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Venous Catheters Infections Associated to *Staphylococcus* Biofilms: Worldwide Problem of the 21st Century

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ABSTRACT

Venous catheters are increasingly used in medical care. However, these devices are easily colonized by microorganisms. *Staphylococcus* spp. are the dominant species in catheter-related infections because of their ability to form biofilms on their surfaces, giving them resistance to antimicrobial agents and leading to recalcitrant chronic infections and therapeutic failure. In addition, the emergence of methicillin-resistant Staphylococci is a global scourge for public health. In this review, an update of our knowledge on staphylococcal biofilms, their formation steps, regulation, resistance to methicillin, their involvements in the worldwide emergence of venous catheters-related infections and treatment strategies for biofilm prevention and eradication have been reported.

INTRODUCTION

Several bacterial species colonize the human body and could be both useful and dangerous for health. Among them, *Staphylococcus aureus*, which is a commensal of the skin, is considered a fearsome pathogen implicated in nosocomial infections [1], especially those related to medical devices such as venous catheters (Figure 1). The emergence of Methicillin-Resistant *S. aureus* (MRSA) is mainly the major clinical problem in hospitals [2]. Its ability to produce biofilm was considered a major virulence factor of pathogenesis [3] causing the failure of these devices [4]. Bacterial colonization on medical devices is the first step in the development of chronic infections and their persistence [5]. Biofilms consist of one or more species adhering to a biotic or abiotic surface and surrounded by an extracellular polysaccharide matrix produced by them [6].In recent years, it has become apparent that their importance in the medical community is crucial. Indeed, what makes these biofilms of paramount importance is their ability to resist antibiotic treatment and immune system attacks leading to therapeutic failure and persistence of infections [7]. In addition, biofilms are involved not only in the colonization of surfaces but they represent a reservoir of dissemination of bacteria in the body [8]. Venous catheters are often used for infusion, administration of drug and nutrition. However, the inappropriate use of these medical devices can lead to catheter-related infections, increasing morbidity and mortality, length of hospital stay and the cost of the care [9].

***Staphylococcus* species and their virulence factors involved in biofilm formation**

Staphylococcus spp. are Gram-positive cocci, non-motile, non-sporulated, aero-anaerobic facultative, classified into two main groups, coagulase positive

staphylococci and, most of them, coagulase negative staphylococci "CNS" [10] and are part of the natural flora of the skin and mucous membranes of humans [11].

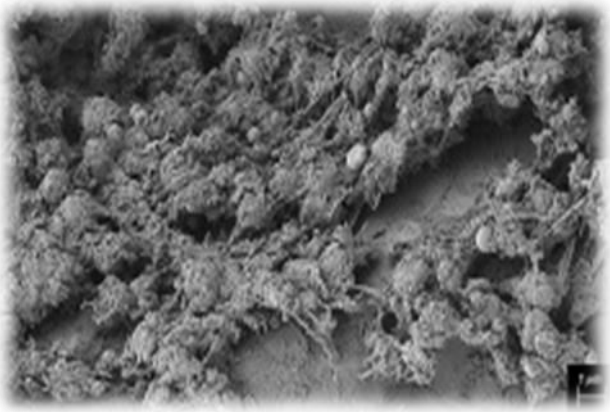

Figure 1: Scanning electron microscopy (SEM) examination of MRSA biofilm formation on central venous catheter implanted in rats [137].

Staphylococcus spp. are opportunistic human pathogens with remarkable adaptability [12]. *Staphylococcus epidermidis* and *S. aureus* are the main cause of catheters-related infections due to their virulence factors, mainly biofilm formation [11]. In recent years, the clinical emergence of methicillin-resistant *Staphylococcus* strains has created many therapeutic challenges for microbiologists and clinicians [10]. When intravenous catheters are implanted in patients, they are rapidly coated with body fluid proteins, such as fibrinogen, fibronectin and collagen, facilitating adhesion of *Staphylococcus* cells expressing on their cell wall surface proteins called the Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMS), that bind specifically to the above mentioned proteins [13,14]. The production of Polysaccharide Intercellular Adhesin (PIA) by enzymes encoded by *icaABCD* operon and composed of linear N-acetyl glucosamine residues linked to β-1-6 is the main mechanism involved in biofilm formation that may contribute to the persistence of infections [3,15]. This operon is composed of four genes encoding four proteins that are involved sequentially in: synthesis of a poly-N-acetylglucosamine polymer *(icaA)* in the presence of *icaD*, translocation of this polymer to the bacterial surface *(icaC)*, deacetylation of the polymer to allow it to attach to the surface *(icaB)*. However, the *icaR* gene plays the role of regulator of this operon [16]. This mechanism has been described for the first time in *S. epidermidis* [17]. These authors demonstrated that mutation of the transposon at the *ica* operon level of *S. epidermidis* altered PIA production and biofilm formation. The study of Mirzaee *et al.* [18] reported that 12/31 biofilm producers *S. aureus* strains (38.7%) were *ica*A and *ica*D positive. More recently, Manandhan *et al.* [19] reported that 22.9% of the biofilm producers *Staphylococcus* strains possessed the *ica*AD genes. However, some Staphylococci species that do not express PIA can also produce biofilm. For example, Kord *et al.* [20] have shown that 4.8% of *S. epidermidis* strains were biofilm producers despite the absence of the *ica* genes. This suggests that the ability to produce biofilm is not always related to the expression of the *ica* genes and may involves other adhesins in cell aggregation and adhesion [15,21] such as biofilm-associated proteins (Bap) [22], Autolysins [23], fibronectin binding proteins (FnBP) [24], collagen binding proteins (Can) [25], elastin binding proteins (EbpS) [26], Protein A [27] and α-enolase (Eno) [28]. Adhesion factors of *Staphylococcus* species and their role in pathogenesis are represented in table 1.

Regulation of *Staphylococcus* biofilm formation

In Staphylococci, biofilm formation is controlled by two systems. The first is named accessory gene regulator (agr). This system produces auto-inducing peptides that regulate the expression of virulence factors as a function of bacterial density [29,30]. Each *Staphylococcus* species contains an agr operon and a specific auto-inducer peptide that vary in length and sequence. In addition, all these peptides contain a thiolactone nucleus and an N-terminal extremity [31]. The proteins that intervene in the *quorum sensing* are regulated by the *agr*BDCA operon. This operon encodes a two-component system, AgrC and AgrA that will detect the auto-inducing peptides produced by the AgrB and AgrD proteins [29]. When the bacterial density is high, the self-inducing peptide activates the AgrC histidine kinase which in turn phosphorylates the AgrA response regulator. The latter binds the promoter P2 which activates RNAII which in turn encodes the proteins AgrA, AgrB, AgrC and AgrD and to the promoter P3 which activates the transcription of the RNAIII which in turn activates the expression of the genes [32,33]. The second regulator system is called staphylococcal accessory regulator A *(sarA)* that regulates also other virulence factors by

modulation of *agr* expression [34]. Sigma B *(sigB)* is an alternative factor that regulates the expression of survival factors against oxidative stress, antibiotics, temperature variations and other environmental stress [35].

Table 1: Adhesion factors involved in biofilm formation in Staphylococcus spp.

Factors		Gene	Function	Reference
Adhesins	biofilm associated protein	*bap*	Adhesin involved in attachment, adhesion on surfaces, and biofilm formation.	Seng *et al.*, [3].
	Autolysin/adhesion	*atLE*	Peptidoglycan hydrolase that plays a role in the degradation of the bacterial wall facilitates adhesion to polystyrene surfaces and biofilm production.	Porayath *et al.*, [23].
	Fibronectin binding protein	*fnBP A and B*	This adhesin is involved in primary adhesion of staphylococcal strains to surfaces and also in aggregation and intercellular adhesion.	Kırmusaoğlu, [149].
	Collagen binding protein	*can*	This extracellular protein of *S. aureus* binds to collagen and is involved in bacterial adhesion and immune evasion.	Herman-Bausier *et al.*, [25].
	Elastin binding protein	*ebpS*	Protein with a molecular weight of 25 kDa,present on the surface of *S. aureus* cells and promotes the colonization of mammalian tissues, facilitating pathogenesis.	Downer *et al.*, [150] ; Kot *et al.*, [28].
	Protein A	*spa*	This protein expressed by all strains of *S. aureus* is able to block opsonophagocytosis by binding to Fcγ in the presence of antibodies and plays an important role in the immune evasion and pathogenicity of Staphylococci.	Hong *et al.*, [27] ; Lacey *et al.*, [151].
	α-enolase	*eno*	This protein expressed on the surface of Staphylococci is able to bind to laminin thus allowing adhesion to the extracellular matrix and tissue colonization of the host.	Kot *et al.*, [28].

Staphylococcal enzymes involved in auto-biofilm dispersion

Biofilm dispersion is caused by the production of extracellular enzymes and surfactants that degrade the polymeric matrix [36]. Among these enzymes, proteases are mainly distinguished because of their ability to degrade extracellular proteins, major components of the matrix [37]. These molecules, regulated by *agr*, *sar*A and *sig*B systems, are considered among the most important virulence factors involved in the development of biofilms [38]. In addition, over-expression of proteases regulated by *sar*A and *sig*B systems has been reported to improve planktonic growth suggesting an inverse relationship between biofilm formation and expression of these enzymes [39]. *S. aureus* express one metalloprotease (aureolysinAur), two cysteine proteases (staphopains SspB and ScpA) and seven serine proteases (SspA and V8) [36]. However, *S. epidermidis* produces fewer proteases such as the metalloprotease SepA, the serine protease Espand the cysteine protease EcpA [38]. These enzymes are involved on the one hand in the acquisition of peptide nutrients but also in the evasion of the immune system of the host by interfering with neutrophils [40] and degradation of specific proteins composing the biofilm matrix in order to release planktonic bacteria. For example, V8 protease has the ability to cleave fibronectin binding protein and Bap. UreolysinAur is able to degrade clumping factor b and Bap. Nevertheless, staphopains A and B allow dispersion without specific protein cleavage [37]. Contrariwise, the metalloprotease SepA of *S. epidermidis* is involved in the intercellular accumulation stage where it cleaves the AtlE autolysin which will release the DNA necessary for the formation of the matrix "eDNA" [38]. Nucleases are the second class of enzymes produced by *Staphylococcus* spp. that degrade extracellular DNA (eDNA). In *S. aureus*, the system SaeRS is involved in nucleases regulation, which protects the bacteria against neutrophils released by the host during their detachment from the biofilm [41]. Nuclease (Nuc) and nuclease 2 (Nuc2) are the most common enzymes produced by *S. aureus* [36]. The implication of nucleases during biofilm dispersion has been demonstrated using nuclease deficient *S. aureus* strains that inhibited the liberation of planktonic bacteria [42]. PSMs (phenol soluble modulins) are peptide surfactants produced by *S. aureus* and *S. epidermidis*, and are also involved in the dispersion of biofilms. Their expression is controlled by the *agr* system [43]. PSMδ and PSMβ are the effector molecules produced by *S. aureus* and *S. epidermidis* respectively [39,44] showed that anti-PSMβ factors inhibited *S. epidermidis* strains spread from catheters, using mouse model of medical device related infection, concluding the importance of these surfactants in biofilm dispersal. Nonetheless, these surfactants are also involved during the stage of maturation of the biofilm and play

a major role in the formation of the three-dimensional structure (thickness, volume, roughness) [45].

Mechanism of *Staphylococcus* biofilm formation on intravenous catheters

Biofilm formation in *S. aureus* occurs in three stages: adhesion/attachment, maturation and dispersion [46]. After insertion of the catheter, the initial adhesion of the bacteria is done through the van der walls, electrostatic and hydrophobic interactions [46]. Once the surface of these devices is covered with host proteins (collagen, fibrinogen, fibronectin, vitronectin), the bacteria express genes that encode the MSCRAMMs that will interact with them [47]. The irreversible adhesion to the catheter is due to the production of the extracellular matrix [48]. Once the attachment is over, the *quorum sensing* regulator *agr* represses the expression of these factors which are useless [39]. During the maturation stage, the bacteria will become intergraded and multiply, producing a heterogeneous exopolysaccharide matrix composed of proteins, eDNA and teichoic acids, thus forming a three-dimensional structure also called "mushroom". Simultaneously, an intercellular aggregation took place after the PIA synthesis [47]. The bacteria organize themselves in the biofilm according to their metabolism and respiratory type. Thus, strict anaerobic bacteria will live at the bottom of the biofilm [48]. In addition, bacterial populations characterized by a very slow growth called “dormant or persistent cells” are observed during the exponential and stationary growth phases. These cells are endowed with a high resistance to antibiotics which can be the cause of the persistence of infections [49].Formation of biofilm is usually followed by a dispersal step. The latter is characterized by the release of planktonic bacteria in order to infect other sites and to form new biofilms [48]. This step can be triggered either by external physical forces (shearing fluids) or by the production of extracellular enzymes such as proteases, nucleases and PSM [37] as described above.

Methicillin-resistant *Staphylococcus* spp. (MRSA)

Staphylococcus aureus is one of the bacteria with an incredible ability to acquire resistance to a large number of antibiotics [50]. In recent years, the emergence of methicillin resistance in these bacteria constitutes a real global problem [51]. Indeed, it has been estimated that a 20% mortality rate is associated with infections caused by methicillin-resistant *S. aureus* (MRSA) [52]. MRSA were reported for the first time in 1961 [53]. Their resistance is due to the presence of the penicillin binding protein PBP2a, which has a low affinity to β-lactams. This protein is encoded by the *mecA* gene, which is carried in a mobile genomic element called staphylococcal cassette chromosome *mec* (SCC*mec*) [54].

Worldwide spread of venous catheter infections associated to *Staphylococcus* spp.

Venous catheters are increasingly used and are considered an essential element in modern medicine [55]. It has been reported that more than 80% of hospitalized patients have a central or peripheral intravascular catheter during their stay in hospital [56]. Indeed, a large number of microorganisms have been implicated in medical device associated infections such as intravenous catheters. *S. aureus* and *S. epidermidis* are the most incriminated species [57]. Their ability to form biofilm makes these infections more complicated [15]. These biofilms can be formed in the catheter lumen or on its outer surface [7] and can cause increase in morbidity, mortality, length of hospital stay and hospitalization costs [58]. In Italy, a retrospective study related to infections associated with central venous catheters was conducted over a period of 4 years (from 2007 to 2010). The prevalence of *S. epidermidis* and *S. aureus* was 12.78% and 10.55% respectively during this period [59]. A recent study in Pakistan showed that 64% of catheters were colonized by Gram positive bacteria and *S. aureus* was the most isolated species (39%) followed by *S. epidermidis* (16%) with the prevalence (59%) of MRSA [60]. MRSA infections have been a huge problem in hospitals since their emergence in the 1980s [61]. Khalil and Azqul [62] reported that CNS strains were the most isolates in catheters and 9.9% of patients hospitalized in cardiac care unit had a catheter related-bloodstream infection. Other recent studies conducted in Sweden, Iran, Belgium, India, USA and Poland reported also that *Staphylococcus* spp. strains were the most isolated from intravascular catheter infections [21,63-67]. In Algeria, a study [68] at the hemodialysis department of the Setif University Hospital recorded a rate of 22.4% Central Venous Catheter Related-Infections (CVC-RI). Among the causative microoganisms, CNS and *S. aureus* were found at a rate of 23.5% each and were all resistant to methicillin and other antibiotics [68]. These authors also mentioned that the weak hand hygiene and disinfection of the

skin before placement of catheters were the cause of 58.8% and 88.8% of CVC-RI respectively. Furthermore, in Mexico, Pérez-Zárate *et al*. [55] demonstrated that hand washing, use of sterile gown and preparation of medicines were considered as risk factors for biofilm formation and catheter related infections. In China, it was found that 57 out of 1523 hospitalized patients, in a kidney intensive care unit, had central venous Catheter Related Blood Stream Infection (CRBSI) and *S. aureus* was incriminated in 10 cases [69]. MRSA was reported the cause of 40% of hemodialysis catheter-related bacteremia in Taipei Veterans General Hospital-Taiwan [70]. Duration of catheterization was reported as major risk factor for biofilm formation and subsequent bacterial dissemination. Indeed, Passerini *et al*. [71] showed that 81% of venous catheters placed in patients for 1 to 14 days were colonized by bacteria in biofilm. In Mexico, another group of researchers found that patients using a longer central venous catheter were more likely to develop a catheter-related infection [56,68] confirmed also that longer duration of catheterization (≥ 10 days) is considered as risk factor of CVC-RI.

Treatment strategies for *Staphylococcus* spp. biofilm related infections

The infections caused by *Staphylococcus* spp. biofilms constitute a major problem of global public health because of the persistence and recalcitrance of these and the therapeutic failure that biofilms confer on them, increasing the mortality rate, morbidity but also hospital costs. For this, the development of anti-biofilms treatment has become a major priority for researchers for their prevention and eradication [46,72]. Several global studies have described several strategies to prevent and treat these biofilms such as nanoparticles, antimicrobial peptides, enzymes, bacteriophages, ultrasound therapy, photodynamic therapy, antimicrobial catheter lock solution, plants extracts and chelating agents [46].

Antimicrobial peptides: Antimicrobial peptides are positively charged cationic molecules, amphipathic and composed of 11 to 50 amino acids [73,74]. The first peptide discovered was LL-37 cathelicidin of human origin [75]. These molecules have a broad spectrum of activity (Gram negative and positive bacteria, fungi). They act by permeabilization of the cell membranes which leads to the appearance of pores thus leading to bacterial lysis [76]. Recent studies reported the anti-biofilm effect of these peptides. For example, Zapotoczna *et al*. [77] recorded that the peptide D-Bac8c^{2,5Leu} alone or combined with antibiotics seem to be a potential agent to treat *S. aureus* intravenous catheter related infections [78]. Demonstrated the capacity of the peptide Brevinin-1GHa (skin secretion of the frog *Hylarana guentheri*) to destabilize the cell membrane and eliminate the biofilm of *S. aureus*. Shurko *et al*. [79] showed also the strong inhibition of *S. aureus* biofilm by two peptides LL-13 and LL-17, which derivates from LL-37. Recently, a Dutch study showed that the combination of teicoplanin with SAAP-148 or SAAP-276 peptides showed a strong interaction with *S. aureus* biofilms [80]. Another Dutch study reported that SAAP-148 peptide seems to have an excellent antibacterial and anti-biofilm activities against MRSA biofilms and persisters cells [81]. The peptide derivatives (SPLUNC1 Δα4 SPLUNC1 Δα4M1) showed significantly higher anti-biofilm activity than the SPLUNC1 parent molecule and this is due to the α4 fraction, which has this activity. The latter can be improved by increasing the cationic and Tryptophan content (improvement of hydrophobicity and amphipathicity) [82]. Other peptides showed an anti-biofilm activity against *Staphylococcus* spp. as 17BIPHE2 (derivates from the human peptide LL-37) [83],citropin 1.1 (isolated from the frog *Litoria citropa*) and temporin A (isolated from the frog *Rana temporaria*) [84], peptide FLIP7 (isolated from maggots *Calliphora vicina*) [85], KR-12 and KE-18 [86], tachyplesin I (isolated from horseshoe crab) [87].

Nanoparticles: In recent years, it has emerged that nanotechnology is a promising way to treat infections. This technology uses metal nanoparticles that allow the elimination of bacteria by interacting with their components such as proteins, nucleic acids, peptides. This interaction causes the release of reactive oxygen, hydrogen peroxide (H_2O_2), hydroxyl radical (*OH) that disrupt cell membranes leading to bacterial lysis[88]. Moreover, these nanoparticles are characterized by their small size and high surface-to-mass ratio facilitating interactions [46,89]. Demonstrated that rhamnolipids (RL) coated with silver (Ag) and iron oxide (Fe_3O_4) exhibited important activity against *S. aureus* biofilms. This is due to the release of the reactive oxygen molecules. In addition, these RLs modify the hydrophobicity of the surfaces reducing bacterial adhesion [90]. Showed that the synergistic

combination of silver and antibiotics (fosfomycin, daptomycin, vancomycin, oxacillin) has eradicated *S. aureus* biofilm. [88] demonstrated recently the promising effect of Ag-thymol (ATNPs), Ag-usnic acid (AUNPs), Cu-thymol (CTNPs), and Cu-usnic acid (CUNPs) on MRSA biofilms. Other studies demonstrated also the anti-biofilm activity of Nitric Oxide "NO" [91], Zink oxide "ZnO" [92], gold [93] and SiO_2-Gentamicin nanohybrids[94].

Enzymes: Bacterial enzymes allow the dispersion of biofilms by acting on several components of the matrix (proteins, exopolysaccharids, eDNA). The extraction and purification of these enzymes can be used as a new alternative for the prevention and eradication of biofilms. Several groups of enzymes have been studied [37]. The first group is Proteases. In 2013, a serine protease V8 was reported as a promising enzyme to disturb staphylococcal biofilm [95]. Elchinger *et al.* [96] reported also that lavourzyme protease had a significant activity against *S. epidermidis* biofilm.The enzymes lysostaphin, α-amylase, bromelain, and papain tested against *S. aureus* biofilms (cultured in 10% human plasma) showed, after 2 and 24 hours of treatment, a decrease in biofilm biomass: up to 76% for lysostaphin, 97% for α-amylase and 98% for bromelain and papain. The three last proteases seem to be promising agents for biofilm treatment [97]. Similarly, it was reported that the proteinase K (2 µg/ml) inhibited the biofilm of bap-positive *S. aureus* V329, but it showed any effect on their planktonic cells [6]. Deoxyribonucleases can also be used as anti-biofilm agents. The nuclease NucB, isolated from *Bacillus licheniformis* was effective against staphylococcal biofilms [98]. DNase I allowed the disturbance of *S. aureus* biofilm by degradation of eDNA [99]. Another group of enzymes have also been used as a means of dispersing biofilms: glycoside hydrolases. The same study cited previously showed that the Dispersin B disrupted *S. aureus* biofilms by degrading poly (1,6) -N-acetyl-D-glucosamine polysaccharide [99]. Flemming *et al.* [100] showed also the dispersion of *S. aureus* biofilm by adding α-amylase (cleaves glycosidic link α (1,4) and cellulase (cleaves glycosidic link β(1,4). The enzyme that cleaves hyaluronic acid of the animal matrix, hyaluronidase is also able to disrupt aggregates forming biofilms [101]. The effect of Poly-N-Acetylglucosamine (PNAG) depolymerase (DA7) against staphylococcal biofilms have been recently demonstrated by [102]. This hydrolase was able to destabilize the biofilm by destruction of PNAG, the major polysaccharide of the matrix.

Antimicrobial catheter lock solutions: The antimicrobial catheter lock is considered as a novel strategy to treat intravascular catheter infection [103]. Antiseptics such as ethanol and Taurolock have been reported to be effective at eradicating biofilms aged three days or more [104]. The antimicrobial agents ML: 8 (1% v/v) and Citrox (1% v/v), which are generally used in the treatment of periodontal infections, were able to reduce staphylococcal biofilm biomass (> 97%) after only 24 hours of treatment [105]. Recent Spanish study showed that the administration of the combination of ethanol (40%) and heparin (60 IU) during 72 hours was effective *in vitro* against staphylococcal biofilm [106].Fibrinolytic agents as plasmin, streptokinase, and nattokinase (alone or combined with antibiotics) were successfully efficient in disturbance of *S. aureus* biofilms in rat model of intravascular catheter infection *in vivo* [103]. Bhatt *et al.* [107] demonstrated the anti-biofilm effect of a novel antibiotic-free formed from gas Plasma-Activated Disinfectant (PAD). This agent allowed reducing the biomass of the staphylococcal biofilm (MRSA and *S. epidermidis*) after 1 hour and a re-growth of the bacteria was not observed after 24 hours of incubation, making it a promising means for the eradication of biofilms. Other studies reported also the antimicrobial catheter lock solutions [108-111].

Antibodies: Since the production of new antibiotic molecules has become limited in recent years, researchers have resumed their interest in treating monoclonal antibodies as an alternative for preventing biofilm and treating infections that directly targets the pathogen [47,112]. An American study showed that PhnD-specific antibodies (Phosphonate ABC transporter substrate binding protein) inhibited *S. aureus* biofilm formation by preventing their fixation and aggregation [113]. Den Reijer *et al.* [114] showed that the immunogenic IsdA and SA0688 seem to be potential novel agents to prevent or treat *Staphylococcus* biofilm-associated infections. The *in vivo* effect of human antibody TRL1068 alone or with vancomycin against MRSA biofilms was investigated using a model of catheter-induced aortic valve infective endocarditis in rats. The results showed significant reductions in biofilm [112].

Bacteriophages: Bacteriophages are natural viruses that infect and replicate inside host bacteria, causing cell lysis and the release of new virions that infect other target bacteria. As a result, phages can enter biofilms; regulate the cell growth, making them a promising strategy for treating bacterial infections [115-116]. These phages have more advantages unlike antibiotics such as their activity on multi-drug resistant strains, low harmful effect on eukaryotic host cells, rapid reproduction and ease of isolation and low cost of production but mainly their high anti-biofilm potential [116]. In 2017, an American study investigated the anti-biofilm potential of phage lysin CF-301 against staphylococcal biofilms. The authors showed that this phage-lysin disrupted biofilm with minimal biofilm eradicating concentration 90 (MBEC90) values of 0.25 µg/ml (MRSA). CF-301 had an anti-biofilm activity at very low concentrations against MRSA biofilms formed on catheters, but also on mixed biofilms (*S. aureus* and *S. epidermidis*) [117]. Tkhilaishvili *et al*. [118] showed that bacteriophage Sb-1 had a synergistic effect with specific antibiotics ((fosfomycin, rifampin, vancomycin, daptomycin or ciprofloxacin) for the eradication of MRSA strains by degrading their extracellular matrix. The synergistic effect of the combination of *PYO* phage and antibiotics was also studied by Dickey and Perrot [119]. Other studies have also reported the potential anti-biofilm effect of phages [102,115,120,121].

Plants extracts: Since antiquity, humans use plants and their extracts as drugs for the treatment of infections [122]. In fact, these plants had several anti-inflammatory, antibacterial, antithrombotic, anti-allergic, hepato-protective and anti-carcinogenic activities [46]. The plant components that have strong antibacterial activity are classified as phytoalexins[123]. In addition, essential oils are also considered effective alternatives to antibiotics and antiseptics [124]. These aromatic oils are extracted from aromatic plants and contain various secondary molecules that play a protective role for plants [125]. In recent years, several studies have been interested in the anti-biofilm activities of plant extracts. For example, in 2016, an Iranian study demonstrated *in vitro* a strong anti-biofilm activity of coriander essential oil (*Coriandrum sativum L.*) against *S. aureus* with a Minimal Inhibitory Concentration (MIC) of 0.8 µl /ml [122]. Gandhi *et al*. [123] studied the effect of *Sesbania grandiflora* extracts against *S. aureus* biofilm and observed that this plant, which contains several molecules (alkaloids, flavonoids, saponins and tannins), inhibited carbohydrates and proteins (major components of the *S. aureus* biofilm matrix) thereby reducing biofilm formation. Khan *et al*. [126]. Found that the ethanolic extract of *Zanthoxylum armatum* fruits inhibited the quorum sensing of *S. aureus* (IC50= 32–256 µg/ml). Another recent Chinese study showed that peppermint essential oil inhibited *S. aureus* biofilm formation at 0.25 mg/ml and eradicated it at concentration ≥4 mg/ml [124]. Other studies have reported the anti-biofilm activity of *Aloe vera* [127], *Allium stipitatum* [128], *Syzygium cumini* L. Skeels [129] extracts, and *Lippia sidoides*, *Thymus vulgaris* and *Pimenta pseudochariophyllus* essential oils [130].

Metal chelators, sulfhydryl compounds: Another group of molecules with antimicrobial activity is the chelating agents that act on the biofilm by sequestering metal ions (iron, magnesium and calcium). These metal ions play an important role in the production of many proteins involved in biofilm formation such as adhesins and proteases [131]. Maisetta *et al*. [132] demonstrated that Ethylenediamine Tetra-Acetic Acid (EDTA) combined with the peptide (temporin 1Tb) was able to kill *S. epidermidis* in biofilm on silicone catheters . Martinez-Andrade *et al*. [133] showed during their *in-vitro* study that 17% EDTA combined with silver nanoparticles (AgNPs) had antimicrobial activity against *S. aureus* biofilms. In addition, this cationic chelator destabilizes biofilms and prevents their adhesion [134]. An Australian study reported that the iron chelator "deferiprone" seems to be a promising molecule to treat *Staphylococcus* biofilms by disrupting the synthesis of the PIA [72]. This iron chelator in combination with antibiotics eradicated the *S. epidermidis* biofilm [135]. Another study recorded also the anti-biofilm effect of iron chelators 2,2′-dipyridyl (2DP) and 1,2,3,4,6-Penta-O-Galloyl-b-D-Glucopyranose (PGG) on *S. aureus* biofilm formation [136]. On the other hand, other molecules such as sulfhydryl compounds such as dithiothreitol, beta-mercaptoethanol and cysteine also appear to have a significant antibacterial effect against *S. aureus* biofilm. These molecules are able to inhibit the formation of PIA, major component of the matrix of Staphylococci [46,72]. The study cited previously demonstrated that the use of a sulfhydryl compound (L-cycteine) at a concentration of

78µM in combination with the peptide 1Tb had reduced the biosynthesis of *S. epidermidis* ATCC 35984 PIA[132].

Nitric oxide: Nitric oxide (NO) is a diatomic and lipophilic gaseous molecule that plays a role in cellular signaling and immunity. This molecule has also an anti-inflammatory, bactericidal and bacteriostatic effect [137]. Furthermore, the NO signaling molecule plays a major role in dispersion of biofilms, which has been associated with a conditional reduction of c-di-GMP leading to matrix degradation and bacterial motility [138]. Several recent studies reported its positive effect on *Staphylococcus* biofilm making it a good alternative in therapeutic [137,139,140].

Photodynamic, Ultrasound and Laser Shock waves therapy: Photodynamic therapy is a new antibacterial alternative using photosensitizing molecules activated in the presence of O_2 by the light generating an oxidation of the biological molecules causing the destruction of the target microorganism. The advantage of this therapy is the lack of selection of resistance, but also, the low cost of treatment and the low risk of side effects [141]. Several studies reported that the photosensitizer 5-aminolevulinicacid (alone or combined with antibiotics) [142], $ZnPc^{n+}$ [143], Chlorin e6 (Ce6) [144], RB (RB-aPDT) (alone or combined with gentamicin) [141] and toluidine blue O [145] seem to be potential alternatives to treat *S. aureus* biofilm related infections. Other therapies such as ultrasound and laser shock waves seem also to give good results in the eradication of biofilms. The advantage with these physical methods is that they are non-invasive, do not cost much and the risk of inducing bacterial resistance is very low [146-148].

CONCLUSION

This review allowed us to point out that the emergence of catheter-related infections caused by staphylococcal biofilm, particularly SARM, and linked to high mortality and morbidity, is a universal public health problem. Surveillance of these infections has become a challenge for clinicians and microbiologists. Measures and training on hygiene rules and placement of venous catheters should be taken to control the onset of these infections and in particular to ensure patient safety. In addition, the major problem with these infections is that the biofilm confers on bacterial populations increased resistance to antibiotics causing a therapeutic failure. As a result, the development of new strategies as an alternative to antibiotics has become a real challenge for the control and eradication of staphylococcal biofilms in recent years. Despite the discovery of many strategies, unfortunately, some of them cannot be administered to patients such as chelators, nanoparticles toxic at certain doses and bacteriophages. Further research should be based on natural molecules such as antimicrobial peptides, which look promising for the treatment of infections. In addition, several researchers have so far demonstrated the *in vitro* anti-biofilm potential of most molecules and therapies (alone or in combination). However, additional studies need to be conducted to validate these findings, such as cytotoxicity, genotoxicity and pharmacology of the molecules, as well as *in vivo* studies.

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