



HAL
open science

Central venous catheters and biofilms: where do we stand in 2017?

Marie Gomet, Fabrice Compain, Christophe Beloin, David Lebeaux

► To cite this version:

Marie Gomet, Fabrice Compain, Christophe Beloin, David Lebeaux. Central venous catheters and biofilms: where do we stand in 2017?. *APMIS*, 2017, Special Issue: Biofilm Infections, 125 (4), pp.365-375. 10.1111/apm.12665 . pasteur-01555423

HAL Id: pasteur-01555423

<https://pasteur.hal.science/pasteur-01555423>

Submitted on 5 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Biofilm special issue

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

3 Marie Gominet¹⁻², Fabrice Compain²⁻³, Christophe Beloin⁴, David Lebeaux¹⁻²

4

5 1- Service de Microbiologie, Unité Mobile de Microbiologie Clinique, Assistance

6 Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France.

7 2- Université Paris Descartes, Paris, France

8 3- Service de Microbiologie, Assistance Publique-Hôpitaux de Paris, Hôpital Européen

9 Georges Pompidou, Paris, France.

10 4- Institut Pasteur, Unité de Génétique des Biofilms, Département de Microbiologie,

11 Paris, France

12

13 **Corresponding author:** David Lebeaux, Service de Microbiologie, Hôpital Européen Georges

14 POMPIDOU, 20 rue Leblanc, 75015 PARIS. Phone: +33 1.56.09.39.51. Fax: +33 1.56.09.24.46.

15 david.lebeaux@aphp.fr

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 ≤ 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, 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.

19

20

21

22

23

24

25

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.

BIBLIOGRAPHY

1. Mermel LA. Prevention of intravascular catheter-related infections. *Ann Intern Med.* 2000 Mar 7;132(5):391–402.
2. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, et al. Clinical Practice Guidelines for the Diagnosis and Management of Intravascular Catheter-Related Infection: 2009 Update by the Infectious Diseases Society of America. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2009 Jul 1;49(1):1–45.
3. Enquête nationale de prévalence des infections nosocomiales et des traitements anti-infectieux en établissements de santé, France, mai-juin 2012 / 2013 / Maladies infectieuses / Rapports et synthèses / Publications et outils / Accueil [Internet]. [cited 2016 Jul 13]. Available from: <http://www.invs.sante.fr/Publications-et-outils/Rapports-et-syntheses/Maladies-infectieuses/2013/Enquete-nationale-de-prevalence-des-infections-nosocomiales-et-des-traitements-anti-infectieux-en-etablissements-de-sante-France-mai-juin-2012>
4. Guidelines for the Prevention of Intravascular Catheter-Related Infections [Internet]. [cited 2016 Apr 26]. Available from: <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5110a1.htm>
5. Gahlot R, Nigam C, Kumar V, Yadav G, Anupurba S. Catheter-related bloodstream infections. *Int J Crit Illn Inj Sci.* 2014;4(2):162–7.
6. Soufir L, Timsit JF, Mahe C, Carlet J, Regnier B, Chevret S. Attributable morbidity and mortality of catheter-related septicemia in critically ill patients: a matched, risk-adjusted, cohort study. *Infect Control Hosp Epidemiol.* 1999 Jun;20(6):396–401.
7. Lebeaux D, Chauhan A, Létoffé S, Fischer F, de Reuse H, Beloin C, et al. pH-mediated potentiation of aminoglycosides kills bacterial persisters and eradicates in vivo biofilms. *J Infect Dis.* 2014 Nov 1;210(9):1357–66.
8. Lebeaux D, Larroque B, Gellen-Dautremer J, Leflon-Guibout V, Dreyer C, Bialek S, et al. Clinical outcome after a totally implantable venous access port-related infection in cancer patients: a prospective study and review of the literature. *Medicine (Baltimore).* 2012 Nov;91(6):309–18.
9. Hollenbeak CS. The cost of catheter-related bloodstream infections: implications for the value of prevention. *J Infus Nurs Off Publ Infus Nurses Soc.* 2011 Oct;34(5):309–13.
10. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science.* 1999 May 21;284(5418):1318–22.
11. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infect Immun.* 1982 Jul;37(1):318–26.
12. Donlan RM. Biofilms and device-associated infections. *Emerg Infect Dis.* 2001;7(2):277–81.
13. Murga R, Miller JM, Donlan RM. Biofilm formation by gram-negative bacteria on central venous catheter connectors: effect of conditioning films in a laboratory model. *J Clin Microbiol.* 2001 Jun;39(6):2294–7.
14. Raad I, Costerton W, Sabharwal U, Sacilowski M, Anaissie E, Bodey GP. Ultrastructural analysis of indwelling vascular catheters: a quantitative relationship between luminal colonization and duration of placement. *J Infect Dis.* 1993 Aug;168(2):400–7.

15. El Helou G, Viola GM, Hachem R, Han XY, Raad II. Rapidly growing mycobacterial bloodstream infections. *Lancet Infect Dis*. 2013 Feb;13(2):166–74.
16. Safdar N, Maki DG. The pathogenesis of catheter-related bloodstream infection with noncuffed short-term central venous catheters. *Intensive Care Med*. 2004 Jan;30(1):62–7.
17. Lebeaux D, Fernández-Hidalgo N, Chauhan A, Lee S, Ghigo J-M, Almirante B, et al. Management of infections related to totally implantable venous-access ports: challenges and perspectives. *Lancet Infect Dis*. 2014 Feb;14(2):146–59.
18. Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, et al. Bacterial biofilms in nature and disease. *Annu Rev Microbiol*. 1987;41:435–64.
19. Donlan RM. Biofilms: Microbial Life on Surfaces. *Emerg Infect Dis*. 2002 Sep;8(9):881–90.
20. Flemming H-C, Wingender J. The biofilm matrix. *Nat Rev Microbiol*. 2010 Sep;8(9):623–33.
21. Costerton JW, Geesey GG, Cheng KJ. How bacteria stick. *Sci Am*. 1978 Jan;238(1):86–95.
22. Körstgens V, Flemming HC, Wingender J, Borchard W. Influence of calcium ions on the mechanical properties of a model biofilm of mucoid *Pseudomonas aeruginosa*. *Water Sci Technol J Int Assoc Water Pollut Res*. 2001;43(6):49–57.
23. Lebeaux D, Ghigo J-M, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. *Microbiol Mol Biol Rev MMBR*. 2014 Sep;78(3):510–43.
24. Lorente L, Henry C, Martín MM, Jiménez A, Mora ML. Central venous catheter-related infection in a prospective and observational study of 2,595 catheters. *Crit Care*. 2005;9(6):R631–5.
25. Mermel LA, McCormick RD, Springman SR, Maki DG. The pathogenesis and epidemiology of catheter-related infection with pulmonary artery Swan-Ganz catheters: a prospective study utilizing molecular subtyping. *Am J Med*. 1991 Sep 16;91(3B):197S – 205S.
26. Raad II, Hohn DC, Gilbreath BJ, Suleiman N, Hill LA, Brusco PA, et al. Prevention of central venous catheter-related infections by using maximal sterile barrier precautions during insertion. *Infect Control Hosp Epidemiol*. 1994 Apr;15(4 Pt 1):231–8.
27. Randolph AG, Cook DJ, Gonzales CA, Pribble CG. Ultrasound guidance for placement of central venous catheters: a meta-analysis of the literature. *Crit Care Med*. 1996 Dec;24(12):2053–8.
28. Chaiyakunapruk N, Veenstra DL, Lipsky BA, Saint S. Chlorhexidine compared with povidone-iodine solution for vascular catheter-site care: a meta-analysis. *Ann Intern Med*. 2002 Jun 4;136(11):792–801.
29. Mimoz O, Lucet J-C, Kerforne T, Pascal J, Souweine B, Goudet V, et al. Skin antisepsis with chlorhexidine-alcohol versus povidone iodine-alcohol, with and without skin scrubbing, for prevention of intravascular-catheter-related infection (CLEAN): an open-label, multicentre, randomised, controlled, two-by-two factorial trial. *Lancet Lond Engl*. 2015 Nov 21;386(10008):2069–77.

30. Sherertz RJ, Ely EW, Westbrook DM, Gledhill KS, Streed SA, Kiger B, et al. Education of physicians-in-training can decrease the risk for vascular catheter infection. *Ann Intern Med*. 2000 Apr 18;132(8):641–8.
31. Faubion WC, Wesley JR, Khalidi N, Silva J. Total parenteral nutrition catheter sepsis: impact of the team approach. *JPEN J Parenter Enteral Nutr*. 1986 Dec;10(6):642–5.
32. Frampton GK, Harris P, Cooper K, Cooper T, Cleland J, Jones J, et al. Educational interventions for preventing vascular catheter bloodstream infections in critical care: evidence map, systematic review and economic evaluation. *Health Technol Assess Winch Engl*. 2014 Feb;18(15):1–365.
33. Armstrong CW, Mayhall CG, Miller KB, Newsome HH, Sugerman HJ, Dalton HP, et al. Prospective study of catheter replacement and other risk factors for infection of hyperalimentation catheters. *J Infect Dis*. 1986 Nov;154(5):808–16.
34. Eggimann P, Harbarth S, Constantin MN, Touveneau S, Chevrolet JC, Pittet D. Impact of a prevention strategy targeted at vascular-access care on incidence of infections acquired in intensive care. *Lancet Lond Engl*. 2000 May 27;355(9218):1864–8.
35. Brunelle D. Impact of a dedicated infusion therapy team on the reduction of catheter-related nosocomial infections. *J Infus Nurs Off Publ Infus Nurses Soc*. 2003 Dec;26(6):362–6.
36. Miller JM, Goetz AM, Squier C, Muder RR. Reduction in nosocomial intravenous device-related bacteremias after institution of an intravenous therapy team. *J Intraven Nurs Off Publ Intraven Nurses Soc*. 1996 Apr;19(2):103–6.
37. Timsit J-F, Schwebel C, Bouadma L, Geffroy A, Garrouste-Orgeas M, Pease S, et al. Chlorhexidine-impregnated sponges and less frequent dressing changes for prevention of catheter-related infections in critically ill adults: a randomized controlled trial. *JAMA*. 2009 Mar 25;301(12):1231–41.
38. Ho KM, Litton E. Use of chlorhexidine-impregnated dressing to prevent vascular and epidural catheter colonization and infection: a meta-analysis. *J Antimicrob Chemother*. 2006 Aug 1;58(2):281–7.
39. Arvaniti K, Lathyris D, Clouva-Molyvdas P, Haidich A-B, Mouloudi E, Synnefaki E, et al. Comparison of Oligon catheters and chlorhexidine-impregnated sponges with standard multilumen central venous catheters for prevention of associated colonization and infections in intensive care unit patients: a multicenter, randomized, controlled study. *Crit Care Med*. 2012 Feb;40(2):420–9.
40. Sesso R, Barbosa D, Leme IL, Sader H, Canziani ME, Manfredi S, et al. Staphylococcus aureus prophylaxis in hemodialysis patients using central venous catheter: effect of mupirocin ointment. *J Am Soc Nephrol JASN*. 1998 Jun;9(6):1085–92.
41. Zakrzewska-Bode A, Muyltjens HL, Liem KD, Hoogkamp-Korstanje JA. Mupirocin resistance in coagulase-negative staphylococci, after topical prophylaxis for the reduction of colonization of central venous catheters. *J Hosp Infect*. 1995 Nov;31(3):189–93.
42. Rao SP, Oreopoulos DG. Unusual complications of a polyurethane PD catheter. *Perit Dial Int J Int Soc Perit Dial*. 1997 Aug;17(4):410–2.

43. O'Grady NP, Alexander M, Burns LA, Dellinger EP, Garland J, Heard SO, et al. Summary of Recommendations: Guidelines for the Prevention of Intravascular Catheter-related Infections. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2011 May 1;52(9):1087–99.
44. Veenstra DL, Saint S, Saha S, Lumley T, Sullivan SD. Efficacy of antiseptic-impregnated central venous catheters in preventing catheter-related bloodstream infection: a meta-analysis. *JAMA*. 1999 Jan 20;281(3):261–7.
45. Maki DG, Stolz SM, Wheeler S, Mermel LA. Prevention of central venous catheter-related bloodstream infection by use of an antiseptic-impregnated catheter. A randomized, controlled trial. *Ann Intern Med*. 1997 Aug 15;127(4):257–66.
46. Darouiche RO, Raad II, Heard SO, Thornby JI, Wenker OC, Gabrielli A, et al. A comparison of two antimicrobial-impregnated central venous catheters. Catheter Study Group. *N Engl J Med*. 1999 Jan 7;340(1):1–8.
47. Sánchez-Gómez S, Martínez-de-Tejada G. Antimicrobial Peptides as Anti-biofilm Agents in Medical Implants. *Curr Top Med Chem*. 2016 Jul 13;
48. Campos RP, do Nascimento MM, Chula DC, Riella MC. Minocycline-EDTA lock solution prevents catheter-related bacteremia in hemodialysis. *J Am Soc Nephrol JASN*. 2011 Oct;22(10):1939–45.
49. Chatzinikolaou I, Zipf TF, Hanna H, Umphrey J, Roberts WM, Sherertz R, et al. Minocycline-ethylenediaminetetraacetate lock solution for the prevention of implantable port infections in children with cancer. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2003 Jan 1;36(1):116–9.
50. Raad I, Hachem R, Tcholakian RK, Sherertz R. Efficacy of minocycline and EDTA lock solution in preventing catheter-related bacteremia, septic phlebitis, and endocarditis in rabbits. *Antimicrob Agents Chemother*. 2002 Feb;46(2):327–32.
51. Liu H, Liu H, Deng J, Chen L, Yuan L, Wu Y. Preventing catheter-related bacteremia with taurolidine-citrate catheter locks: a systematic review and meta-analysis. *Blood Purif*. 2014;37(3):179–87.
52. Kavosi Z, Sarikhani Khorrami M, Keshavarz K, Jafari A, Hashemi Meshkini A, Safaei HR, et al. Is Taurolidine-citrate an effective and cost-effective hemodialysis catheter lock solution? A systematic review and cost-effectiveness analysis. *Med J Islam Repub Iran*. 2016;30:347.
53. Souweine B, Lautrette A, Gruson D, Canet E, Klouche K, Argaud L, et al. Ethanol lock and risk of hemodialysis catheter infection in critically ill patients. A randomized controlled trial. *Am J Respir Crit Care Med*. 2015 May 1;191(9):1024–32.
54. Chauhan A, Bernardin A, Mussard W, Kriegel I, Estève M, Ghigo J-M, et al. Preventing Biofilm Formation and Associated Occlusion by Biomimetic Glycocalyxlike Polymer in Central Venous Catheters. *J Infect Dis*. 2014 Nov 1;210(9):1347–56.
55. Smith RS, Zhang Z, Bouchard M, Li J, Lapp HS, Brotske GR, et al. Vascular catheters with a nonleaching poly-sulfobetaine surface modification reduce thrombus formation and microbial attachment. *Sci Transl Med*. 2012 Sep 26;4(153):153ra132.
56. Darouiche RO, Green BG, Donovan WH, Chen D, Schwartz M, Merritt J, et al. Multicenter randomized controlled trial of bacterial interference for prevention of urinary tract infection in patients with neurogenic bladder. *Urology*. 2011 Aug;78(2):341–6.

57. Darouiche RO, Donovan WH, Del Terzo M, Thornby JI, Rudy DC, Hull RA. Pilot trial of bacterial interference for preventing urinary tract infection. *Urology*. 2001 Sep;58(3):339–44.
58. Rendueles O, Ghigo J-M. Multi-species biofilms: how to avoid unfriendly neighbors. *FEMS Microbiol Rev*. 2012 Sep;36(5):972–89.
59. Rendueles O, Ghigo J-M. Mechanisms of Competition in Biofilm Communities. *Microbiol Spectr*. 2015 Jun;3(3).
60. Seifert H, Cornely O, Seggewiss K, Decker M, Stefanik D, Wisplinghoff H, et al. Bloodstream Infection in Neutropenic Cancer Patients Related to Short-Term Nontunnelled Catheters Determined by Quantitative Blood Cultures, Differential Time to Positivity, and Molecular Epidemiological Typing with Pulsed-Field Gel Electrophoresis. *J Clin Microbiol*. 2003 Jan;41(1):118–23.
61. García X, Sabatier C, Ferrer R, Fontanals D, Duarte M, Colomina M, et al. Differential time to positivity of blood cultures: a valid method for diagnosing catheter-related bloodstream infections in the intensive care unit. *Med Intensiva Soc Esp Med Intensiva Unidades Coronarias*. 2012 Apr;36(3):169–76.
62. Gowardman JR, Jeffries P, Lassig-Smith M, Stuart J, Jarrett P, Deans R, et al. A comparative assessment of two conservative methods for the diagnosis of catheter-related infection in critically ill patients. *Intensive Care Med*. 2013 Jan;39(1):109–16.
63. Raad I. Intravascular-catheter-related infections. *Lancet Lond Engl*. 1998 Mar 21;351(9106):893–8.
64. Eggimann P. Diagnosis of intravascular catheter infection. *Curr Opin Infect Dis*. 2007 Aug;20(4):353–9.
65. Safdar N, Fine JP, Maki DG. Meta-analysis: methods for diagnosing intravascular device-related bloodstream infection. *Ann Intern Med*. 2005 Mar 15;142(6):451–66.
66. Bouza E, Alcalá L, Muñoz P, Martín-Rabadán P, Guembe M, Rodríguez-Créixems M, et al. Can microbiologists help to assess catheter involvement in candidaemic patients before removal? *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2013 Feb;19(2):E129–35.
67. Kaasch AJ, Rieg S, Hellmich M, Kern WV, Seifert H. Differential time to positivity is not predictive for central line-related *Staphylococcus aureus* bloodstream infection in routine clinical care. *J Infect*. 2014 Jan;68(1):58–61.
68. Bouza E, Alvarado N, Alcalá L, Sánchez-Conde M, Pérez MJ, Muñoz P, et al. A Prospective, Randomized, and Comparative Study of 3 Different Methods for the Diagnosis of Intravascular Catheter Colonization. *Clin Infect Dis*. 2005 Apr 15;40(8):1096–100.
69. Guembe M, Martín-Rabadán P, Echenagusia A, Camúñez F, Rodríguez-Rosales G, Simó G, et al. Value of superficial cultures for prediction of catheter-related bloodstream infection in long-term catheters: a prospective study. *J Clin Microbiol*. 2013 Sep;51(9):3025–30.
70. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. *N Engl J Med*. 1977 Jun 9;296(23):1305–9.

71. Brun-Buisson C, Abrouk F, Legrand P, Huet Y, Larabi S, Rapin M. Diagnosis of central venous catheter-related sepsis. Critical level of quantitative tip cultures. *Arch Intern Med*. 1987 May;147(5):873–7.
72. Erb S, Frei R, Schregenberger K, Dangel M, Nogarth D, Widmer AF. Sonication for Diagnosis of Catheter-Related Infection Is Not Better Than Traditional Roll-Plate Culture: A Prospective Cohort Study With 975 Central Venous Catheters. *Clin Infect Dis*. 2014 Aug 15;59(4):541–4.
73. Slobbe L, Barzouhi A el, Boersma E, Rijnders BJA. Comparison of the Roll Plate Method to the Sonication Method To Diagnose Catheter Colonization and Bacteremia in Patients with Long-Term Tunnelled Catheters: a Randomized Prospective Study. *J Clin Microbiol*. 2009 Apr 1;47(4):885–8.
74. Douard MC, Arlet G, Longuet P, Troje C, Rouveau M, Ponscarne D, et al. Diagnosis of venous access port-related infections. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 1999 Nov;29(5):1197–202.
75. Guembe M, Martín-Rabadán P, Echenagusia A, Camúñez F, Rodríguez-Rosales G, Simó G, et al. How Should Long-Term Tunneled Central Venous Catheters Be Managed in Microbiology Laboratories in Order To Provide an Accurate Diagnosis of Colonization? *J Clin Microbiol*. 2012 Mar 1;50(3):1003–7.
76. Bouza E, Martín-Rabadán P, Echenagusia A, Camúñez F, Rodríguez-Rosales G, Simó G, et al. Diagnosis of venous access port colonization requires cultures from multiple sites: should guidelines be amended? *Diagn Microbiol Infect Dis*. 2014 Feb;78(2):162–7.
77. Afshari A, Schrenzel J, Ieven M, Harbarth S. Bench-to-bedside review: Rapid molecular diagnostics for bloodstream infection--a new frontier? *Crit Care Lond Engl*. 2012;16(3):222.
78. Murray PR, Masur H. Current Approaches to the Diagnosis of Bacterial and Fungal Bloodstream Infections for the ICU. *Crit Care Med*. 2012 Dec;40(12):3277–82.
79. Fenollar F, Raoult D. Molecular diagnosis of bloodstream infections caused by non-cultivable bacteria. *Int J Antimicrob Agents*. 2007 Nov;30 Suppl 1:S7–15.
80. Larsen MK, Thomsen TR, Moser C, Høiby N, Nielsen PH. Use of cultivation-dependent and -independent techniques to assess contamination of central venous catheters: a pilot study. *BMC Clin Pathol*. 2008 Oct 28;8:10.
81. Campanac C, Pineau L, Payard A, Baziard-Mouysset G, Roques C. Interactions between Biocide Cationic Agents and Bacterial Biofilms. *Antimicrob Agents Chemother*. 2002 May 1;46(5):1469–74.
82. De Beer D, Stoodley P, Roe F, Lewandowski Z. Effects of biofilm structures on oxygen distribution and mass transport. *Biotechnol Bioeng*. 1994 May 1;43(11):1131–8.
83. Lewis K. Persister cells, dormancy and infectious disease. *Nat Rev Microbiol*. 2007 Jan;5(1):48–56.
84. Fernandez-Hidalgo N, Almirante B, Calleja R, Ruiz I, Planes AM, Rodriguez D, et al. Antibiotic-lock therapy for long-term intravascular catheter-related bacteraemia: results of an open, non-comparative study. *J Antimicrob Chemother*. 2006 Jun;57(6):1172–80.

85. Rijnders BJ, Van Wijngaerden E, Vandecasteele SJ, Stas M, Peetermans WE. Treatment of long-term intravascular catheter-related bacteraemia with antibiotic lock: randomized, placebo-controlled trial. *J Antimicrob Chemother.* 2005 Jan;55(1):90–4.
86. Fortún J, Grill F, Martín-Dávila P, Blázquez J, Tato M, Sánchez-Corral J, et al. Treatment of long-term intravascular catheter-related bacteraemia with antibiotic-lock therapy. *J Antimicrob Chemother.* 2006 Oct;58(4):816–21.
87. Walraven CJ, Lee SA. Antifungal lock therapy. *Antimicrob Agents Chemother.* 2013 Jan;57(1):1–8.
88. Peters BM, Ward RM, Rane HS, Lee SA, Noverr MC. Efficacy of ethanol against *Candida albicans* and *Staphylococcus aureus* polymicrobial biofilms. *Antimicrob Agents Chemother.* 2013 Jan;57(1):74–82.
89. Chambers ST, Peddie B, Pithie A. Ethanol disinfection of plastic-adherent micro-organisms. *J Hosp Infect.* 2006 Jun;63(2):193–6.
90. Balestrino D, Souweine B, Charbonnel N, Lautrette A, Aumeran C, Traoré O, et al. Eradication of microorganisms embedded in biofilm by an ethanol-based catheter lock solution. *Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc.* 2009 Oct;24(10):3204–9.
91. Rane HS, Bernardo SM, Walraven CJ, Lee SA. In vitro analyses of ethanol activity against *Candida albicans* biofilms. *Antimicrob Agents Chemother.* 2012 Aug;56(8):4487–9.
92. Onland W, Shin CE, Fustar S, Rushing T, Wong W-Y. Ethanol-lock technique for persistent bacteremia of long-term intravascular devices in pediatric patients. *Arch Pediatr Adolesc Med.* 2006 Oct;160(10):1049–53.
93. Dannenberg C, Bierbach U, Rothe A, Beer J, Körholz D. Ethanol-lock technique in the treatment of bloodstream infections in pediatric oncology patients with broviac catheter. *J Pediatr Hematol Oncol.* 2003 Aug;25(8):616–21.
94. Valentine KM. Ethanol lock therapy for catheter-associated blood stream infections in a pediatric intensive care unit. *Pediatr Crit Care Med J Soc Crit Care Med World Fed Pediatr Intensive Crit Care Soc.* 2011 Nov;12(6):e292–6.
95. Okshevsky M, Regina VR, Meyer RL. Extracellular DNA as a target for biofilm control. *Curr Opin Biotechnol.* 2015 Jun;33:73–80.
96. Darouiche RO, Mansouri MD, Gawande PV, Madhyastha S. Antimicrobial and antibiofilm efficacy of triclosan and DispersinB combination. *J Antimicrob Chemother.* 2009 Jul;64(1):88–93.
97. Bayer AS, Park S, Ramos MC, Nast CC, Eftekhari F, Schiller NL. Effects of alginate on the natural history and antibiotic therapy of experimental endocarditis caused by mucoid *Pseudomonas aeruginosa*. *Infect Immun.* 1992 Oct;60(10):3979–85.
98. Chauhan A, Lebeaux D, Ghigo J-M, Beloin C. Full and Broad-Spectrum In Vivo Eradication of Catheter-Associated Biofilms Using Gentamicin-EDTA Antibiotic Lock Therapy. *Antimicrob Agents Chemother.* 2012 Dec 1;56(12):6310–8.

99. Duong HTT, Jung K, Kutty SK, Agustina S, Adnan NNM, Basuki JS, et al. Nanoparticle (star polymer) delivery of nitric oxide effectively negates *Pseudomonas aeruginosa* biofilm formation. *Biomacromolecules*. 2014 Jul 14;15(7):2583–9.
100. Lu Y, Slomberg DL, Schoenfisch MH. Nitric oxide-releasing chitosan oligosaccharides as antibacterial agents. *Biomaterials*. 2014 Feb;35(5):1716–24.
101. Verderosa AD, Mansour SC, de la Fuente-Núñez C, Hancock REW, Fairfull-Smith KE. Synthesis and Evaluation of Ciprofloxacin-Nitroxide Conjugates as Anti-Biofilm Agents. *Mol Basel Switz*. 2016;21(7).
102. Craven M, Kasper SH, Canfield MJ, Diaz-Morales RR, Hrabie JA, Cady NC, et al. Nitric oxide-releasing polyacrylonitrile disperses biofilms formed by wound-relevant pathogenic bacteria. *J Appl Microbiol*. 2016 Apr;120(4):1085–99.
103. Barraud N, Kjelleberg S, Rice SA. Dispersal from Microbial Biofilms. *Microbiol Spectr*. 2015 Dec;3(6).
104. Estrela AB, Abraham W-R. Combining Biofilm-Controlling Compounds and Antibiotics as a Promising New Way to Control Biofilm Infections. *Pharmaceuticals*. 2010 May 11;3(5):1374–93.
105. Blackledge MS, Worthington RJ, Melander C. Biologically-Inspired Strategies for Combating Bacterial Biofilms. *Curr Opin Pharmacol*. 2013 Oct;13(5):699–706.
106. Ha D-G, O'Toole GA. c-di-GMP and its Effects on Biofilm Formation and Dispersion: a *Pseudomonas Aeruginosa* Review. *Microbiol Spectr*. 2015 Apr;3(2):MB – 0003–2014.
107. Sambanthamoorthy K, Luo C, Pattabiraman N, Feng X, Koestler B, Waters CM, et al. Identification of small molecules inhibiting diguanylate cyclases to control bacterial biofilm development. *Biofouling*. 2014 Jan;30(1):17–28.
108. Hong-Geller E, Micheva-Viteva SN. Targeting Bacterial Persistence to Develop Therapeutics Against Infectious Disease. In: Vallisuta O, Olimat S, editors. *Drug Discovery and Development - From Molecules to Medicine* [Internet]. InTech; 2015 [cited 2016 Aug 28]. Available from: <http://www.intechopen.com/books/drug-discovery-and-development-from-molecules-to-medicine/targeting-bacterial-persistence-to-develop-therapeutics-against-infectious-disease>
109. Wood TK. Combatting bacterial persister cells. *Biotechnol Bioeng*. 2016 Mar;113(3):476–83.
110. Allison KR, Brynildsen MP, Collins JJ. Metabolite-enabled eradication of bacterial persisters by aminoglycosides. *Nature*. 2011 May 12;473(7346):216–20.
111. Taber HW, Mueller JP, Miller PF, Arrow AS. Bacterial uptake of aminoglycoside antibiotics. *Microbiol Rev*. 1987 Dec;51(4):439–57.
112. Schlessinger D. Failure of aminoglycoside antibiotics to kill anaerobic, low-pH, and resistant cultures. *Clin Microbiol Rev*. 1988 Jan;1(1):54–9.
113. Sabath LD, Toftegaard I. Rapid Microassays for Clindamycin and Gentamicin When Present Together and the Effect of pH and of Each on the Antibacterial Activity of the Other. *Antimicrob Agents Chemother*. 1974 Jul;6(1):54–9.

114. Conlon BP, Nakayasu ES, Fleck LE, LaFleur MD, Isabella VM, Coleman K, et al. Activated ClpP kills persisters and eradicates a chronic biofilm infection. *Nature*. 2013 Nov 21;503(7476):365–70.
115. Marques CNH, Morozov A, Planzos P, Zelaya HM. The Fatty Acid Signaling Molecule cis-2-Decenoic Acid Increases Metabolic Activity and Reverts Persister Cells to an Antimicrobial-Susceptible State. *Appl Environ Microbiol*. 2014 Nov;80(22):6976–91.
116. Chowdhury N, Wood TL, Martínez-Vázquez M, García-Contreras R, Wood TK. DNA-crosslinker cisplatin eradicates bacterial persister cells. *Biotechnol Bioeng*. 2016 Sep 1;113(9):1984–92.
117. Ghosh C, Manjunath GB, Konai MM, Uppu DSSM, Hoque J, Paramanandham K, et al. Aryl-Alkyl-Lysines: Agents That Kill Planktonic Cells, Persister Cells, Biofilms of MRSA and Protect Mice from Skin-Infection. *PloS One*. 2015;10(12):e0144094.
118. Kim W, Conery AL, Rajamuthiah R, Fuchs BB, Ausubel FM, Mylonakis E. Identification of an Antimicrobial Agent Effective against Methicillin-Resistant *Staphylococcus aureus* Persisters Using a Fluorescence-Based Screening Strategy. *PloS One*. 2015;10(6):e0127640.
119. Motlagh AM, Bhattacharjee AS, Goel R. Biofilm control with natural and genetically-modified phages. *World J Microbiol Biotechnol*. 2016 Apr;32(4):67.
120. Lungren MP, Donlan RM, Kankotia R, Paxton BE, Falk I, Christensen D, et al. Bacteriophage K antimicrobial-lock technique for treatment of *Staphylococcus aureus* central venous catheter-related infection: a leporine model efficacy analysis. *J Vasc Interv Radiol JVIR*. 2014 Oct;25(10):1627–32.
121. Lungren MP, Christensen D, Kankotia R, Falk I, Paxton BE, Kim CY. Bacteriophage K for reduction of *Staphylococcus aureus* biofilm on central venous catheter material. *Bacteriophage*. 2013 Oct 1;3(4):e26825.