

Biofilms and intravascular catheter related bloodstream infections

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Intravascular catheters (IVCs) are the most frequently used medical devices in hospitals. However, IVCs are associated with life threatening infection, bloodstream infection (IVC-BSI), which is one of main sources of hospital acquired infections and continue to be associated with morbidity, mortality and additional medical cost [1]. In order to prevent IVC-BSI, it is important to understand the process of microbial deposition on IVC surfaces and how to avoid microbial deposition occurring. The precursor to bloodstream infection is usually catheter colonisation; microbes found deposited on the extra- or intra- luminal surfaces of catheters are the principal source and cause of bloodstream infections [2]. Post microbial deposition, biofilms will be produced and these are thought to be responsible for persistent infections that are recalcitrant to systemic antibiotic therapy [1].

This review is to provide an in-depth synthesis and critique of the literature as pertaining to the role of microbial attachment in IVC-BSI. We discuss the knowledge gained from microbial research in other (non-IVC) medical and non-medical applications that may be helpful in understanding the IVC context. In addition, published theory and data regarding microbial colonisation and biofilm development specifically in IVCs is reviewed. Prevention strategies for IVC-BSIs are presented. Finally, deficits in our current knowledge regarding the role of initial microbial-attachment in IVC-BSIs are identified and areas for future research are highlighted. It is hoped that this review will provide a platform for the further development of IVC-BSIs theory, in addition to opportunities for prevention, diagnostic and treatment innovation.

Keywords: Biofilm; Microbial attachment; Bloodstream infections; Antibiotic-resistance; Intravascular catheter

1. Introduction

Intravascular catheters (IVCs) occupy a very important place in the day-to-day provision of healthcare in hospitals. Nearly 300 million IVCs are used yearly in USA [3]. Along with their undoubted advantages, however, the application of IVCs is also associated with life-threatening infections [4]. The IVC-BSI rate is 3-8 % in the USA and it is estimated that additional \$300 million to \$2.3 billion expenditure is needed for management of patients with IVC-BSI [5, 6]. In order to prevent IVC-related infections, it is important to understand the process of microbial deposition on IVC surfaces and how to avoid microbial deposition occurring. The precursor to bloodstream infection is usually catheter colonisation; microbes found deposited on the extra or intra luminal surfaces of catheters are the principal source and cause of bloodstream infections [2]. Post microbial deposition, biofilms will be produced and these are thought to be responsible for persistent infections that are recalcitrant to systemic antibiotic therapy [1].

This review is to provide an in-depth synthesis and critique of the literature as pertaining to the role of bacterial attachment in IVC-related infection. We discuss the knowledge gained from microbial research in other (non-IVC) medical and non-medical applications that may be helpful in understanding the IVC context. In addition, published theory and data regarding microbial colonisation and biofilm development specifically in IVCs is reviewed. Prevention strategies for IVC-related infections are presented. Finally, deficits in our current knowledge regarding the role of initial microbial-attachment in IVC-related infection are identified and areas for future research are highlighted. It is hoped that this review will provide a platform for the further development of IVC-related infection theory, in addition to opportunities for prevention, diagnostic and treatment innovation.

2. Microbes and their routes of IVC-related infection

There are four possible pathways leading to IVC-related infection (Fig. 1).

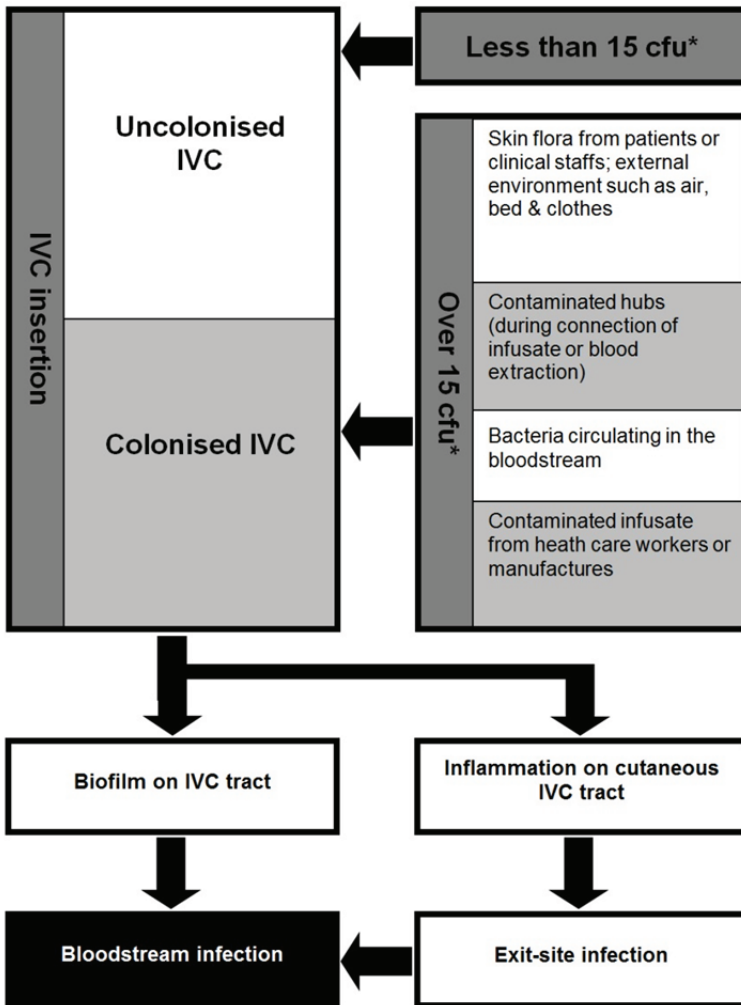


Fig. 1 Potential routes of intravascular catheter (IVC)-related infection. Semiquantitative tip culture is considered colonized if the plate grew $15 \geq$ CFU (colony-forming units).

The first is migration of microbes down the catheter tract, that is, through the ‘wound’ created to insert the catheter. These microbes may be from the patients’ skin, from contaminated disinfectant or healthcare workers’ hands. The process may happen on insertion, if the catheter is contaminated and then introduced into the patient or at any time while the catheter is *in situ* via microbial migration. The insertion of an IVC provides a potential portal of entry for bacteria to cross from an unsterile external environment to the normally sterile blood. The second route is via the catheter hub which might become contaminated during connection of fluids and medicines administration or during extraction of blood by health care workers or patients’ skin flora. Recently, Nishikawa reported that bacterial contamination was more common in the hub area than indwelling catheter segments, and the hub seems an important risk in post insertion care, in addition to adequate aseptic technique on catheter insertion [7]. The third route is for catheters to be contaminated directly by bacteria circulating in the bloodstream. That is, the patient has an existing bloodstream infection, and microbes

are able to attach on to the indwelling catheter as they pass by the device. The fourth is that of contaminated infusate, which may occur at the manufacturing stage (intrinsic) or during manipulation by healthcare workers (extrinsic). It was recently explored that infusates other than water including heparin have great potential to form crystals in the intraluminal surface of IVCs, which would induce bacterial attachment and colonisation [7].

Microbial attachment on the IVC surface is likely to be followed by biofilm development and maturation (Fig. 2) and then dispersion of microbial cells from the biofilm into bloodstream.

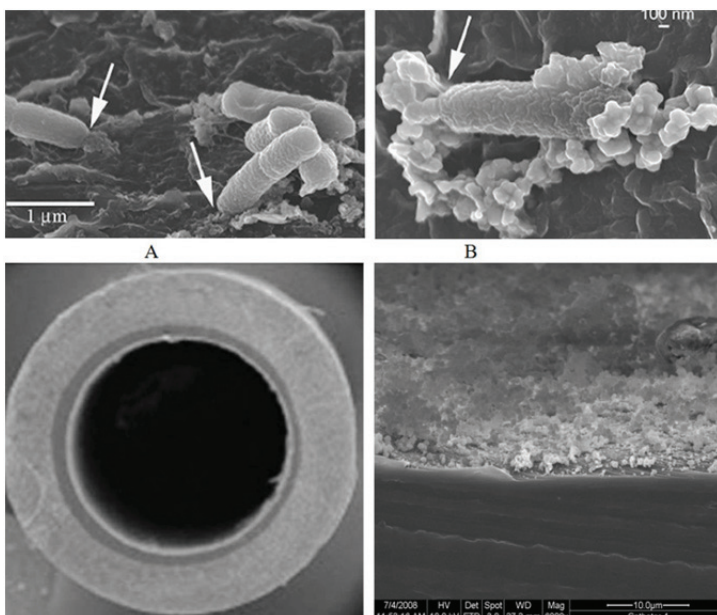


Fig. 2 Scanning electron micrograph of intravascular catheter’s (IVC’s) surfaces: (A, B) Bacteria seeded on deposits on the internal surface of IVC [7]. Arrows show where bacteria adhered to the deposits. (C, D) Scanning electron micrograph of biofilms on an IVC.

The most frequently isolated bacteria from IVCs are coagulase-negative staphylococci and *Staphylococcus aureus*. These bacteria can originate from the cutaneous flora of the patient or the hands of medical personnel and then reach the patients’ tissues and organs via the blood, causing serious infections and high mortal rates (details are given in Table 1). Thus the infectious route for these organisms is likely through skin-bloodstream, i.e. the bacterium enters the bloodstream through IVC wounds in the skin and cause subsequent infection in other organs. The second most common pathogens for IVC-related infections are Gram-negative bacilli. These

microorganisms are generally acquired from the local hospital environment, such as *Enterobacter spp.*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, and *Citrobacter freundii* [8]. Fungi, such as *Candida species*, from the hands of medical personnel, contaminated infusions or parenteral nutrition, are also important pathogens isolated from catheters [9]. Initially IVCs are often primarily colonised by a single microorganism species, but multiple species enter subsequent to the development of biofilms [10]. However, all the microbes reported are isolated by culture-dependent methods which bias microbes who favour the growth media and grow fast under standard laboratory conditions. In addition, some microbial species may compete with others for nutrients or they may even inhibit other microbes from growing and the sensitivity of the semi-quantitative method may also be reduced when the patient is receiving antibiotic treatment.

Table 1 Distribution of pathogens isolated from catheter related infections and associated crude mortality rates.

Catheter type	No. of catheters examined	Colonisation rate (%)	BSI rate (%)	Mortality (%)	Bacteria species	Reference
AC	2949	0.68	0.34	12.98	Coagulase-negative staphylococci 60 % Staphylococcus aureus 10 % Escherichia coli 30 %	[11]
CVC	309	8.7	0.3	7.8	Coagulase-negative staphylococci 70 % Staphylococcus aureus 12 % Others 18 %	[12]
CVC	2692	3.8	5.2	11	Coagulase-negative staphylococci 12 % Staphylococcus aureus 9 % Others 79 %	[13]
CVC	311	NA	8.6	11	Coagulase-negative staphylococci 22 % Pseudomonas 18 % Others 60 %	[14]
CVC	156	36.1	37.2	28.7	MSSA 23 % MRSA 18 % Others 59 %	[15]
CVC	90	25	3.3	NA	Coagulase-negative staphylococci 57 % MRSA 29% Others 14 %	[16]
CVC	60	13.3	1.7	9.6	Coagulase-negative staphylococci 75 % Pseudomonas 12.5 % E. coli 12.5 %	[17]
PICC	279	13.6	1.4	NA	Coagulase-negative staphylococci 77 % Others 23 %	[18]

BSI, bloodstream infection; AC, arterial catheter; CoNS, coagulase-negative staphylococci; CVC, central venous catheter; N/A, not available; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; PICC, peripherally inserted central catheter.

Microorganisms embedded in biofilms often present different phenotypic and genotypic characteristics than when grown planktonically [19]. They are able to obtain and concentrate a number of different nutrients from the environment [19]; they are resistant to a number of antimicrobial agents and have very low metabolic rates [20]. The biofilm mode can facilitate dissemination of organisms. Microorganisms may also exhibit different virulence phenotypes when growing within a biofilm and these phenotypes may not have been detected from IVCs in the past because traditional hospital diagnostics involve growth of organisms on rich nutrient media under planktonic conditions [21]. Rather than amorphous aggregates, biofilms are complex structured communities in which physiological conditions such as nutrients and oxygen availability vary at different depths [22]. Therefore the microorganisms at different depths are phenotypically, morphologically and functionally different. In addition to the effects of microbial attachment, antimicrobial resistance and virulence phenotype produce, Gram-negative bacteria within biofilms have been shown to release endotoxins [23].

Biofilm formation and survival require cell-cell communication which is known as quorum sensing. In fact, quorum sensing is regulation of gene expression in response to fluctuations in cell-population density. Quorum sensing microbes produce and release chemical signal molecules called autoinducers that increase in concentration as a function of cell density [24]. The most well described quorum sensing molecules are N-Acyl homoserine lactones (AHL) in Gram-negative bacteria, oligopeptides in Gram-positive bacteria and autoinducer-2 (AI-2) in both Gram-negative and Gram-positive bacteria [25]. It is generally believed that quorum sensing plays an important role in modelling expression genes associated with production of enzymes, virulence, metabolites and biofilm development [24]. For instance, quorum sensing in *Pseudomonas aeruginosa* control and express a series of toxins, virulence factors, biofilm formation and the interaction with innate immune system which all involve in the pathogenesis of infections [26]. However, quorum sensing molecules in *Staphylococcus* such as *agr* and *luxS* were found to be inactive or down-regulated when biofilm and biofilm-associate infections in *Staphylococcus* developed [27]. It is therefore important to determine and identify quorum sensing signals and the associated regulated pathways. Several reviews on quorum sensing have been published recently. Steindler and Venturi demonstrated the detection of quorum sensing molecules [28]. Dickschat gave an overview about quorum sensing molecules which mediate the formation of biofilms [24]. Asad and Opal summarised quorum sensing systems in bacterial pathogens and their relationships with bacterial infection

[29]. Most of studies to date focus only on specific quorum sensing signals in single bacterial species, although bacterial communities are actually more complex in the real world.

3. Effect of IVC materials on IVC-related infections

The material used to construct the IVC would affect the risk of biofilm formation and subsequent infections. The commonly used biomaterials include silicone, polytetrafluoroethylene, polyurethane, and polyvinyl chloride [30]. Kaplan et al. [30] used a mouse model to investigate *S. aureus* infection with different IVC biomaterials and found that silicone rods had a greater risk for *S. aureus* infection than did polytetrafluoroethylene rods when both were inserted subcutaneously. Sherertz et al. [31] reported that silicone IVCs had a greater risk of infection compared with polytetrafluoroethylene, polyurethane, and polyvinyl chloride IVCs. The presence of IVCs, regardless of biomaterial, may also engender or alter specific immune response. A study by Zimmerli et al. [32] showed neutrophils exposed to some IVC biomaterials had decreased bactericidal activity compared with blood or peritoneal exudate neutrophils. More recently, Kaplan et al. [30] found that supernatant obtained after the association of neutrophils with IVC biomaterials induced chemotaxis by fresh neutrophils. The study also discovered that successive waves of neutrophils moving towards an infected foreign body became successively less effective in their ability to kill microorganisms. Finally, deposition of adherence proteins, such as albumin, fibrinogen, Hageman factor, high molecular weight kininogen, fibronectin, laminin, thrombospondin, collagen, and immunoglobulins on IVC surfaces [33] together with the attachment of bacterial cells, result in changing IVC surface conditioning, which promotes receptor-mediated attachment of microorganisms. The biomaterials used in IVC manufacture also affect host protein deposition, and subsequent bacterial attachment, colonisation, biofilm formation and infections.

4. Clinical importance of biofilms

4.1 Biofilms and the resistance of antimicrobial agents

In comparison to planktonic bacteria, microorganisms within biofilms have an increased resistance to antimicrobial agents including antibiotics, disinfectants and germicides [34]. Although the reasons for the increased tolerance are unknown, some researchers believe that antimicrobials may be readily inactivated or fail to penetrate the biofilm [35]. In addition, bacteria can exchange plasmids, which may carry resistance factors by conjugation within biofilms. Hausner and Wuertz [36] described the plasmid transfer between *E. coli* and *Alcaligenes eutrophus* in laboratory-grown biofilms. Some microbiologists believe that the physical proximity of cells within microcolonies in biofilms would favour conjugation over the same process among planktonic organisms [37]. Ehlers and Bouwer discovered that conjugation rates between different species of *Pseudomonas* were significantly higher in biofilms than in the same organisms under planktonic growth conditions [38]. The resistance traits of microorganisms within biofilms could spread to other microorganisms in the biofilm.

4.2 Biofilms and resistance to defence mechanisms of the body

It is believed that direct immune responses only occur against antigens on the outer surface of the biofilm, and that antibodies and other serum often fail to penetrate the biofilm. Ward et al. [39] proved this hypothesis using a rabbit model. The experimental results showed that bacterial growth within a biofilm on an implanted peritoneal device was unaffected by the vaccinated animal's immune system. Despite the high antibody titres of vaccinated animals (1,000-fold-higher than normal) it was found that the polysaccharide layer prevented antibodies from reaching the surface of bacterial cells within the biofilm. In addition, it has been shown that phagocytes ineffectively engulf bacteria growing within a complex polysaccharide matrix attached to a solid surface [40]. Subsequently, phagocytes release large amounts of pro-inflammatory enzymes and cytokines, leading to inflammation and destruction of nearby tissues. Shiao and Wu [41] found that extracellular slime produced by *S. epidermidis* interfered with macrophage phagocytic activity. Yasuda et al. [42] observed that resuspended *E. coli* cells in a biofilm were as sensitive to phagocytosis as non-biofilm bacteria but were less sensitive to the killing activity of the human polymorphonuclear leukocytes *in vitro*. It is believed that microbial cells detached from a biofilm would lead to an acute infection if they can overcome immunological and non-specific defence mechanisms of the body [43].

As a biofilm develops, some microorganisms may individually detach from biofilm, and aggregates or clusters may also detach or slough off [44]. This detachment is considered as a universal process for all biofilms. Laboratory studies have shown that increases in shear stress, as would occur during changes in direction or rate of IVC infusate or injection flow, result in an increase in the rate of cell erosion from the biofilm [45]. Such detachment of microorganisms or aggregates would lead to changes of microbial cell concentration within the blood stream [46]. It is these detached microorganisms that are most likely to cause bloodstream infection.

5. Other factors involving in IVC-related infections

The differential infection patterns of some IVC types are related to the subsequent infection rates. A recent study, in critically ill patients with short term arterial catheters (321 catheters), showed femoral placed arterial catheters, and those inserted in the operating theatre or emergency room were more likely to be colonised by bacteria, than those in the radial artery and inserted in the intensive care unit [47]. The flow dynamics of the circulation and patient variables were also suggested as factors that could influence the properties of bacterial attachment and hence biofilm density on IVCs between central and arterial device types [47].

Infection profiles at differing IVC insertion body sites was also demonstrated in a recent study where in comparison to the internal jugular and subclavian sites, the femoral insertion site carried a greater risk of infection by Gram-negative bacteria and yeasts [48]. The microbial flora found at or around the femoral site was also different from those found at upper body sites. A different colonisation density between critically ill male and female patients was reported with IVCs at the internal jugular site, in female patients a significant lower colonisation risk than male patients [47]. It was theorized that infection from the internal jugular site in female patients is easier to prevent due to lower hair growth [47]. It is more likely that bacteria attach in male patients with IVCs inserted at the internal jugular site, or in all patients at the femoral, and it is therefore more important to prevent bacterial attachment and infection in these groups.

6. IVC-related infection control strategies

The simplest concept of IVC related infections control is the initial prevention of bacterial attachment and eventually minimisation of biofilm formation using antimicrobial agents [49]. Several strategies have been proposed using antimicrobial agents to prevent biofilm formation on IVCs and evaluated in clinical studies. Most importantly, these include skin antisepsis, silver ion device coatings, antiseptic hubs, antimicrobial flush/lock solutions, and antimicrobial impregnation or device coatings. Silver acts as an antibacterial agent by preventing the attachment and growth of microorganisms [50]. A silver-impregnated subcutaneous collagen cuff that acts both an antimicrobial deterrent and physical barrier to migrating bacteria has been developed. The cuff is usually placed at the interface of the skin insertion site and the proximal subcutaneous space [50]. The use of the silver-impregnated subcutaneous collagen cuff has been reported to decrease the risk of IVC associated colonisation [51]. However Raad noted that this silver cuff was ineffective in preventing IVC-related infection for long-term catheters (more than 10 days). It was suggested the silver ions chelated to the cuff are released completely within 3-7 days [52] and thus are only effective for short-term catheters.

An antiseptic chamber-containing hub model has been designed and examined to protect against hub colonisation since initial colonisation of the hub is thought to be another primary route for IVC-related infection and biofilm formation [53]. This hub model has two components. One is a female component which consisting of a plastic cylinder, with latex rubber closures at both ends limiting the chamber to 0.2 ml of 3 % iodinated alcohol. The other male component is a 20 gauge needle that connects the female component to the administration set. When the two portions are linked, the needle passes through the antiseptic chamber and is sterilized by contact with the antiseptic solution [53]. The report from this study showed that this hub model reduced the IVC colonisation [53]. This hub is currently only available in Europe and is expensive.

Various antimicrobial flush/lock solutions containing anticoagulants and antimicrobial agents have been used in IVC lumens. These solutions have been mainly assessed in long-term catheters in which luminal colonisation secondary to hub colonisation is thought to be the leading source of infection and biofilm formation [8]. It might be assumed that these solutions kill bacteria before they attach to the IVC surface. In a study using 126 randomly assigned oncology paediatric patients, Henrickson et al. [54] examined the effectiveness of three antimicrobial agents as the antibiotic lock solutions, i.e. Heparin (10 IU/ml), heparin-vancomycin (25 µg/ml) and heparin-vancomycin-ciprofloxacin (2 µg/ml). The authors found that use of heparin-vancomycin-ciprofloxacin significantly reduced colonisation on catheters when compared to the other two antimicrobial agents [54]. A combination of minocycline hydrochloride and EDTA was used in another study and this combination is synergistic against resistant Gram-positive and Gram-negative bacteria and *C. albican* [55]. A number of other antibiotic lock solutions have been used and compared. For example, gentamicin and citrate [56], cefotaxime and heparin [57], tauridone and citrate [58], and heparin. Subsequently, Garland et al. [59] reported a randomized study with critically ill neonates and found an 80% reduction in peripherally inserted IVC-related bloodstream infection with vancomycin lock performed for 20 or 60 minutes twice a day. Vancomycin, however, is not recommended by the Centres for Disease Control and Prevention guidelines to be used as prophylactic agent in the prevention of IVC colonisation since it could contribute to the emergence of vancomycin-resistant enterococci [1]. Although antimicrobial flush or lock solutions could reduce the risk of IVC-related infection and biofilm formation, they could also increase the risk of emergence of antimicrobial resistance.

Coating and impregnating of catheters with antimicrobial agents that are released from both the internal and external surfaces is commonly used on short-term IVCs, and has been shown to provide effective control of colonisation of catheters [60]. In addition, antimicrobial coating provides broad-spectrum antibacterial activity and also remains stable on short-term catheters. Several studies on the application of antibiotic-impregnated catheters have shown the efficacy

of this method. Maki et al. reported that central venous catheters coated with chlorhexidine-silver sulfadiazine could contribute to a 44 % reduction in IVC colonisation and 79 % reduction in IVC-BSI [61]. Catheters coated with minocycline and rifampicin also significantly reduced IVC-related colonisation and subsequent bloodstream infections [62]. In addition, these catheters are cost-effective, and reduce the need for IVC removal. The advantages of these catheters were more evident in the first 10 days of IVC use [63]. However, the risk of emergence of resistance to antimicrobial agents has been raised as a consideration by clinicians when these catheters are used. In addition, they require additional costs which may be prohibitive, particularly in developing countries.

7. Conclusion

IVC-BSI remains a major problem in health care associated with morbidity, mortality and additional medical cost. Microbial biofilm formation make these infections more complicated as the detached microbial cells from the biofilm can lead to acute infection and these microorganisms are highly resistant to a large number of antimicrobial agents. Despite a number of research studies, there is almost no knowledge of the relationship between biofilms and patient symptoms or outcomes due to the usual approach to use only planktonic detaching methods. A better understanding of the interaction of the host, the IVC, and the microbe would be valuable in managing biofilm development and IVC related infections. Although the mechanisms for biofilm formation are not well understood, the current understanding of the biological basis of IVCs and microbial communities within the biofilms suggests this is an important component in the pathogenesis of IVC-related infections. Current knowledge about the most likely responsible organisms for IVC-BSI is determined by traditional surface culture techniques. Early detection and adequate treatment of causative pathogens within 24 hours is critical for a favourable outcome, yet the majority of patients with suspected catheter related infection, yield negative diagnostic investigations, necessitating empiric, rather than optimal antimicrobial therapy [64]. For example, in a study of 631 intensive care unit (ICU) catheters, 207 (33%) were removed due to clinical signs of CRI, yet definitive diagnosis from matched catheter and blood cultures was only achieved in 27 (13%), and catheter tip colonisation in 114 (55%) of suspected cases [65]. Whilst modern microbiological techniques are able to detect biofilm and explore its contents, they are currently not available or unfeasible to use in the busy hospital environment. It will be important in future years that new techniques are validated and developed in research environment and eventually those superior methods to detect microbial colonisation and biofilm at the bedside.

Improved knowledge of the mechanism of bacterial attachment on IVCs could lead to the design of superior strategies to prevent IVC-BSI. Reducing bacterial attachment is one of main focus for current research on prevention of IVC-related infections. It is known that microorganisms have various strategies to attach to IVC surfaces and this is an area to explore to improve understanding of IVC-colonisation. Microbial colonisation on IVCs could be avoided if successful strategies to prevent attachment of these organisms are identified including novel antiseptics with longer and stronger antimicrobial activity and new biomaterial for IVCs which inhibit microbial colonisation and attachment without promoting antimicrobial resistance. Unfortunately, to date, there is no IVC biomaterial currently available which can completely avoid the organisms' attachment and colonisation. The application of quorum sensing inhibitors might be a promising way to inhibit bacterial attachment and subsequent infection processes. However, our understanding in quorum sensing systems is still very limited and more studies need to be done. Decreasing the frequency of IVC surface colonisation and bacterial attachment is an approach on which a large body of knowledge already exists. However, further studies of the bacterial communities on IVCs may reveal novel insights and provide further information as to how manipulation of the bacteria could modulate both IVC colonisation and bloodstream infection.

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