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Review

Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control

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Biofilms are of great importance in infection control and healthcare-associated infections owing to their inherent tolerance and 'resistance' to antimicrobial therapies. Biofilms have been shown to develop on medical device surfaces, and dispersal of single and clustered cells implies a significant risk of microbial dissemination within the host and increased risk of infection. Although routine microbiological testing assists with the diagnosis of a clinical infection, there is no 'gold standard' available to reveal the presence of microbial biofilm from samples collected within clinical settings. Furthermore, such limiting factors as viable but non-culturable micro-organisms and small-colony variants often prevent successful detection. In order to increase the chances of detection and provide a more accurate diagnosis, a combination of microbiological culture techniques and molecular methods should be employed. Measures such as antimicrobial coating and surface alterations of medical devices provide promising opportunities in the prevention of biofilm formation on medical devices.

Introduction

Healthcare-associated infections (HCAIs) can occur in care homes, hospitals or in a patient's own home (van Kleef *et al.*, 2013), with a prevalence level of 6.4 % and 1 000 000 cases reported each year in England (HPA, 2012a). Medical device-related infections pose a huge financial burden on healthcare services and are associated with increased patient morbidity and mortality (Donlan, 2008); not surprisingly, HCAIs are of significant economic concern (NAO, 2000). The most commonly reported HCAIs involve ventilator-associated pneumonia (VAP) and lower respiratory tract infections (22.8 % of cases), catheter-associated urinary tract infections (CAUTIs; 17.2 % of cases) and surgical-site infections (SSIs; 15.7 % of cases (HPA, 2012a). The micro-organisms most frequently associated with HCAIs include Gram-positive bacteria, such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*; Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, and yeasts, particularly *Candida* species (Donlan, 2001). It is the growth of these micro-organisms within biofilms that has posed a challenge in treating HCAIs, owing to the association of biofilms with increased resistance to anti-microbial therapies. Biofilms are communities of micro-organisms that can attach to both abiotic and biotic surfaces and have therefore been implicated in the development of wound infections, non-healing wounds and medical device-related infections (Vinh & Embil, 2005; Seth *et al.*, 2012; Percival *et al.*, 2012).

A major feature of the biofilm is the self-produced extracellular polymeric substances (EPS) (Lindsay & von Holy, 2006). EPS mainly consists of polysaccharides, nucleic acids (extracellular DNA) and proteins, which help to protect the micro-organisms from external threats, including immune system components and antimicrobials (Percival *et al.*, 2010).

The growth of micro-organisms within a biofilm has been associated with a number of chronic infections. *P. aeruginosa* forms biofilms in the lungs of patients with cystic fibrosis (CF) and, despite the aggressive use of antibiotics, colonization is often a life-long problem (Anderson *et al.*, 2008), leading to chronic inflammation and lung tissue damage (Høiby *et al.*, 2010). Biofilm-forming *P. aeruginosa* also has a role to play in the persistence of cutaneous wound infections and has been shown to form biofilms in both

Abbreviations: CAUTI, catheter-associated urinary tract infection; CF, cystic fibrosis; EPS, extracellular polymeric substances; ETT, endotracheal tube; HCAI, healthcare-associated infection; MRSA, meticillin-resistant *Staphylococcus aureus*; QS, quorum sensing; SCV, small-colony variant; SSI, surgical-site infection; VAP, ventilator-associated pneumonia.

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human and veterinary wounds (Chincholikar & Pal, 2002; Westgate *et al.*, 2010). In particular, chronic venous leg ulcers have often been shown to harbour *P. aeruginosa*, with *P. aeruginosa*-infected chronic wounds appearing larger than *P. aeruginosa*-negative wounds (Kirketerp-Møller *et al.*, 2008). It is also interesting to note that bacteria isolated from acute and chronic wounds have been shown to display higher biofilm-forming potential than bacteria isolated from normal skin (Westgate *et al.*, 2010).

The association of biofilms and medical device-related infections was first recognized in 1972 (Johanson *et al.*, 1972), biofilms being commonly associated with a wide range of polymeric medical devices, such as catheters and cardiac pacemakers (Marrie *et al.*, 1982; Peters *et al.*, 1982; Hall-Stoodley *et al.*, 2004). The emergence of biofilm-related infections due to the widespread use of medical devices in healthcare settings has given rise to the term 'polymer-associated infection'.

The aim of this review is to provide an update on HCAIs and the role biofilms play in increasing medical device-associated infection risk and decreasing antimicrobial effectiveness.

HCAIs

HCAIs occur as a result of infection by a number of agents, most commonly bacteria, but also fungi, parasites, viruses and prions (see Table 1); the most widely publicized source of HCAIs is the hospital 'superbug' meticillin-resistant *S. aureus* (MRSA), which is a common cause of septicaemia or bacteraemia in clinical settings (HPA, 2012b).

There are several risk factors for the development of HCAIs, including long hospital stay, immunocompromised patient (following chemotherapy for instance), invasive surgery and home wound management (HPA, 2012a).

In order to further understand the risk of developing an healthcare-associated infection (HCAI), it is important to understand the routes of transmission to the host. Micro-organisms can be acquired from several reservoirs, such as human skin, water and food sources (Percival & Walker, 1999). Micro-organisms reach the new host either directly by contact with the infected person or indirectly, due to airborne contamination, consumption of contaminated food or contact with contaminated surfaces. The new host can come into contact with the micro-organisms through inhalation, ingestion, breaks in the skin barrier following surgery or insertion of intravenous lines, or through mucous membranes, including the eyes, mouth and nose. Indeed, HCAIs such as SSIs can be avoided if conscientious hygiene procedures are practised (Percival *et al.*, 2014a).

Biofilms: formation, dispersal and the risk of dissemination

Formation

Biofilm formation comprises several stages: reversible attachment, irreversible attachment, colonization, maturation and dispersion.

Micro-organisms living within a biofilm possess specific mechanisms that allow initial surface attachment, the development of a community structure and ecosystem, and subsequent detachment from the biofilm. The attachment of micro-organisms to a surface can be facilitated by factors such as increased shear forces, bacterial motility, and electrostatic interactions between the micro-organism and surface. In a state of 'reversible attachment' there is thought to be equilibrium between attached and free-floating micro-organisms. However, there are features of the microbial cell surface that promote the attachment process to the surface, including flagella, pili, fimbriae and glycocalyx (Donlan, 2001). In terms of microbial attachment to medical devices,

Table 1. An overview of the most commonly isolated micro-organisms found in biofilm-related HCAIs

HCAI	Micro-organism	Reference
Medical device-related		
CAUTI	Coagulase-negative staphylococci (CNS), *C. albicans, A. baumannii, P. mirabilis, E. coli, K. pneumoniae, P. aeruginosa, S. aureus, S. epidermidis*	Douglas (2003); Chakravarti *et al.* (2005); Holá *et al.* (2010); Wang *et al.* (2010); Choe *et al.* (2012); Djeribi *et al.* (2012); Singhai *et al.* (2012)
Central-line-associated septicaemia	CNS, *C. albicans, K. pneumoniae, P. aeruginosa, S. aureus, S. epidermidis*	Douglas (2003); Larsen *et al.* (2008); Pannanusorn *et al.* (2013); Singhai *et al.* (2012)
VAP	*Candida, K. pneumoniae, P. aeruginosa, S. aureus, S. epidermidis*	Bauer *et al.* (2002); Singhai *et al.* (2012); Vandecandelaere *et al.* (2012)
Surgical-site infection		
Surgical wound, prosthesis-related infection	*Candida, E. coli, Staphylococcus* spp., MRSA, *P. aeruginosa, S. aureus, S. epidermidis*	Roggenkamp *et al.* (1998); Douglas (2003); Seifert *et al.* (2005); Kathju *et al.* (2009); Kiedrowski & Horswill (2011); Stoodley *et al.* (2011); Edmiston *et al.* (2013)

the adherence of bacteria to biomaterials through cell-surface and biomaterial-surface interactions has been reported. For example, staphylococcal species display cell-surface proteins, namely staphylococcal surface protein-1 and -2 (SSP-1 and SSP-2) (von Eiff *et al.*, 1999), localized on the cell surface on a fimbria-like polymer and linked with the adhesion of *S. epidermidis* to polystyrene (Veenstra *et al.*, 1996). In addition, the capsular polysaccharide/adhesin has a role to play in the adherence of clinical isolates of coagulase-negative staphylococci to biomaterials (Muller *et al.*, 1993). Furthermore, the protein autolysin (AtlE) in *S. epidermidis* has been linked with the adhesion of this micro-organism to a polymer surface; this protein confers not only the ability to adhere to a polystyrene surface but also the ability to bind to vitronectin, thus demonstrating a role for cellular adhesion to plasma protein-coated polymer surfaces during the later stages of bacterial adherence (Heilmann *et al.*, 1997).

As the cell population density of the developing biofilm fluctuates, the gene expression of cells within the biofilm is regulated by a process known as quorum sensing (QS). Through this system, bacteria release chemical signals called autoinducers, which are constitutively produced and increase in concentration as the density of the biofilm increases. As the concentration of these autoinducers reaches a critical threshold, alterations in gene expression occur, leading to an array of physiological processes, including motility, sporulation and release of virulence factors necessary for survival (Lindsay & von Holy, 2006; Mangwani *et al.*, 2012). Gram-negative bacteria release molecules called acylhomoserine lactones, whereas Gram-positive bacteria release oligopeptide molecules (Lindsay & von Holy, 2006). Some of the well-studied QS molecules, for example *N*-(3-oxo-dodecanoyl)-L-homoserine, are associated with *Pseudomomas aeruginosa* biofilm. In addition, the *N*-(3-oxo-dodecanoyl)-L-homoserine QS molecule has been reported to increase *Pseudomomas aeruginosa* biofilm virulence and repress host immune responses (Driscoll *et al.*, 2007). Given that *Pseudomomas aeruginosa* has been implicated in a number of pathological processes, particularly CF, these QS molecules have since been the target for drug development using QS inhibitors (Hentzer *et al.*, 2003).

Biofilm dispersal and the risk of dissemination

The process known as biofilm dispersal promotes dissemination within the host, as parts of the biofilm slough off and are able to colonize new sites, posing a severe threat to the host (Donelli, 2006). Biofilm dispersal is a process at the latter end of the biofilm life cycle whereby cells that were once part of a complex, relatively static, slow-growing micro-community within the biofilm become differentiated, often highly motile micro-organisms (McDougald *et al.*, 2012). These dispersed cells are able to attach to new surfaces and initiate biofilm growth. It is important to note that these are indeed specialized cells and are different from bacteria that slough off from the biofilm or that are disturbed through adverse environmental conditions.

One of the intracellular mechanisms responsible for dispersal within a biofilm is the secondary messenger molecule cyclic-di-GMP (c-di-GMP) (Karatan & Watnick, 2009). This is an intracellular molecule that controls the transition from biofilm to planktonic phenotypes. More specifically, it has been reported that a reduction in intracellular c-di-GMP can lead to dispersal in some micro-organisms. In addition, the genes associated with motility, such as those involved in flagellum formation, are upregulated (McDougald *et al.*, 2012). It is important to note that dispersal can also affect non-motile micro-organisms. In the case of *S. aureus*, not only has the repression of the *agr*-related QS regulatory gene been shown to play a role in biofilm formation, but its activation has also been reported to induce the release of *S. aureus* cells from the biofilm (Boles & Horswill, 2008).

There are multiple factors that initiate dispersal, including changes in nutrients, temperature and oxygen levels. The presence of other micro-organisms within the biofilm can influence dispersal through chemical signals such as acylhomoserine lactones, diffusible fatty acids and peptides (Hall-Stoodley *et al.*, 2004; Kaplan, 2010).

Regarding SSIs, dissemination of micro-organisms can occur through contaminated medical instruments or the transfer of micro-organisms from the patient's surrounding skin or the skin of the healthcare professional. In terms of medical devices, contamination can occur through contact with skin, contaminated water or other external sources. When a biofilm develops on living tissues or medical devices, it is possible for detached cells to cause systemic infection, particularly if the host immune response is compromised (Donlan, 2001).

Mechanisms of biofilm resistance

Micro-organisms that grow within the biofilm state are thought to possess several mechanisms that increase resistance to external antimicrobial treatments as compared with bacteria in the planktonic state.

One of the theories aimed at understanding this recalcitrance involves the slow or incomplete penetration of antimicrobial agents through the EPS matrix of the biofilm (Francolini & Donelli, 2010). The matrix barrier can also act as a defence mechanism against other external stimuli such as UV light and dehydration (Hall-Stoodley *et al.*, 2004). The EPS matrix has also been shown to neutralize and dilute antimicrobial substances (Hall-Stoodley *et al.*, 2004). Indeed, it has been reported that mature biofilms (over 7 days old) are resistant to 500–5000 times the concentration of bactericidal agents necessary to successfully kill planktonic cells of the same organism (Khoury *et al.*, 1992). Although incomplete penetration of the matrix barrier has been well recorded and reviewed, this resistance mechanism is not effective against all antimicrobials. Singh

and colleagues tested the efficacy of oxacillin, cefotaxime, amikacin, ciprofloxacin and vancomycin against *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 35984 in 48 h laboratory-grown biofilms. The results of this study demonstrated a significant reduction in the penetration of oxacillin, cefotaxime and vancomycin, while amikacin and ciprofloxacin showed no significant reduction in biofilm penetration (Singh *et al.*, 2010).

Another theory involves the slow growth rate within areas of the biofilm, which is thought to hamper the actions of many antimicrobials that require a certain degree of cellular activity in order to function (Hall-Stoodley *et al.*, 2004). It has also been suggested that phenotypic variants commonly referred to as 'persister cells' confer resistance within the biofilm owing to their slow rate of growth. Although these persister cells lack the genetic traits that resemble those of antibiotic resistance, they show high levels of multidrug tolerance. (Spoering & Lewis, 2001; Hall-Stoodley *et al.*, 2004; Lewis, 2008, 2010; Percival *et al.*, 2011).

Other mechanisms that are thought to play a role in the antimicrobial resistance acquired by certain micro-organisms within biofilms include the presence of efflux pumps, with the expression of several gene-encoding efflux pumps being increased in biofilms (Soto, 2013). Furthermore, plasmid exchange occurs at a higher rate in biofilms, increasing the chances of developing naturally occurring and antimicrobial-induced resistance (Hausner & Wuertz, 1999). Finally, it is thought that an altered micro-environment within a biofilm, such as nutrient depletion and reduced oxygen levels, may also reduce the efficacy of antimicrobials (Francolini & Donelli, 2010).

Biofilms and HCAI

The initial contamination of the medical device most likely occurs from a small number of micro-organisms, which are often transferred to the device in question via the patient's or healthcare workers' skin, contaminated water or other external environmental sources (von Eiff *et al.*, 1999, 2005).

Whilst a range of micro-organisms has been implicated in medical device-related infections, *S. epidermidis* and *S. aureus* are most commonly associated with biofilms formed on medical devices and are widely acknowledged as a major source of HCAIs (von Eiff *et al.*, 1999, 2005; Götz, 2002; Vuong *et al.*, 2004). Indeed, according to some authors, nearly 80 % of the bacteria involved in material-associated infections are *S. epidermidis* (von Eiff *et al.*, 1999).

In addition to the staphylococcal species, the identification of multidrug-resistant Gram-negative bacteria, particularly *A. baumannii*, *E. coli, K. pneumoniae* and *P. aeruginosa*, is becoming more widespread in long-term care facilities and acute care hospitals. In fact, these species are often the cause of biofilm-based HCAIs, including CAUTI (Niveditha *et al.*, 2012).

Central venous catheters

Central venous catheters are utilized to deliver fluids, blood products, nutritional solutions or medications, and for access in dialysis treatment (Percival & Kite, 2007; Donlan, 2008). Both the outer part of the catheter and the catheter lumen can become contaminated, so resulting in biofilm formation, with the duration that the catheter is *in situ* impacting on the location and the degree of colonization (Donlan, 2008). Within the first week of catheterization, extraluminal biofilm is considered the major cause of catheter-associated bloodstream infections.

On the contrary, vascular catheters that had been *in situ* for over 30 days showed evidence of predominantly luminal colonization and biofilm formation (Raad *et al.*, 1993). Therefore, patients who require long-term use of such devices for intravenous access, such as bone marrow transplant patients, are at a greater risk of bloodstream infection (Donlan *et al.*, 2001). It has also been noted that catheter colonization and biofilm formation on central venous catheters occurs early. Anaissie and colleagues found that microbial colonization and biofilm formation occurred as early as 1 day after catheter insertion in a cohort of adult cancer patients whose central venous catheters were removed. These authors also found that this was a universal occurrence and was not related to the clinical status of the patient or the microbiological findings from the catheter (Anaissie, *et al.*, 1995).

Urinary catheters

Urinary catheters are tubular latex or silicone devices that are used to measure urine output, collect urine during surgery, prevent urine retention or control urinary incontinence (Kaye & Hessen, 1994). For patients undergoing catheterization, the risk of developing a catheter-associated infection increases by approximately 10 % each day the catheter is in place. Biofilms can readily develop on both the inner and outer surfaces of urinary catheters (Donlan, 2001), and the ascending colonization cannot be avoided solely through hygiene measures. Therefore, in order to prevent such infections, it is important for clinicians to utilize catheters only when necessary and to avoid catheterization for extended periods of time (Talsma, 2007). The contaminating bacteria may originate from those organisms that colonize the periurethral skin, which migrate into the bladder via the mucoid layer that forms between the epithelial surface or the urethra and the catheter (Stickler, 2008). Contamination of the urine within the catheter drainage bag can also be a source of bacteria that can then go on to cause infection (Stickler, 2008). Often, the main strategy against CAUTI is the removal and replacement of the catheter. However, frequent disruption of the catheter and replacement can lead to further complications; shedding of parts of the biofilm from the indwelling device enables the spread of infecting bacteria to previously uncolonized sites.

Stickler described how the production of urease by some bacteria, particularly *P. mirabilis*, causes a rise in the

urinary pH, enhancing the formation of crystalline biofilms within the urinary catheter. These crystalline biofilms can form on the outer surface of the catheter, around the balloon and catheter tip, which can lead to trauma to the bladder and urethral epithelia (Stickler, 2008). Furthermore, when the catheter balloon is deflated, debris may be shed from the biofilm on the balloon surface; biofilm debris is then able to cause blockage in the bladder due to stone formation. The crystalline biofilm can also cause blockage of the catheter lumen, preventing the flow of urine through the catheter (Stickler, 2008; Percival *et al.*, 2009).

VAP and endotracheal tubes (ETTs)

VAP has been reported to be prevalent after 48–72 h in patients who have been intubated and are on mechanical ventilation. VAP has major implications for both the patient and the healthcare system, leading to prolonged hospital stay and increased healthcare costs (Diaz *et al.*, 2005; Palmer, 2009). The risk of developing VAP following intubation with mechanical ventilation is increased 6- to 20-fold, with mortality rates ranging from 24 to 76 %, significantly higher than mortality rates for urinary tract and skin infections (1–4 %) (Chastre & Fagon, 2002; Craven & Hjalmarson, 2010). VAP can be classified as either early-onset ($<$5 days hospitalization) or late-onset ($\geqslant$5 days hospitalization) based on the risk of infection with multidrug-resistant pathogens. ETTs have been reported to be a factor in the acquisition of VAP (Depuydt *et al.*, 2006; Augustyn, 2007; Amin, 2009; Inglis *et al.*, 1989; Bauer *et al.*, 2002; Ramirez *et al.*, 2007). Biofilms proliferate very quickly on ETTs, with a study that reports they form within 24 h (Bauer *et al.*, 2002). A correlation between the micro-organisms found in the lower respiratory tract and the ETT has been reported (Adair *et al.*, 1999).

The micro-organisms that have been documented to colonize ETTs and grow in the form of a biofilm are numerous, including the multidrug-resistant bacterium MRSA and Gram-negative bacilli such as *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *Acinetobacter* spp. (Inglis, 1989; Bauer *et al.*, 2002; Ramirez *et al.*, 2007). Vandecandelaere and colleagues identified micro-organisms in biofilm form on ETTs using a combination of traditional culture techniques and 16S rRNA sequencing. The results revealed the presence of a diverse range of micro-organisms, from the common oral-associated microflora to more clinically relevant isolates, including *Acinetobacter* spp., *P. aeruginosa*, *S. aureus* and *S. epidermidis* (Vandecandelaere *et al.*, 2012; Vandecandelaere & Coenye, 2015). These authors not only highlighted the importance of utilizing multiple techniques for the identification of biofilms but also emphasized the microbial diversity within biofilms in ETTs.

The surveillance of microbiological activity in patients following intubation is an important way to assess the subtleties of host–pathogen interactions in terms of the development of VAP and thus determine effective treatment pathways. Depuydt and coworkers took weekly tracheal aspirates for the detection of VAP due to multidrug-resistant pathogens and determined that multidrug-resistant pathogens were associated with 69 % of VAP episodes, and that this led to appropriate antibiotic coverage in 89 % of cases (Depuydt *et al.*, 2008). Inadequate antimicrobial coverage or the delayed action of antimicrobials increases the risk of VAP-associated mortality, and therefore understanding the microbiological differences between early- and late-onset VAP is of great importance. The presence of multidrug-resistant pathogens has been reported to be associated with late-onset VAP (Trouillet *et al.*, 1998). Although a more recent study identified an association of Gram-negative bacilli with late-onset VAP as compared with early-onset, no significant differences between early- and late-onset VAP were found for specific pathogens such as MRSA, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*. This study highlighted the importance of using antimicrobial therapies to target multidrug-resistant pathogens at early-onset VAP.

SSIs

SSIs are those wound infections that occur following a surgical procedure (Graves, 2004). SSIs can occur due to the contamination of a wound by micro-organisms derived from the patient's own skin.

The most frequent micro-organism associated with SSI is *S. aureus*, commonly found amongst the normal flora of the skin. Kathju and colleagues demonstrated by confocal microscopy the presence of bacilli and cocci within biofilms on explanted sutures taken from a chronic SSI. Further investigation using fluorescence *in situ* hybridization identified components of the biofilm to be *Staphylococcus* using a *Staphylococcus*-specific probe (Kathju *et al.*, 2009). A more recent study investigated the presence of biofilms on two different types of suture, absorbable and non-absorbable, from both infected and non-infected wounds. Using traditional culture methods, this study identified *Corynebacterium* to be the most commonly isolated micro-organism from non-infected wounds, followed by *Bacillus* spp. and *S. epidermidis*. The micro-organisms that predominated on sutures from infected wounds included *S. epidermidis*, *S. aureus*, MRSA and *P. aeruginosa*, to name a few. The authors found a significant difference in the presence of biofilms in infected wounds as compared with uninfected wounds. Despite this, 66.6 % of uninfected wound-derived sutures were positive for biofilms (Edmiston *et al.*, 2013, 2015). To help prevent the risk of developing SSI, it is important that medical professionals adhere to NICE guidelines regarding hygiene procedures. Despite this, 5 % of patients that undergo surgery still develop SSI (NICE, 2008).

In chronic wounds in particular, it has been proposed that the ratio of the planktonic : biofilm phenotypes is shifted in favour of the biofilm phenotype, thus resulting in delayed wound healing (Percival *et al.*, 2012). Furthermore, the role

of wound dressings as a 'bioreactor' in the upregulation of the biofilm phenotype within a wound has been hypothesized (Thomas *et al.*, 2011). Although the primary use of a wound dressing in the treatment of a wound is to prevent further colonization of micro-organisms from the external environment, this hypothesis, termed the 'ping-pong' effect, describes a dressing-covered wound bed as a 'static biofilm reactor' that promotes biofilm predominance. More specifically, the underside of the wound dressing is said to act as an additional reservoir for both planktonic and biofilm isolates.

Detection and diagnosis of medical biofilms

To date, there are no detection methods available for the diagnosis of a biofilm within a clinical setting. The use of traditional culture methods to determine colonization is not indicative of biofilm growth (Hall-Stoodley *et al.*, 2012). Furthermore, negative results from swab samples may not necessarily imply the absence of an infection, but could possibly be due to the slow growth rate within a biofilm of species that cannot be detected within the usual detection range (Lindsay & von Holy, 2006).

One of the barriers that can make successful diagnosis difficult is the emergence of small-colony variants (SCVs). SCVs are a subpopulation of biofilm bacteria that produce small colonies, develop resistance to antimicrobial action and can evade detection owing to their slow growth rate (Neut *et al.*, 2007). Given that SCVs have a reduced metabolic rate, they can be easily missed during routine microbiological cultures, which are commonly grown on agar plates for 48 h (Neut *et al.*, 2007). This is a particular problem in patients who have undergone joint replacement surgery, owing to misdiagnosis of pain associated with late-onset infection as being caused by other surgical complications. These patients often develop a gradual onset of joint pain without the clinical signs and symptoms of infection, such as fever, surgical-site wound drainage, redness or swelling.

S. aureus infections are of particular interest in terms of the problems associated with SCVs (Kahl, 2014). Since SCVs and normal *S. aureus* appear very similar on Gram stain, it is not possible to distinguish between normal and variant growth types, making diagnosis difficult using standard microbiological techniques (Vaudaux *et al.*, 2006). Infection of human endothelial cells with both WT and SCV strains of *S. aureus* has demonstrated that SCVs are able to survive within host cell lysosomes and that certain SCVs are also able to withstand the bactericidal activity within these lysosomes (Schröder *et al.*, 2006). A 6-year prospective, longitudinal study (Kahl *et al.*, 2003) demonstrated the presence of *S. aureus* SCVs in the airways of patients with CF, demonstrating persistent infection, with SCVs persisting longer in the airways than normal *S. aureus*. Seifert and colleagues have also reported a case of medical device-related bloodstream infection caused by *S. aureus* SCVs; recurrent infection with this *S. aureus* subpopulation in a patient with a pace maker demonstrates the difficulties faced by clinicians in terms of diagnosis and treatment of SCV infections (Seifert *et al.*, 2005).

Other bacteria causing persistent infections have been isolated as SCVs. For instance, *P. aeruginosa* SCVs have been isolated from the lungs of patents with CF (von Götz *et al.*, 2004), while *E. coli* SCVs have been identified in chronic prosthetic hip infections (Roggenkamp *et al.*, 1998). A good example of the importance of SCVs in persistent infections and, in this case, the potential failure of reimplantation of joint replacements, is given in the work of Neut and colleagues regarding *P. aeruginosa* biofilm formation on gentamicin-loaded bone cement (Neut *et al.*, 2005). In this study, although there was a 44 % reduction in bacterial viability, results showed the development of SCVs with a decreased sensitivity towards gentamicin and enhanced EPS production, which overall reduced the susceptibility to antibiotics.

Concerning the difficulties in detecting causative agents of biofilm-based infections, a possible explanation can be found in recent data demonstrating that *S. aureus* can enter the viable but not culturable (VBNC) state in biofilms associated with central venous catheters that are negative on standard microbiological assays. Furthermore, it has been reported that vancomycin, quinupristin/dalfopristin and daptomycin can induce a true VBNC state or persistence in *S. aureus* cells embedded in biofilms (Zandri *et al.*, 2012; Pasquaroli *et al.*, 2013, 2014).

To improve the diagnosis of device-related infections, methods such as the sonication of suspected infected implants may improve culture positivity (Achermann *et al.*, 2010). In addition to this, more sophisticated molecular methods of identification, including PCR and fluorescence *in situ* hybridization, are now being used to identify bacteria in complex biological samples, and are proving to be a more accurate means of detection. Several publications indicate differences between culture and molecular diagnostic methods. For example, in otitis media with effusion, pathogens are identified around 25–30 % of the time by culture methods and 80–100 % of the time by culture-independent molecular methods (Hall-Stoodley *et al.*, 2006).

Biofilm control: preventive measures and future perspectives

With ever-increasing evidence of the presence of biofilms in HCAIs, current research has focused upon more sophisticated methods of sterilization and the modification of medical devices in order to prevent microbial growth and biofilm formation (Table 2).

The addition of antimicrobials to the surface of medical devices such as catheters has been the focus of much research and can be addressed in three ways: the agent can be applied as a thin film on the surface of the catheter, ionically bound to the surface, or bound to the surface within a polymer matrix (Shintani, 2004). A number of

Table 2. Anti-biofilm technologies

Technology	Mechanism	Reference
Surface modification		
Silver	Antimicrobial	Rello *et al.* (2010); Chakravarti *et al.* (2005); Raad *et al.* (2011)
Hydrogels	Reduction of bacterial adhesion	Ahearn *et al.* (2000); Lai & Fontecchio (2002)
Antifouling polyurethanes	Reduction of bacterial adhesion	Francolini *et al.* (2014)
Antibiotics: minocycline/rifampicin	Antimicrobial	Darouiche *et al.* (1999)
Nanomodification: *Rhizopus arrhisus* lipase, trimethylsilane plasma coating	Reduction of bacterial adhesion	Machado *et al.* (2012); Ma *et al.* (2012a)
Small molecules		
Chelating agents: tetrasodium EDTA	Interference with metal ions important in biofilm formation	Percival *et al.* (2005)
Antivirulence compounds	Inhibits bacterial gene expression	Ma *et al.* (2012b)
Bioactive molecules/enzymes		
Bacteriophage virus	Antimicrobial	Yilmaz *et al.* (2013)
Bioactive peptides: human β-defensin 3	Antimicrobial	Huang *et al.* (2012); Zhu *et al.* (2013)
Enzymic detergents	Antimicrobial	Ren *et al.* (2013)
Dispersin B	Antimicrobial	Kaplan *et al.* (2004); Donelli *et al.* (2007); Gawande *et al.* (2014)

factors influence the effectiveness of catheters, for example, treated with an anti-infective agent; the solubility, the hydrophilicity and the affinity to surrounding tissue are all factors that affect the release and duration of anti-infective activity (Shintani, 2004).

Early studies into antimicrobial-impregnated devices, such as that by Darouiche and colleagues, compared the effectiveness of both minocycline/rifampicin- and chlorhexidine/silver sulfadiazine-impregnated central venous catheters in terms of their action against catheter colonization and bloodstream infection. The authors found that catheters impregnated with minocycline/rifampicin were associated with a lower rate of infection than catheters impregnated with chlorhexidine/silver sulfadiazine. However, the chlorhexidine/silver sulfadiazine-impregnated catheters were only treated with the antimicrobials on the external surface, whereas the comparator catheters were treated with minocycline/rifampicin on both the luminal and external surfaces. Furthermore, the concentration and availability of the antimicrobials used in the minocycline/rifampicin catheters was considerably higher than those in the chlorhexidine/silver sulfadiazine-treated catheters (Darouiche *et al.*, 1999; Yousif *et al.*, 2015).

It is well known that silver has been widely employed as an antimicrobial with a broad spectrum of activity that has been shown to be efficacious on biofilms. Conventional approaches mainly consist in the deposition of metallic silver on the device polymer surface. In this regard, silver-impregnated ETTs delay microbial colonization in animal studies (Olson *et al.*, 2002), and this effect has been replicated in human studies. Furthermore, a randomized control study reported that patients intubated for 24 h or longer with a silver-coated ETT indeed had significantly lower colonization rates than control groups and confirmed that reduction in microbial bacterial colonization and biofilm formation could lower the incidence of VAP (Kollef *et al.*, 2008). Interesting results in this field are also produced by directly incorporating silver ions in polymeric substrates, in order to obtain medical devices refractory to microbial colonization. In fact, preclinical studies have shown that the presence of silver-impregnated ETTs can have a positive effect in reducing colonization and biofilm development of *P. aeruginosa* in an *in vitro* model of the early pathogenesis of VAP (Rello *et al.*, 2010). Despite these promising *in vitro* results, larger randomized controlled trials, such as that of Pickard and colleagues, demonstrated that silver alloy-coated catheters were not effective in reducing the incidence of CAUTI, casting doubt over the routine use of antimicrobial-treated catheters (Pickard *et al.*, 2012).

Francolini and coworkers have successfully demonstrated the anti-biofilm activity of silver ion-incorporated polyurethanes, which are polymers particularly suitable for the development of various medical devices, such as cardiovascular implants, vascular grafts, catheters and artificial heart-assisting devices (Francolini *et al.*, 2010). On the other hand, the use of silver-nanoparticle-impregnated central venous catheters had no significant effect on catheter colonization, catheter-related bloodstream infection incidence or mortality in critically ill patients (Antonelli *et al.*, 2012). Finally, the bactericidal effect of an electric field applied to a catheter fitted with silver electrodes has also proven an effective adjunct to silver-treated urinary catheters; Chakravarti *et al.* (2005) demonstrated how crystalline biofilm formation can be temporarily inhibited by the release of heightened concentrations of silver ions, capable of inhibiting bacterial growth and preventing encrustation caused by *P. mirabilis*. More recent studies, such as that of Raad and colleagues, tested the efficiency of

antimicrobial gardine- and gendine-coated ETTs against silver-coated ETTs *in vitro* and showed that MRSA, *P. aeruginosa*, *C. albicans* and *K. pneumoniae* biofilm growth could be completely inhibited for up to 2 weeks compared with the silver-coated ETTs (Raad *et al.*, 2011).

The modification of a medical device surface with a hydrogel is one way in which biocompatibility can be achieved; a hydrogel is a hydrophilic polymer that has a capacity to absorb large quantities of water and therefore can promote a soft surface that helps minimize microbial colonization (Shintani, 2004). A number of studies, both *in vivo* and *in vitro*, have demonstrated the effectiveness of hydrogel and silver–hydrogel catheters through reduced microbial colonization (Bull *et al.*, 1991; Ahearn *et al.*, 2000; Lai & Fontecchio, 2002).

More recently, modifications to ETT surfaces at the nanoscale level have also been researched. A recent study by Machado and co-workers explored the effect of nanomodified ETT on *S. aureus* biofilm formation (Machado *et al.*, 2012). This study showcased the creation of a textured, nanomodified surface using a *Rhizopus arrhisus* lipase that was able to enzymically degrade the polyvinylchloride material of the ETT. The nanomodified ETT was exposed to a constant flow of *S. aureus* medium and incorporated in an airway model. The results showed significantly reduced colony-forming units (c.f.u.) ml^{-1} for bacteria in the nanomodified ETT when compared with the untreated control. In addition, there was an increase in protein absorption by the nanomodified ETT, which the authors hypothesized may prevent the colonization and formation of biofilms (Machado *et al.*, 2012).

Given the knowledge that QS systems have a major role to play in both biofilm development and microbial virulence, much attention has been focused on QS regulators in the formation and resilience of biofilm-based medical device-related infections. The *S. epidermidis* QS regulator *agr* has been shown to be involved in biofilm detachment, and an isogenic *agr* mutant showed increased biofilm development (Vuong *et al.*, 2004). Interestingly, by disabling the *agr* QS regulator, *S. epidermidis* seems to generate an increased capacity for biofilm development on medical devices. The aim of QS inhibitors is to enhance the susceptibility of the biofilm to antimicrobials (Bjarnsholt & Givskov, 2008). Three main pharmacological targets of QS systems include the signal generator, the QS molecule itself and the signal receptor (Rasmussen & Givskov, 2006). Christensen and colleagues showed that *P. aeruginosa* biofilms in an *in vivo* mouse model could be disrupted by the use of the antibiotic tobramycin and several QS molecules, including furanone and horseradish juice extract. Synergy was seen between both treatments, and the presence of QS inhibitor molecules increased the susceptibility of the *P. aeruginosa* biofilm to tobramycin (Christensen *et al.*, 2012).

The use of photodynamic therapy has been shown to have an antimicrobial effect on antibiotic-resistant *P. aeruginosa* and clinical MRSA biofilms grown on ETTs (Biel *et al.*, 2011; Percival *et al.*, 2014b). Biel *et al.* (2011) used a methylene blue-based photosensitizer in the lumen of the infected ETT before exposing the ETT to light from a fibre optic diffuser at 644 nm wavelength, resulting in a 99.9 % reduction in polymicrobial biofilm growth.

The use of bioactive molecules and enzymes is a novel strategy in the prevention of biofilm growth on implanted materials. Ren and colleagues used an artificial biofilm model to assess various detergents for the ability to remove *E. coli* from flexible endoscopes. This study revealed that more bacterial biofilm was found using the enzymic detergent treatment than a non-enzymic detergent treatment (Ren *et al.*, 2013).

A very recent study by Gawande and colleagues demonstrated the efficacy of a naturally occurring enzyme-based gel on chronic wound-associated micro-organisms (Gawande *et al.*, 2014). This novel gel, Dispersin-B-KSL-W, contains the juvenile periodontitis-associated *Aggregatibacter actinomycetemcomitans*-derived enzyme Dispersin-B, which inhibits biofilm formation and disperses pre-formed biofilms. In addition to this, the gel contains a broad-spectrum cationic antimicrobial decapeptide named KSL-W. The authors demonstrated that the novel Dispersin-B-KSL-W gel significantly reduced counts of MRSA, *S. epidermidis*, CoNS, *A. baumannii* and *K. pneumoniae*, when compared with a control and the commercially available Silver-Sept wound gel. This study highlights a potential role for the combination and naturally occurring enzymes and broad-spectrum antimicrobials in the treatment of biofilm-containing wounds in pressure ulcers (Gawande *et al.*, 2014).

Future research should aim to increase our understanding of microbial biofilms and their interactions with biotic and abiotic surfaces, and to establish possible control strategies such as the use of antimicrobial-treated medical devices and locks for biofilm prevention and control. An ideal indwelling medical device would possess surfaces that are similar to those of a healthy human, limiting bacterial adhesion and thus preventing infection. To achieve biocompatibility, the surface of the medical device should be smooth and uniform to allow the growth of healthy tissue and evasion of invading pathogens. In addition to the use of anti-infective agents, consideration of the surface physico-chemical properties of the medical devices is also key and can help to overcome the limitations associated with medical devices pre-treated with antimicrobials.

Future perspectives

Biofilms are of great importance in control of healthcare-associated and other infections. This is not only due to their ability to act as a safe-haven for those micro-organisms that are of public health significance, but also due to their inherent tolerance of and ‘resistance’ to antimicrobials. The diagnosing of a biofilm infection represents an area of grave concern, with identification

significantly easier on abiotic surfaces following removal of a medical device than on biotic surfaces. For example, evidencing a biofilm within a chronic wound is complex and presently there is no 'gold standard' for that (Percival *et al.*, 2014c). With this in mind, focus upon diagnostic methods that incorporate routine microbiological procedures with more sophisticated methods that are low-cost, reliable and easily incorporated into routine clinical laboratory testing should be considered. Nevertheless, prevention of a biofilm represents the most important aspect in reference to HCAIs. This can be achieved by a number of techniques, in particular, devices that incorporate antimicrobial coatings and surface alterations or pharmacological inhibitors of QS molecules provide promising opportunities. Consequently, for HCAI, there is still a major need to further develop the understanding of microbial interaction with biotic and abiotic surfaces and of how the adverse environment of the host affects microbial survival, proliferation and recalcitrance.

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