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Molecular Epidemiology of Clostridium difficile infection

Clostridium difficile is the most commonly acquired nosocomial pathogen in the United States. Of all patients on antibiotics, 10-25% will develop *C. difficile* infection (CDI) and some of these may develop a severe complication, known as pseudomembranous colitis. The overall goal of this project was to understand *C. difficile* epidemiology at the Bozeman Deaconess Hospital (BDH). Prompt detection of pathogenic strains with molecular biology techniques can aid in the rapid intervention of CDI cases. In this project, we were given de-identified specimens (stool samples) from patients who were suspected to have CDI. Specimens were plated onto selective media and presumptive single colonies of *C. difficile* were isolated. I verified through PCR that isolates were indeed *C. difficile* and that they carried genes encoding for at least one of the *C. difficile* toxins, TcdA and TcdB. I also helped differentiate between strains using PCR ribotyping, which is a general genotyping method for *C. difficile*. However, the presence/absence of toxin genes and PCR ribotyping are not highly discriminant methods to differentiate between strains. To do so, I used a technique called multi-locus variable-number tandem-repeat analysis (MLVA) to detect sub-groups within individual PCR ribotypes. Due to inconsistent results with MLVA, we then used a technique called High Resolution Melt Curve Analysis (HRMCA) to differentiate between strains. A fluorescent dsDNA dye, called SYBER green, was included in the initial PCR and was detected when released from the melting DNA. Using HRMCA, we could quickly and accurately discriminate between strains of the same PCR ribotype.