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1 Biofilm special issue

2 Central venous catheters and biofilms: where do we stand in 2016?

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16

17 **Running head:** central venous catheters and biofilms

18

19 **Key-words:** catheter-related bloodstream infections, antimicrobial lock therapy, persisters, skin

20 antiseptics

21

## 1 **Abstract**

2 The use of central venous catheters (CVC) is associated with a risk of microbial colonization and  
3 subsequent potentially severe infection. Microbial contamination of the catheter leads to the  
4 development of a microbial consortia associated with the CVC surface and embedded in an  
5 extracellular matrix, named biofilm. This biofilm provides bacterial cells ability to survive to  
6 antimicrobial agents and the host immune system and to disseminate to other sites of the body.

7 The best preventive strategy is to avoid any unnecessary catheterization or to reduce indwelling  
8 duration when a CVC is required. Beside aseptic care and antibiotic-impregnated catheters (like  
9 minocycline/rifampin), preventive locks can be proposed in some cases, whereas non biocidal  
10 approaches are under active research like anti-adhesive or competitive interactions strategies.

11 When the diagnosis of catheter-related bloodstream infection (CRBSI) is suspected on clinical  
12 symptoms, it requires a microbiological confirmation by paired blood cultures in order to avoid  
13 unnecessary catheter removal.

14 The treatment of CRBSI relies on catheter removal and systemic antimicrobials. However, antibiotic  
15 lock technic (ALT) can be used as an attempt to eradicate biofilm formed on the inside lumen of the  
16 catheter in case of uncomplicated long-term catheter-related BSI caused by coagulase-negative  
17 staphylococci (CoNS) or *Enterobacteriaceae*. Recently, promising strategies have been developed to  
18 improve biofilm eradication ; they rely on matrix degradation or destabilization or the development of  
19 anti-persister compounds, targeting the most tolerant bacterial cells inside the biofilm.

20 Understanding biofilm formation at the molecular level may help us to develop new approaches to  
21 prevent or treat these frequent infections.

22

23

# 1 Introduction

2 A central venous catheter (CVC) is a device inserted in a large vein, used to inject parenteral nutrition,  
3 blood products or fluids that would harm a smaller peripheral vein, such as antineoplastic  
4 chemotherapy. CVC can also be used to perform hemodialysis, obtain blood tests (specifically the  
5 "central venous oxygen saturation"), and measure central venous pressure. Main types of CVC include  
6 non-tunnelled and tunnelled catheters, totally implantable venous access ports (TIVAP) and PICC-lines  
7 (peripherally inserted central catheters). As they improve patients' care, the use of CVC and other  
8 implanted devices is constantly increasing in modern medicine. In the United States, 15 million CVC-  
9 days (*i.e.*, the total number of days of exposure to CVCs for all patients in the selected population  
10 during the selected time period) are recorded in intensive care units (ICUs) each year (1).

11 The use of CVC is associated with a risk of colonization and subsequent infection (2). In a french  
12 nationwide study led in 2012 by the Institut National de Veille Sanitaire (INVS), bloodstream infections  
13 (BSIs) were the fourth cause of hospital-acquired infections, and 33% of them were related to a CVC  
14 (3). With an average incidence of CVC-associated BSIs in the United States of 5.3 per 1000 catheter-  
15 days in the ICU, approximately 80 000 CVC-related BSIs occur in ICUs each year in the USA (4). Of note,  
16 the relative risk for catheter-related bloodstream infections (CRBSI) is up to 64 times greater with CVCs  
17 than with peripheral venous catheters (5).

18 Even if the attributable mortality after CRBSI is still a subject of methodological debates (3,4,6),  
19 morbidity is well documented and includes severe sepsis and septic shock, septic thrombophlebitis,  
20 endocarditis and haematogenous spreading (7,8). Beside these clinical complications, economic  
21 impact is important as healthcare-associated infections lead to increased costs, hospital length of stay  
22 and antimicrobial treatments. The cost of CRBSIs ranges between \$33,000 and \$75,000, depending on  
23 the type of ICU (9).

24 Microbial contamination leads to the development of complex bacterial or fungal biofilm communities,  
25 which are potential sources of BSI. A biofilm is an assemblage of microbial cells that is associated with

1 a surface and encased in a self-produced extracellular matrix (10). From this biofilm, planktonic (*i.e.*  
2 free-swimming) cells can be released, causing a BSI.

3 Biofilms on CVC have first been described in 1982, during an outbreak of *Staphylococcus epidermidis*  
4 BSI (11). In this study, polysaccharide-mediated microbial adhesion to catheters was significantly  
5 associated with clinical strains, as compared to skin isolates. Since this pioneer report, numerous  
6 studies confirmed the involvement of biofilm in the pathogenesis of CVC-related infections and the  
7 epidemiological importance of this phenomenon.

8

9 With extensive use of CVCs, CRBSIs will continue to pose a serious threat unless preventive strategies,  
10 diagnostic techniques, and treatment modalities are improved to address and tackle the  
11 physiopathologic mechanisms of CVC-associated biofilm formation. The aims of this review are to  
12 precise characteristics of biofilm formation on CVCs and to give some clues about how to prevent its  
13 formation and to improve its eradication.

14

## 1 Biofilm formation on central venous catheters

2 A biofilm is a microbial consortia associated with a surface and embedded in an extracellular matrix.  
3 Microbial contamination, colonization, and biofilm formation on catheter surfaces can occur as soon  
4 as 24 hours after insertion and involve several steps (12).

5 After catheter insertion, the surface of the device is immediately covered by a conditioning film made  
6 of organic macromolecules such as pyruvate, glucose and fibrinogen (13). Microbial contamination of  
7 the catheter surface can originate from the skin microbiota, the catheter hub, haematogenous seeding  
8 from another infected site, or, less frequently, from a non-sterile infusate (14). Extra luminal  
9 contamination from the skin prevails in the first 10 days of catheter insertion and therefore mostly  
10 concerns short-term catheters (inserted for  $\leq 14$  days). On the other hand, intra luminal  
11 contamination, mainly from the hub, increases with duration of catheterization and concerns long-  
12 term catheters (15,16). Depending on the source of contamination, in order of prevalence, the 4  
13 groups of microbes that most commonly cause CRBSI are as follows : coagulase-negative *staphylococci*,  
14 *Staphylococcus aureus*, enteric Gram-negative bacilli and *Candida* spp. (2,17).

15

16 Microbial adhesion on catheter is increased by the prior formation of the conditioning film. It also  
17 depends on physico-chemical characteristics (including surface roughness, hydrophobicity and  
18 electrostatic interactions (18)) and on properties of the microbial cell (presence of adhesins such as  
19 fimbriae, flagella, or surface-associated polysaccharides or proteins that helps anchoring the cell on  
20 catheter surface (19)). These bacterial pioneers facilitate the arrival of other pathogens by providing  
21 more diverse adhesion sites. They multiply and produce the extracellular matrix (ECM) that holds the  
22 biofilm together, leading to an irreversible attachment to the catheter surface (20).

23 The ECM is composed of water, polysaccharides, proteins, extracellular DNA and lipids (12,18,20,21).

24 ECM provides mechanical stability to biofilms, improve their adhesion onto catheters and form a  
25 cohesive, three-dimensional polymer network that interconnects biofilm cells. It also protects  
26 microbial cells against external aggressions including host immune defences and antibiotics (20).

1 Biofilm architecture can also be strongly influenced by the interaction of anionic extracellular  
2 polymeric substance (EPS), containing carboxylic groups, with multivalent cations. For example,  $\text{Ca}^{2+}$   
3 ions can form a bridge between polyanionic alginate molecules, increasing mechanical stability (22).

4 In several Gram-negative bacteria, intracellular level of the second messenger cyclic di-GMP is involved  
5 in the switch from planktonic to biofilm life-style. Furthermore, cell-to-cell communication  
6 mechanisms (such as quorum sensing, QS) plays a key-role in biofilm maturation and matrix production  
7 (20).

8 Once a mature biofilm is established, planktonic bacteria may disperse from the community, cause  
9 CRBSI and spread to other sites of the body. The most problematic feature of mature biofilms in case  
10 of catheter-related infection is an increased ability of biofilm cells to survive to antimicrobial agents  
11 and the host immune system (23). This ability, called biofilm tolerance towards antibiotics, is the main  
12 reason explaining the difficulty to eradicate biofilms and control biofilm-related infections, and is the  
13 topic of a dedicated review of this biofilm issue.

14 Considering the difficulties to eradicate device-related bacterial infections, preventive strategies are  
15 cornerstone to tackle catheter-associated biofilm formation.

16

17

## 1 Preventing biofilm formation on central venous catheters

2 Measures to minimize the risk of catheter colonization and infection should meet a triple specification:  
3 efficacy, patient's safety and cost-effectiveness. In this regard, the best preventive strategy is to avoid  
4 any unnecessary catheterization or to reduce indwelling duration when a CVC is required.

5 Subclavian site should be preferred to the jugular and femoral venous accesses to reduce infection risk  
6 (24). Maximal sterile barrier precautions (handwashing, sterile gloves, large drape, sterile gown, mask,  
7 and cap) during CVC insertion significantly reduce the incidence of CRBSI, as compared with standard  
8 precautions (e.g., sterile gloves and small drapes) (25,26).

9 In a meta-analysis of eight studies, the use of bedside ultrasound for the placement of CVCs  
10 significantly reduced mechanical complications compared with the standard landmark placement  
11 technique (relative risk [RR] = 0.22; 95% confidence interval [CI] = 0.10--0.45) but did not significantly  
12 reduced the risk of infection (27). Skin antisepsis before catheter insertion with 2% chlorhexidine-  
13 alcohol has been demonstrated to significantly reduce CRBSI, as compared with povidone iodine-  
14 alcohol (28,29).

15

16 After insertion, risk of infection declines following standardization of aseptic care (30–32) whereas  
17 insertion and maintenance of intravascular catheters by inexperienced staff might increase the risk for  
18 catheter colonization and CRBSI (33,34). Furthermore, establishment of an experienced infusion  
19 therapy team to insert and maintain catheters decreases the rate of CRBSI by up to eight times (35). An  
20 infusion therapy team is also cost-effective, particularly in medical centres with high rates of catheter-  
21 related infections (36). Use of a chlorhexidine gluconate-impregnated sponge (CHGIS) in intravascular  
22 catheter dressings reduce catheter-related infections and is cost-effective (37–39).

23 Mupirocin ointment on the insertion sites of CVCs reduced the risk for CRBSI (40) but it has been  
24 associated with mupirocin resistance (41), and might adversely affect the integrity of polyurethane  
25 catheters (42). Such a preventive approach is not recommended (43).

1

2 If incidence density is still high after all these measures have been implemented, antimicrobial-coated  
3 catheters can be proposed, but only for short-term catheters, as antimicrobial activity decreases over  
4 time. One meta-analysis (44) demonstrated that catheters coated with chlorhexidine/silver  
5 sulfadiazine reduced the risk for CRBSI compared with standard noncoated catheters (45). However,  
6 in a multicenter randomized trial, CVCs impregnated on both the external and internal surfaces with  
7 minocycline/rifampin were associated with lower rates of CRBSI when compared with the first-  
8 generation chlorhexidine-silver sulfadiazine impregnated catheters (46). Due to their broad range  
9 spectrum of bactericidal activity, antimicrobial peptides (AMPs) have demonstrated a strong potential  
10 as anti-biofilm agents. Catheters coated by AMPs killing bacteria upon contact or via controlled release  
11 may therefore constitute promising future strategies to prevent catheter colonization (47).

12

13 Beside antimicrobial-impregnated catheters, preventive locks (instillation of highly concentrated  
14 antibiotic solutions left to dwell in the catheter for 12 to 24h) can be proposed for long-term catheters.  
15 The use of antibiotic (such as vancomycin or gentamicin) catheter flush or lock solutions could lead to  
16 the emergence of antibiotic-resistant organisms. However, non-antibiotic locks have been developed  
17 such as minocycline and edetic acid (EDTA), which has been successful to prevent catheter-related  
18 infection (48–50). Other non-antibiotic substances have been proposed such as ethanol or taurolidine-  
19 citrate but large studies are still needed to fully elucidate the benefits and the risks of such approaches  
20 (51–53).

21

22 Based on current knowledge on biofilm formation, non-biocidal approaches to prevent catheter  
23 colonization and CRBSI are under active research and may bring new preventive strategies. For  
24 instance, treatment of abiotic surfaces through the adsorption of different bacterial polysaccharides  
25 has a long-lasting antiadhesive effect and significantly inhibits mature biofilm development of a broad  
26 spectrum of pathogenic bacteria (54). The nonleaching polymeric sulfobetaine (polySB), which

1 coordinates water molecules on the catheter surface, reduced *in vitro* and *in vivo* adherence on both  
2 the external and the internal surfaces of polySB-modified catheters compared to unmodified catheters  
3 (55). Coating of TIVAP by methyl-cellulose, a polymer with both eukaryotic and bacterial cells anti-  
4 adhesive property, was also efficient in reducing adhesion and biofilm formation of *Pseudomonas*  
5 *aeruginosa* and *S. aureus* in a rat model of TIVAP colonization (54). Finally, a seducing prophylaxis, as  
6 was shown for urinary catheter (56,57), would be to contaminate the catheter by a preventive  
7 microbiota that would interfere with colonization by pathogenic bacteria. Indeed, adhering bacteria  
8 can prevent entry of incoming bacteria into already formed biofilms by several mechanisms including  
9 downregulation of adhesion and biofilm formation genes ; molecules interfering with bacterial quorum  
10 sensing ; degradation of components of the matrix ; production of dispersion signals (58,59).

11 At the moment, these data are limited to research, probably because of high costs associated with the  
12 development of compounds or devices amenable for clinical studies as well as non-adapted legislation  
13 to use bacterial interference to fight device-related infections.

14

15 Failure of prevention leads to catheter colonization and CRBSI, whose diagnosis relies on the  
16 association of clinical and microbiological synergy. Choosing the best tools to make diagnosis of CRBSI  
17 is important to treat rapidly with the right molecules or to avoid unnecessary removal of the catheter  
18 when possible.

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## 1 **Diagnosis**

2 The diagnosis of CRBSI and associated biofilm may be difficult and relies on clinical as well as laboratory  
3 examination. It is suspected when the patient presents fever and/or local cutaneous signs (erythema,  
4 swelling, tenderness, purulent drainage around the catheter exit-site and tunnel or port-pocket  
5 infections), which are nonetheless not specific or sensitive enough. Complications like haematogenous  
6 dissemination can be severe.

7 When suspected by clinical symptoms, the diagnosis of CRBSI requires a microbiological confirmation.  
8 Catheter-sparing diagnostic procedures are justified when the causal link between catheter and fever  
9 or BSI is not demonstrated, for instance in a patient with isolated fever and no local signs of infection.

10 The only validated catheter-sparing method is the use of paired blood culture, *i.e.* consecutive sample  
11 of blood from the CVC (central blood culture) and from a peripheral vein (peripheral blood culture).

12 CRBSI is demonstrated when colony counts are at least 3-fold higher in quantitative central blood  
13 cultures than in peripheral blood culture, (2,60–64). This method has 74-84% sensitivity and 98-100%  
14 specificity (65). Differential time to positivity, whereby a non-quantitative central blood culture that  
15 becomes positive at least 2 hours earlier than the peripheral blood culture is also currently available  
16 with most automated systems (86-92% sensitivity ; 79-87% specificity) (65). However, the  
17 interpretation of such methods can be hampered by blood culture contamination during sample  
18 because of suboptimal skin or blood bottles disinfection, or because of previous antimicrobial therapy  
19 (that may reduce both sensitivity and specificity of these tests). Paired blood cultures perform poorly  
20 with specific pathogens such as *Candida* spp. (66) and *S. aureus*. Kaasch *et al.* observed a poor  
21 diagnostic performance (sensitivity 37%, specificity 77%, Positive Predictive Value 46%, Negative  
22 Predictive Value 70%), in patients with CRBSI caused by *S. aureus* (67).

23 Beside, semiquantitative superficial cultures proved to be a useful diagnostic tool for ruling out CRBSI  
24 among patients with short- and long-term catheters, including TIVAPs. In case of BSI, skin samples  
25 (obtained by rubbing the area around the insertion site with a dry cotton swab), and inner hub samples

1 (obtained by using alginate swabs that are introduced into the hub and rubbed repeatedly against its  
2 inner surface) have a Negative Predictive Value between 93.5% and 96.4%, according to 2 prospective  
3 studies (68,69).

4 After catheter has been removed, the definition of catheter-related infection is based on the  
5 association of clinical signs (local or systemic) and a significant catheter colonization. Two methods are  
6 currently used to perform catheter tip culture. The Maki's roll-plate semi-quantitative culture method  
7 (70) is based on rolling the distal tip back and forth on an agar plate. At least fifteen colony forming  
8 units (CFU) from a 5-cm segment of catheter tip define a catheter colonization (45-84% sensitivity ;  
9 85% specificity) (4). However, this method virtually only detects extra luminal and distal colonization,  
10 and may not retrieve organisms that are strongly held in the biofilm layer. To improve the detection  
11 of biofilm bacteria, quantitative culture techniques (threshold:  $\geq 10^3$  CFU/catheter segment) have been  
12 developed (71), including the catheter sonication or vortexing (dislodging organisms from the external  
13 and internal surfaces of the catheters and possibly releasing organisms embedded within the biofilm  
14 layer). Many studies have compared these techniques and no difference has been seen for the most  
15 (68,72,73). However, catheter tip culture appears to be not sensitive enough to diagnose TIVAP-related  
16 infections. Indeed, one study reported that after TIVAP removal, tip culture was only 46% sensitive,  
17 whereas septum culture was 93.3% sensitive for confirming the diagnosis of TIVAP-related BSI (74).  
18 Bouza *et al* reported that combination of cultures of multiple sites of TIVAP was the best technique to  
19 diagnose TIVAP colonization (75,76). Another frequent challenge is to diagnose CRBSI in patients who  
20 already received systemic antibiotics, leading to false-negative results of blood or catheter tip culture.

21

22 Based on these limits, several methods have been proposed to improve our ability to detect catheter-  
23 associated biofilms. Even if electron microscopy is seducing to diagnose biofilm on the catheter  
24 surface, it is time-consuming, expensive and does not allow any microbial identification or antibiotic  
25 susceptibility testing. Studies by transmission and scanning electron microscopy have shown that

1 almost all indwelling vascular catheters, even those for which quantitative catheter cultures are  
2 negative, are colonized by micro-organisms.

3 Molecular methods improved the detection and identification of microorganisms, including in biofilm,  
4 both on the inner and the outer surface of catheters (77–79). One study reported combination of  
5 molecular methods to bring additional benefit (DNA extraction followed by gene amplification)  
6 (80). The comparison of the culture-based method with results from the molecular analysis using clone  
7 libraries, DGGE (denaturant gradient gel electrophoresis) and sequencing showed that the same  
8 bacteria could be identified using molecular and culture methods. However, molecular approaches  
9 were more sensitive than culture approaches and detected bacterial colonization in 78% of tested  
10 catheters (versus 39% with cultivation method) with different microorganisms on internal and external  
11 site of the catheter and frequent polymicrobial biofilms. Even if promising, these molecular approaches  
12 are difficult to translate in routine for several reasons. First, due to the lack of validated thresholds, it  
13 is difficult to distinguish contamination from colonization. Furthermore, the detection of bacterial DNA  
14 can be related to dead cells with no significant clinical impact.

15 So far, the diagnosis of CRBSI mostly relies on the association of clinical signs and laboratory findings.  
16 According to the clinical status of the patient, the type of catheter, the identified microorganism,  
17 different strategies to treat CRBSIs can be proposed.

18

19

## 1 **Eradication**

2 The hallmark of mature biofilm is their extreme tolerance towards antimicrobial agents and in  
3 particular antibiotics (23). This ability, called biofilm tolerance towards antibiotics, is the topic of a  
4 dedicated review of this biofilm issue. Main mechanisms of this phenomenon include : a reduced  
5 antibiotic penetration because of higher cell density and presence of the ECM (81), difference in gene  
6 expression such as multidrug efflux pumps and stress-response regulons and reduced growth and  
7 metabolic activity in the depth of biofilm. Indeed, oxygen levels and nutrients such as carbon sources  
8 are depleted near the centre of biofilm (82). Even if the above-mentioned mechanisms are important,  
9 it nowadays appears that most of biofilm tolerance towards antibiotics is due to the presence of  
10 persister cells, microbial cells that have entered a non- or extremely slow-growing physiological state.  
11 Persister cells are present in biofilms and planktonic cultures (0.001% to 0.1% of a bacterial population)  
12 and are the leading cause of infection recurrence (83).

13 Because of this extreme tolerance and the difficulty to eradicate biofilms, removal of the catheter and  
14 systemic antibiotic treatment is the gold-standard of CRBSI treatment (2).

15 However, due to the patient's general condition, some catheters are considered more precious such  
16 as long-term dialysis catheters or TIVAP. In these particular situations, the risk-benefit balance must  
17 be evaluated and a conservative treatment can be proposed, including antimicrobial lock therapy (ALT)  
18 (2). ALT consists in the instillation of highly concentrated antibiotic solutions (up to 1,000 times the  
19 MIC) left to dwell in the catheter for 12 to 24h. In the largest published study, ALT combined with  
20 systemic antibiotics seems to be effective for treating CRBSI (82% were cured), especially in Gram-  
21 negative and CoNS episodes, but treatment failure was observed in 9/10 cases due to *S. aureus* and  
22 1/5 case due to *P. aeruginosa* (84). In 21 open trials of ALT for CRBSI involving long-term catheters,  
23 with or without concomitant parenteral therapy, catheter salvage without relapse was obtained in  
24 77% of episodes (2). Two controlled clinical trials of the use of ALT together included only 92 patients,  
25 and treatment was successful in 58% of the control subjects and 75% of the patients treated with ALT  
26 (85,86). Currently, IDSA recommends the use of ALT associated with systemic antibiotics for the

1 conservative treatment of uncomplicated long-term catheter-related BSI caused by CoNS or  
2 *Enterobacteriaceae*.

3 Regarding CRBSIs caused by *Candida* species, current guidelines recommend catheter removal. In  
4 situations where the need for catheter salvage appears to outweigh the risks, echinocandins, liposomal  
5 amphotericin B and ethanol appear to be the most promising antifungal lock therapy strategies (87).

6 Ethanol locks have also been proposed for the treatment of bacterial CRBSI due to its activity on a  
7 broad range of microorganisms, and its anti-biofilm activity. It has been shown to both prevent and  
8 treat CRBSI. Two-hour exposure to 70% ethanol is required to kill established biofilms of Gram-positive  
9 or -negative bacteria, and *Candida* species *in vitro* (88–91). Many retrospective paediatric studies  
10 suggest that using the ethanol-lock technique for persistent catheter related infections in children with  
11 long-term intravascular devices is effective in salvaging the line, with a low rate of recurrences (92–  
12 94).

13 Considering the non-optimal efficiency of ALT (risk of failure and clinical evidence against its use for  
14 some species like *S. aureus*), the need for alternative or novel strategies has become of paramount  
15 importance. Based on theoretical benefits against antibiotic tolerance, several therapeutics have been  
16 evaluated to eradicate biofilm : anti-matrix, jamming with biofilm formation and maturation signals  
17 and anti-persisters approaches.

18 Degradation of the matrix is a way to weaken biofilm and several strategies have been proposed. First,  
19 enzymes can degrade various components of the EPS. The use of nucleases as an anti-biofilm strategy  
20 has been explored against a number of bacterial strains (95). The glycoside hydrolase dispersin B has  
21 demonstrated activity *in vivo*, lowering the rate of catheter colonization by *S. aureus* in combination  
22 with triclosan in a rabbit model of infection (96). Alginate lyase degrades a polysaccharide known as  
23 alginate and has also demonstrated *in vivo* efficacy, enhancing the clearance of mucoid *P.*  
24 *aeruginosa* when coadministered with amikacin in a rabbit model of endocarditis (97). Another  
25 strategy relies on the use of cation chelators (such as EDTA) that destabilizes the matrix. One recent  
26 study demonstrated that use of tetrasodium EDTA (30 mg/ml) as an adjuvant to gentamicin (5 mg/ml)

1 ALT, associated with systemic antibiotics, completely eradicated Gram-positive and Gram-negative  
2 bacterial biofilms in TIVAP implanted in rats (98).

3 Nitric oxide (NO), an endogenous product of anaerobic metabolism, which is known to induce  
4 dispersal, has recently shown promising effects, alone as nanoparticles or polymers formulation, or in  
5 combination notably with ciprofloxacin, to fight mature biofilms (99–103).

6 Jamming with biofilm formation and maturation signals is also currently explored through the use of  
7 phytochemicals to interfere with bacterial quorum sensing (QS) signalling pathways (104,105). Natural  
8 biofilm-controlling compounds have been demonstrated as inhibitors of quorum sensing like acyl  
9 homoserine lactones (AHLs) for Gram-negative bacteria or auto inducing peptides (AIPs) for Gram-  
10 positive bacteria. Both Gram-positive and Gram-negative bacteria utilize autoinducer-2 (AI-2). Bis-  
11 (3'5')-cyclic di-guanylic acid (c-di-GMP) is believed to be a ubiquitous second messenger signal  
12 molecule and has been implicated in biofilm dispersion in *P. aeruginosa*, where elevated c-di-GMP  
13 levels inhibited effective surface detachment (106). Some molecules that antagonize the enzymes that  
14 synthesize c-di-GMP and inhibit biofilm formation by *P. aeruginosa* and *Acinetobacter baumannii* are  
15 reported (107).

16 Considering that persisters are responsible for the most part of biofilm tolerance towards antibiotics,  
17 the identification of a potent broad-range anti-persister compound is nowadays considered as a quest  
18 for a holy grail (108,109).

19 Among molecules that have been found to somehow awake or sensitize persisters, Allison *et al* (110)  
20 showed that gentamicin was significantly potentiated against persisters by specific metabolites that  
21 enter upper glycolysis (glucose, mannitol and fructose), as well as pyruvate *in vitro*, whereas  
22 gentamicin was effective *in vivo* in combination with mannitol against *Escherichia coli*, and in  
23 combination with fructose against *S. aureus*. pH-mediated susceptibility of bacteria to aminoglycosides  
24 has been described few decades ago (111–113). Inspired by these data, one study showed that pH-  
25 mediated potentiation of aminoglycosides by L-arginine (a non-toxic basic amino acid) is effective

1 against both *in vitro* and *in vivo* biofilms produced by Gram-positive and Gram-negative nosocomial  
2 pathogens (7).

3 Another example of molecule sensitizing persister is acyldepsipeptide antibiotic (ADEP4), which can  
4 activate the ClpP protease, which degrades over 400 intracellular targets, forcing cells to self-digest  
5 and die. Combining ADEP4 with rifampin may lead to complete eradication of *S. aureus* biofilms *in vitro*  
6 and in a mouse model of a chronic infection (114). Cis-2-decenoic acid has also been shown to increase  
7 metabolic activity of biofilm persister cells of *E. coli* and *P. aeruginosa* and to potentiate activity of  
8 ciprofloxacin (115).

9 Furthermore, some molecules capable of directly killing persisters have also been identified. For  
10 example, DNA cross-linker agents such as mitomycin or cisplatin (116) or compounds increasing  
11 membrane permeability such as Aryl Alkyl Lysines (117) or the molecule NH125 (118) have been shown  
12 to efficiently kill persisters from methicillin-resistant *S. aureus*.

13 Lastly, since the emergence of multidrug resistant pathogens, bacteriophages return to daylight and  
14 are seducing agents against biofilm due to their quick diffusion in the biofilm matrix (119). For instance,  
15 an experimental study demonstrated *in vitro* efficacy of bacteriophage on decreasing mean colony  
16 forming units in silicon discs inoculated with *S. aureus* strains ; in a rabbit model, scanning electron  
17 microscopy demonstrated that biofilms had disappeared of the surface of the catheter after treatment  
18 (120,121).

19 For each of these above-mentioned strategies, clinical studies are now required before allowing  
20 translation to routine.

21

22

23

## 1 **Conclusion**

2 CRBSI is one of the major cause of nosocomial infection and a challenge to treat. The degree of severity  
3 and the persistence of infections are worsened when microorganisms form biofilms. In contrast to  
4 infections caused by planktonic bacteria that respond relatively well to standard antibiotic therapy,  
5 biofilm-forming bacteria tend to cause chronic infections whereby infections persist despite seemingly  
6 adequate antibiotic therapy.

7 Antimicrobial lock therapy (ALT) appears a promising approach for treatment of catheter associated  
8 BSI. To counteract the biofilm tolerance problems, innovative strategies like use of phytochemicals  
9 agents or anti-biofilm peptides are currently explored.

10 Understanding downstream processes at the molecular level in biofilm formation will help us to  
11 develop new approach to treating these infections.

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