Surface-attached cells, biofilms and biocide susceptibility: Implications for hospital cleaning and disinfection


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Surface-attached cells, biofilms and biocide susceptibility: implications for hospital cleaning and disinfection

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Summary

Microbes tend to attach to available surfaces and readily form biofilms, which is problematic in healthcare settings. Biofilms are traditionally associated with wet or damp surfaces such as indwelling medical devices and tubing on medical equipment. However, microbes can survive for extended periods in a desiccated state on dry hospital surfaces, and biofilms have recently been discovered on dry hospital surfaces. Microbes attached to surfaces and in biofilms are less susceptible to biocides, antibiotics and physical stress. Thus, surface attachment and/or biofilm formation may explain how vegetative bacteria can survive on surfaces for weeks to months (or more), interfere with attempts to recover microbes through environmental sampling, and provide a mixed bacterial population for the horizontal transfer of resistance genes. The capacity of existing detergent formulations and disinfectants to disrupt biofilms may have an important and previously unrecognized role in determining their effectiveness in the field, which should be reflected in testing standards. There is a need for further research to elucidate the nature and physiology of microbes on dry hospital surfaces, specifically the prevalence and composition of biofilms. This will inform new approaches to hospital cleaning and disinfection, including novel surfaces that reduce microbial attachment and improve microbial detachment, and methods to augment the activity of biocides against surface-attached microbes such as bacteriophages and antimicrobial peptides. Future strategies to address environmental contamination on hospital surfaces should consider the presence of microbes attached to surfaces, including biofilms.
Introduction

Microbes tend to attach to available surfaces and form biofilms readily.1–3 Biofilms are problematic in healthcare settings, where they are thought to be involved in 65% of nosocomial infections, and are usually reported in relation to indwelling medical devices and prostheses, water lines and tubing on endoscopes, and on wounds.1–3 In these settings, biofilm persistence can be prolonged, periodically ‘sloughing off’ and releasing planktonic bacteria that may act as a source of infection. Biofilms are a common problem on liquid—hard surface interfaces, and in areas of a hospital that are usually wet or damp, such as taps and sink drains. The recent problems caused by Pseudomonas aeruginosa in water supplied to intensive care units, which resulted in changes to UK national guidance, illustrates this problem.4

A biofilm is a community of micro-organisms attached to a substrate producing extracellular polymeric substances (EPS) and exhibiting an altered phenotype compared with corresponding planktonic cells, especially regarding growth, gene transcription, protein production and intercellular interaction.1–3,5,6 Biofilms comprising various micro-organisms, including bacteria, viruses, fungi and other micro-organisms, can form on almost any biological or inanimate surface, and have been identified in various industrial and clinical settings.7,8 Not all microbes attached to surfaces meet the definition of a biofilm, and the transition from a planktonic culture through surface attachment to an established biofilm is likely to be a continuum rather than a stepwise process (Figure 1).1–3

Microbes including bacterial spores, vegetative bacteria, fungi and viruses can also survive on dry surfaces for extended periods.8–10 Contaminated environmental surfaces are an increasingly recognized reservoir in the transmission of certain healthcare-associated pathogens.11–13 Whilst this extended survival is not surprising for the metabolically inert bacterial endospores, survival of some vegetative bacteria that is measured in years rather than days challenges our understanding of bacterial physiology.10,14 The structural and physiological state of microbes dried on to hospital surfaces has not been studied in detail, but it seems likely that bacteria attach to surfaces to some degree, and may form biofilms. Indeed, a recent study from Australia by Vickery et al.15 ‘destructively sampled’ (i.e. cut the materials out of the hospital environment and undertook laboratory analysis) several hospital surfaces after cleaning and bleach disinfection. Scanning electron microscopy was used to examine the surfaces for biofilms, which were identified on five of six surfaces. Furthermore, viable meticillin-resistant Staphylococcus aureus (MRSA) was identified in the biofilm on three of the surfaces.

This article will review in-vitro studies that explore the structure, physiology and biocide susceptibility of microbes dried on to hard surfaces in the context of surface attachment and biofilm establishment, and discuss the potential implications for hospital cleaning and disinfection.16

Search strategy

Pubmed was searched with no date restrictions using the search terms ‘biofilm and biocide’, ‘biofilm and reduced susceptibility’, ‘biofilm and [MRSA, VRE, C. difficile, Acinetobacter, E. coli, Pseudomonas]’ and ‘susceptibility planktonic biofilm biocide’ (see Figure 1). The reference lists of articles identified via the Pubmed searches were hand searched to identify other relevant literature.

Resistance and reduced susceptibility

Biofilms constitute a protected mode of growth, allowing bacteria to survive in hostile environments and conferring
reduced susceptibility to dehydra
tion, phagocytosis, metal
icity, acid exposure, antibiotics and biocides. Microbes
attached to surfaces that have not formed an established
biofilm appear to represent an intermediate step, with reduced
susceptibility to biocides compared with planktonic cells, but
increased susceptibility relative to biofilms (Figure 1).}

**Mechanisms of reduced susceptibility**

Causes of reduced susceptibility to antimicrobial agents in
biofilms are multi-factorial, including reduced penetration
(particularly due to changes in cell density and the produc-
tion of EPS), slow growth (and subsequent reduced metabolism
of antimicrobial agents), modulation of the stress response and
other metabolic processes, and changes in quorum
sensing.\textsuperscript{5,21−23} It seems likely that these mechanisms also
explain reduced biocide susceptibility in surface-attached cells
that have not yet formed biofilms.

**Biocide susceptibility**

Many studies have evaluated the impact of established
biofilms on biocide susceptibility, and fewer studies have
examined the susceptibility of surface-attached cells that have
not yet formed established biofilms (Figure 1). Table I sum-
marizes studies that have investigated organisms and biocides
relevant to disinfection in healthcare settings that include data
comparing susceptibility in planktonic culture with surface-
attached cells or biofilms. Studies have evaluated a
range of organisms (both alone and in combination), various
suspending media, and several methods of attaching cells to
surfaces and producing biofilms on different substrates; all of
these factors are likely to influence biocide susceptibility. One
important factor is the maturation of the biofilms tested,
which ranges from cells attached to surfaces for hours to
weeks old.\textsuperscript{24,22} Furthermore, some studies have controlled
for cell density in attached cells or biofilms compared with
planktonic culture.\textsuperscript{26} Thus, although several studies have sug-
gested that cell density alone does not explain the reduced
susceptibility of biofilms to biocides, it is difficult to be certain
of the impact of the biofilm phenotype independent of cell
density in many studies.\textsuperscript{25,27−29} A number of different
approaches have been taken to quantify growth, including both
direct microbial culture and indirect measures, such as live/
dead viability assays.\textsuperscript{20,30−32} Finally, different approaches to
compare susceptibility in planktonic culture and biofilms have
included measuring the amount of biocide required to inhibit
growth [minimum inhibitory concentration (MIC)] or kill cells
[minum mum bactericidal concentration (MBC)],\textsuperscript{7,18,20,33} or
measuring the survival time at a given concentration of
biocide;\textsuperscript{20,32,34,35} this makes comparison of studies difficult.

Notwithstanding difficulties in comparing studies, the phase
of the surface-attached cells influences biocide susceptibility.
In general, bacteria in planktonic culture are more susceptible
than attached cells, which are, in turn, more susceptible than
established biofilms (Figure 1).\textsuperscript{18−20} Meanwhile, detached
biofilm cells revert to the susceptible phenotypic state.\textsuperscript{19,36,37}
Similarly, growth phase affects biocide susceptibility of
planktonic culture.\textsuperscript{38−40} Reduced susceptibility in surface-
attached cells ranges widely from two-fold to >1000-fo
d.\textsuperscript{26,41} For example, clinical isolates of MRSA and
*P. aeruginosa* were grown as biofilms on discs of common ma-
terials in the hospital environment, and treated with three
commonly used hospital biocides: benzalkonium chloride (1%
w/v), chlorhexidine gluconate (4% w/v) and triclosan (1% w/
v).\textsuperscript{7} The MBCs of all biocides for planktonic cultures of both
organisms were considerably lower than the concentrations
recommended for use by the manufacturer. However, when
isolates were grown as biofilms, the biocides were ineffective
at killing bacteria at the concentrations recommended for use.
The MBCs of all three biocides were found to be 10−1000-fold
higher than the same isolates grown in planktonic culture for
MRSA and *P. aeruginosa*. Following biocide treatment, up to
11% of cells in MRSA biofilms survived, and up to 80% of cells in
*P. aeruginosa* biofilms survived. Another study evaluated the
susceptibility of four *Candida* spp. and two *Escherichia coli*
strains to sodium hypochlorite, ethanol, hydrogen peroxide and
iodine.\textsuperscript{20} Strains were tested in planktonic culture, as attached
cells and as biofilms in microtitre plates. Whilst susceptibility
varied by organism and biocide, biofilms were less susceptible
than attached cells, which were less susceptible than plank-
tonic cells. MICs for biofilms were up to >10-fold higher for 5-
min and 24-h exposures compared with planktonic cells. These
studies suggest that although biocides may be effective against
planktonic populations of bacteria, some biocides currently
used in hospitals may be ineffective against nosocomial path-
ogens when attached to surfaces or in biofilms, and thus fail to
control this reservoir for hospital-acquired infection.

However, whilst surface-attached microbes and biofilms are
generally less susceptible to biocides than bacteria in plankto-
nic culture, the degree of reduced susceptibility is not al-
ways this stark. For example, a study reported no difference
between planktonic culture and biofilms of *Klebsiella pneu-
moniae* exposed to sodium hypochlorite and monochlor-
amine.\textsuperscript{32} Other studies have not identified reduced
susceptibility for all biocides or organisms tested.\textsuperscript{20,24,36,41} It is
difficult to determine the relative importance of organism,
biocide and testing conditions in these studies that found little
or no reduced biocide susceptibility associated with biofilms.

The composition of the biofilm also influences susceptibility.
For example, high-nutrient, high-density biofilms are less sus-
ceptible to biocides than low-nutrient, low-density bio-
films.\textsuperscript{18,31,49} This seems particularly important in the context of
biocides that may be present on hospital surfaces, which are likely
to be low-nutrient, low-density biofilms in most cases. However,
gross contamination with body fluids could provide an environ-
ment in which high-nutrient, high-density biofilms could form on
hospital surfaces. Indeed, three-quarters of the biofilms reported
by Vickery et al.\textsuperscript{50} had very thick EPS despite having a low density of
microbes in most cases, perhaps in response to desiccation.\textsuperscript{15,46,47}

The microbial ecology of the biofilm is another factor
influencing susceptibility. Biofilms composed of multiple spe-
cies are less susceptible than single-species biofilms, although
this is not always the case with the corresponding planktonic
cultures.\textsuperscript{19,36,37,48,49}

Some biocides are more effective than others at inactivating
bacteria in biofilms, although conflicting data have been re-
ported, which may be explained by differences in experimental
conditions.\textsuperscript{20,24,35,49,50} In one study, susceptibility varied by
phase, organism and biocide.\textsuperscript{20} In another study, the oxidizing
agents sodium hypochlorite and peroxycyanic acid were more effec-
tive than a range of other chemicals (including alcohols,
biganides, halogens, phenols and quaternary ammonium
compounds) for inactivating *P. aeruginosa* and *S. aureus* bio-
films.\textsuperscript{35} In other studies, sodium hypochlorite was more
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<td>Condell 2012</td>
<td><em>Salmonella enterica</em> (189)</td>
<td>Seven common food contact surface biocides</td>
<td>Tested in planktonic culture, dried on surfaces and as established high-nutrient (2-day) or low-nutrient (7-day) biofilms on microtitre plates</td>
<td>Susceptibility rank: high-nutrient biofilm &lt; low-nutrient biofilm &lt; surface dried &lt; planktonic culture</td>
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<td>Behnke 2012</td>
<td><em>Pseudomonas aeruginosa</em> (1); <em>Burkholderia cepacia</em> (1)</td>
<td>Chlorine dioxide</td>
<td>Tested in single- and binary-species planktonic culture, attached (4-day) and detached biofilm</td>
<td>Susceptibility rank: attached biofilm &lt; detached biofilm = planktonic cells. Binary cultures were less susceptible than single-species cultures</td>
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<td>Xing 2012</td>
<td><em>Staphylococcus aureus</em> (13)</td>
<td>Chlorhexidine and harmaline</td>
<td>Tested in planktonic culture and in 2-day biofilms on microtitre plates</td>
<td>Biofilms were 10 to &gt;100 times less susceptible to chlorhexidine and &gt;2 times less susceptible to harmaline. Synergy noted for most strains</td>
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<td>Leung 2012</td>
<td><em>Candida spp.</em> (4); <em>Escherichia coli</em> (2)</td>
<td>Sodium hypochlorite, ethanol, hydrogen peroxide and iodine</td>
<td>Tested in planktonic culture (low- and high-titre), attached cells (90 min) and 1-day biofilm in microtitre plates; 24-h and 5-min contact times compared</td>
<td>Susceptibility rank: biofilm &lt; attached cells &lt; high-titre planktonic cells &lt; low-titre planktonic cells. MICs for biofilm vs planktonic cells up to &gt;10-fold higher for 5-min and 24-h exposures</td>
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<td>Behnke 2011</td>
<td><em>P. aeruginosa</em> (1); <em>B. cepacia</em> (1)</td>
<td>Sodium hypochlorite</td>
<td>Tested in single- and binary-species planktonic cultures, attached (4-day) and detached biofilms</td>
<td>Susceptibility rank: attached biofilm &lt; detached biofilm = planktonic cells. Binary-species cultures were less susceptible than single-species cultures for attached and detached biofilms, but the reverse was true for planktonic cells</td>
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<td>Xu 2011</td>
<td><em>Neisseria gonorrhoeae</em> (3)</td>
<td>Atmospheric pressure non-equilibrium plasma</td>
<td>Tested dried on glass surfaces or 4-day biofilm on glass</td>
<td>Bacteria in biofilm survived approximately twice as long as bacteria dried on surfaces</td>
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<td>Wong 2010</td>
<td><em>S. enterica</em> (1)</td>
<td>Six biocides</td>
<td>Tested in planktonic culture or 3-day biofilm on microtitre plates</td>
<td>Bacteria in biofilm were less susceptible than planktonic cells for all but sodium hypochlorite</td>
</tr>
<tr>
<td>Tote 2010</td>
<td><em>S. aureus</em> (1); <em>P. aeruginosa</em> (1)</td>
<td>12 biocides</td>
<td>Tested in planktonic culture or in 1-day (<em>P. aeruginosa</em>) or 3-day (<em>S. aureus</em>) biofilm on microtitre plates</td>
<td>Most disinfectants tested did not eliminate bacteria in the biofilm after 60-min contact. Only hydrogen peroxide and chlorine had an impact on the biofilm matrix</td>
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<tr>
<td>Lee 2009</td>
<td>Meticillin-resistant <em>S. aureus</em> (2)</td>
<td>Three denture-cleaning biocides</td>
<td>Tested in planktonic culture, sessile biofilm (4 h), established biofilm (24 h) or mature biofilm (120 h) on resin</td>
<td>Two of three biocides were less effective for the inactivation of bacteria in biofilm. NaOCl was the most effective against biofilm</td>
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<td>Hendry 2009</td>
<td><em>S. aureus</em> (1); meticillin-resistant <em>S. aureus</em> (1); <em>P. aeruginosa</em> (1); <em>E. coli</em> (1); <em>Candida albicans</em> (1)</td>
<td>Eucalyptus oil, '1,8-cineole' and chlorhexidine</td>
<td>Tested in planktonic culture or 2-day biofilm on microtitre plates</td>
<td>Biofilm MICs and MBCs were 10 to &gt;100 times less susceptible than planktonic culture. Synergy between chlorhexidine and the other agents was noted against some organisms</td>
</tr>
<tr>
<td>Smith 2008</td>
<td>Meticillin-resistant <em>S. aureus</em> (8); <em>P. aeruginosa</em> (8)</td>
<td>Benzalkonium chloride, triclosan and chlorhexidine</td>
<td>Tested in planktonic culture or 1-day biofilms on metal or plastic discs</td>
<td>MBCs for MRSA biofilms were 100 to 1000 times greater than for planktonic cells; MBCs for <em>P. aeruginosa</em> biofilm were 10 to 100 times greater than for planktonic cells</td>
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<tr>
<td>Brandle 2008</td>
<td><em>Enterococcus faecalis</em> (1); <em>Streptococcus sobrinus</em> (1); <em>C. albicans</em> (1); <em>Actinomyces naeslundii</em> (1), <em>Fusobacterium nucleatum</em> (1)</td>
<td>Calcium hydroxide</td>
<td>Tested in planktonic culture, adherent cells, single-species 5-day biofilm and mixed-species 5-day biofilm on dentin and detached biofilm</td>
<td>Susceptibility rank: mixed species biofilm &lt; single-species biofilm &lt; adherent &lt; planktonic = detached biofilm</td>
</tr>
<tr>
<td>Nett 2008</td>
<td><em>C. albicans</em> (2); <em>Candida parapsilosis</em> (2); <em>Candida glabrata</em> (1)</td>
<td>Ethanol, hydrogen peroxide and sodium dodecyl sulphate</td>
<td>Tested in planktonic culture, planktonic culture with adjustment to match the cell density of the biofilm and 1-day biofilm on microtitre plates</td>
<td>Concentrations required to inhibit growth in biofilm were 2- to 10-fold higher; lower concentrations of hydrogen peroxide prevented biofilm formation than the other agents tested</td>
</tr>
<tr>
<td>Karpanen 2008</td>
<td><em>Staphylococcus epidermidis</em> (2)</td>
<td>Chlorhexidine gluconate, tea tree oil and thymol</td>
<td>Tested in planktonic culture or 3-day biofilm on microtitre plates</td>
<td>MiCs/MBCs were elevated up to 16-fold for biofilm; synergy was noted between chlorhexidine and eucalyptus oil</td>
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<tr>
<td>Bjarnsholt 2007</td>
<td><em>P. aeruginosa</em> (1)</td>
<td>Silver</td>
<td>Tested in planktonic culture or 4-day biofilm</td>
<td>Biofilm was 10–100 times less susceptible than planktonic cells</td>
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<td>Tabak 2007</td>
<td><em>Salmonella typhimurium</em> (3)</td>
<td>Triclosan</td>
<td>Tested in planktonic (log and stationary phase) culture and in 1-day biofilm on microtitre plates</td>
<td>Susceptibility rank: biofilm &lt; stationary phase planktonic &lt; log phase planktonic. 8-log difference in bacteria surviving in biofilm vs planktonic log phase</td>
</tr>
<tr>
<td>Surdeau 2006</td>
<td><em>E. coli</em> (1); <em>Enterococcus hirae</em> (1); <em>P. aeruginosa</em> (1); <em>S. aureus</em> (1)</td>
<td>Novel disinfectant (Oxsil 320N)</td>
<td>Tested in planktonic culture and 1-day biofilm on stainless steel</td>
<td>Disinfectant concentration required to achieve a 5-log reduction was approximately 10 times more for biofilm vs planktonic culture</td>
</tr>
<tr>
<td>Theraud 2004</td>
<td>Five fungi from patient (3) and environment (3)</td>
<td>Five antiseptics, three disinfectants and UVC</td>
<td>Tested in single- and mixed-species planktonic culture, and single- and mixed-species 1-day biofilms on microtitre plates</td>
<td>UVC and 3% hydrogen peroxide were not fungicidal in initial suspension tests. Agents were less effective against mixed suspensions. Only chlorhexidine was effective against biofilms</td>
</tr>
<tr>
<td>Simoes 2003</td>
<td><em>Pseudomonas flourescens</em> (1)</td>
<td>Orthophthalaldehyde</td>
<td>Tested in planktonic culture and 6-day biofilm on glass</td>
<td>Biofilm was less susceptible than planktonic cells based on respiratory activity</td>
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<td>Bardouniotis 2003</td>
<td><em>Mycobacterium fortuitum</em> (1); <em>Mycobacterium marinum</em> (1)</td>
<td>Seven biocides</td>
<td>Tested in planktonic culture and biofilm on microtitre plate assessed over 14 days</td>
<td>MBECs were up to 40-fold higher than MBCs for <em>M. fortuitum</em>, but not for <em>M. marinum</em></td>
</tr>
<tr>
<td>Elvers 2002</td>
<td><em>Alcaligenes denitrificans</em> (1); <em>Pseudomonas alcaligenes</em> (1); <em>Stenotrophomonas maltophilia</em> (1); <em>Flavobacterium indologenes</em> (1); <em>Fusarium oxysporum</em> (1); <em>Fusobacterium solani</em> (1); <em>Rhodotorula glutinis</em> (1)</td>
<td>One biocide (isothiazolone compound)</td>
<td>Tested in single-species planktonic culture, and single- and mixed-species 1-day biofilms on glass</td>
<td>Biofilms were less susceptible than planktonic cells. Mixed-species biofilm, particularly for the bacterial species, offered greater protection</td>
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</table>
effective than chlorhexidine for inactivating *Enterococcus faecium* and MRSA in biofilms,\(^{24,50}\) whereas chlorhexidine was found to be effective against yeast biofilms when sodium hypochlorite was not effective.\(^{30}\) In general, oxidizing agents target multiple biofilm components and microbial targets, whereas other biocides such as chlorhexidine only target cell wall components; thus, oxidizing agents tend to have a higher level of efficacy against biofilms.\(^ {20,24,35,49,50}\) The variations in performance of biocides under different experimental conditions may have implications for practice, where the same biocide could have a different impact on biofilms in different settings.

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<td>Peng 2002(^{31})</td>
<td><em>Bacillus cereus</em> (1)</td>
<td>Sodium hypochlorite and quaternary ammonium compounds</td>
<td>Tested in planktonic culture, attached to stainless steel chips (4 h) and 8-day biofilm on stainless steel with or without milk</td>
<td>Susceptibility rank: milk biofilm &lt; biofilm &lt; attached &lt; planktonic. 5-log difference between planktonic cells and milk biofilm</td>
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<td>Bardouniotis 2001(^{108})</td>
<td><em>Mycobacterium phlei</em> (1)</td>
<td>Seven biocides</td>
<td>Tested in planktonic culture and 5-day biofilm on microtitre plate</td>
<td>MBECs were higher than MBCs after 30-min and 120-min exposure to most agents tested</td>
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<td>Joseph 2001(^{12})</td>
<td><em>Salmonella</em> spp. (2)</td>
<td>Chlorine and iodine</td>
<td>Tested in planktonic culture and 10-day biofilms on plastic, cement and stainless steel</td>
<td>Biofilms were less susceptible to both disinfectants; survival time no more than 10 min in suspension vs &gt; 25 min in biofilm</td>
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<tr>
<td>Joseph 2001(^{12})</td>
<td><em>Salmonella</em> spp. (2)</td>
<td>Chlorine and iodine</td>
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<td>Cochrant 2000(^{19})</td>
<td><em>P. aeruginosa</em> (1)</td>
<td>Monochloramine and hydrogen peroxide</td>
<td>Tested in planktonic culture and 3-h to 3-day biofilms on alginate beads and glass slides</td>
<td>Biofilms were less susceptible to both disinfectants. Reduced diffusion of biocide in biofilm did not explain reduced susceptibility</td>
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<td>Elasri 1999(^{53})</td>
<td><em>P. aeruginosa</em> (1)</td>
<td>UVA, UVB and UVC</td>
<td>Strain tested in planktonic culture or biofilm in alginate beads assessed over 1 day</td>
<td>Biofilm transmitted only a small amount of UV radiation (13% of UVC, 31% of UVB and 33% of UVA), meaning biofilm was less susceptible than planktonic cells</td>
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<td>Das 1998(^{43})</td>
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<td>Five biocides</td>
<td>Tested in planktonic culture and 6–24-h biofilms on microtitre plates</td>
<td>Biofilms were up to 33-fold less susceptible to the disinfectants tested, apart from chloroxylenol and cetrimide (E. coli only)</td>
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<td>Stewart 1998(^{55})</td>
<td><em>Enterobacter aerogenes</em> (1)</td>
<td>Four biocides</td>
<td>Tested in planktonic culture and high- and low-density biofilms on alginate beads assessed over 5 h</td>
<td>Susceptibility rank: high-density biofilm &lt; low-density biofilm &lt; planktonic</td>
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<td>Yu 1993(^{42})</td>
<td><em>Klebsiella pneumoniae</em> (1)</td>
<td>Sodium hypochlorite and monochloramine</td>
<td>Tested in planktonic culture and biofilm on stainless steel discs</td>
<td>No difference identified between planktonic and biofilm cells</td>
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<td>Eginton 1998(^{56})</td>
<td><em>S. epidermidis</em> (1); <em>P. aeruginosa</em> (1)</td>
<td>Sodium hypochlorite and dodgen; SDS and Tween-80</td>
<td>Tested in planktonic culture and 16-h biofilms on glass and stainless steel</td>
<td>Biofilms were up to &gt;1000-fold less susceptible than planktonic cells; attachment to the surfaces was loosened</td>
</tr>
<tr>
<td>LeChevallier 1988(^{75})</td>
<td><em>Pseudomonas picketti</em>, <em>Pseudomonas paucimobilitis Moraxella</em>; <em>K. pneumoniae</em> (1)</td>
<td>Hypochlorous acid, hypochlorite, chlorine dioxide and monochloramine</td>
<td>Tested in planktonic culture and 3-week biofilms on granular activated carbon, metal or glass</td>
<td>Biofilms were 150 to 3000 times less susceptible to hypochlorous acid, and 2- to 100-fold less susceptible to monochloramine</td>
</tr>
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MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MBEC, minimal biofilm eradicating concentration; UV, ultraviolet.

Search strategy: Pubmed search for ‘susceptibility planktonic biofilm biocide’ performed on 15\(^{th}\) November 2013. Of 44 results, 35 were selected for review and 21 were included. A further 10 articles were included following review of the reference lists. Articles were included if they tested organisms and biocides relevant to disinfection in healthcare facilities, and included data comparing planktonic with surface-attached and/or biofilm mode susceptibility.

\(^a\) Population from de-ionized water system: composition 70% *P. picketti*; 18% *Moraxella* spp.; 12% *P. paucimobilitis*. 
Comparing biocides may be further confounded by the ‘dose–response’ type relationship that has been shown between biofilm susceptibility and biocide concentration. For example, one study showed that 10% hydrogen peroxide was considerably more effective for inactivating bacteria in biofilms compared with 6% hydrogen peroxide. Biofilms have also been shown to reduce the susceptibility of microbes to physical processes such as exposure to ultraviolet (UV) radiation, most likely due to poor penetration of UV into the biofilm. This may have implications for automated room disinfection systems using UV radiation. To the authors’ knowledge, no studies have evaluated the impact of hydrogen-peroxide-based automated room disinfection systems against biofilms, although emerging data suggest that liquid hydrogen peroxide, as an oxidizing agent, targets both the biofilm matrix and microbes in the biofilm.

Aside from the inactivation of microbes attached to surfaces, the chemical properties of biocides also seem to be important in terms of preventing, promoting or dismantling biofilms. One study showed that only sodium hypochlorite and hydrogen peroxide damaged both the bacteria within the biofilm and the biofilm matrix itself. Also, hydrogen peroxide was more effective than other agents at preventing Candida spp. biofilm formation. In another study, exposure to chlorhexidine and benzalkonium chloride inhibited biofilm formation for E. coli, K. pneumoniae and P. aeruginosa, but promoted biofilm formation in Staphylococcus epidermidis, suggesting that microbial factors are important. It is possible, therefore, that one microbe in a biofilm may be inactivated by a biocide, but another less susceptible microbe may survive and then grow to replace the microbe that was inactivated.

**Antibiotic susceptibility**

Bacteria in biofilms are usually less susceptible to antibiotics than bacteria in planktonic culture, and many of the mechanisms for reduced susceptibility to biocides and antibiotics are shared. Furthermore, bacteria acquired from surfaces in biofilm mode with reduced biocide susceptibility may retain reduced susceptibility to antibiotics.

**Physical removal**

The protected mode of growth offers physical protection to cells within biofilms, and makes the physical breakdown of biofilms challenging. Although biofilm attachment appears to be loosened by some biocides, several studies have illustrated how difficult it can be to remove bacteria in biofilms through cleaning and/or inactivation through disinfection. For example, regular and extended detergent cleaning did not remove a Bacillus cereus biofilm *in vitro*; a modified procedure including heating to 70 °C was required. Clearly, heating to 70 °C is not feasible for the cleaning and disinfection of hospital surfaces in clinical areas. Similarly, attached, viable Pseudomonas fragi were detected on stainless steel surfaces after two cleaning and disinfection procedures were tested under ‘worst-case’ conditions at 50% in-use disinfectant concentrations. An acid-detergent-based method was more effective at removing attached cells than an alkaline-detergent-based method. However, these studies were performed using mature biofilms which may not be representative of the biofilms present on hospital surfaces.

Surface-attached cells and biofilms are clearly not the only reason for failures in hospital disinfection, given the difficulty in achieving adequate distribution and contact time using manual methods. However, both reduced biocide susceptibility (Table I) and increasing resilience to physical removal by cleaning are likely to contribute to failures in hospital cleaning. This could partly explain why disinfectants that are effective for the inactivation of planktonic bacteria in laboratory tests are not effective for the eradication of a considerably lower load of the same bacterial species from hospital surfaces. In support of this, it is noteworthy that the biofilms identified by Vickery et al. were on surfaces that had been cleaned with detergent and then disinfected using 500 ppm chlorine. These findings may have implications for infection control practices within hospitals, and on the choice of appropriate disinfectants used to decontaminate surfaces.

The presence of biofilms on dry hospital surfaces could also interfere with attempts to recover microbes through environmental sampling. This could mean that an environmental reservoir of a pathogen remains undetected, or the concentration of contamination and degree of associated risk is underestimated.

**Persistence**

Vegetative bacteria dried on to surfaces can survive for weeks to months (or more) *in vitro*, despite the lack of a nutrient source or water (aside from ambient humidity). Biofilms may explain this surprising propensity of vegetative bacteria. This is supported by a recent study which found that biofilm-forming strains of Acinetobacter baumannii survived for longer on dry surfaces than non-biofilm-forming strains (36 vs 15 days; P < 0.001). In-vitro studies evaluating the persistence of dried inocula did not supply any water or nutrients. However, in the hospital environment, daily and terminal cleaning or disinfection does provide a supply of water, and some bacteria may be able to metabolize some constituent parts of detergents and even disinfectants, providing a nutrient source for the growth in biofilms.

**Transfer of plasmids and development of antimicrobial resistance**

Biofilms are suited for horizontal gene dissemination because they are a mixed population at high bacterial density, which facilitates metabolic activity in the harshest environments, albeit at a reduced rate. Horizontal transfer of plasmids does occur through conjugation, as illustrated by the transfer of extended-spectrum β-lactamase (CTX-M-15) and carbapenemase (NDM-1) plasmids between Enterobacteriaceae when dried on surfaces. Furthermore, the mutation rate (the rate at which DNA replication mistakes occur during cell division) of bacteria in biofilms is increased. Thus, both horizontal transfer of resistance determinants such as plasmids and increased mutation rates could result in the acquisition or development of reduced susceptibility to antimicrobial agents and other important microbial capabilities, such as increased virulence.

**Tackling surface-attached cells and biofilms**

Surface-attached cells, especially established biofilms, present a difficult challenge to hospital cleaning and disinfection,
combining protection from physical removal with reduced susceptibility to biocides (Table I). A number of different approaches are available to tackle surface-attached cells and biofilms. Using physical methods to dislodge detached bacteria, which can be aided by the use of a detergent, can be effective in removing established biofilms and preventing the development of biofilms. However, detergent cleaning alone may not be sufficient to remove biofilms. Tackling the microbes in the biofilm alone (e.g., using some disinfectants or attempts to interfere with quorum sensing) can be effective, but may not reach microbes protected deep in the biofilm matrix. Tackling the biofilm matrix alone (e.g., using enzymatic digestion) will help to reach microbes protected within the biofilm matrix and interrupt persistence of the biofilm, but will not necessarily have direct microbiocidal activity. Thus, tackling both the microbes in the biofilm and the biofilm matrix simultaneously (using oxidizing disinfectants or combination approaches) offers the potential to reach microbes protected deep in the matrix and interrupt the persistence of the biofilm. In addition, some biocides have the ability to reduce biofilm formation, which can be assisted by choosing surface materials that do not readily support biofilm formation.

**Biocides and biocide adjuvants**

Differences between biocides appear to influence their activity against bacteria attached to surfaces and may also promote, prevent or dismantle biofilms. Thus, biocides with the highest activity against bacteria attached to surfaces, and ideally those with the ability to prevent biofilm formation and dismantle existing biofilms, should be selected. Emerging data indicate that oxidizing agents may possess more of these properties than other agents. Similarly, detergent formulations that are better at physical removal should be selected, although there is a paucity of data on the capacity of currently available detergents to address surface-attached cells.

Several novel approaches also warrant consideration as potential additives to hospital detergents or disinfectants to augment their effectiveness against biofilms. Firstly, certain enzymes such as DNase and dispersinB have been shown to dissolve the biofilm matrix. For example, detergents supplemented with high concentrations of enzymes were effective against hydrated biofilms, whereas detergents supplemented with low concentrations of enzymes were not. Secondly, quorum-sensing inhibitors have proven successful in increasing antimicrobial susceptibility. In one study, a quorum-sensing inhibitor, was found to enhance the effects of copper sulphate on biofilms of Pseudomonas syringae. Thirdly, recently discovered human antimicrobial peptides also have antibiofilm activities. For example, a range of antimicrobial peptides tested against multi-drug-resistant A. baumannii demonstrated direct antimicrobial activity, and enhanced the activity of a range of other antimicrobial agents. However, the addition of enzymes, quorum-sensing inhibitors or antimicrobial peptides into a cleaning or disinfection solution would result in chemical residues on surfaces with associated health and safety implications, so are not recommended without further study. Another approach is the inclusion of bacteriophages, which have been found to disrupt biofilms. For example, Streptococcus pyogenes biofilms were degraded by PlyC, a bacteriophage-encoded endolysin, which also acted synergistically with a range of antimicrobial agents. However, the therapeutic use of bacteriophages in human medicine and, by implication, in the clinical environment is controversial due to potential for the rapid development of resistance and the risk that the introduced bacteriophages may play an unintended role in horizontal gene transfer.

**Surface modification to prevent biofilm formation**

Some surface materials are more prone to biofilm formation than others. A recent study reviewed attempted to modify the chemical or physical surface properties of medical devices to inhibit or prevent microbial adhesion. These include ‘liquid glass’ (silicon dioxide), Sharklet pattern, advanced polymer coatings [e.g. polyethylene glycol (PEG), super-hydrophobic/phlic and zwitterionic] and diamond-like carbon films. Whilst these technologies have the potential to reduce biofilm deposition on hospital surfaces, they are at an early stage of development. The feasibility and cost-effectiveness of scaling up these technologies for use on hospital surfaces needs to be evaluated.

Another approach is the implementation of antimicrobial surfaces. Options include metals such as copper and silver, or chemicals such as organosilanes with quaternary ammonium groups and light-activated antimicrobials. Copper is the most-studied candidate for antimicrobial surfaces, and has been shown to inactivate microbes and DNA deposited on surfaces and may reduce the transmission of pathogens in the hospital setting. However, the presence of a conditioning film can greatly reduce the efficacy of antimicrobial surfaces. Thus, an antimicrobial surface that combines reduced biofilm formation with direct antimicrobial activity is a promising area for future research. Another challenge in developing an antimicrobial surface for hospitals is the requirement for multiple different surface types (from fabric to hard surfaces) with a range of required functions. Thus, there is unlikely to be a single agent or surface structure that is suitable for all applications.

**Implications for susceptibility testing**

Surface-attached cells and biofilms are a more accurate reflection of the occurrence of bacteria in nature than planktonic cells. However, planktonic culture remains the current model for many microbiological studies and testing standards including susceptibility testing. Although quantitative surface tests for evaluation of the bactericidal activity of chemical disinfectants do exist (e.g. BS EN 13697:2001), none have been published for EPS-producing biofilms. Future testing should specify the use of surface-attached cells and consider the use of biofilm models to ensure that the disinfectants tested are as effective in the ‘real world’ as in laboratory tests. It seems likely that low-nutrient, low-density surface-attached cells would be more appropriate than high-nutrient, high-density established biofilms. Most in-vitro studies measured growth over a 24-h period to evaluate the impact of a chemical bioocide to determine the MIC or MBC, using methodology often used to test antibiotic susceptibility (Table I). One study compared the MICs of four common biocides for E. coli and various Candida spp. with a ‘contact time’ of 5 min and 24 h. Unsurprisingly, the concentration required to inhibit growth within 5 min was considerably greater than the concentration required to inhibit growth over
24 h. Thus, as biocides are only applied for a short period in practice, evaluating the impact of a biocide over a short contact time as per most published biocide testing standards is more suitable for in-vitro biocide studies than measuring the MIC or MBC when microbes are grown in varying concentrations of biocide.

Further research is required to evaluate the prevalence and composition of biofilms in situ on hard and soft hospital surfaces, to develop in-vitro models that are representative of those likely to be found on hospital surfaces, and to optimize methods to tackle biofilms on hospital surfaces, which may include new cleaning and disinfection agents and adjuvants, new technologies (such as microfibre or automated room disinfection technology), and surface modification.15

Conclusion

Surface-attached cells are likely to be common on dry hospital surfaces, and there is evidence that they also harbour established biofilms. The variety of methods used to create and evaluate in-vitro biofilms makes it difficult to compare studies evaluating antibiofilm biocide activity. Nonetheless, microbes attached to surfaces, especially established biofilms, are less susceptible to chemical biocides, UV radiation and antibiotics than their corresponding planktonic bacteria. The phase of the surface-attached microbes influences susceptibility; attached cells are more susceptible to biocides than established biofilms; low-density, nutrient-limited biofilms make less of an impact on biocide susceptibility than high-density, high-nutrient biofilms; and bioicides are less effective for inactivating bacteria in mixed-species biofilms than in single-species biofilms. Biode-specific issues also influence susceptibility in terms of activity against bacteria in biofilms, and the prevention, promotion and dismantling of biofilms. Reduced susceptibility to biocides combined with protection from physical removal through cleaning is likely to contribute to failures in hospital cleaning and disinfection.

Biofilms may explain why vegetative bacteria can survive for unusually long periods (weeks to months) on dry hospital surfaces. Also, the presence of surface-attached bacteria and biofilms is likely to interfere with attempts to recover bacteria from hospital surfaces, and may lead to underestimation of both the prevalence of contamination with pathogens and the number of bacteria that are on surfaces. This has important implications, particularly for hospital outbreak investigation. Biofilms provide a mixed bacterial community where the horizontal transfer of resistance genes may occur. Attempts to tackle surface-attached microbes and biofilms on hospital surfaces should include: identification and selection of biocide and detergents with the best all-round performance, including the ability to inactivate surface-attached cells and biofilms; ensuring that in-vitro tests are developed to model surface-attached microbes likely to be encountered in the field; harnessing surface science to develop a hospital environment that reduces the chance of biofilm formation; and further research to develop novel approaches to augment the activity of biocides against surface-attached microbes, including established biofilms.

Conflict of interest statement

JAO is employed part-time by Bioquell, and JAGS, JC and SY are employed by Bioquell. All other authors have no conflicts of interest to declare.

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