes-Soares, H., Linhares, I. M., Jayaram, A., Ledger, W. J., & Forney, L. J. (2013). Influence of vaginal bacteria and D- and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase inducer: implications for protection against upper genital tract infections. *MBio, 4*(4). doi:10.1128/mBio.00460-13

Woese, C. R. (1987). Bacterial evolution. *Microbiol Rev, 51*(2), 221-271. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2439888

Woese, C. R., Stackebrandt, E., Macke, T. J., & Fox, G. E. (1985). A phylogenetic definition of the major eubacterial taxa. *Syst Appl Microbiol, 6*, 143-151. doi:10.1016/s0723-2020(85)80047-3

Wolrath, H., Boren, H., Hallen, A., & Forsum, U. (2002). Trimethylamine content in vaginal secretion and its relation to bacterial vaginosis. *APMIS, 110*(11), 819-824. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12588422

Xia, J., & Wishart, D. S. (2011). Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. *Nat Protoc, 6*(6), 743-760. doi:10.1038/nprot.2011.319

Yamamoto, T., Zhou, X., Williams, C. J., Hochwalt, A., & Forney, L. J. (2009). Bacterial populations in the vaginas of healthy adolescent women. *J Pediatr Adolesc Gynecol, 22*(1), 11-18. doi:10.1016/j.jpag.2008.01.073

Yarlett, N., Goldberg, B., Moharrami, M. A., & Bacchi, C. J. (1993). Trichomonas vaginalis: characterization of ornithine decarboxylase. *Biochem J, 293 (Pt 2)*, 487-493. doi:10.1042/bj2930487

Yeoman, C. J., Yildirim, S., Thomas, S. M., Durkin, A. S., Torralba, M., Sutton, G., . . . Wilson, B. A. (2010). Comparative genomics of Gardnerella vaginalis strains reveals substantial differences in metabolic and virulence potential. *PLoS One, 5*(8), e12411. doi:10.1371/journal.pone.0012411

Yeoman, C. J. C., N.; Yildirim, S.; Miller, M.E.B.; Kent, A.; Stumpf, R.; Leigh, S.R.; Nelson, K.E.; White, B.A.; Wilson, B.A. (2011). Towards an Evolutionary Model of Animal-Associated Microbiomes. *Entropy, 13*, 570-594. doi:http://doi.org/10.3390/e13030570

Yildirim, S., Yeoman, C. J., Janga, S. C., Thomas, S. M., Ho, M., Leigh, S. R., . . . Stumpf, R. M. (2014). Primate vaginal microbiomes exhibit species specificity without universal Lactobacillus dominance. *The ISME Journal, 8*(12), 2431-2444. doi:10.1038/ismej.2014.90

APPENDIX A

CO-AUTHORED PUBLICATIONS

frontiers in Physiology

ORIGINAL RESEARCH
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Vaginal biogenic amines: biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis?

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Bacterial vaginosis (BV) is the most common vaginal disorder among reproductive age women. One clinical indicator of BV is a "fishy" odor. This odor has been associated with increases in several biogenic amines (BAs) that may serve as important biomarkers. Within the vagina, BA production has been linked to various vaginal taxa, yet their genetic capability to synthesize BAs is unknown. Using a bioinformatics approach, we show that relatively few vaginal taxa are predicted to be capable of producing BAs. Many of these taxa (*Dialister, Prevotella*, *Parvimonas, Megasphaera, Peptostreptococcus,* and *Veillonella* spp.) are more abundant in the vaginal microbial community state type (CST) IV, which is depleted in lactobacilli. Several of the major *Lactobacillus* species (*L. crispatus, L. jensenii,* and *L. gasseri*) were identified as possessing gene sequences for proteins predicted to be capable of putrescine production. Finally, we show in a small cross sectional study of 37 women that the BAs putrescine, cadaverine and tyramine are significantly higher in CST IV over CSTs I and III. These data support the hypothesis that BA production is conducted by few vaginal taxa and may be important to the outgrowth of BV-associated (vaginal dysbiosis) vaginal bacteria.

Keywords: acid stress, polyamine, malodor, bacterial vaginosis, cadaverine

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Introduction

The microbial community (microbiome) that colonizes the vagina of healthy women are typically dominated by one of several bacterial species of the genus *Lactobacillus* (Wolrath et al., 2002; Ravel et al., 2011; Macklaim et al., 2012). Therein, lactobacilli produce lactic acid creating an acidic environment (pH 2.8–4.2) that is inhospitable to many non-*Lactobacillus* commensals and potential vaginal pathogens (Amsel et al., 1983; Graver and Wade, 2011; O'Hanlon et al., 2011). This represents a classic form of niche-construction (Yeoman et al., 2011) recapitulated by human colonic and ruminal lactobacilli during gastrointestinal acidosis events (Allison et al., 1975; Bongaerts et al., 2000). Along with lactic acid, the lactobacilli may produce various antimicrobials (Aroutcheva et al., 2001; Anokhina et al., 2007; Rönnqvist et al., 2007) and toxin attenuating molecules (Cadieux et al., 2009; Li et al., 2011) that collectively are thought to constitute one of the primary barriers to vaginal diseases like bacterial vaginosis (BV) (Atassi and Servin, 2010).

BV is the most common disorder of the vagina in reproductive-aged women (Lefèvre et al., 1985) having been estimated to occur in almost one-third of U.S. women between 2001 and 2004 (Allsworth and Peipert, 2007). Clinical signs of BV include an amine or "fishy" vaginal odor, a creamy gray discharge, an elevated pH and/or the presence of superficial squamous cells with peripheral clumps of bacteria (clue cells) (Amsel et al., 1983). The signs are also observed alongside significant reductions in vaginal lactobacilli, which are replaced by an outgrowth of diverse, strict and facultative anaerobic bacterial taxa that commonly includes *Gardnerella vaginalis, Dialister* spp., *Atopobium* spp., *Prevotella* spp., *Mobiluncus* spp. and others (Spiegel et al., 1980; Amsel et al., 1983). These microbiological features may be causally linked as reductions in *Lactobacillus* spp. correspond to decreased vaginal concentrations of lactic acid and significant increases in vaginal pH (pH > 4.5) that provide a more hospitable environment for BV-associated species (O'Hanlon et al., 2011). However, while the depletion of vaginal lactobacilli and outgrowth of anaerobes is a characterizing co-feature of BV, it has been shown that ∼27% of reproductive-age women exhibit vaginal microbiome deplete of *Lactobacillus* spp. (Ravel et al., 2011). This *Lactobacillus*-deplete vaginal microbiome is recognized as one of the five community state types (CSTs), termed by Ravel et al. as CST IV (Ravel et al., 2011) (**Table 1**). Recent findings have shown women with vaginal CST IV may persist in this state for extended periods of time without reporting symptoms of BV, regardless of their perception of whether those symptoms are present or not (Gajer et al., 2012). These findings may indicate a multi-staged, multi-microbial pathway to BV, whereby the protective features of the lactobacilli must be overcome prior to the chance exposure to, and colonization of less acid-sensitive organism(s) capable of eliciting additional clinical features (Lambert et al., 2013).

One clinical feature of BV, malodor, has been linked to increases in vaginal biogenic amines (BAs), including the polyamines putrescine, cadaverine, and trimethylamine (Yeoman et al., 2013). BAs are organic compounds with one or more amine (NH_2) group(s), and may represent useful biomarkers of BV (Blankenstein et al., 2015). Other common BAs include tyramine, agmatine, spermine, and spermidine, the latter having also been observed at low relative concentrations in the vaginal metabolome (Yeoman et al., 2013). BAs are primarily produced via specific amino acid decarboxylation pathways (Shah and Swiatlo, 2008) (**Figure 1**). In *Escherichia coli* and many *Pseudomonas* species, putrescine is synthesized from arginine or ornithine using one of two major pathways: (i) decarboxylation of arginine to agmatine by arginine decarboxylase (encoded by gene s*peA*; Enzyme Commission number, E.C. 4.1.1.19) and then to putrescine either directly by agmatinase (s*peB* gene; E.C. 3.5.3.11) or via N-carbamoylputrescine as catalyzed by agmatine deiminase (E.C. 3.5.3.12) and then N-carbamoylputrescine amidohydrolase (*AguB* gene; E.C. 3.5.1.53), or (ii) decarboxylation of ornithine to putrescine via ornithine decarboxylase (*speC* gene; E.C. 4.1.1.17) (Tabor and Tabor, 1985) (**Figure 1**). These two putrescine biosynthesis pathways have been shown to operate simultaneously in many bacteria (Tabor and Tabor, 1985; Craciun and Balskus, 2012). Cadaverine and tyramine biosynthesis is less commonly described among bacterial species, although this could be a reflection of the relatively limited investigations in non-model species. *E. coli* synthesizes cadaverine during anaerobic growth at low pH in the presence of its precursor, lysine, as catalyzed by lysine decarboxylase (*cadA* gene; E.C. 4.1.1.18) (Watson et al., 1992). Tyramine is synthesized by various *Enterococcus* species by the decarboxylation of tyrosine (Fernandez de Palencia et al., 2011). Perhaps the best-studied BA in the context of BV is trimethylamine (TMA). TMA is most commonly produced by the reduction of trimethylamine oxide (TMAO), a reaction catalyzed by trimethylamine N-oxide reductase (E.C. 1.7.2.3). TMA can also be synthesized from choline by choline trimethylamine-lyase (Craciun and Balskus, 2012), N,N,N-trimethylglycine via betaine reductase (E.C. 1.21.4.4), and ergothioneine by ergothionase (Muramatsu et al., 2013). A previous study showed strains of vaginal *Mobiluncus* species, including both *M. mulieris* and *M. curtisii* were able to produce TMA through the reduction of TMAO, and weakly through the reduction of choline (Cruden and Galask, 1988). While various bacterial species have been shown to be capable of producing BAs, aside from *Mobiluncus* spp., and the vaginal parasite, *Trichomonas vaginalis*, which has been shown to encode an ornithine decarboxylase (Yarlett et al., 1993), little is known about their production by the vaginal microbiome.

In addition to their potential as biomarkers of BV, BAs may also be important to facilitating the outgrowth of BV-associated vaginal taxa. This hypothesis is based on the following observations: (i) amino-acid decarboxylation involves the consumption of intracellular hydrogen ions and is a well-described bacterial acid resistance and mitigation mechanism (Kanjee and Houry, 2013); and (ii) the growth and resistance to host immunological defenses of some bacteria, including the urogenital pathogen, *Neisseria gonorrhoeae*, has been shown to be improved in the presence of various BAs (Strøm et al., 1979; Goytia and Shafer, 2010; Nasrallah et al., 2011; Jelsbak et al., 2012). It is also noteworthy that BAs have been correlated with

TABLE 1 | Community state types (CSTs) of the vagina.

I (*L. crispatus*)		II (*L. gasseri*)		III (*L. iners*)		IV (Diverse group)		V (*L. jensenii*)	
Women	**pH**	**Women**	**pH**	**Women**	**pH**	**Women**	**pH**	**Women**	**pH**
27%	4.0 ± 0.3	6%	5.0 ± 0.7	34%	4.4 ± 0.6	27%	5.3 ± 0.6	5%	4.7 ± 0.4

Adapted from Ravel et al. (2011).

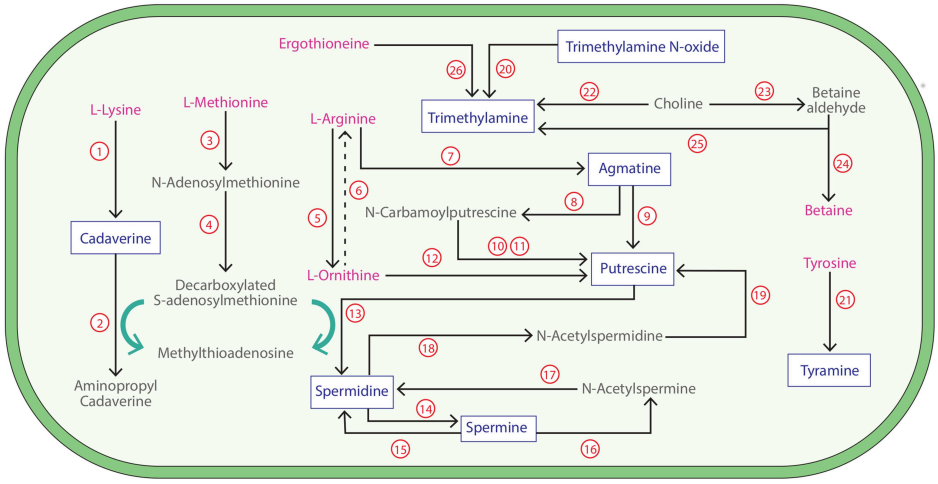

FIGURE 1 | Biogenic amine synthesis pathways in bacteria. The dominant biogenic amine (BA) synthesis pathways involving the production of BAs (blue) from amino acids (pink) and associated derivatives in bacterial species are shown. Pathways are as follows (genes searched are shown in bold and underlined): **1. Lysine decarboxylase (Enzyme Commission number, E.C. 4.1.1.18)**; 2. Cadaverine aminopropyl-transferase (E.C. 2.5.1.-); 3. Methionine adenosyltransferase (E.C. 2.5.1.6); 4. S-adenosylmethionine decarboxylase (E.C. 4.1.1.50); **5. Arginase (E.C. 3.5.3.1)**; 6. Multiple enzyme reactions; **7. Arginine decarboxylase (E.C. 4.1.1.19)**; **8. Agmatine deiminase (E.C. 3.5.3.12)**; **9. Agmatinase (E.C. 3.5.3.11)**; **10. N-Carbamoylputrescine amidohydrolase (E.C. 3.5.1.53)**; 11. Putrescine transcarbamylase (E.C. 2.1.3.6); **12. Ornithine decarboxylase (E.C. 4.1.1.17)**; **13. Spermidine synthase (E.C. 2.5.1.16)**; **14. Spermine synthase (E.C. 2.5.1.22)**; **15. Spermine oxidase (E.C. 1.5.3.16)**; 16. Spermine acetyltransferase (E.C. 2.3.1.57); 17. Acetylpolyamine oxidase (EC 1.5.3.13); 18. Spermidine acetyltransferase (E.C 2.3.1.57); 19. Acetylpolyamine oxidase (E.C.1.5.3.13); **20. Trimethylamine N-oxide reductase (E.C. 1.7.2.3)**; **21. Tyrosine decarboxylase (E.C. 4.1.1.25)**; **22. Choline trimethylamine-lyase (E.C. 4.3.99.4)**; 23. Choline dehydrogenase (E.C. 1.1.99.1); 24. Betaine aldehyde dehydrogenase (E.C. 1.2.1.8); **25. Betaine reductase (E.C. 1.21.4.4)**; **26. Ergothionase (E.C. not applicable)**.

numerous host disease states (Löser et al., 1990; Paik et al., 2006; Pegg, 2009; Brooks, 2013). Here we put forward a novel conceptual hypothesis of vaginal dysbiosis via bacterial BA-production (detailed in **Figure 2**) that precedes BV development.

Methods

Strains and Genomes

Our search database included 50 urogenital isolates whose genomic data was available in GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Taxonomy was guided toward the major taxa identified by Ravel et al. (2011) in their comprehensive evaluation of the vaginal microbiome of reproductive age women, and taxa in the studies of Yeoman et al. (2013) and Srinivasan et al. (2012) as being prominent and associated with odor or BA production. Where representative genomes of vaginal isolates were not available, sequenced isolates sourced from other body niches were utilized as available ($n = 7$), or, if not available, all protein-coding sequences within that genus ($n = 7$) were utilized. For example, *Bifidobacterium dentium* Bd1 was sequenced as part of the Human Microbiome Project, but previous literature has shown four other *Bifidobacterium* species are commonly isolated from the vagina (Korshunov et al., 1999). Therefore, protein-coding data of gastrointestinal isolates of *Bifidobacterium bifidum*, *B. breve*, *B. adolescentis*, and *B. longum* were used. Elsewhere, no complete genomic data or inventory of vaginal *Sneathia* species/isolates have been reported, so we utilized all available *Sneathia* protein coding data in GenBank. All available protein-coding sequences of selected bacterial taxa were downloaded from GenBank.

Identification of Biogenic Amine Producing Genes in Vaginal Bacteria

Functionally characterized gene sequences of biogenic amine-synthesizing proteins (BSPs), including arginine decarboxylase, agmatine deiminase, N-carbamoylputrescine amidohydrolase, agmatinase, lysine decarboxylase, ornithine decarboxylase, tyrosine decarboxylase, trimethylamine N-oxide reductase, ergothionase, choline trimethylamine-lyase, betaine reductase, spermidine synthase, and spermine synthase (**Table S1**) were obtained from GenBank and used in a stand-alone BLASTP search against a database of the protein coding sequences of vaginal taxa. The BA-synthesizing protein sequences used in the study are available in the Supplementary Materials. BLASTP alignments with ≥45% sequence identity across ≥80% of the query sequence, or ≥35% sequence identity across ≥90% of the

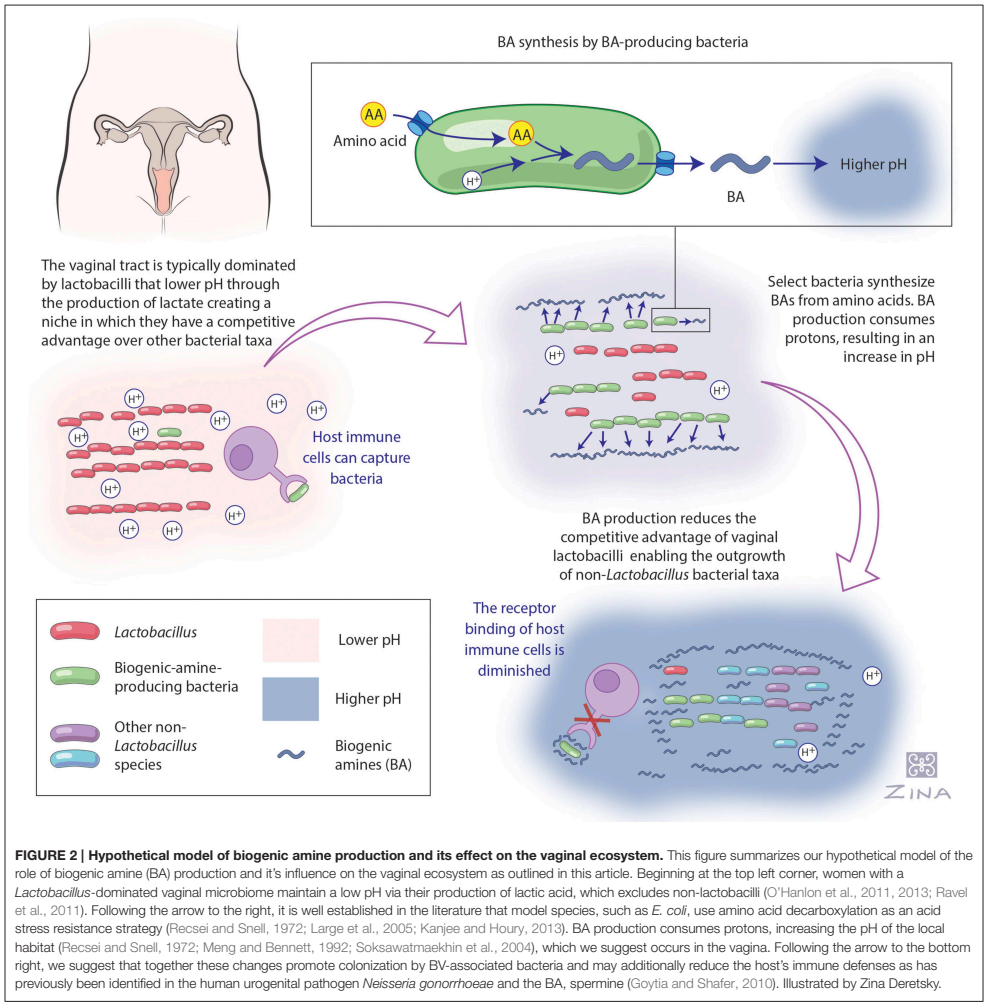

FIGURE 2 | Hypothetical model of biogenic amine production and its effect on the vaginal ecosystem. This figure summarizes our hypothetical model of the role of biogenic amine (BA) production and it's influence on the vaginal ecosystem as outlined in this article. Beginning at the top left corner, women with a *Lactobacillus*-dominated vaginal microbiome maintain a low pH via their production of lactic acid, which excludes non-lactobacilli (O'Hanlon et al., 2011, 2013; Ravel et al., 2011). Following the arrow to the right, it is well established in the literature that model species, such as *E. coli*, use amino acid decarboxylation as an acid stress resistance strategy (Recsei and Snell, 1972; Large et al., 2005; Kanjee and Houry, 2013). BA production consumes protons, increasing the pH of the local habitat (Recsei and Snell, 1972; Meng and Bennett, 1992; Soksawatmaekhin et al., 2004), which we suggest occurs in the vagina. Following the arrow to the bottom right, we suggest that together these changes promote colonization by BV-associated bacteria and may additionally reduce the host's immune defenses as has previously been identified in the human urogenital pathogen *Neisseria gonorrhoeae* and the BA, spermine (Goytia and Shafer, 2010). Illustrated by Zina Deretsky.

query sequence with an expected value (*E*-value) $\leq 10^{-30}$ were determined to be a positive indication of BSPs among the genetic infrastructure of the query microbe. All protein sequences were also searched using hidden markov models (HMMs) corresponding to the BSPs downloaded from the TIGRFAM (http://www.jcvi.org/cgi-bin/tigrfams/index.cgi) or Pfam (http://pfam.xfam.org/) databases (**Table S1**) using HMMer (http://hmmer.janelia.org/). HMM hits that had *E*-values below their prescribed trusted cut-offs were determined to be a positive indication of these genes in the query protein sequences. Data were visualized using heat maps that were produced using the *gplots* package (Warnes et al., 2009) constructed in R v.3.1.2 (Ihaka and Gentleman, 1996). Results were color coded in the heat map based on the extent of evidence supporting the presence of each BSP in each respective genome.

Determining Biogenic Amine Producing Taxa among CSTs with Stratification by pH

Data on the fine scale taxonomic composition and vaginal pH of 394 women were obtained from Ravel et al. (2011). Taxa

corresponding to those identified as encoding putative BSPs were interrogated. The Ravel dataset was stratified by CST and pH so that the relative abundances of the 16S rRNA gene sequence reads from BSP-encoding taxa were averaged for each of the five CSTs and then further categorized into ether low or high pH. We determined pH 4.0–4.5 was "low" and pH 4.6–7.0 was "high," based on pH ranges used to diagnose BV based on Amsel's criteria (Amsel et al., 1983). Significance between groups was determined with the Mann-Whitney-Wilcoxon test with Bonferroni correction used for multiple testing where $p < 0.05$ performed in R v.3.1.2 (Ihaka and Gentleman, 1996).

Threshold Analysis of Vaginal Taxa along the pH Gradient

To further understand the association of BSP-encoding taxa with pH in the vagina, threshold indicator taxa analysis (TITAN) was conducted. TITAN was used to assess the association of taxa to pH using the dataset obtained from Ravel et al. (2011). TITAN uses IndVal (Indicator Value) scores to capture the strength-of-association between a particular species along a gradient (Dufrene and Legendre, 1997). The probability of obtaining an equal or larger IndVal score from random data is estimated by comparing the magnitude of each observed IndVal score with those generated by randomly assigning membership along the variable gradient via permutations. Bootstraps were used to compute the confidence interval of the change point location along the gradient for each taxa. Confidence intervals for each IndVal were generated using 500 permutations of the data. The bacterial abundance dataset was log10(x + 1) transformed prior to analysis to reduce the influence of highly variable taxa and rare operational taxonomic units (OTUs) (<5 occurrences across women) were removed (Baker and King, 2010). TITAN analyses were performed using the *mvpart* package in R v.3.1.2 (Ihaka and Gentleman, 1996) with code provided in Baker and King (2010).

Determining Biogenic Amine Levels Corresponding to CSTs

Sample Set for Measuring Biogenic Amine Levels

Analyses were performed on self-collected mid-vaginal swab (Copan flocked nylon elution-swab and Starplex double headed rayon swab) samples collected from 37 non-pregnant, non-lactating women, aged 18–45 years recruited for a single visit to the Center for Health Behavior Research (CHBR) at the University of Maryland School of Public Health (UMSPH) as part of a previously published study (Brotman et al., 2014). Women had to be healthy as determined by medical history, with absence of acute or chronic illnesses. In addition, participants were excluded if they had used an antibiotic or anti-mycotic in the prior 30 days or reported a known history of other drug or alcohol dependence in the prior 12 months. All participants provided written informed consent. Ethical approval was obtained from the Institutional Review Boards of the University of Maryland Baltimore (UMB) and the UMSPH.

Sample Preparation

Samples were eluted from swabs in sterile molecular water and subjected to both gas chromatography mass spectrometry (GC/MS) and liquid chromatography mass spectrometry (LC/MS) with Orbitrap Elite accurate mass platforms (Thermo Scientific, Waltham, MA, USA). Sample processing was performed by Metabolon (Durham, NC, 27713) using an automated MicroLab STAR® system (Hamilton Company, Reno, NV, USA). Recovery standards were added prior to the first step in the extraction process for QC purposes. Sample preparation was conducted using a proprietary series of organic and aqueous extractions to remove the protein fraction while allowing maximum recovery of small molecules. The resulting extract was divided into two fractions: one for analysis by LC and one for analysis by GC. Samples were placed briefly on a TurboVap®(Zymark, Hopkinton, MA, USA) to remove the organic solvent. Each sample was then frozen and dried under vacuum. Samples were then prepared for the appropriate instrument, either LC/MS or GC/MS.

Liquid Chromatography Mass Spectrometry

LC/MS measurements were conducted on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a ThermoFisher Scientific Orbitrap Elite high resolution/accurate mass spectrometer, which consisted of a heated electrospray ionization (HESI) source and Orbitrap mass analyzer operated at 30,000 mass resolution. The sample extract was dried then reconstituted in LC-compatible solvents, each of which contained eight or more injection standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion optimized conditions and the other using basic negative ion optimized conditions in two independent injections using separate dedicated columns. Extracts reconstituted in acidic conditions were gradient eluted using water and methanol containing 0.1% formic acid, while the basic extracts, which also used water/methanol, contained 6.5 mM ammonium bicarbonate. The MS analysis alternated between MS and data-dependent MS^2 scans using dynamic exclusion. Raw data files were archived and extracted as described below.

Gas Chromatography Mass Spectrometry

Samples for GC/MS analysis were re-dried under vacuum desiccation for a minimum of 24 h prior to being derivatized under dried nitrogen using bistrimethyl-silyl-triflouroacetamide (BSTFA). The GC column was 5% phenyl and the temperature ramp is from 40 to 300°C in a 16 min period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization. The instrument was tuned and calibrated for mass resolution and mass accuracy prior to use. The information output from the raw data files was automatically extracted as described below.

Data Extraction

The data extraction of the raw MS data files yielded information that could be loaded into a relational database and manipulated without resorting to binary large object (BLOB) manipulation.

Once in the database the information was examined and peaks were identified using Metabolon's proprietary peak integration software, and component parts were stored in a separate and specifically designed complex data structure (Ryals et al., 2007).

Compound Identification

Spectra corresponding to each BA were identified by comparison to library entries of purified BA standards and their distinction from more than 1000 other commercially available purified standard compounds. The combination of chromatographic properties and mass spectra gave an indication of a match to the specific BA compound or an isobaric entity. Results were manually curated to ensure that data were accurate and to remove any system artifacts, mis-assignments, and background noise.

Results

Distribution of Biogenic Amine Synthesizing Proteins among Common Vaginal Taxa

Previous studies have shown correlative relationships among the vaginal odor characteristic of BV, BAs including putrescine, cadaverine, and trimethylamine (TMA) and particular bacterial taxa (Chen et al., 1979, 1982; Sanderson et al., 1983; Cruden and Galask, 1988; Wolrath et al., 2002; Srinivasan et al., 2012; Yeoman et al., 2013). To examine the potential for common vaginal taxa to produce these BAs, we examined the protein coding genes of 65 common vaginal bacterial taxa for the presence of biogenic BSPs by alignment to functionally characterized BSPs and searches with hidden markov models (HMMs) built for BSPs (see Methods). Interestingly, very few vaginal taxa possessed BSPs for the biosynthesis of the major BAs (**Figure 2**). *E. coli* 83972 was the only taxa predicted to be capable of producing putrescine, cadaverine, and TMA (**Figure 3**). *E. coli* 83972 also appeared to be capable of producing agmatine, spermine and spermidine. All examined strains of *Dialister micraerophilus, Veilonella* spp., *Proteus mirabilis*, *Janthinobacterium* spp. and common lactobacilli, *L. crispatus, L. gasseri*, and *L. jensenii* (but not *L. iners*) each encoded BSP homologs of genes capable of producing putrescine through the decarboxylation of ornithine (**Figure 3**). *Prevotella amnii, P. bivia, P. buccalis, P. disiens*, *P. oralis* possessed putative BSPs allowing the production of agmatine, and eventually putrescine through the decarboxylation of arginine. Two *Eggerthella* spp. *B. longum*, *B. breve*, *Mycoplasma* spp., *Porphyromonas uenonis*, *Parvimonas micra* and *Gemella* spp. also possessed one or more gene homologs of BSPs on the pathway conversion of arginine to agmatine and then putrescine. Only the taxa *E. coli* 83972, *P. mirabilis*, and *Janthinobacterium* spp. were found to possess putative BSPs encoding lysine decarboxylases for the biosynthesis of cadaverine (**Figure 3**). It was noteworthy that in each instance the lysine decarboxylase genes were incorrectly annotated as arginine decarboxylases. *E. coli* 83972, *Megasphaera genomosp type I, Anaeroglobus geminatus* F0357, along with all examined *Dialister microaerophilus, Veillonella* and *Leptotrichia* spp. possessed spermidine and/or spermine synthase genes. Spermidine and spermine synthase genes were not readily distinguishable from one another bioinformatically. *D. micraerophilus* DSM 19965 was the only taxon predicted to possess a tyrosine decarboxylase enabling the production of the monoamine tyramine.

The BA trimethylamine (TMA) is synthesized through four known pathways (**Figure 1**). Interestingly, only *E. coli* 83972 was found to possess a homolog of the trimethylamine N-oxide reductase, the only currently described enzyme capable of synthesizing TMA from trimethylamine N-oxide (TMAO) (Strøm et al., 1979). This was unexpected given previous findings that TMA was produced in cultures containing TMAO by vaginal *Mobiluncus* spp. (Cruden and Galask, 1988). To reconcile this discrepancy, we more intensively examined the genomes of the vaginal *Mobiluncus* isolates. Weaker alignments were found between the characterized TMAO reductase and a gene annotated in each *M. curtisii* genome as a dimethyl sulfoxide reductase α-subunit (28% protein identity, over 95% of the query, $E = 1 \times 10^{-66}$). Similar matches were not found in representative *M. mulieris* genomes and were not supported by HMM examinations. Broader searches among all available sequences from the *Mobiluncus* genus also did not reveal any candidate homologs. Additionally, available *Mobiluncus* spp. genomes did not encode a homolog of the choline trimethylamine-lyase required for the production of TMA from choline, also reported by Cruden and Galask (1988). However, homologs of BSPs encoding choline trimethylamine-lyase were observed in *E. coli* 83972, *Megasphaera genomosp type I*, and various *Leptotrichia* spp. TMA could also be synthesized by *P. micra, Peptostreptococcus anaerobius*, and various *Leptotrichia* and *Gemella* spp. using betaine reductase. *P. mirabilis* was the only taxon found to possess putative BSPs encoding ergothionase, which catalyzes conversion of ergothioneine to thiolurocanic acid and TMA (Craciun and Balskus, 2012).

BSP-encoding Taxa Associated with Increased pH and CST IV

To determine the relationship of the various BSP-possessing taxa identified above to the growth of BV-associated bacteria, we tested their relative abundances in the various CSTs using data provided by Ravel and colleagues on 394 reproductive-aged women (Ravel et al., 2011). Because *Mobiluncus* spp. had previously been shown to produce TMA (Cruden and Galask, 1988), we also included this taxa in our analyses. BSP-encoding taxa were present in vaginal microbiome from all CSTs. However, there was a clear difference in the abundance and type of BSP-encoding taxa between CSTs (**Figure 4**). Women categorized into the low-*Lactobacillus* CST IV, had higher levels of predicted putrescine-producers, *Prevotella, Dialister,* and *Veillonella* spp. and TMA producers, *Peptostreptococcus*, *Parvimonas,* and *Megasphaera* spp. as well as *Mobiluncus* spp. (**Figure 4**).

As pH is considered an important barrier to the outgrowth of BV-associated bacteria, and the production of BAs may impact pH directly, we then stratified the data for each CST by pH (pH 4.0–4.5 "low" and pH 4.6–7.0 "high") and re-examined the relative abundances of each BSP-encoding taxa. These pH categories were determined based on the known pH ranges of CST groups representing women (**Table 1**) (Ravel et al., 2011) and from current clinical criteria (Amsel et al., 1983).

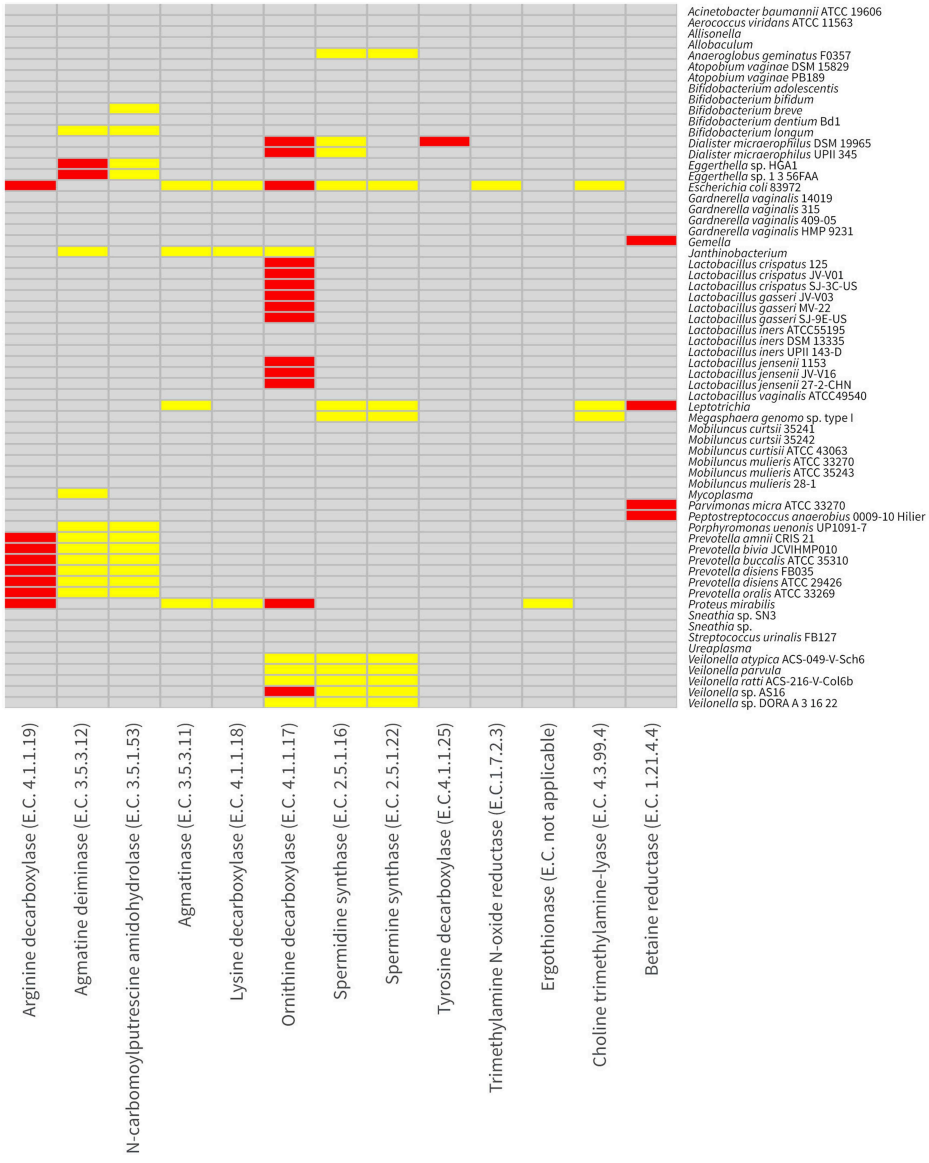

FIGURE 3 | Distribution of biogenic amine-synthesizing proteins (BSPs) in vaginal taxa. The distribution of BSPs in the 65 vaginal taxa examined is presented as a heat map. Gray indicates no evidence of the BSP shown on the horizontal axis in the taxon shown on the vertical axis. Yellow indicates a strong alignment to a characterized homolog or to a corresponding hidden markov model (HMM), while red indicates both a strong alignment to a characterized homolog and a corresponding HMM. Enzyme numbers (E.C.) are shown with BSPs.

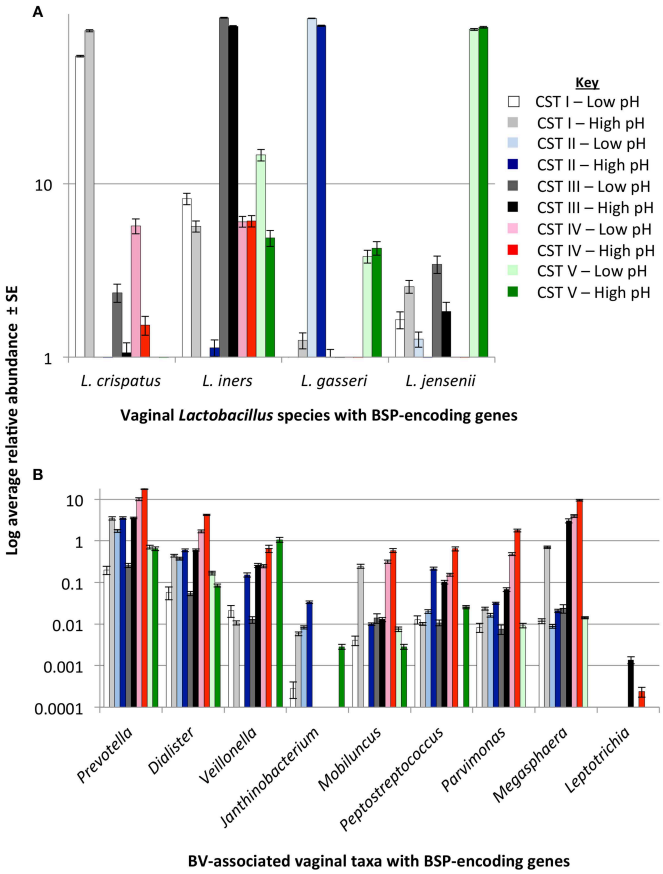

FIGURE 4 | BSP-encoding gene presence stratified by community state type (CST) and pH. Bar charts display average log scaled relative abundances ± standard error (SE) of bacterial taxa in the vaginal microbiome of healthy women with biogenic amine-synthesizing proteins (BSP) encoding genes as identified from GenBank. Taxa are grouped by *Lactobacillus* species **(A)** and BV-associated anaerobes **(B)** shown in relation to CST and vaginal pH. Abundance data was taken from Ravel et al. (2011). Significant differences between groups are shown in **Table 2**.

Prevotella and *Dialister* spp. were significantly more abundant in CST I, III, and IV when pH was 4.6 or greater (**Figure 4**, **Table 2**). In the "high" pH category for CST III, *Veillonella*, *Parvimonas* and *Megasphaera* species were also significantly more abundant (**Figure 4**, **Table 2**). As expected, *Lactobacillus crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii* displayed a decreasing trend with increasing pH while BV-associated BSP-encoding taxa displayed the opposite trend (**Figure S1**).

The relationship between pH and vaginal taxa was further explored with TITAN. TITAN provides an understanding of the community response to gradients in the surrounding habitat and can be used to assess community thresholds. We compared vaginal taxa along the gradient of pH observed in the vagina of 394 women and identified the value of greatest synchronous decline in bacterial taxa occurs at pH 4.4 (**Figure 5**, **Table 3**). The individual taxa that contributed strongly and negatively to increases in pH included all of the dominant *Lactobacillus* spp. and with the exception of a *Clostridium* spp. were the only genera that had significantly negative decline with increases in pH (**Figure 5**, **Table S2**). Those taxa which

TABLE 2 | Difference in low and high pH for BSP-encoding taxa by CST.

Species/Genus	Testing between low and high pH groups				
	CST I	CST II	CST III	CST IV	CST V
L. crispatus					
L. iners					
L. gasseri					
L. jensenii					
Prevotella			**		
Dialister	*		**		
Veillonella					
Janthinobacterium			NA	NA	NA
Mobiluncus					
Peptostreptococcus					
Parvimonas			*		
Megasphaera			**		
Leptotrichia	NA	NA		NA	NA

*The presence of biogenic amine-synthesizing proteins (BSPs) obtained bioinformatically as grouped by community state types (CSTs). Results obtained from Mann-Whitney-Wilcoxon testing with Bonferroni corrections between groups as shown in **Figure 2**. **P < 0.01; *P < 0.05. P-values were corrected for multiple testing. Not applicable due to insufficient observations (NA).*

responded significantly and positively to higher pH included the BSP-encoding species from the genera *Prevotella, Dialister, Parvimonas, Megasphaera, Mobiluncus*, *Peptostreptococcus,* and *Veillonella* spp. (**Figure 5**, **Table S2**). In addition, species from the *Peptoniphilus, Anaerococcus, Atopobium, Sneathia,* and *Finegoldia* genera were more common where pH > 4.6 (**Figure 5**, **Table S2**).

Putrescine and Cadaverine Are Higher in CST IV Vaginal Microbiome

To determine the actual changes in vaginal BA levels associated with CSTs, we performed a cross-sectional study on the vaginal metabolome of 37 women found to represent the three most common CSTs (CST I, CST III, and CST IV). Women with CST IV had higher abundances of the BAs cadaverine, putrescine, and agmatine over the other CSTs (**Figure 6**, **Table 4**). All precursor amino acids and derivatives including the amino acids lysine, methionine, ornithine, arginine and tyrosine were lower in CST IV over CSTs I and III (**Figure 6**, **Table 4**). These trends in BAs and amino acids were significant between CST IV and CST I but not CST IV and CST III (**Table 4**). Additionally, the triamine, spermidine, and tetraamine, spermine, were higher in women with the CST I vaginal microbiome (**Figure 6**, **Table 4**). Our analyses did not detect TMA, although it was not clear if this was methodological rather than a true absence.

Discussion

BAs are produced by all eukaryotes but only by select bacteria (Pegg, 2013). They are compounds that have one or more amino group ($-NH_2$) and are often derived from amino acid precursors (Tabor and Tabor, 1985). In bacteria, BAs are involved in many essential reactions relevant to transcription, translation, growth and metabolism (Tabor and Tabor, 1985; Wallace et al., 2003; Wortham et al., 2007). Recently it has been realized that BAs are associated with a number of other functions in prokaryotes specific to the manifestation and symptoms of infections, including improved acid resistance, protection from oxidative stress and host immunological defenses, promotion of biofilm formation, and control of membrane permeability (Tabor and Tabor, 1985; Dela Vega and Delcour, 1996; Jung and Kim, 2003; Patel et al., 2006; Wortham et al., 2007; Shah and Swiatlo, 2008; Goytia and Shafer, 2010; Zhao and Houry, 2010; Nasrallah et al., 2011; Jelsbak et al., 2012). Correspondingly, BAs have been associated with various host disease states (Löser et al., 1990; Paik et al., 2006; Pegg, 2009; Brooks, 2013). There is currently no consensus as to the common function of BAs in bacteria, yet their presence and production has been shown to be a factor in the manifestation of several bacterially mediated diseases. We hypothesize this may also be the case with BV (outlined in **Figure 2**).

BA synthesis as indicated by the presence of genes encoding BSPs were observed sporadically across vaginal taxa. BA synthesis is coupled to amino acid decarboxylation, which has been shown to be a protective mechanism developed to maintain the intracellular pH homeostasis of various bacteria (including *E. coli* and some *Lactobacillus* spp.) when growing under acidic conditions (Jung and Kim, 2003; Azcarate-Peril et al., 2004; Large et al., 2005; Cid et al., 2008; Kanjee and Houry, 2013). The decarboxylase enzymes are typically induced by the presence of the amino acid precursor when confronted with an acidic environment (Yarlett et al., 1993). The decarboxylation reaction consumes a proton, which cumulatively results in an increased pH within the cytoplasm creating a transmembrane pH gradient (Molenaar et al., 1993). The decarboxylase enzymes function in co-operation with an amino acid/BA antiporter system that moves the BAs to the outside of the cell in exchange for extracellular amino acids (Yarlett et al., 1993). This massive extrusion of the basic BA product coupled by the uptake of the amino acid substrate and proton motive force may also contribute to increases in the pH of the extracellular milieu (Meng and Bennett, 1992; Molenaar et al., 1993; Bearson et al., 1998). Two of these systems, well understood in *E. coli*, produce cadaverine and putrescine through the decarboxylation of lysine and ornithine, respectively (Kanjee and Houry, 2013). A number of studies have identified increased concentrations of these BAs and others in women with BV (Wolrath et al., 2002; Baker and King, 2010; Sakamoto et al., 2012; Lambert et al., 2013). Together, eight genera and multiple species including the most common *Lactobacillus* spp. encoded either lysine or ornithine decarboxylase or both. The BSP encoding ornithine decarboxylase was the most common system we identified across the 64 vaginal taxa investigated in our analysis and several other systems were common to multiple species within a genus. All vaginal lactobacilli with the exception of *L. vaginalis* and *L. iners* were identified as having ≥35% sequence identity to the ornithine decarboxylase protein sequences from non-vaginal *L. brevis* and *L. saerimneri* (formerly *Lactobacillus* sp. strain 30a) (Guirard

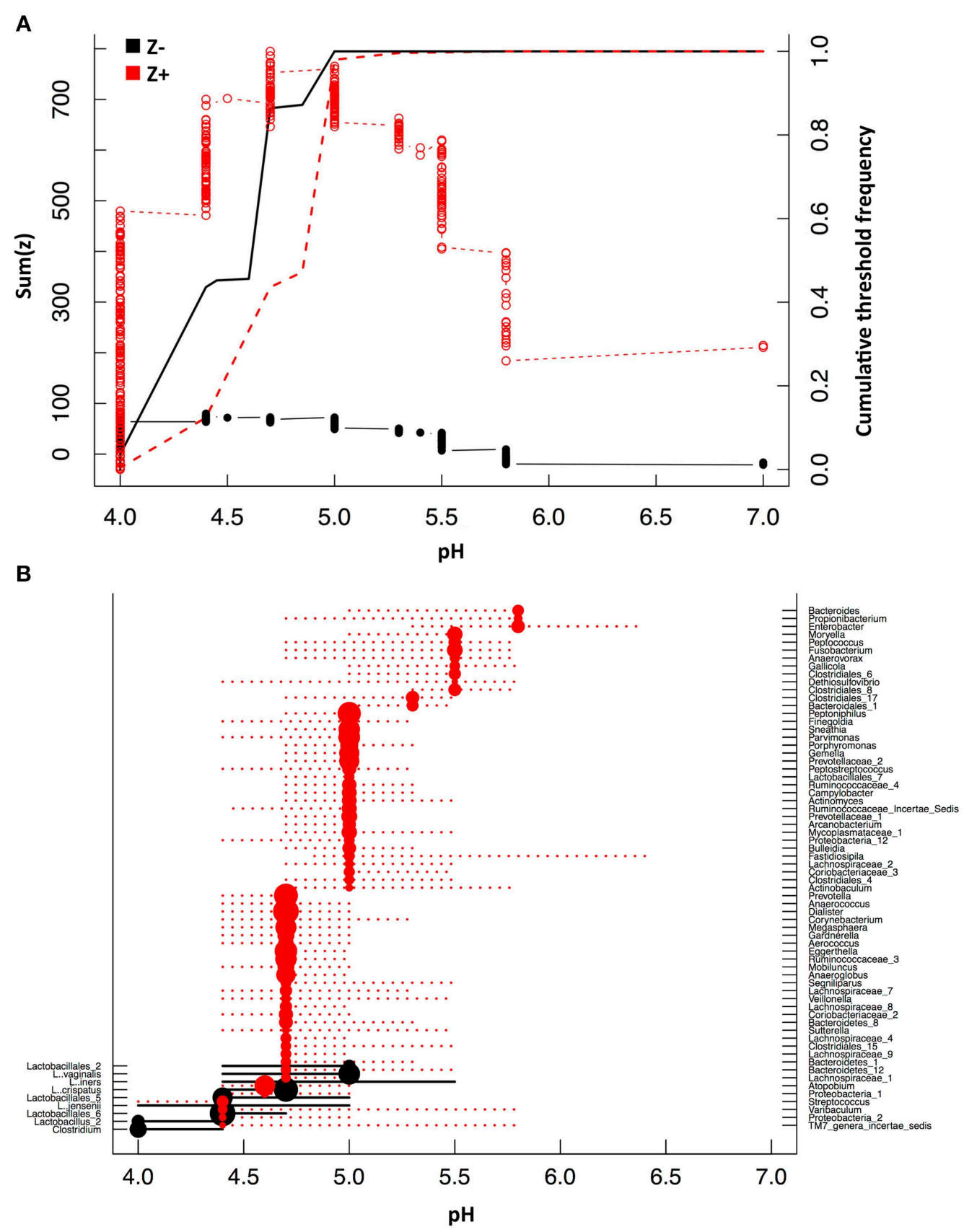

FIGURE 5 | Threshold indicator taxa analysis (TITAN) with pH. Community thresholds as determined by TITAN. The cumulative sum of Z− (taxa responding negatively to higher levels of pH) and Z+ (taxa responding positively to higher levels of pH) scores are displayed in relation to pH **(A)**. Change points (dots) and 95% and 5% confidence intervals (dotted lines) for taxa along the pH gradient are displayed **(B)** ($P < 0.05$, purity > 0.07, reliability > 0.7 for 500 bootstrap and 250 permutation replicates). Size of change point symbol (dots) is proportional to the magnitude of the taxa response.

and Snell, 1980; Romano et al., 2013). Like these two species, many foodborne lactobacilli have been identified as being capable of ornithine decarboxylase (Arena and Manca de Nadra, 2001; Pereira et al., 2001; Azcarate-Peril et al., 2004). These enzymes are likely to be used periodically as a way of overcoming acid stress. However, we suggest that non-lactobacilli are capable of stronger production, which may cause a feedback inhibition of the enzymes in the lactobacilli that reduces their ability to outcompete other species in acidic conditions. The literature suggests that these systems operate most efficiently under mild acid stress conditions (pH 4.0–5.0) and are optimally induced during anaerobic conditions at pH > 5.0 (Kanjee and Houry, 2013). These conditions are consistent with the typical pH and anaerobic environment of the vagina during BV development (Ravel et al., 2011).

We hypothesize that BA production may mitigate the acidic barrier that favors vaginal lactobacilli. The BAs may also have a more direct effect on the growth of vaginal lactobacilli. A previous study revealed that the growth of several *Lactobacillus* species was stimulated by spermine and spermidine (Guirard and Snell, 1964), both of which we found to be significantly greater in the *L. crispatus*-dominated CST I and significantly lower in low-*Lactobacillus* CST IV. Additionally, elevated concentrations of putrescine as found in CST IV women in this study

have previously been identified as inhibitors of growth of representative lactobacilli (Guirard and Snell, 1964).

BA production may also be important to other morbidities associated with BV, including increased risks of various STIs. Previous studies have shown host defenses to be less effective in the presence of BAs. Goytia and Schafer (Goytia and Shafer, 2010) revealed that the genital tract pathogen, *N. gonorrhoeae*, was more resistant to the impact of host mediated defenses in the presence of the BA, spermine. Similarly, the growth of *Legionella pneumophila* is enhanced in the presence of cadaverine, putrescine, spermine, and spermidine (Nasrallah et al., 2011), and expression of the virulence loci of *Salmonella typhimirium* is stimulated in the presence of putrescine and spermidine (Jelsbak et al., 2012). BAs have also been shown to initiate biofilm formation in a number of relevant disease pathogens, including *Vibrio cholera*, *Yersinia pestis, E. coli,* and *N. gonorrhoeae* (Patel et al., 2006; McGinnis et al., 2009; Parker et al., 2012; Sakamoto et al., 2012; Goytia et al., 2013; Karatan and Michael, 2013). Biofilms are a significant mechanism of disease and have been associated with BV (Swidsinski et al., 2013).

Various studies have previously described the presence of BAs in the vagina (Chen et al., 1979, 1982; Sanderson et al., 1983; Wolrath et al., 2002; Yeoman et al., 2013), wherein BAs have been associated with the characteristic vaginal odor of BV (Srinivasan et al., 2012; Yeoman et al., 2013). Consequently, they have been considered as a potential biomarker of BV (Chen et al., 1982; Wolrath et al., 2002; Sobel et al., 2012). Two recent studies have identified correlative relationships between particular vaginal taxa and either odor (Srinivasan et al., 2012; Yeoman et al., 2013) or directly to the relative concentration of certain BAs (Yeoman et al., 2013). Srinivasan et al. (2012) found associations between *Eggerthella* spp., *Leptotrichia amnionii*, *G. vaginalis*, *D. micraerophilus*, *Prevotella bivia*, *P. disiens*, *Porphyromonas asaccharolytica*, *P. micra*, and BVAB1 (BV-associated bacteria 1) each being correlated with clinically diagnosed vaginal odor. Several of these same genera (*Dialister* spp. with both putrescine and cadaverine; *Prevotella* spp., and *Porphyromonas* spp. with putrescine) were found to have Spearman's rank order correlations with specific BAs in the study of Yeoman

TABLE 3 | Community level threshold indicator analysis (TITAN) for pH.

	pH thresholds	5%	50%	95%
Sum z−	4.4	4.4	4.7	5.0
Sum z+	4.7	4.4	5.0	5.0

Threshold indicator analysis (TITAN) identifies the response of vaginal bacteria in relation to pH to estimate a community threshold. The cumulative sums of z− (taxa responding negatively to higher levels of pH) and z+ (taxa responding positively to higher levels of pH) scores are displayed. The thresholds are based on the sum of the z− and z+. Associated percentiles correspond to the frequency distribution of thresholds from 500 bootstrap replicates. Abundance data was taken from Ravel et al. (2011).

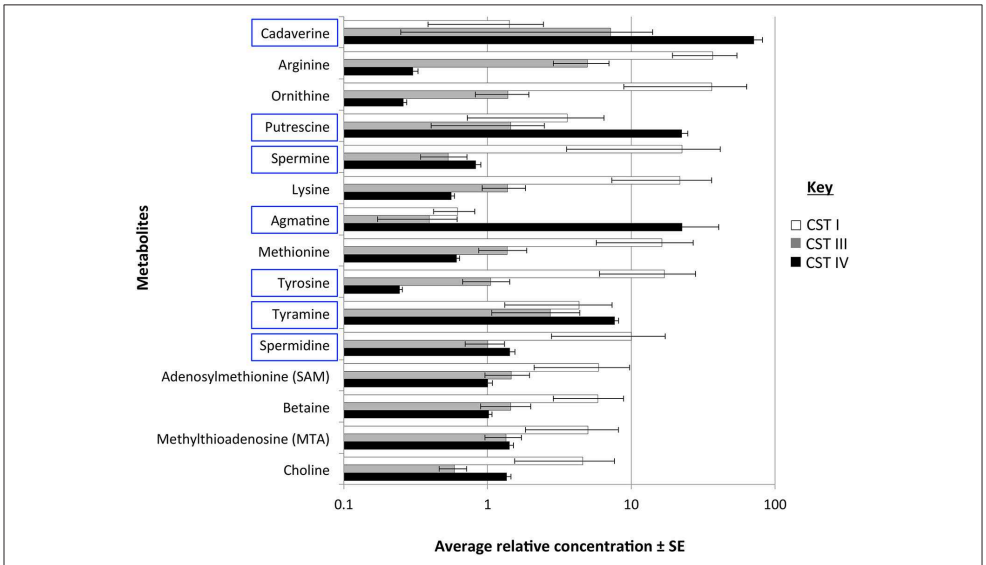

FIGURE 6 | Metabolite presence in the vagina associated with community state types (CSTS). Bar chart displays the log average relative concentration of metabolites ± standard error (SE) involved in biogenic amine biosynthetic pathways (as shown in **Figure 1**). BAs are highlighted in blue rectangles. Metabolites were measured from the vagina of 37 women. Bar colors indicate the CSTs of the vaginal microbiome. Significant differences between groups are shown in **Table 4**.

TABLE 4 | Difference in metabolite concentration between CSTs.

Metabolites	Testing groups		
	CST I, III	CST I, IV	CST III, IV
Adenosylmethionine (SAM)			
Agmatine		*	
Arginine		**	
Betaine			
Cadaverine		**	
Choline			
Lysine		**	
Methionine		**	
Methylthioadenosine (MTA)			
Ornithine		**	
Putrescine		**	
Spermidine			
Spermine			
Tyramine			
Tyrosine		**	

Difference observed between community state types (CSTs). Results obtained from Mann-Whitney-Wilcoxon testing with Bonferroni correction between groups as shown in ***Figure 6****. **P < 0.01; *P < 0.05.*

et al. (2013). The same study also found strong correlations between both *Streptococcus* spp. and *Mycoplasma* spp. and cadaverine and between both *Mobiluncus* spp. and *Anaeroglobus* spp. with putrescine. Macklaim et al. (2013) in their recent investigation of the vaginal meta-transcriptome detected the expression of an arginine decarboxylase (putrescine) by *P. amnii*, and spermidine synthase genes by *Dialister* and *Megasphaera* genera. However, the only study providing physiological evidence for the production of a BA, TMA, revealed it to be produced by vaginal *Mobiluncus* species, and not by vaginal *Bacteroides* or *Gardnerella* species (Cruden and Galask, 1988). Our analyses support several of these correlative associations and all of the transcriptional observations of Macklaim et al. (2013). Discrepancies between correlative relationships and the genetic ability to biosynthesize BAs (i.e., correlations with *G. vaginalis*, *Streptococcus*, *Anaeroglobus,* and *Mobiluncus*) illustrate the limitations of correlative analyses and the difficulties in separating cause and effect. However, our findings are not consistent with the findings of Cruden and colleagues (Cruden and Galask, 1988). It is more difficult to reconcile this discrepancy, though it may be explainable by alternate and currently unrecognized pathways for the biosynthesis of TMA from either TMAO or choline or perhaps the trimethylamine N-oxide reductase and choline trimethylamine-lyase genes present in the *Mobiluncus* are sufficiently diverged to escape our bioinformatic approach. In either case, this is worthy of further investigation. Our results did support TMA production by a variety of other species, through the metabolism of TMAO, choline, and N,N,N-trimethylglycine, and from ergothione. We also observed weaker but full length matches in various *Veillonella* and *Janthinobacterium* spp., *Prevotella buccalis, P. disiens*, and *Acinetobacter baumanii* that may worthy of further interrogation.

Our additional findings demonstrate that several BA-producing taxa are enriched within the low-*Lactobacillus*, CST IV vaginal microbiome. In addition, taxa including *Prevotella, Megasphaera, Parvimonas,* and *Veillonella* spp. are found in maximum abundance when pH exceeds pH 4.6. Threshold analysis suggests a community change point occurs at pH 4.4 which displays a correlation between decreasing abundances of *Lactobacillus* spp. and increasing abundances of BV-associated bacteria with many of these taxa identified as BA-producers. This supports the hypothesis that BA production is an important factor for the mitigation of one of the most widely described barriers to vaginal pathogens, vaginal pH. Although *Lactobacillus* spp. are predicted to synthesize putrescine, we also show that cadaverine and tyramine are enriched when *Lactobacillus* spp. are depleted (CST IV), while spermine and spermidine are enriched within other CSTs.

Based on the data provided in this study and other observations in the literature, we hypothesize that the microbial production of BAs is more than just a biomarker for BV. We put forward a novel conceptual model of the role of BAs in the vagina and suggest they are an important metabolic feature for overcoming pH and facilitating the outgrowth of BV-associated bacteria. For women categorized as CST IV, the production of BAs by BV-associated vaginal taxa was more common than what we observed in other CSTs. This may represent a vulnerable CST for the vagina, whereby it is at a greater risk of colonization by microbe(s) capable of eliciting the additional signs or symptoms of BV. This may include subpopulations of *G. vaginalis*, who have been repeatedly linked to BV, are able to recapitulate the clue cell symptom in murine models (Gilbert et al., 2013), and are the major colonizing bacterial taxa of clue cells in humans (Cook et al., 1989). We also hypothesize that the agents that are capable of eliciting symptoms of BV would not be able to competitively colonize the more typical *Lactobacillus*-dominated vaginal ecosystem, consistent with the existing dogma. The current understanding of BV lacks a clear, mechanistic pathway for its development. The hypothesis proposed herein suggests the microbial synthesis of BAs has a major role in predisposing the vagina to BV development. In support of our hypothesis, we have shown through the analysis of available datasets, that genes encoding enzymes predicted to be capable of synthesizing BAs are present in a number of taxa. The bioinformatics approach used in this study provides a window into the potential of these bacteria to produce BAs, with limitations in our ability to accurately determine the activity of these genes. For this reason, this information warrants further investigations with culture-based determination of enzymes and BAs from bacteria relevant to the vaginal microbiome and associated with BV. One curiosity was the determination of putative genes capable of putrescine-production in many lactobacilli with an opposite correlation in our observation with metabolite data associated with CST groupings. Overall, we observed that the production of BAs is more common in the low-*Lactobacillus* CST IV, also termed "asymptomatic BV." Taking this data together, we hypothesize that the production of BAs in the vagina increases the ability

of BA-producing taxa to competitively colonize the vagina, putting the host at a greater risk of BV development. These findings may help to reshape our understanding of BV and shed a mechanistic light on particular microbes that may be important to vaginal dysbiosis, potentially at the earliest stage of BV.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fphys.2015.00253

Figure S1 | Abundance trends of vaginal bacteria in relation to vaginal pH. Graphs display the average relative abundance of Lactobacillus spp. and bacteria associated with bacterial vaginosis as pH increases. Abundance data was taken from Ravel et al. (2011).

Table S1 | Characterized homologs and HMMs utilized in this study.

Table S2 | TITAN results for individual taxa response to pH.

References

Allison, M. J., Robinson, I. M., Dougherty, R. W., and Bucklin, J. A. (1975). Grain overload in cattle and sheep: changes in microbial populations in the cecum and rumen. *Am. J. Vet. Res.* 36, 181–185.

Allsworth, J. E., and Peipert, J. F. (2007). Prevalence of bacterial vaginosis: 2001–2004 National Health and Nutrition Examination Survey data. *Obstet. Gynecol.* 109, 114–120. doi: 10.1097/01.AOG.0000247627.84791.91

Amsel, R., Totten, P. A., Spiegel, C. A., Chen, K. C., Eschenbach, D., and Holmes, K. K. (1983). Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am. J. Med.* 74, 14–22. doi: 10.1016/0002-9343(83)91112-9

Anokhina, I. V., Kravtsov, E. G., Protsenko, A. V., Yashina, N. V., Yermolaev, A. V., Chesnokova, V. L., et al. (2007). Bactericidal activity of culture fluid components of *Lactobacillus fermentum* strain 90 TS-4 (21) clone 3, and their capacity to modulate adhesion of Candida albicans yeast-like fungi to vaginal epithelial cells. *Bull. Exp. Biol. Med.* 143, 359–362. doi: 10.1007/s10517-007-0112-0

Arena, M. E., and Manca de Nadra, M. C. (2001). Biogenic amine production by *Lactobacillus*. *J. Appl. Microbiol.* 90, 158–162. doi: 10.1046/j.1365-2672.2001.01223.x

Aroutcheva, A., Gariti, D., Simon, M., Shott, S., Faro, J., Simoes, J. A., et al. (2001). Defense factors of vaginal lactobacilli. *Am. J. Obstet. Gynecol.* 185, 375–379. doi: 10.1067/mob.2001.115867

Atassi, F., and Servin, A. L. (2010). Individual and co-operative roles of lactic acid and hydrogen peroxide in the killing activity of enteric strain *Lactobacillus johnsonii* NCC933 and vaginal strain *Lactobacillus gasseri* KS120.1 against enteric, uropathogenic and vaginosis-associated pathogens. *FEMS Microbiol. Lett.* 304, 29–38. doi: 10.1111/j.1574-6968.2009.01887.x

Azcarate-Peril, M. A., Altermann, E., Hoover-Fitzula, R. L., Cano, R. J., and Klaenhammer, T. R. (2004). Identification and inactivation of genetic loci involved with *Lactobacillus acidophilus* acid tolerance. *Appl. Environ. Microbiol.* 70, 5315–5322. doi: 10.1128/AEM.70.9.5315-5322.2004

Baker, M. E., and King, R. S. (2010). A new method for detecting and interpreting biodiversity and ecological community thresholds. *Methods Ecol. Evol.* 1, 25–37. doi: 10.1111/j.2041-210X.2009.00007.x

Bearson, B. L., Wilson, L., and Foster, J. W. (1998). A low pH-inducible, PhoPQ-dependent acid tolerance response protects *Salmonella typhimurium* against inorganic acid stress. *J. Bacteriol.* 180, 2409–2417.

Blankenstein, T., Lytton, S. D., Leidl, B., Atweh, E., Friese, K., and Mylonas, I. (2015). Point-of-care (POC) diagnosis of bacterial vaginosis (BV) using VGTest™ ion mobility spectrometry (IMS) in a routine ambulatory care gynecology clinic. *Arch. Gynecol. Obstet.* 292, 355–362. doi: 10.1007/s00404-014-3613-x

Bongaerts, G., Bakkeren, J., Severijnen, R., Sperl, W., Willems, H., Naber, T., et al. (2000). Lactobacilli and acidosis in children with short small bowel. *J. Pediatr. Gastroenterol. Nutr.* 30, 288–293. doi: 10.1097/00005176-200003000-00014

Bonnin-Jusserand, M., Grandvalet, C., Rieu, A., Weidmann, S., and Alexandre, H. (2012). Tyrosine-containing peptides are precursors of tyramine produced by *Lactobacillus plantarum* strain IR BL0076 isolated from wine. *BMC Microbiol.* 12:199. doi: 10.1186/1471-2180-12-199

Brooks, W. H. (2013). Increased polyamines alter chromatin and stabilize autoantigens in autoimmune diseases. *Front. Immunol.* 4:91. doi: 10.3389/fimmu.2013.00091

Brotman, R. M., He, X., Gajer, P., Fadrosh, D., Sharma, E., Mongodin, E. F., et al. (2014). Association between cigarette smoking and the vaginal microbiota: a pilot study. *BMC Infect. Dis.* 14:471. doi: 10.1186/1471-2334-14-471

Cadieux, P. A., Burton, J. P., Devillard, E., and Reid, G. (2009). *Lactobacillus* by-products inhibit the growth and virulence of uropathogenic *Escherichia coli*. *J. Physiol. Pharmacol.* 60(Suppl. 6), 13–18. Available online at: http://www.jpp.krakow.pl/journal/archive/12_09_s6/pdf/13_12_09_s6_article.pdf

Chen, K. C., Amsel, R., Eschenbach, D. A., and Holmes, K. K. (1982). Biochemical diagnosis of vaginitis: determination of diamines in vaginal fluid. *J. Infect. Dis.* 145, 337–345. doi: 10.1093/infdis/145.3.337

Chen, K. C., Forsyth, P. S., Buchanan, T. M., and Holmes, K. K. (1979). Amine content of vaginal fluid from untreated and treated patients with nonspecific vaginitis. *J. Clin. Invest.* 63, 828–835. doi: 10.1172/JCI109382

Cid, S. B., Miguélez-Arrizado, M. J., Becker, B., Holzapfel, W. H., and Vidal-Carou, M. C. (2008). Amino acid decarboxylation by *Lactobacillus curvatus* CTC273 affected by the pH and glucose availability. *Food Microbiol.* 25, 269–277. doi: 10.1016/j.fm.2007.10.013

Cook, R. L., Reid, G., Pond, D. G., Schmitt, C. A., and Sobel, J. D. (1989). Clue cells in bacterial vaginosis: immunofluorescent identification of the adherent gram-negative bacteria as *Gardnerella vaginalis*. *J. Infect. Dis.* 160, 490–496. doi: 10.1093/infdis/160.3.490

Craciun, S., and Balskus, E. P. (2012). Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. *Proc. Natl. Acad. Sci. U.S.A.* 109, 21307–21312. doi: 10.1073/pnas.1215689109

Cruden, D. L., and Galask, R. P. (1988). Reduction of trimethylamine oxide to trimethylamine by Mobiluncus strains isolated from patients with bicterial Vaginosis. *Microb. Ecol.* 1, 95–100. doi: 10.3109/08910608809140187

Dela Vega, A. L., and Delcour, A. H. (1996). Polyamines decrease *Escherichia coli* outer membrane permeability. *J. Bacteriol.* 178, 3715–3721.

De Las Rivas, B., González, R., Landete, J. M., and Muñoz, R. (2008). Characterization of a second ornithine decarboxylase isolated from *Morganella morganii*. *J. Food Prot.* 71, 657–661. Available online at: http://www.ingentaconnect.com/content/iafp/jfp/2008/00000071/00000

003/art00033?token=004714215c39437a63736a6f3547652176666c24532a766f644a467b4d616d3f4e4b340

Dufrene, M., and Legendre, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol. Monogr.* 67, 345–366. doi: 10.2307/2963459

Fernandez de Palencia, P., Fernandez, M., Mohedano, M. L., Ladero, V., Quevedo, C., Alvarez, M. A., et al. (2011). Role of tyramine synthesis by food-borne *Enterococcus durans* in adaptation to the gastrointestinal tract environment. *Appl. Environ. Microbiol.* 77, 699–702. doi: 10.1128/AEM.01411-10

Gajer, P., Brotman, R. M., Bai, G., Sakamoto, J., Schutte, U. M., Zhong, X., et al. (2012). Temporal dynamics of the human vaginal microbiota. *Sci. Transl. Med.* 4:132ra52. doi: 10.1126/scitranslmed.3003605

Gilbert, N. M., Lewis, W. G., and Lewis, A. L. (2013). Clinical features of bacterial vaginosis in a murine model of vaginal infection with *Gardnerella vaginalis*. *PLoS ONE* 8:e59539. doi: 10.1371/journal.pone.0059539

Giles, T. N., and Graham, D. E. (2007). Characterization of an acid-dependent arginine decarboxylase enzyme from *Chlamydophila pneumoniae*. *J. Bacteriol.* 189, 7376–7383. doi: 10.1128/JB.00772-07

Goda, S., Sakuraba, H., Kawarabayasi, Y., and Ohshima, T. (2005). The first archaeal agmatinase from anaerobic hyperthermophilic archaeon *Pyrococcus horikoshii*: cloning, expression, and characterization. *Biochim. Biophys. Acta* 1748, 110–115. doi: 10.1016/j.bbapap.2004.12.010

Goytia, M., Dhulipala, V. L., and Shafer, W. M. (2013). Spermine impairs biofilm formation by *Neisseria gonorrhoeae*. *FEMS Microbiol. Lett.* 343, 64–69. doi: 10.1111/1574-6968.12130

Goytia, M., and Shafer, W. M. (2010). Polyamines can increase resistance of *Neisseria gonorrhoeae* to mediators of the innate human host defense. *Infect. Immun.* 78, 3187–3195. doi: 10.1128/IAI.01301-09

Graver, M. A., and Wade, J. J. (2011). The role of acidification in the inhibition of *Neisseria gonorrhoeae* by vaginal lactobacilli during anaerobic growth. *Ann. Clin. Microbiol. Antimicrob.* 10:8. doi: 10.1186/1476-0711-10-8

Guirard, B. M., and Snell, E. E. (1964). Effect of polyamine structure on growth stimulation + spermine + spermidine content of lactic acid bacteria. *J. Bacteriol.* 88, 72–80.

Guirard, B. M., and Snell, E. E. (1980). Purification and properties of ornithine decarboxylase from Lactobacillus sp. 30a. *J. Biol. Chem.* 255, 5960–5964.

Hamasaki-Katagiri, N., Katagiri, Y., Tabor, C. W., and Tabor, H. (1998). Spermine is not essential for growth of *Saccharomyces cerevisiae*: identification of the SPE4 gene (spermine synthase) and characterization of a spe4 deletion mutant. *Gene* 210, 195–201. doi: 10.1016/S0378-1119(98)00027-4

Ihaka, R., and Gentleman, R. (1996). R: a language for data analysis and graphics. *J. Comput. Graph. Stat.* 5, 299–314.

Jelsbak, L., Thomsen, L. E., Wallrodt, I., Jensen, P. R., and Olsen, J. E. (2012). Polyamines are required for virulence in *Salmonella enterica* serovar Typhimurium. *PLoS ONE* 7:e36149. doi: 10.1371/journal.pone.0036149

Jones, J. E., Causey, C. P., Lovelace, L., Knuckley, B., Flick, H., Lebioda, L., et al. (2010). Characterization and inactivation of an agmatine deiminase from *Helicobacter pylori*. *Bioorg. Chem.* 38, 62–73. doi: 10.1016/j.bioorg.2009.11.004

Jung, I. L., and Kim, I. G. (2003). Polyamines and glutamate decarboxylase-based acid resistance in *Escherichia coli*. *J. Biol. Chem.* 278, 22846–22852. doi: 10.1074/jbc.M212055200

Kanjee, U., and Houry, W. A. (2013). Mechanisms of acid resistance in *Escherichia coli*. *Annu. Rev. Microbiol.* 67, 65–81. doi: 10.1146/annurev-micro-092412-155708

Karatan, E., and Michael, A. J. (2013). A wider role for polyamines in biofilm formation. *Biotechnol. Lett.* 35, 1715–1717. doi: 10.1007/s10529-013-1286-3

Kezmarsky, N. D., Xu, H., Graham, D. E., and White, R. H. (2005). Identification and characterization of a L-tyrosine decarboxylase in *Methanocaldococcus jannaschii*. *Biochim. Biophys. Acta* 1722, 175–182. doi: 10.1016/j.bbagen.2004.12.003

Kikuchi, Y., Kojima, H., Tanaka, T., Takatsuka, Y., and Kamio, Y. (1997). Characterization of a second lysine decarboxylase isolated from *Escherichia coli*. *J. Bacteriol.* 179, 4486–4492.

Korshunov, V. M., Gudieva, Z. A., Efimov, B. A., Pikina, A. P., Smeianov, V. V., Reid, G., et al. (1999). [The vaginal Bifidobacterium flora in women of reproductive age]. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 4, 74–78.

Lambert, J. A., John, S., Sobel, J. D., and Akins, R. A. (2013). Longitudinal analysis of vaginal microbiome dynamics in women with recurrent bacterial vaginosis: recognition of the conversion process. *PLoS ONE* 8:e82599. doi: 10.1371/journal.pone.0082599

Large, T. M., Walk, S. T., and Whittam, T. S. (2005). Variation in acid resistance among shiga toxin-producing clones of pathogenic *Escherichia coli*. *Appl. Environ. Microbiol.* 71, 2493–2500. doi: 10.1128/AEM.71.5.2493-2500.2005

Lee, M. J., Huang, C. Y., Sun, Y. J., and Huang, H. (2005). Cloning and characterization of spermidine synthase and its implication in polyamine biosynthesis in *Helicobacter pylori* strain 26695. *Protein Expr. Purif.* 43, 140–148. doi: 10.1016/j.pep.2005.04.017

Lefèvre, J. C., Jean, M., Averous, S., Viraben, R., Blanc, C., Bauriaud, R., et al. (1985). [Etiology of bacterial vaginosis (non-specific vaginitis)]. *J. Gynecol. Obstet. Biol. Reprod.* 14, 703–708.

Li, J., Wang, W., Xu, S. X., Magarvey, N. A., and McCormick, J. K. (2011). *Lactobacillus reuteri*-produced cyclic dipeptides quench agr-mediated expression of toxic shock syndrome toxin-1 in staphylococci. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3360–3365. doi: 10.1073/pnas.1017431108

Liu, F., Xu, W., Du, L., Wang, D., Zhu, Y., Geng, Z., et al. (2014). Heterologous expression and characterization of tyrosine decarboxylase from *Enterococcus faecalis* R612Z1 and *Enterococcus faecium* R615Z1. *J. Food Prot.* 77, 592–598. doi: 10.4315/0362-028X.JFP-13-326

Löser, C., Fölsch, U. R., Paprotny, C., and Creutzfeldt, W. (1990). Polyamines in colorectal cancer. Evaluation of polyamine concentrations in the colon tissue, serum, and urine of 50 patients with colorectal cancer. *Cancer* 65, 958–966.

Macklaim, J. M., Cohen, C. R., Donders, G., Gloor, G. B., Hill, J. E., Parham, G. P., et al. (2012). Exploring a road map to counter misconceptions about the cervicovaginal microbiome and disease. *Reprod. Sci.* 19, 1154–1162. doi: 10.1177/1933719112446075

Macklaim, J. M., Fernandes, A. D., Di Bella, J. M., Hammond, J. A., Reid, G., and Gloor, G. B. (2013). Comparative meta-RNA-seq of the vaginal microbiota and differential expression by Lactobacillus iners in health and dysbiosis. *Microbiome* 1:12. doi: 10.1186/2049-2618-1-12

McGinnis, M. W., Parker, Z. M., Walter, N. E., Rutkovsky, A. C., Cartaya-Marin, C., and Karatan, E. (2009). Spermidine regulates *Vibrio cholerae* biofilm formation via transport and signaling pathways. *FEMS Microbiol. Lett.* 299, 166–174. doi: 10.1111/j.1574-6968.2009.01744.x

Meng, S. Y., and Bennett, G. N. (1992). Nucleotide sequence of the *Escherichia coli* cad operon: a system for neutralization of low extracellular pH. *J. Bacteriol.* 174, 2659–2669.

Meyer, M., Granderath, K., and Andreesen, J. R. (1995). Purification and characterization of protein PB of betaine reductase and its relationship to the corresponding proteins glycine reductase and sarcosine reductase from *Eubacterium acidaminophilum*. *Eur. J. Biochem.* 234, 184–191. doi: 10.1111/j.1432-1033.1995.184_c.x

Molenaar, D., Bosscher, J. S., ten Brink, B., Driessen, A. J., and Konings, W. N. (1993). Generation of a proton motive force by histidine decarboxylation and electrogenic histidine/histamine antiport in *Lactobacillus buchneri*. *J. Bacteriol.* 175, 2864–2870.

Muramatsu, H., Matsuo, H., Okada, N., Ueda, M., Yamamoto, H., Kato, S., et al. (2013). Characterization of ergothionase from Burkholderia sp. HME13 and its application to enzymatic quantification of ergothioneine. *Appl. Microbiol. Biotechnol.* 97, 5389–5400. doi: 10.1007/s00253-012-4442-0

Nakada, Y., and Itoh, Y. (2003). Identification of the putrescine biosynthetic genes in *Pseudomonas aeruginosa* and characterization of agmatine deiminase and N-carbamoylputrescine amidohydrolase of the arginine decarboxylase pathway. *Microbiology* 149, 707–714. doi: 10.1099/mic.0.26009-0

Nasrallah, G. K., Riveroll, A. L., Chong, A., Murray, L. E., Lewis, P. J., and Garduño, R. A. (2011). *Legionella pneumophila* requires polyamines for optimal intracellular growth. *J. Bacteriol.* 193, 4346–4360. doi: 10.1128/JB.01506-10

O'Hanlon, D. E., Moench, T. R., and Cone, R. A. (2011). In vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid but not hydrogen peroxide. *BMC Infect. Dis.* 11:200. doi: 10.1186/1471-2334-11-200

O'Hanlon, D. E., Moench, T. R., and Cone, R. A. (2013). Vaginal pH and microbicidal lactic acid when Lactobacilli dominate the microbiota. *PLoS ONE* 8:e80074. doi: 10.1371/journal.pone.0080074

Paik, M. J., Lee, S., Cho, K. H., and Kim, K. R. (2006). Urinary polyamines and N-acetylated polyamines in four patients with Alzheimer's disease as their N-ethoxycarbonyl-N-pentafluoropropionyl derivatives by gas

chromatography-mass spectrometry in selected ion monitoring mode. *Anal. Chim. Acta* 576, 55–60. doi: 10.1016/j.aca.2006.01.070

Parker, Z. M., Pendergraft, S. S., Sobieraj, J., McGinnis, M. M., and Karatan, E. (2012). Elevated levels of the norspermidine synthesis enzyme NspC enhance *Vibrio cholerae* biofilm formation without affecting intracellular norspermidine concentrations. *FEMS Microbiol. Lett.* 329, 18–27. doi: 10.1111/j.1574-6968.2012.02498.x

Patel, C. N., Wortham, B. W., Lines, J. L., Fetherston, J. D., Perry, R. D., and Oliveira, M. A. (2006). Polyamines are essential for the formation of plague biofilm. *J. Bacteriol.* 188, 2355–2363. doi: 10.1128/JB.188.7.2355-2363.2006

Pegg, A. E. (2009). Mammalian polyamine metabolism and function. *IUBMB Life* 61, 880–894. doi: 10.1002/iub.230

Pegg, A. E. (2013). Toxicity of polyamines and their metabolic products. *Chem. Res. Toxicol.* 26, 1782–1800. doi: 10.1021/tx400316s

Pereira, C. I., Crespo, M. T., and Romão, M. V. (2001). Evidence for proteolytic activity and biogenic amines production in *Lactobacillus curvatus* and *L. homohiochii*. *Int. J. Food Microbiol.* 68, 211–216. doi: 10.1016/S0168-1605(01)00534-7

Ravel, J., Gajer, P., Abdo, Z., Schneider, G. M., Koenig, S. S., McCulle, S. L., et al. (2011). Vaginal microbiome of reproductive-age women. *Proc. Natl. Acad. Sci. U.S.A.* 108(Suppl. 1), 4680–4687. doi: 10.1073/pnas.1002611107

Recsei, P. A., and Snell, E. E. (1972). Histidine decarboxylaseless mutants of Lactobacillus 30a: isolation and growth properties. *J. Bacteriol.* 112, 624–626.

Romano, A., Trip, H., Lolkema, J. S., and Lucas, P. M. (2013). Three-component lysine/ornithine decarboxylation system in *Lactobacillus saerimneri* 30a. *J. Bacteriol.* 195, 1249–1254. doi: 10.1128/JB.02070-12

Romano, A., Trip, H., Lonvaud-Funel, A., Lolkema, J. S., and Lucas, P. M. (2012). Evidence of two functionally distinct ornithine decarboxylation systems in lactic acid bacteria. *Appl. Environ. Microbiol.* 78, 1953–1961. doi: 10.1128/AEM.07161-11

Rönnqvist, D., Forsgren-Brusk, U., Husmark, U., and Grahn-Håkansson, E. (2007). *Lactobacillus fermentum* Ess-1 with unique growth inhibition of vulvo-vaginal candidiasis pathogens. *J. Med. Microbiol.* 56, 1500–1504. doi: 10.1099/jmm.0.47226-0

Ryals, J., Lawton, K., Stevens, D., and Milburn, M. (2007). Metabolon, Inc. *Pharmacogenomics* 8, 863–866. doi: 10.2217/14622416.8.7.863

Sakamoto, A., Terui, Y., Yamamoto, T., Kasahara, T., Nakamura, M., Tomitori, H., et al. (2012). Enhanced biofilm formation and/or cell viability by polyamines through stimulation of response regulators UvrY and CpxR in the two-component signal transducing systems, and ribosome recycling factor. *Int. J. Biochem. Cell Biol.* 44, 1877–1886. doi: 10.1016/j.biocel.2012.07.010

Sanderson, B. E., White, E., and Baldson, M. J. (1983). Amine content of vaginal fluid from patients with trichomoniasis and gardnerella associated non-specific vaginitis. *Br. J. Vener. Dis.* 59, 302–305. doi: 10.1136/sti.59.5.302

Shah, P., and Swiatlo, E. (2008). A multifaceted role for polyamines in bacterial pathogens. *Mol. Microbiol.* 68, 4–16. doi: 10.1111/j.1365-2958.2008.06126.x

Sobel, J. D., Karpas, Z., and Lorber, A. (2012). Diagnosing vaginal infections through measurement of biogenic amines by ion mobility spectrometry. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 163, 81–84. doi: 10.1016/j.ejogrb.2012.03.022

Soksawatmaekhin, W., Kuraishi, A., Sakata, K., Kashiwagi, K., and Igarashi, K. (2004). Excretion and uptake of cadaverine by CadB and its physiological functions in *Escherichia coli*. *Mol. Microbiol.* 51, 1401–1412. doi: 10.1046/j.1365-2958.2003.03913.x

Spiegel, C. A., Amsel, R., Eschenbach, D., Schoenknecht, F., and Holmes, K. K. (1980). Anaerobic bacteria in nonspecific vaginitis. *N. Engl. J. Med.* 303, 601–607. doi: 10.1056/NEJM198009113031102

Srinivasan, S., Hoffman, N. G., Morgan, M. T., Matsen, F. A., Fiedler, T. L., Hall, R. W., et al. (2012). Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS ONE* 7:e37818. doi: 10.1371/journal.pone.0037818

Strøm, A. R., Olafsen, J. A., and Larsen, H. (1979). Trimethylamine oxide: a terminal electron acceptor in anaerobic respiration of bacteria. *J. Gen. Microbiol.* 112, 315–320. doi: 10.1099/00221287-112-2-315

Sugawara, A., Matsui, D., Yamada, M., Asano, Y., and Isobe, K. (2015). New enzymatic methods for selective assay of l-lysine using an l-lysine specific decarboxylase/oxidase from *Burkholderia* sp. AIU 395. *J. Biosci. Bioeng.* 119, 369–374. doi: 10.1016/j.jbiosc.2014.08.013

Swidsinski, A., Verstraelen, H., Loening-Baucke, V., Swidsinski, S., Mendling, W., and Halwani, Z. (2013). Presence of a polymicrobial endometrial biofilm in patients with bacterial vaginosis. *PLoS ONE* 8:e53997. doi: 10.1371/journal.pone.0053997

Tabor, C. W., and Tabor, H. (1985). Polyamines in microorganisms. *Microbiol. Rev.* 49, 81–99.

Takatsuka, Y., Yamaguchi, Y., Ono, M., and Kamio, Y. (2000). Gene cloning and molecular characterization of lysine decarboxylase from *Selenomonas ruminantium* delineate its evolutionary relationship to ornithine decarboxylases from eukaryotes. *J. Bacteriol.* 182, 6732–6741. doi: 10.1128/JB.182.23.6732-6741.2000

Valenzuela, M., Caceres, A., Almarza, O., Bravo, D., Soto, S., Cerda, O., et al. (2014). Characterization of the arginine decarboxylase gene (ORF HP0422, speA) involved in acid tolerance in *Helicobacter pylori*. *Helicobacter* 19, 182–193. doi: 10.1111/hel.12115

Wallace, H. M., Fraser, A. V., and Hughes, A. (2003). A perspective of polyamine metabolism. *Biochem. J.* 376, 1–14. doi: 10.1042/bj20031327

Warnes, G. R., Bolker, B., Bonebakker, L., Gentleman, R., Huber, W., Liaw, A., et al. (2009). *gplots: Various R programming Tools for Plotting Data*. Seattle, WA: R package version 2.

Watson, N., Dunyak, D. S., Rosey, E. L., Slonczewski, J. L., and Olson, E. R. (1992). Identification of elements involved in transcriptional regulation of the *Escherichia coli* cad operon by external pH. *J. Bacteriol.* 174, 530–540.

Wolrath, H., Borén, H., Hallen, A., and Forsum, U. (2002). Trimethylamine content in vaginal secretion and its relation to bacterial vaginosis. *APMIS* 110, 819–824. doi: 10.1034/j.1600-0463.2002.1101108.x

Wortham, B. W., Patel, C. N., and Oliveira, M. A. (2007). Polyamines in bacteria: pleiotropic effects yet specific mechanisms. *Adv. Exp. Med. Biol.* 603, 106–115. doi: 10.1007/978-0-387-72124-8_9

Yamamoto, I., Okubo, N., and Ishimoto, M. (1986). Further characterization of trimethylamine N-oxide reductase from *Escherichia coli*, a molybdoprotein. *J. Biochem.* 99, 1773–1779.

Yarlett, N., Goldberg, B., Moharrami, M. A., and Bacchi, C. J. (1993). Trichomonas-vaginalis - characterization of ornithine decarboxylase. *Biochem. J.* 293, 487–493. doi: 10.1042/bj2930487

Yeoman, C. J., Chia, N., Yildirim, S.,Miller, M. E. B., Kent, A., Stumpf, R., et al. (2011). Towards an evolutionary model of animal-associated microbiomes. *Entropy* 13, 570–594. doi: 10.3390/e13030570

Yeoman, C. J., Thomas, S. M., Miller, M. E., Ulanov, A. V., Torralba, M., Lucas, S., et al. (2013). A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. *PLoS ONE* 8:e56111. doi: 10.1371/journal.pone.0056111

Zhang, K., and Ni, Y. (2014). Tyrosine decarboxylase from *Lactobacillus brevis*: soluble expression and characterization. *Protein Expr. Purif.* 94, 33–39. doi: 10.1016/j.pep.2013.10.018

Zhao, B., and Houry, W. A. (2010). Acid stress response in enteropathogenic gammaproteobacteria: an aptitude for survival. *Biochem. Cell Biol.* 88, 301–314. doi: 10.1139/O09-182

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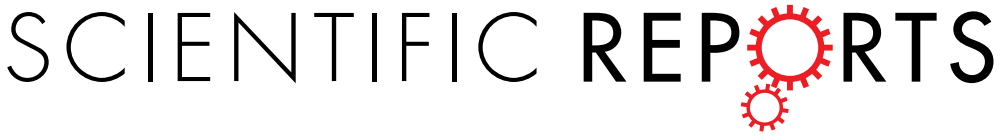

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Cigarette smoking is associated with an altered vaginal tract metabolomic profile

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Cigarette smoking has been associated with both the diagnosis of bacterial vaginosis (BV) and a vaginal microbiota lacking protective *Lactobacillus* spp. As the mechanism linking smoking with vaginal microbiota and BV is unclear, we sought to compare the vaginal metabolomes of smokers and non-smokers (17 smokers/19 non-smokers). Metabolomic profiles were determined by gas and liquid chromatography mass spectrometry in a cross-sectional study. Analysis of the 16S rRNA gene populations revealed samples clustered into three community state types (CSTs) ---- CST-I (*L. crispatus*-dominated), CST-III (*L. iners*-dominated) or CST-IV (low-*Lactobacillus*). We identified 607 metabolites, including 12 that differed significantly (q-value < 0.05) between smokers and non-smokers. Nicotine, and the breakdown metabolites cotinine and hydroxycotinine were substantially higher in smokers, as expected. Among women categorized to CST-IV, biogenic amines, including agmatine, cadaverine, putrescine, tryptamine and tyramine were substantially higher in smokers, while dipeptides were lower in smokers. These biogenic amines are known to affect the virulence of infective pathogens and contribute to vaginal malodor. Our data suggest that cigarette smoking is associated with differences in important vaginal metabolites, and women who smoke, and particularly women who are also depauperate for *Lactobacillus* spp., may have increased susceptibilities to urogenital infections and increased malodor.

According to the U.S. National Health Interview Survey, 13.9 percent of U.S. women reported that they smoked cigarettes in 2016[1], and it has been well-documented that women who smoke have greater risks for adverse reproductive health outcomes, including premature birth, delivery of low birth weight infants, certain birth defects, and ectopic pregnancy[2–6]. Smokers are also more susceptible to bacterial infections than non-smokers[7], however, few studies have investigated the mechanisms linking smoking and gynecologic infections. Smoking has been shown in large observational studies to be a dose-dependent risk factor for the diagnosis of bacterial vaginosis (BV)[8–13], as well as significantly associated with risk of other genital infections in females including *Trichomonas vaginalis*[14], herpes simplex virus type 2 (HSV-2)[15] and *Chlamydia trachomatis*[16,17]. Smoking has long been implicated in higher risk for oral and genital human papillomavirus (HPV) prevalence and viral load, progression to cervical pre-cancer, and vulval intraepithelial neoplasia[18–23]. Smoking is associated with damaged cervical epithelium through DNA modification and suppressed local and systemic immune responses[24], both of which may increase susceptibility to a wide range of female reproductive tract infections[25,26]. Nicotine's major metabolite, cotinine, has been shown to become concentrated in cervical mucus, providing evidence that smoking can directly affect the vaginal and cervical epithelium[27,28]. Smoking cessation was also associated with dramatic changes in the gut microbiota in one study[29].

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Variable	SS (trace)	Pseudo-F	p-value	Proportion (%)	Cumulative Proportion (%)
Individual model test					
CST	3181.00	9.94	0.002	22.62	NA
Smoking status	880.65	2.27	0.074	6.26	NA
Race	1120.00	2.94	0.033	7.96	NA
Education level	1216.90	3.22	0.038	8.65	NA
Fitted model with selected variables, adjusted $R^2 = 0.26$					
CST	3181.00	9.94	0.001	22.62	22.62
Race	817.71	2.68	0.037	5.82	28.44
Education level	533.18	1.79	0.11	3.79	32.23
Smoking status	292.20	0.98	0.37	2.08	34.31

Table 1. Significantly fitted predicator variables to vaginal metabolites. Distance based linear modelling (DISTLM) was performed on vaginal metabolites fitted with participant behavioral variables listed in Table 2. DISTLM was conducted with adjusted R2 over 9,999 permutations. DISTLM identifies the best-fit model based on all the available variables that best-explain the composition of vaginal metabolites. The visual display of these data as represented by the distance-based redundancy analysis (dbRDA) plot is displayed in Fig. 3.

In a 2014 study, our research group confirmed that the composition of the vaginal microbiota is strongly associated with smoking[30]. In that study, we compared the vaginal microbiota, as determined by 16S rRNA gene amplicon sequencing, between smokers and non-smokers. We reported that women who had a vaginal microbiota that were lacking significant numbers of *Lactobacillus* spp. were 25-fold more likely to report current smoking than women with a *L. crispatus*-dominated microbiota. Most *Lactobacillus* spp. are thought to provide broad-spectrum protection to pathogenic infections through the production of lactic acid and bacteriocins[31,32]. The lactic acid reduces the vaginal pH to $\leq$4.0 and is a potent bactericide and virucide[31,33–36]. Indeed, epidemiologic studies have shown that a relatively low level of *Lactobacillus* spp. and the presence of a wide array of strict and facultative anaerobes in the vaginal microbiota, as observed in the clinical diagnosis of BV, is associated with increased incidence of HIV and other sexually transmitted infections (STIs)[15,37–43].

Despite advances in understanding the epidemiologic links between smoking and women's reproductive health, the mechanistic processes by which smoking affects the vaginal microenvironment and BV is unclear. The effect of smoking on the function of the microbiome can be investigated by assessing the metabolome. The metabolome is the set of small (<1 kDa) molecule chemicals, which includes host and microbially-produced and modified biomolecules, as well as exogenous chemicals[44]. Measurement of the metabolomic profile from any biological sample is expected to contain numerous low molecular mass molecules with different physicochemical characteristics and concentration ranges[44].

The metabolome is an important characteristic of the vaginal microenvironment and differences in some metabolites are associated with functional variations of the vaginal microbiota[45–48]. For example, the presence of certain chemicals or metabolites have been shown *in vitro* to directly reduce or inhibit the growth of select bacterial species[49–51]. Biosynthesis of biogenic amines (BAs) (cadaverine, putrescine, spermine, spermidine, trimethylamine, and tyramine) may allow various bacteria to survive in low pH environments[52], like that found in the healthy *Lactobacillus*-dominated vagina. BAs have also been shown to enhance growth rates of various pathogenic bacteria, such as *Neisseria gonorrhoeae*, and shield them from host innate immunological defenses[53–55]. The recent applications of metabolomics with *in vivo* samples have identified relationships between bacterial species and vaginal metabolomic profiles. In particular *Dialister*, *Mobiluncus*, *Atopobium*, *Prevotella*, *Mycoplasma* and *Gardnerella* species were correlated with the presence of several metabolites along with vaginal odor and discharge[45–48]. We have hypothesized that BAs may be an important feature in the destabilization of *Lactobacillus* spp.-dominant vaginal microbiota and the initiation of BV, as well as the characteristic malodor of BV[56]. The decarboxylation of amino acid precursors to form BAs results in increased pH and may increase the risk for BV[57].

In this study, we sought to characterize vaginal microbiota functional differences between smokers and non-smokers by investigating the vaginal metabolome. These data may allow enhanced understanding of the mechanism by which smoking may increase the risk of urogenital infections and may contribute to our understanding of the effect of smoking on the female reproductive tract.

Results

In total, 607 compounds were identified in the vaginal metabolome of 36 women. PCA indicated that the metabolomic profiles clustered by both smoking status and bacterial community state type (CST) (Figure S1). CST accounted for 23% of the metabolite variation observed in the complete data set (distance based linear model, DISTLM $F = 9.9397$, $P_{PERM} = 0.0001$; Table 1), while smoking status explained 6%. However, the concentrations of 12 metabolites were significantly different between smokers and non-smokers (q-value = <0.05; Fig. 1A, Table S1). Tobacco constituents and their primary breakdown products (nicotine, cotinine and hydroxycotinine) represented the strongest differences between smokers and non-smokers with non-smokers having a 2- to 12-fold reduction in these compounds (q-value = <0.05). After adjusting for the influence of CST, five of the metabolites persisted in their differences between smokers and non-smokers (Figs 1B, 2).

Further, 142 metabolites had abundances that were marginally significantly different between smokers and non-smokers (q-value between 0.05 and 0.10) without adjustment for CST (Table S2). These compounds are

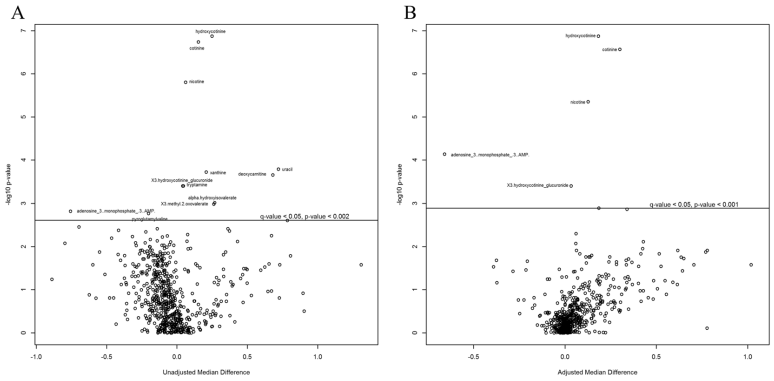

Figure 1. Compounds differing between smokers and non-smokers with and without adjustment for CST. Volcano plots display $-\log_{10}$ (p-value) and the median difference in concentration between smokers and non-smokers when unadjusted (**A**) and adjusted for community state type (**B**). Quantile regression was conducted on centered and scaled metabolite concentrations. Significance testing was conducted with Wilcoxon rank sum test and corrected for multiple comparisons. Metabolites that differed significantly where q-value $<=0.05$ between smokers and non-smokers are shown above the line in each plot.

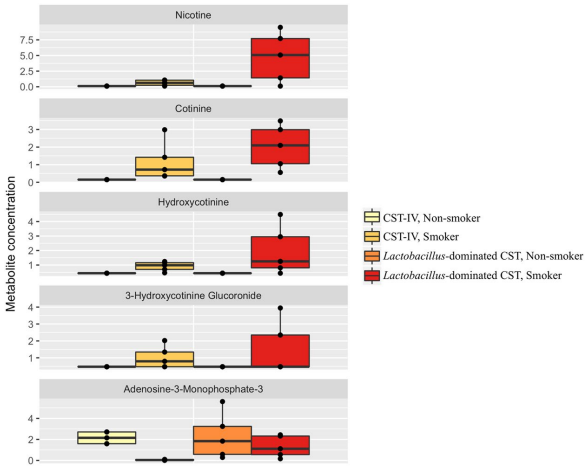

Figure 2. Vaginal metabolites that differ between smokers and non-smokers. Boxplots display metabolites identified as significantly (q-value = <0.05) different in the vagina of smokers and non-smokers when unadjusted and adjusted for the impact of bacterial community state type (CST). Samples categorized as CST-I (*L. crispatus*-dominated) and CST-III (*L. iners*-dominated) were grouped and compared with CST-IV (low-*Lactobacillus* spp.). Quantile regression was conducted on centered and scaled metabolite concentrations. Significance testing was conducted with Wilcoxon rank sum test and corrected for multiple comparisons.

involved in amino acid metabolism, including some essential for bodily functions such as lysine, tryptophan, leucine, isoleucine, histidine and methionine as well as many amino acid dipeptides required for protein hydrolysis. Interestingly, the greatest differences in the composition and abundance of the metabolomes between smokers and non-smokers were among biogenic amines, including, cadaverine (fold change in non-smokers, FC = −53.19), putrescine (FC = −15.80), agmatine (FC = −15.49), tyramine (FC = −5.15) and tryptamine (FC = −4.43), which were much higher in smokers (Table S2, Figure S2).

	Non-smoker		Smoker		
	n	%	n	%	p-value[1]
Participant details					
Age					0.011
19–28	16	44	4	11	
29–38	1	3	6	17	
39–48	2	6	7	19	
Marital status					0.066
Single, never married	17	47	8	22	
Separated, divorce, widowed	1	3	5	14	
Married	1	3	3	8	
Race					0.170
Asian/Pacific Islander	3	8	1	3	
White	8	22	4	11	
African American/black	4	11	10	28	
Hispanic	3	8	0	0	
Multi-racial	1	3	1	3	
Other	0	0	1	3	
Education level					1.000
High School, up to 12 years	0	0	1	3	
College and graduate, > 12 years	19	53	16	44	
Clinical					
CST					0.011
I, *L. crispatus*-dominated	13	36	4	11	
III, *L. iners*-dominated	4	11	3	8	
IV, Low-*Lactobacillus* spp.	2	6	10	28	
Nugent's Gram stain score					0.000
0–3	17	47	8	22	
4–6	2	6	2	6	
7–9	0	0	7	19	
Vaginal pH					0.004
<=4.0	10	28	4	11	
4.1–5.5	1	5	1	3	
4.6–5.0	4	11	2	6	
>=5.1	4	11	10	28	
Self-reported symptoms					
Vaginal odor					0.969
No	15	42	16	44	
Yes	4	11	1	3	
Vaginal irritation, 24 hours prior					1.000
No	19	53	17	47	
Yes	0	0	0	0	
Vaginal itching, 24 hours prior					1.000
No	19	53	17	47	
Yes	0	0	0	0	
Vaginal burning, 24 hours prior					1.000
No	19	53	17	47	
Yes	0	0	0	0	
Pain urinating, 24 hours prior					1.000
No	19	53	17	47	
Yes	0	0	0	0	
Vaginal discharge, 24 hours prior					0.847
No	14	39	14	39	
Yes	5	14	3	8	

Continued

	Non-smoker		Smoker		
	n	%	n	%	p-value[1]
Participant details					
Hygiene					
Vaginal douche, 2 months prior					0.108
No	18	50	9	25	
Yes	1	3	7	19	
Not recorded	0	0	1	3	
Menstruating currently					0.075
No	9	25	9	25	
Yes	10	28	8	22	
Tampon or pad, 24 hours prior					0.456
No pad, no tampon	10	28	10	28	
Pad only	2	6	1	3	
Tampon only	4	11	2	6	
Tampon and pad	2	6	1	3	
Not recorded	1	3	3	8	
Sexual behaviors					
Lifetime number of sexual partners					0.25
0–6	13	36	3	8	
7+	6	17	12	33	
Not recorded	0	0	2	6	
Number of sex partners, 2 months prior					0.000
0	4	11	2	6	
1	12	33	14	39	
2	3	8	1	3	
Vaginal intercourse with a condom, 24 hours prior					0.673
No vaginal intercourse	14	39	9	25	
Vaginal intercourse no condom	3	8	6	17	
Vaginal intercourse with condom	2	6	2	6	
Anal intercourse, 24 hours prior					1.000
No	19	53	17	47	
Yes	0	0	0	0	
Sex toy use, 24 hours prior					1.000
No	19	53	17	47	
Yes	0	0	0	0	
Lubricant use, 24 hours prior					1.000
No	19	53	17	47	
Yes	0	0	0	0	
Partner type					0.000
Regular	10	28	12	33	
Occasional	2	6	0	0	
New	1	3	0	0	
Not recorded	6	17	5	14	
Receptive oral sex, 24 hours prior					0.543
Yes	2	6	1	3	
No	17	47	16	44	
Digital penetration, 24 hours prior					0.555
Yes	3	8	2	6	
No	16	44	15	42	
Thong use, 24 hours prior					0.000
Yes	6	17	3	8	
No	13	36	13	36	
Not recorded	0	0	1	3	

Table 2. Factors associated with smoking status, Baltimore, MD (n=36) [1]p-value determined using Fisher's exact test.

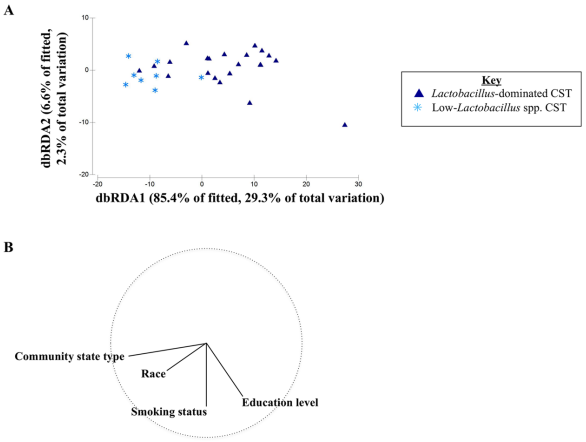

Figure 3. Participant predictor variables fitted to vaginal metabolites. Distance-based linear modelling (DISTLM) was conducted on log-transformed Euclidean distance matrix of vaginal metabolites. All variables were included in the final model that when applied to the data cloud of vaginal metabolites (Table 1). Distance-based redundancy analysis (dbRDA) plot (**A**) displays the metabolite composition fitted to the four variables labeled with the strongest variable, CST. Variable vectors indicating strength and direction are identified (**B**).

The associations observed between smoking and the vaginal metabolome may be explained in part by participant-specific variables, such as sexual behaviors, health, race and other confounding factors. We utilized a distance-based linear modeling (DISTLM) approach to explain the contribution of confounding participant variables to the vaginal metabolome (Table 1). Due to the relatively small sample size of our study, the model was limited to a total of four variables, which we selected as the best fit from all potential models with the inclusion of smoking status and CST. The model including CST, race, education level, and smoking status, had an adjustedR^2 of 0.26, and these variables explained 34% of the observed variation in the vaginal metabolomic profiles (Table 1). Using this analytical approach based on the full metabolome, smoking status did not show a statistically significant relationship to the full spectrum of vaginal metabolites after adjustment for CST, race, and education, although in some cases, when the associations are large and the p-values do not reach significance, it may be due to the lack of power associated with the relatively small sample size (Fig. 3, Table 1).

In addition to the multivariate analyses above, we also sought to directly contrast the metabolome of *Lactobacillus*-dominated CST-I/III and the low-*Lactobacillus*/diverse anaerobes observed in CST-IV. To increase the power of significance testing, we combined *L. crispatus*-dominated CST-I and *L. iners*-dominated CST-III. In bivariate analysis, there was a significant difference (q-value $<=0.05$) in the concentrations of 67% of all identified vaginal metabolites between CST-I/III and CST-IV (Table S3). After adjusting for the influence of a woman being a current smoker, the abundance of 54% of vaginal metabolites still differed significantly (q-value $<=0.05$). The majority of the metabolites that were elevated in the *Lactobacillus*-dominated CSTs were amino acids, lipids, peptides and especially dipeptides (Fig. 4). Carbohydrates, mannitol, lactate, xylulose and fucose were higher in the *Lactobacillus*-dominated CSTs compared to CST-IV (Table S3). The glycine conjugate of benzoic acid, hippurate, was also markedly higher (34-fold) in *Lactobacillus*-dominated communities. The BAs (cadaverine, putrescine, agmatine, tyramine, tryptamine) and the straight chain fatty acid deoxycarnitine were also observed to be significantly higher in CST-IV compared to *Lactobacillus*-dominant communities (Table S3). Correlations with pH measurement and BA concentrations were positive, yet lacked statistical significance (Table S4, Figure S3).

Further, among women in CST-IV, the BAs were markedly higher in smokers versus non-smokers (q-value: <0.05, Cadaverine (89-fold), Putrescine (26-fold), Agamatine (26-fold), Tyramine (10-fold) and Trypatamine (7-fold), Figure S2).

Discussion

Our group and others have previously reported that female smokers are more likely to display a low-*Lactobacillus* CST-IV vaginal microbiota and are at increased risk for morbidities such as bacterial vaginosis[9,30] and sexually transmitted infections[39]. To obtain greater insight into this relationship, we assessed the vaginal metabolome of smokers and non-smokers. Using a comprehensive metabolomics approach and multiple analyses of the data, we identified an extensive and diverse range of vaginal metabolites for which profiles were affected by both the microbiology and smoking status. Bacterial composition (CST) was the most pronounced driver of the vaginal metabolome in our model and was associated with changes in 57% of all metabolites, suggesting vaginal microbiota are the major drivers of the vaginal metabolome. We therefore carefully controlled for CST in multivariate

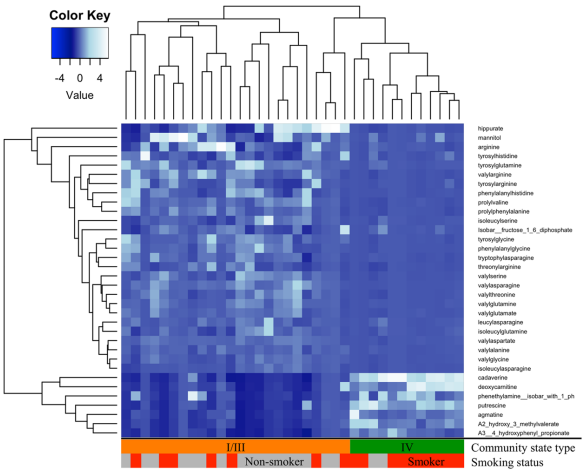

Figure 4. Metabolites differ between CST. Heatmap displays selection of metabolites with a high fold change identified as significantly (q-value = <0.05) different in the vagina of bacterial community state type (CST) when unadjusted and adjusted for the impact of smoking status. Low-*Lactobacillus* CST-IV and *Lactobacillus*-dominated CST-I and CST-III are grouped. Quantile regression was conducted on centered and scaled metabolite concentrations. Significance testing was conducted with Wilcoxon rank sum test and corrected for multiple comparisons.

analysis and also conducted stratified analysis based on CST so that we could directly contrast smokers versus non-smokers, while essentially holding CST constant. As expected, nicotine and its breakdown products were markedly elevated in the vagina of smokers. In humans, 70–80% of nicotine is converted to cotinine followed by conversion to hydroxycotinine[58]. Hydroxycotinine is a well-known biomarker identified in the urine, plasma and serum of individuals exposed to both active and passive smoking[59–61]. Previous studies have identified nicotine and cotinine in the cervical mucus of smokers[27] with good correlation to concentrations in blood and urine[62,63]. Our findings are consistent with the vaginal metabolome contributing to the mechanism linking smoking to the microbiome.

Another key finding was that we observed significant increases in the abundance of various BAs among smokers relative to non-smokers, which was far more pronounced in women with a low-*Lactobacillus* CST-IV. BAs are unique molecules that carry one or more amine groups (NH_2). They are essential to mammalian and bacterial physiology, tightly controlled in cellular metabolisms. Cadaverine, putrescine, agmatine and tryptamine have roles in the metabolism of essential amino acids, including tryptophan and lysine. Their accumulation indicates upheaval or alterations in these metabolic systems. In particular, the odors of the amines cadaverine and putrescine are foul-smelling to humans, identifiable as indicators of tissue decomposition associated with death or bacterial contamination[64,65]. Several of these BAs, including cadaverine and putrescine, have previously been correlated with diagnosis of BV[45,66,67] and implicated in the associated 'fishy' vaginal malodor of BV[46,47].

Our group recently suggested a hypothetical model for the displacement of vaginal *Lactobacillus* spp. and increased risk of BV and urogenital infection with BAs[68]. We hypothesize that in the vaginal canal, BAs may favor non-*Lactobacillus* species, while also increasing the vaginal pH, collectively enabling colonization by a more diverse community, as is observed with CST-IV. Our hypothesis is based on two observations. First, the amino-acid decarboxylation reactions that produce BAs involve the consumption of intracellular hydrogen ions and is a well-described bacterial acid resistance and mitigation mechanism[52]. The consumption of hydrogen ions increases the pH of the local habitat[52], overcoming what is widely considered the primary barrier to pathogen outgrowth and a clinically-recognized symptom of BV. Second, the growth of several pathogens, including the urogenital pathogen, *Neisseria gonorrhoeae*, and their resistance to host immunological defenses has been shown to be superior in the presence of various BAs[53–55,69]. In this study, we observed that lactate was lower and BAs were higher, in low-*Lactobacillus* CST-IV participants as expected. However, lactate was higher in *Lactobacillus*-dominated CST-I/III even when BA concentrations were low. Lactate concentration includes both protonated lactic acid (LAH) and lactate anion (LA^-) with the former being recognized as the active microbicidal form capable of inactivating BV-associated bacteria[31,70]. Lactate concentrations increase with increasing hydrogen ion concentrations[31] and therefore the reduction in lactate and increase in BAs further supports our hypotheses, and suggest that bacteria present in low-*Lactobacillus* CST-IV use available hydrogen ions to perform amino acid decarboxylation and produce BAs, thus resisting acid stress[56].

Our results identifying the presence of nicotine breakdown products in smokers may reflect differences in the degree of transport and/or accumulation of nicotine and its derivatives in the vagina by CST. These differences may relate to the co-variation in pH with CST[33]. Absorption of nicotine across biological membranes (such as cervicovaginal endothelial/epithelial cells) has previously been shown to be pH dependent[56,71]. Nicotine is a weak base ($pK_a = 8.0$) and in acidic environments nicotine does not rapidly cross membranes[68]. Cotinine and hydroxycotinine are more acidic with a $pK_a = 4.8$ and 4.3, respectively[72]. Previous studies which have measured both in serum and vaginal samples identified higher levels of nicotine in the cervical mucus compared with serum samples, yet cotinine values were similar[27,63]. Nicotine may be selectively concentrated in the vagina because the majority (72%) of women display a vaginal pH of 4.0 to 4.6[33]. Conversely, women with low-*Lactobacillus* CST-IV vaginal microbiota have a vaginal pH of 5.3 ± 0.6[33] and, consistently displayed comparatively lower levels of nicotine. Alternatively, these findings may indicate microbiological metabolism of nicotine in the more diverse and low-*Lactobacillus* state of CST-IV.

Similarly, hippurate, a normal excretory product of urine that is increased with exposure to phenolic compounds and toluene, a byproduct of cigarette smoke[73], was increased in *Lactobacillus*-dominated CST-I/III over CST-IV and also in non-smokers over smokers. Hippurate is a known substrate of *Gardnerella vaginalis*[74], an organism often found in higher abundances when *Lactobacillus* spp. are low and commonly associated with symptomatic BV[75–77]. Hippurate is decreased in the low-*Lactobacillus* CST-IV and this may suggest that microbial utilization of hippurate and therefore smoking may move the vaginal environment closer to favor the proliferation of *G. vaginalis*.

Beyond metabolites directly affiliated with nicotine metabolism, we observed substantive shifts in dipeptides and biogenic amines. More than 150 dipeptides in smokers or CST-IV participants were significantly decreased relative to non-smokers and those with *Lactobacillus* spp.-dominated vaginal microbiota. In a previous study, Ghartey and colleagues noted significant reductions in vaginal dipeptides among women who delivered infants preterm, for which CST-IV and BV are important risk factors[78]. Dipeptides refer to one or more amino acid joined by a peptide bond with important roles in protein metabolism and cell signaling. CST-I and III women had higher concentrations of dipeptides relative to CST-IV, suggesting *Lactobacillus* spp. dominance may be important to this phenotype. Dipeptides are constituents of the peptidoglycan cell wall of bacteria and are made during its' synthesis. Therefore, some dipeptides, such as muramyl dipeptides, serve as signal to the immune system in mammals and play a direct role in the regulation of inflammation[79]. Their production has also been indicated in *Lactobacillus* spp. and other bacteria as a mean of quorum sensing and cellular signaling[80]. *Lactobacillus* spp. may exploit dipeptides to increase their osmotolerance[81], or produce dipeptides for amensalistic purposes. Various *Lactobacillus* spp. have been noted to produce cyclic dipeptides with anti-fungal[80,82–84], anti-viral[85] and anti-bacterial[80,86] properties. This includes the production of dipeptides by a vaginal isolate of *L. reuteri* that disrupts the virulence capabilities of *Staphylococcus aureus* involved in toxic shock syndrome[67]. The increase in dipeptides in non-smokers and *Lactobacillus* spp.-dominant CSTs may be reflective of increased production by *Lactobacillus* spp. of these bioactive compounds. Conversely, the reduction in dipeptides in women with a low-*Lactobacillus* CST-IV vaginal microbiota may be a result of the increased proteolytic activity from bacteria present in this CST. Many BV-associated bacteria have been correlated with, or shown to be capable of secreting proteases, which break down proteins into amino acids[87–91]. Aside from reducing the number of detected dipeptides in the vaginal tract, proteases may also have the effect of inactivating proteins important to host defenses and make host tissues more susceptible to other organsims' virulence factors[87].

The breakdown compounds of a number of drugs, such as cocaine (norbenzoylecgonine, benzoylecogonine), antidepressants (escitalopram), common painkillers (acetaminophen glucoronide, ibuprofen) and decongestants (pseudoephedrine) were each observed in one or more samples in the study. This indicates that some drugs could possibly be assessed from vaginal metabolomic profiles. There is relatively little known about the relationship between individual drugs and their impact on the vaginal microbiome. Cocaine use has been associated with shifts in bacterial phyla in the gut[92] and additionally has been associated with a greater likelihood of contracting sexually transmitted infections[93,94]. The use of antidepressants has been linked to menstrual disorders and hormonal changes in women[95,96] both of which may cause indirect shifts in the vaginal microbiota. As these studies may suggest a potential impact of drugs and medications and their byproducts on the vaginal microbiota, further exploration is needed to make any conclusions.

Our data are consistent with recent studies of the vaginal metabolome[45,47,97]. In prior studies, the BAs (cadaverine, putrescine and tyramine) were consistently higher in women with BV[45,47,48,97]. We did not detect trimethylamine in our samples as has previously been identified and we suspect this is due to its high volatility[45,47,48,97]. Srinivasan *et al.* reported the same issue with their lack of trimethylamine detection using similar methods yet reported lower levels of trimethylamine oxide (an intermediary product) in women with BV using separate methods[97]. Therefore, there are limitations to the use of metabolomics, including particle resolution, compound sensitivity, polarity and volatility[98]. Identification of detected metabolites is further constrained by comparison to facility-built databases[98,99]. However, this is best achieved by utilizing standardized collection, preparation and[98] pairing liquid chromatography (LC), gas chromatography (GC) and mass spectrometry (MS) which can enhance identification of analytes with differing characteristics[98].

Aside from biogenic amines, McMillian *et al.* reported alpha-hydroxyisovalerate and gamma-hydroxybutyrate (GHB) as associated with BV and high bacterial diversity[47]. We also observed higher levels of alpha-hydroxyisovalerate and GHB in low-*Lactobacillus* CST-IV and additionally we observed a trend towards increases in smokers. Alpha-hydroxyisovalerate was positively correlated with diverse BV-associated bacteria such as *Atopobium, G. vaginalis, Dialister* and *Gemella*. McMillian *et al.* went on to show how *G. vaginalis*, a vaginal bacterial species commonly associated with BV is a producer of GHB[47]. Srinivasan *et al.* also identified 12-hydroxyeicosatetraenoic acid (12-HETE) in cases of BV and we also detected a significantly high abundance of this compound in women from CST-IV over the *Lactobacillus*-dominant CSTs (I or III), although it was not associated with smoking status[97].

A limitation of our study was the small sample size and the distribution of smokers and non-smokers within each community state type (17 CST-I, 7 CST-III, 12 CST-IV participants). As a result, we have relatively reduced power in some of these statistical tests in stratified analyses. Some of the assumptions of traditional parametric statistical tests are sensitive to small sample sizes, and the use of non-parametric statistical tests, as was employed here, can overcome these assumptions with increased robustness to skewness and outliers. The use of a false discovery rate provided a further method for conservative interpretation of these results. We combined *L. crispatus*-dominated CST-I and *L. iners*-dominated CST-III because in this study, succinate was the only metabolite with abundance significantly different between samples belonging to CST-I compared to CST-III. Combining the two CSTs allowed us to increase our statistical power by comparing CST-I/III to CST-IV, however combining the *L. crispatus* and *L.iners*-dominated communities may not be functionally optimal. There is a growing body of research focused on *L. iners*[32,100–103] that aims to evaluate its protective value as part of the vaginal microbiota. *L. iners* is commonly detected in healthy women, and interestingly in women with BV, as well as being among the first *Lactobacillus* spp. to recover after antibiotic treatment for BV[104]. Further, it is often considered to be a tipping point for some women at risk for BV[104]. Despite the small sample size, this study is unique in its use of metabolomics and microbial abundance data in combination with extensive participant behavioral data and its rigorous evaluation of smoking status by using quantification of carbon monoxide exhaled and cotinine in saliva. The depth of the analyses performed in this study, and the results obtained, may inform where to focus resources in a larger confirmatory study.

Conclusions

It is well-documented that a vaginal microbiota dominated by *Lactobacillus* spp. is associated with reduced risk for urogenital infections, including sexually transmitted infections (STIs) and urinary tract (UTIs) infections[105]. In this study, we determined that overall smoking did not affect the vaginal metabolome after controlling for CST, but several key metabolites were elevated in smokers. Among women who were smokers and depauperate for *Lactobacillus* spp. (classified as CST-IV), we observed that they had significantly more perturbed metabolic profile than other CSTs when compared to their non-smoking counterparts. Biogenic amines were elevated in smokers and these metabolites have known roles in anaerobic bacterial proliferation, immune- and stress-resistance with a significant link to the development of BV, and possibly other reproductive tract infections. Women who smoke may have increased susceptibility to reproductive tract infections due to the observed increase in concentrations of BAs and the finding was even more pronounced among women who had low levels of *Lactobacillus* spp.

The metabolite profile of the vagina was strongly influenced by the resident microbiota as well as cigarette smoking in epidemiologic analyses that controlled for possible confounders. Detection of nicotine and its breakdown products in the vagina may serve as molecular biomarkers of smoking. Our results suggest that smoking is associated with several important metabolites present in the vagina that may have implications for women's health. This study serves as a pilot for the development of future studies of the mechanisms linking smoking to poor gynecologic and reproductive health outcomes.

Methods

Sample Collection. Forty women self-collected mid-vaginal swabs during a single visit at the Center for Health Behavior Research at the University of Maryland School of Public Health (UMSPH). The study has previously been described[30]. In brief, smoking burden was determined by saliva cotinine testing, carbon monoxide exhalation levels and self-report of smoking habits on a set of comprehensive behavioral surveys. Participants were excluded if they had used an antibiotic or antimycotic in the 30 days prior. Four women were excluded from this analysis due to poor DNA quality affecting the normalization of the vaginal metabolome dataset (final sample size for analysis was 36 (17 smokers and 19 non-smokers) (Table 2). All participants provided written informed consent, and ethical approval was obtained from the Institutional Review Boards of the University of Maryland Baltimore (UMB), Montana State University and the UMSPH. All samples were collected and analysed in accordance with the relevant guidelines and regulations.

Sample Preparation for Metabolomics. Vaginal samples were eluted from swabs (Starplex rayon swab) in 200 µl phosphate buffered saline (PBS) and subjected to both gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS) with Orbitrap Elite accurate mass platforms (Thermo Scientific, Waltham, MA, USA). Sample processing and analysis was performed by Metabolon (Durham, NC, USA) using an automated MicroLab STAR® system (Hamilton Company, Reno, NV, USA). Recovery standards were added prior to the first step in the extraction process for quality control purposes. Sample preparation was conducted using a proprietary series of organic and aqueous extractions to remove the protein fraction while allowing maximum recovery of small molecules. The resulting extract was divided into two fractions: one for analysis by LC and one for analysis by GC. Samples were placed briefly on a TurboVap® (Zymark, Hopkinton, MA, USA) to remove the organic solvent. Each sample was then frozen and dried under vacuum. Samples were then prepared for the appropriate instrument, either LC-MS or GC-MS.

Liquid Chromatography and Gas Chromatography Mass Spectrometry. LC-MS measurements were conducted on a Waters ACQUITY ultra-performance liquid chromatograph (UPLC) and a ThermoFisher Scientific Orbitrap Elite high resolution/accurate mass spectrometer (Thermo Scientific, Waltham, MA, USA), which consisted of a heated electrospray ionization (HESI) source and orbitrap mass analyzer operated at a resolution 30,000 mass. The sample extract was dried then reconstituted in LC-compatible solvents, each of which contained eight or more injection standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion optimized conditions and the other using basic negative ion optimized conditions in two independent injections using separate dedicated columns. Extracts

reconstituted in acidic conditions were gradient eluted using water and methanol containing 0.1% formic acid, while the basic extracts, which also used water/methanol, contained 6.5 mM ammonium bicarbonate. The MS analysis alternated between MS and data-dependent MS^2 scans using dynamic exclusion.

Samples for GC-MS analysis were re-dried under vacuum desiccation for a minimum of 24 hours prior to being derivatized under dried nitrogen using bistrimethyl-silyl-triflouroacetamide (BSTFA). The GC column was 5% phenyl and the temperature ramp is from 40° to 300 °C in a 16 min period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization. The instrument was tuned and calibrated for mass resolution and mass accuracy prior to use. Peaks in the GC-MS and LC-MS data were identified using Metabolon's proprietary peak integration software to resolve sample metabolite peaks over noise. Complete details of methods describing metabolomic profiling are described in Lawton *et al.* (2008)[106].

Compound Identification and Preparation. Spectra corresponding to each metabolite were identified by comparison to library entries of purified metabolite standards and their distinction from more than 1,000 other commercially available purified standard compounds. The combination of chromatographic properties and mass spectra gave an indication of a match to the specific biogenic amine compound or an isobaric entity. Results were manually curated to ensure that data were accurate and to remove any system artifacts, miss assignments, and background noise.

Taxonomic Assignment and Community State Type Profiling. DNA extraction, PCR amplification and sequencing of 16S rRNA gene amplicons from the vaginal tract of study participants were conducted in a prior study[30]. Briefly, the V1-V3 region of the 16S rRNA gene was PCR amplified using the primers 27F-YM + 3[107] and 534R[57] and pyrosequenced using a Roche 454 FLX instrument. Species level assignments of *Lactobacillus* were performed using higher order Markov Chain models using the software speciateIT (speciateIT.sourceforge.net)[33]. For each sample, community state types (CSTs) were assigned to individual samples based on diversity and relative abundances of different phylotypes as defined in the work of Gajer and Brotman *et al.*[108]. The vaginal microbiota samples were categorized into CST-I (*L. crispatus*-dominated), CST-III (*L. iners*-dominated) and CST-IV (low-*Lactobacillus*/high strict and facultative anaerobes) (Table 2) Two other well-documented CSTs, CST-II (*L. gasseri*-dominated) and CST-V (*L. jensenii*-dominated) were not identified in this limited sample and are less commonly found even in larger surveys of women[33].

Data Analyses. The metabolomic dataset was normalized to DNA concentration and missing values were imputed with the minimum detected value for that compound which essentially assigns values based on the sensitivity limit[109]. Data were then centered and scaled and the median was set equal to 1 prior to log transformation. Samples were visualized using a principal components analysis (PCA) (Figure S1). We performed quantile regression to estimate the median metabolite concentration differences 1) between smokers and non-smokers, 2) between CSTs and, 3) also with adjustment for confounding factors. P-values were obtained using the Wilcoxon rank-sum test. To correct for multiple tests, false discovery rate (FDR) and q-values were calculated for each compound where $p = <0.05$. Where a p-value estimates the proportion of all tests which will result in false positives (i.e. 5% where p-value = 0.05), a q-value estimates the proportion of significant tests that will result in false positives[110]. Initial investigations between *Lactobacillus*-dominated CST-I and CST-III yielded only one significant difference in the metabolite succinate (q-value = 0.009) with adjustment for smoking status. This metabolite was not identified as differing significantly (q-value = 0.05) in all further analyses, and therefore we grouped CST-I and CST-III for further binary analyses to increase power in comparisons to CST-IV. Fold change (FC) was calculated based on Guo *et al.*[111] where $FC_i = x_i - y_i$ where i is metabolite of interest for the mean value of the control (x) and treatment (y).

We performed distance based linear modeling (DISTLM) by partitioning the distances of compounds using distance-based redundancy analysis (dbRDA)[112], a form of multivariate multiple regression that can be performed directly on a distance or dissimilarity matrix. As the number of participants limits the number of variables that can be included in the model, we planned to include just two variables, in addition to our main factors of CST and smoking status. Initially we ran individual variables against the metabolite data and selected only those variables that were significant ($p <= 0.05$). These were age, race, marital status, education level, thong undergarment in the prior 24 hours, and vaginal douching in the two months prior. The dissimilarity matrix of vaginal metabolites was fitted with four of the variables listed in Table 2 and the final model was selected with the greatest adjusted R^2 value.

Boxplots and heatmaps were constructed using the ggplot2[113] and RColorBrewer (colorbrewer2.org) packages conducted in R version 3.1.2[114]. PCA, correlation coefficients, DISTLM and dbRDA plots were produced in Primer version 6[115].

Data availability. The questionnaire and metabolome data are available at the National Center for Biotechnology Information (NCBI) Database of Genotypes and Phenotypes (dbGaP) under Accession number phs001386.v1.p1. Metagenomic sequence data were submitted to the public NCBI Sequence Read Archive (SRA) with the accession number PRJNA391039.

References

1. National Center for Health Statistics. *Early Release of Selected Estimates Based on Data from the National Health Interview Survey, 2016* (2017).
2. *Fact sheet: Current Cigarette Smoking Among Adults in the United States*. *JAMA* **309** (2013).
3. Eschenbach, D. A. *Diagnosis of Bacterial Vaginosis (Nonspecific Vaginitis): Role of the Laborator.* **6**, 134–138 (1984).
4. Tripathi, R., Dimri, S., Bhalla, P. & Ramji, S. *B*acterial vaginosis and pregnancy outcome. *International Journal of Gynecology & Obstetrics* **83**, 193–195 (2003).
5. van Oostrum, N., De Sutter, P., Meys, J. & Verstraelen, H. Risks associated with bacterial vaginosis in infertility patients: a systematic review and meta-analysis. *Human reproduction* **28**, 1809–1815 (2013).

6. Feldkamp, M. L. *et al*. Case-control study of self reported genitourinary infections and risk of gastroschisis: findings from the national birth defects prevention study, 1997-2003. *Bmj* **336**, 1420–1423 (2008).
7. Bagaitkar, J., Demuth, D. R. & Scott, D. A. *Tobacco use increases susceptibility to bacterial infection*. **4**, 12–10 (2008).
8. Larsson, P. G., Platz-Christensen, J. J. & Sundström, E. Is bacterial vaginosis a sexually transmitted disease? *International journal of STD & AIDS* **2**, 362–364 (1991).
9. Hellberg, D., Nilsson, S. & Mårdh, P. A. Bacterial vaginosis and smoking. *International journal of STD & AIDS* **11**, 603–606 (2000).
10. Hay, P. E. *et al. A longitudinal study of bacterial vaginosis during* pregnancy. *International Journal of Gynecology & Obstetrics* **3**, 320 (1995).
11. Nelson, D. B. *et al*. Vaginal symptoms and bacterial vaginosis (BV): how useful is self-report? Development of a screening tool for predicting BV status. *Epidemiol. Infect.* **135**, 1369–1375 (2007).
12. Miller, G. C., McDermott, R., McCulloch, B., Fairley, C. K. & Muller, R. Predictors of the prevalence of bacterial STI among young disadvantaged Indigenous people in north Queensland, Australia. *Sexually transmitted infections* **79**, 332–335 (2003).
13. Cherpes, T. L., Hillier, S. L., Meyn, L. A., Busch, J. L. & Krohn, M. A. A delicate balance: risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race, and positive herpes simplex virus type 2 serology. *Sex Transm Dis* **35**, 78–83 (2008).
14. Swartzendruber, A., Sales, J. M., Brown, J. L., DiClemente, R. J. & Rose, E. S. Correlates of Incident Trichomonas vaginalis Infections Among African American Female Adolescents. *Sexually transmitted diseases* **41**, 240–245 (2014).
15. Cherpes, T. L., Meyn, L. A., Krohn, M. A. & Hillier, S. L. Risk factors for infection with herpes simplex virus type 2: Role of smoking, douching, uncircumcised males, and vaginal flora. *Sex Transm Dis* **30**, 405–410 (2003).
16. Wangnapi, R. A. *et al*. Prevalence and risk factors for Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis infection in pregnant women in Papua New Guinea. *Sexually transmitted infections* **91**, 194−+ (2015).
17. Marcone, V. *et al*. Epidemiology of Chlamydia trachomatis endocervical infection in a previously unscreened population in Rome, Italy, 2000 to 2009. **17**, 16–23 (2012).
18. Miller, C. S. & White, D. K. Human papillomavirus expression in oral mucosa, premalignant conditions, and squamous cell carcinoma: A retrospective review of the literature. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **82**, 57–68 (1996).
19. Gillison, M. L. *et al*. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. **100**, 407–420 (2008).
20. Castellsagué, X. & Muñoz, N. Chapter 3: Cofactors in human papillomavirus carcinogenesis–role of parity, oral contraceptives, and tobacco smoking. *J. Natl. Cancer Inst. Monographs* 20–28 (2003).
21. Garrett, L. R., Perez-Reyes, N., Smith, P. P. & McDougall, J. K. Interaction of HPV-18 and nitrosomethylurea in the induction of squamous cell carcinoma. *Carcinogenesis* **14**, 329–332 (1993).
22. Nischan, P., Ebeling, K. & Schindler, C. Smoking and Invasive Cervical-Cancer Risk - Results From a Case-Control Study. **128**, 74–77 (1988).
23. Khan, A. M., Freeman-Wang, T., Pisal, N. & Singer, A. Smoking and multicentric vulval intraepithelial neoplasia. *J Obstet Gynaecol* **29**, 123–125 (2009).
24. Arnson, Y., Shoenfeld, Y. & Amital, H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. *J. Autoimmun.* **34**, J258–J265 (2010).
25. Simons, A. M., Phillips, D. H. & Coleman, D. V. Damage to DNA in Cervical Epithelium Related to Smoking. *Tobacco.* **49**, 186 (1994).
26. Bornstein, J., Rahat, M. A. & Abramovici, H. Etiology of Cervical Cancer: Current Concepts. **50**, 146 (1995).
27. McCann, M. F. *et al*. Nicotine and cotinine in the cervical mucus of smokers, passive smokers, and nonsmokers. **1**, 125–129 (1992).
28. Winkelstein, W. Smoking and Cervical-Cancer - Current Status - a Review. **131**, 945–957 (1990).
29. Biedermann, L. *et al*. Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans. **8**, e59260 (2013).
30. Brotman, R. M. *et al*. Association between cigarette smoking and the vaginal microbiota: a pilot study. *BMC Infect. Dis.* **14**, 471 (2014).
31. O'Hanlon, D. E., Moench, T. R. & Cone, R. A. Vaginal pH and Microbicidal Lactic Acid When Lactobacilli Dominate the Microbiota. *PLoS ONE* **8**, ARTN e80074 (2013).
32. Macklaim, J. M., Gloor, G. B., Anukam, K. C., Cribby, S. & Reid, G. At the crossroads of vaginal health and disease, the genome sequence of Lactobacillus iners AB-1. *Proc. Natl. Acad. Sci. USA* **108**, 4688–4695 (2011).
33. Ravel, J. *et al*. Vaginal microbiome of reproductive-age women. *Proc. Natl. Acad. Sci. USA* **108**(Suppl 1), 4680–4687 (2011).
34. Mehta, S. D. *et al*. The Vaginal Microbiota over an 8- to 10-Year Period in a Cohort of HIV-Infected and HIV-Uninfected Women. *PLoS ONE* **10**, e0116894 (2015).
35. Albert, A. Y. K. *et al*. A Study of the Vaginal Microbiome in Healthy Canadian Women Utilizing cpn 60-Based Molecular Profiling Reveals Distinct Gardnerella Subgroup Community State Types. **10**, e0135620 (2015).
36. Srinivasan, S. *et al*. More Than Meets the Eye: Associations of Vaginal Bacteria with Gram Stain Morphotypes Using Molecular Phylogenetic Analysis. **8** (2013).
37. Cherpes, T. L., Meyn, L. A., Krohn, M. A., Lurie, J. G. & Hillier, S. L. Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. *Clin Infect Dis* **37**, 319–325 (2003).
38. Martin, H. L. *et al*. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis* **180**, 1863–1868 (1999).
39. Brotman, R. M. *et al*. Bacterial Vaginosis Assessed by Gram Stain and Diminished Colonization Resistance to Incident Gonococcal, Chlamydial, and Trichomonal Genital Infection. *J. Infect. Dis.* **202**, 1907–1915 (2010).
40. King, C. C. *et al*. Bacterial vaginosis and the natural history of human papillomavirus. *Infectious Diseases in Obstetrics and Gynecology* **2011**, 319460–8 (2011).
41. Gallo, M. F. *et al*. Bacterial Vaginosis, Gonorrhea, and Chlamydial Infection Among Women Attending a Sexually Transmitted Disease Clinic: A Longitudinal Analysis of Possible Causal Links. *Annals of epidemiology* **22**, 213–220 (2012).
42. Balkus, J. E. *et al*. Bacterial vaginosis and the risk of trichomonas vaginalis acquisition among HIV-1-negative women. *Sexually transmitted diseases* **41**, 123–128 (2014).
43. Ghartey, J. P. *et al*. Lactobacillus crispatus Dominant Vaginal Microbiome Is Associated with Inhibitory Activity of Female Genital Tract Secretions against Escherichia coli. **9** (2014).
44. Klupczynska, A., Derezinski, P. & Kokot, Z. J. Metabolomics in Medical Sciences - Trends, Challenges and Perspectives. *Acta Poloniae Pharmaceutica* **72**, 629–641 (2015).
45. Yeoman, C. J. *et al.* A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. **8**, e56111 (2013).
46. Srinivasan, S. *et al.* Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS ONE* **7**, e37818 (2012).
47. McMillan, A. *et al*. A multi-platform metabolomics approach identifies highly specific biomarkers of bacterial diversity in the vagina of pregnant and non-pregnant women. *Scientific Reports* **5**, 14174 (2015).

48. Vitali, B. *et al*. Vaginal microbiome and metabolome highlight specific signatures of bacterial vaginosis. *Eur J Clin Microbiol* 1–10 https://doi.org/10.1007/s10096-015-2490-y (2015).
49. Aroutcheva, A. A., Simoes, J. A. & Faro, S. Antimicrobial protein produced by vaginal Lactobacillus acidophilus that inhibits Gardnerella vaginalis. *Infectious Diseases in Obstetrics and Gynecology* **9**, 33–39 (2001).
50. Turovskiy, Y. *et al.* Susceptibility of Gardnerella vaginalis biofilms to natural antimicrobials subtilosin, epsilon-poly-L-lysine, and lauramide arginine ethyl ester. *Infectious Diseases in Obstetrics and Gynecology* **2012**, 284762 (2012).
51. Braga, P. C., Dal Sasso, M., Culici, M. & Spallino, A. Inhibitory activity of thymol on native and mature Gardnerella vaginalis biofilms: *in vitro* study. *Arzneimittel-Forschung* **60**, 675–681 (2010).
52. Kanjee, U. & Houry, W. A. Mechanisms of acid resistance in *Escherichia coli*. **67**, 65–81 (2013).
53. Nasrallah, G. K. *et al*. Legionella pneumophila requires polyamines for optimal intracellular growth. **193**, 4346–4360 (2011).
54. Jelsbak, L., Thomsen, L. E., Wallrodt, I., Jensen, P. R. & Olsen, J. E. Polyamines are required for virulence in Salmonella enterica serovar Typhimurium. *PLoS ONE* **7**, e36149 (2012).
55. Goytia, M. & Shafer, W. M. Polyamines can increase resistance of Neisseria gonorrhoeae to mediators of the innate human host defense. *Infection and immunity* **78**, 3187–3195 (2010).
56. Nelson, T. M. *et al*. Vaginal biogenic amines: biomarkers of bacterial vaginosis or precursors to vaginal dysbiosis? **6**, 253 (2015).
57. Ravel, J. *et al.* Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. *Microbiome* **1**, 1 (2013).
58. Hukkanen, J., Jacob, P. & Benowitz, N. L. Metabolism and disposition kinetics of nicotine. *Pharmacol. Rev.* **57**, 79–115 (2005).
59. Baheiraei, A., Banihosseini, S. Z., Heshmat, R., Mota, A. & Mohsenifar, A. Association of self-reported passive smoking in pregnant women with cotinine level of maternal urine and umbilical cord blood at delivery. *Paediatr Perinat Epidemiol* **26**, 70–76 (2012).
60. Dempsey, D., Jacob, P. & Benowitz, N. L. Accelerated metabolism of nicotine and cotinine in pregnant smokers. *The Journal of pharmacology and experimental therapeutics* **301**, 594–598 (2002).
61. Benowitz, N. L. Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiol Rev* **18**, 188–204 (1996).
62. Poppe, W. A., Peeters, R., Daenens, P., Ide, P. S. & Vanassche, F. A. Tobacco Smoking and the Uterine Cervix - Cotinine in. *Blood, Urine and Cervical Fluid*. **39**, 110–114 (1995).
63. Schiffman, M. H. *et al*. Biochemical epidemiology of cervical neoplasia: measuring cigarette smoke constituents in the cervix. *Cancer Res* **47**, 3886–3888 (1987).
64. Mietz, J. L. & Karmas, E. Polyamine and histamine content of rockfish, salmon, lobster, and shrimp as an indicator of decomposition. *Journal of the Association of Official Analytical Chemists (USA)* (1978).
65. Hussain, A. *et al*. High-affinity olfactory receptor for the death-associated odor cadaverine. *Proc. Natl. Acad. Sci. USA* **110**, 19579–19584 (2013).
66. Chen, K. C. *et al*. Amine content of vaginal fluid from untreated and treated patients with nonspecific vaginitis. *The Journal of clinical investigation* **63**, 828–835 (1979).
67. Sobel, J. D. *et al*. Diagnosing vaginal infections through measurement of biogenic amines by ion mobility spectrometry. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **163**, 81–84 (2012).
68. Benowitz, N. L. Clinical Pharmacology of Nicotine: Implications for Understanding, Preventing, and Treating Tobacco. *Addiction*. **83**, 531–541 (2008).
69. Strom, A. R. *et al*. Trimethylamine oxide: a terminal electron acceptor in anaerobic respiration of bacteria. *Journal of general microbiology* **112**, 315–320 (1979).
70. O'Hanlon, D. E., Moench, T. R. & Cone, R. A. In vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid but not hydrogen peroxide. *BMC infectious diseases* **11**, 200 (2011).
71. Koumans, E. H. *et al.* The prevalence of bacterial vaginosis in the United States, 2001-2004; associations with symptoms, sexual behaviors, and reproductive health. *Sexually transmitted diseases* **34**, 864–869 (2007).
72. Li, P., Beck, W. D., Callahan, P. M., Terry, A. V. J. & Bartlett, M. G. Quantitation of cotinine and its metabolites in rat plasma and brain tissue by hydrophilic interaction chromatography tandem mass spectrometry (HILIC-MS/MS). *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* **907**, 117–125 (2012).
73. Brunnemann, K. D., Kagan, M. R., Cox, J. E. & Hoffmann, D. Chemical Studies on Tobacco-Smoke.87. Determination of Benzene, Toluene and 1,3-Butadiene in Cigarette-Smoke by Gc-Msd. *Exp Pathol* **37**, 108–113 (1989).
74. Piot, P., Van Dyck, E., Totten, P. A. & Holmes, K. K. Identification of Gardnerella (Haemophilus) vaginalis. **15**, 19–24 (1982).
75. Aroutcheva, A. A., Simoes, J. A., Behbakht, K. & Faro, S. Gardnerella vaginalis isolated from patients with bacterial vaginosis and from patients with healthy vaginal ecosystems. **33**, 1022–1027 (2001).
76. Spiegel, C. A. *et al*. Gardnerella-Vaginalis and Anaerobic-Bacteria in the Etiology of Bacterial (Nonspecific) Vaginosis. 41–46 (1983).
77. Swidsinski, A. *et al*. An adherent Gardnerella vaginalis biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. *Am J Obstet Gynecol* **198**, 97 e1–6 (2008).
78. Leitich, H. *et al.* Bacterial vaginosis as a risk factor for preterm delivery: A meta-analysis. *Am J Obstet Gynecol* **189**, 139–147 (2003).
79. Delbridge, L. M. & O'Riordan, M. X. Innate recognition of intracellular bacteria. *Current opinion in immunology* **19**, 10–16 (2007).
80. Strom, K., Sjogren, J., Broberg, A. & Schnurer, J. *Lactobacillus plantarum MiLAB 393 Produces the Antifungal Cyclic Dipeptides Cyclo(L-Phe-L-Pro) and Cyclo(L-Phe-trans-4-OH-L-Pro) and 3-Phenyllactic Acid*. **68**, 4322–4327 (2002).
81. Piuri, M., Sanchez-Rivas, C. & Ruzal, S. M. Adaptation to high salt in Lactobacillus: role of peptides and proteolytic enzymes. *J. Appl. Microbiol.* **95**, 372–379 (2003).
82. Magnusson, J., Ström, K., Roos, S., Sjögren, J. & Schnürer, J. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. *FEMS Microbiol. Lett.* **219**, 129–135 (2003).
83. Houston, D. R. *et al.* Structure-based exploration of cyclic dipeptide chitinase inhibitors. *J Med Chem* **47**, 5713–5720 (2004).
84. Yang, E. J. & Chang, H. C. Purification of a new antifungal compound produced by Lactobacillus plantarum AF1 isolated from kimchi. *Int J Food Microbiol* **139**, 56–63 (2010).
85. Sinha, S., Srivastava, R., De Clercq, E. & Singh, R. K. Synthesis and Antiviral Properties of Arabino and Ribonucleosides of 1,3-Dideazaadenine, 4-Nitro-1, 3-dideazaadenine and Diketopiperazine. *Nucleosides, Nucleotides & Nucleic Acids* **23**, 1815–1824 (2004).
86. Kwon, O. S., Park, S. H., Yun, B. S., Pyun, Y. R. & Kim, C. J. Cyclo(Dehydroala-L-Leu), an alpha-glucosidase inhibitor from Penicillium sp F70614. **53**, 954–958 (2000).
87. Olmsted, S. S., Meyn, L. A., Rohan, L. C. & Hillier, S. L. Glycosidase and proteinase activity of anaerobic gram-negative bacteria isolated from women with bacterial vaginosis. *Sex Transm Dis* **30**, 257–261 (2003).
88. Wiggins, R., Hicks, S. J., Soothill, P. W., Millar, M. R. & Corfield, A. P. Mucinases and sialidases: their role in the pathogenesis of sexually transmitted infections in the female genital tract. *Sexually transmitted infections* **77**, 402–408 (2001).
89. Briselden, A. M., Moncla, B. J., Stevens, C. E. & Hillier, S. L. Sialidases (neuraminidases) in bacterial vaginosis and bacterial vaginosis-associated microflora. *J. Clin. Microbiol.* **30**, 663–666 (1992).
90. McGregor, J. A. *et al*. Bacterial vaginosis is associated with prematurity and vaginal fluid mucinase and sialidase: Results of a controlled trial of topical clindamycin cream. *Am J Obstet Gynecol* **170**, 1048–1060 (1994).
91. Straus, D. C., Mattingly, S. J., Milligan, T. W., Doran, T. I. & Nealon, T. J. Protease production by clinical isolates of type III group B streptococci. *J. Clin. Microbiol.* **12**, 421–423 (1980).

92. Volpe, G. E. *et al.* Associations of cocaine use and HIV infection with the intestinal microbiota, microbial translocation, and inflammation. *J Stud Alcohol Drugs* **75**, 347–357 (2014).
93. Khan, M. R. *et al.* Non-injection and injection drug use and STI/HIV risk in the United States: the degree to which sexual risk behaviors versus sex with an STI-infected partner account for infection transmission among drug users. *AIDS Behav* **17**, 1185–1194 (2013).
94. Cavazos-Rehg, P. A. *et al.* Risky sexual behaviors and sexually transmitted diseases: a comparison study of cocaine-dependent individuals in treatment versus a community-matched sample. *Aids Patient Care St* **23**, 727–734 (2009).
95. Uguz, F. *et al.* Antidepressants and menstruation disorders in women: a cross-sectional study in three centers. *Gen Hosp Psychiatry* **34**, 529–533 (2012).
96. Urban, R. J. & Veldhuis, J. D. A selective serotonin reuptake inhibitor, fluoxetine hydrochloride, modulates the pulsatile release of prolactin in postmenopausal women. **164**, 147–152 (1991).
97. Srinivasan, S. *et al.* Metabolic signatures of bacterial vaginosis. **6** (2015).
98. Gika, H. G., Theodoridis, G. A., Plumb, R. S. & Wilson, I. D. Current practice of liquid chromatography-mass spectrometry in metabolomics and metabonomics. *Journal of Pharmaceutical and Biomedical Analysis* **87**, 12–25 (2014).
99. Courant, F., Antignac, J.-P., Dervilly-Pinel, G. & Le Bizec, B. Basics of mass spectrometry based metabolomics. **14**, 2369–2388 (2014).
100. Fredricks, D. N. Molecular methods to describe the spectrum and dynamics of the vaginal microbiota. *Anaerobe* **17**, 191–195 (2011).
101. Verstraelen, H. *et al.* Longitudinal analysis of the vaginal microflora in pregnancy suggests that L. crispatus promotes the stability of the normal vaginal microflora and that L. gasseri and/or L. iners are more conducive to the occurrence of abnormal vaginal microflora. *BMC Microbiol.* **9**, 116 (2009).
102. Witkin, S. S. *et al.* Influence of vaginal bacteria and D- and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase inducer: implications for protection against upper genital tract infections. **4** (2013).
103. McMillan, A., Macklaim, J. M., Burton, J. P. & Reid, G. Adhesion of Lactobacillus iners AB-1 to human fibronectin: a key mediator for persistence in the vagina? *Reprod Sci* **20**, 791–796 (2013).
104. Mayer, B. T. *et al.* Rapid and profound shifts in the vaginal microbiota following antibiotic treatment for bacterial vaginosis. *J Infect Dis* **212**, 793–802 (2015).
105. Martin, D. H. The microbiota of the vagina and its influence on women's health and disease. **343**, 2–9 (2012).
106. Lawton, K. A. *et al.* Analysis of the adult human plasma metabolome. https://doi.org/10.2217/14622416.9.4.383 **9**, 383–397 (2008).
107. Frank, J. A. *et al.* Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Applied and Environmental Microbiology* **74**, 2461–2470 (2008).
108. Gajer, P. *et al.* Temporal Dynamics of the Human Vaginal Microbiota. *Sci Transl Med* **4**, 132ra52–132ra52 (2012).
109. Xia, J. & Wishart, D. S. Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. *Nature protocols* **6**, 743–760 (2011).
110. Vinaixa, M. *et al.* A Guideline to Univariate Statistical Analysis for LC/MS-Based Untargeted Metabolomics-Derived Data. *Metabolites* **2**, 775–795 (2012).
111. Guo, L. *et al.* Rat toxicogenomic study reveals analytical consistency across microarray platforms. *Nature biotechnology* **24**, 1162–1169 (2006).
112. Legendre, P. & Anderson, M. J. Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. **69**, 1 (1999).
113. Warnes, G. R. *et al.* gplots: Various R programming tools for plotting data. *R package version* **2** (2009).
114. Ihaka, R. & Gentleman, R. R: a language for data analysis and graphics. **5**, 299–314 (1996).
115. Clarke, K. & Gorley, R. *PRIMER v6: User manual/tutorial: PRIMER E.* (Plymouth) 2006.

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Author Contributions
R.M.B., J.R., J.M.R. and E.G. designed and implemented the study. T.N., J.B., M.S., R.M.B., C.Y., R.M. and D.R. performed analyses of samples and/or data. T.N., R.M.B. and C.Y. wrote the main manuscript text. All authors reviewed the manuscript.

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