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REVIEW ARTICLE

A Consideration of Antibacterial Agent Efficacies in the Treatment and Prevention of Formation of *Staphylococcus aureus* Biofilm

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ABSTRACT

Staphylococcus aureus is a Gram-positive bacterium found frequently on a person's skin and sometimes in their upper respiratory tract. Although regarded primarily as a commensal of the human microbiota *S. aureus* shows the ability to become an opportunistic pathogen. Hence, it is a common cause of skin and lung infections and of food poisoning. *S. aureus* forms biofilms, complex communities of bacteria inside an exopolysaccharide matrix, which adhere to different surfaces, including those associated with hospital-acquired infections such as catheters, shunts and other implanted medical devices. In this instance, the presence of proteins adsorbed to the surface of the biomaterial provides a nutrient source for bacterial growth.

Due to antimicrobial resistance, use of longstanding antibiotics alone is increasingly an ineffective therapeutic intervention for biofilm-related infections. Therefore, a growing concern is the treatment of medical devices in order to prevent antibiotic resistance associated with routine handling of these items in a healthcare setting. Consequently, several different biotechnological approaches have targeted a practical solution to *S. aureus* biofilm formation. These include novel antibiotics administered alone or combined with other compounds, application of natural products like enzymes and antimicrobial peptides, and harnessing of nanoparticles and phage therapy. This brief article provides an overview of each of these cutting-edge methods aimed at inhibition of *S. aureus* biofilms. Development of an effective agent to prevent and treat biofilm formation would represent a significant step forward for infection control of methicillin-resistant *S. aureus* (MRSA) and other antibiotic-resistant strains that provides a major global public health challenge. *J Microbiol Infect Dis 2019; 9(4):167-172.*

Keywords: *Staphylococcus aureus, bacterium, biofilm, infection, antibiotic, resistance*

INTRODUCTION

Biofilm formation is an important contributory factor in the establishment and persistence of bacterial infections, and thus is considered a principal reason for antimicrobial resistance [1]. Fossils belonging to different domains of microorganism, archaea and bacteria, and which date back over three billion years have been shown to contain biofilm. Evidently, biofilm formation is an archaic component of the prokaryotic life cycle as well as a critical determinant of survival across a broad spectrum of niche environments [2]. A biofilm is composed of a self-produced extracellular matrix (ECM) made of exopolysaccharide, commonly described as 'slime', that encloses a community, or colony, of microorganisms. The slime consists of multiple porous layers that contain channels to enable cells in the centre of the colony to receive nutrients and to remove metabolic waste products. Within this elaborate structure antimicrobial resistance is greatly enhanced [3]. Survival in hostile environments, including being subjected to host innate and adaptive immunity, is improved, thereby leading to chronic infection and inflammation. The production of bacterial biofilms is thought to be responsible for over 80% of persistent clinical infections, for which conventional treatments are no longer effective at achieving clearance [4].

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BIOFILM FORMATION

Catheters, shunts and other implanted medical devices provide suitable surfaces on which biofilms formed by both Gram-negative and Gram-positive bacteria can develop [5,6]. Most of these bacteria form part of a healthy person's microbiome, existing as commensals or in a mutually beneficial relationship with their host – usually in the saliva, mouth, gastrointestinal tract, nose, ear canal, mucosa or on the skin – where they help in numerous metabolic activities. However, they retain the potential for pathogenesis and, given the opportunity, their unregulated growth or presence in an atypical location can lead to infection [7]. The species most frequently detected *are Staphylococcus aureus, S. epidermidis, Streptococcus viridans, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa* [6]. The first two of these, the Gram-positive cocci *S. aureus* and S. epidermidis, together are estimated to cause between 50-70% of catheter biofilm infections, around 40-50% of prosthetic heart valve infections and over 85% of septicemia cases [5].

In the process of being used for their intended purpose in a healthcare setting medical devices and biomaterials are prone to contacting – and becoming covered by deposits of – naturally-occurring proteins on or in a patient's skin, tissues or body fluids [6]. This applies especially to blood plasma and serum proteins such as fibronectin, fibrinogen, thrombospondin and vitronectin, all of which may provide a platform for ECM formation [8]. The adherence by ECM components to serum proteins is a fundamental initial stage in the interaction between *S. aureus* and a human host. Once established on the underlying substrate, this bacterium employs a wide range of modes to facilitate colonization and dissemination [9].

There are five main steps involved in biofilm formation; in sequence these are attachment, multiplication, exodus, maturation and dispersal. Following alighting upon a surface, bacteria produce various cell proteins to enable steadfast adherence to different host matrix substrates. The next step, multiplication, happens in the nutrient-rich conditions provided by the presence of serum proteins, when the complex of *S. aureus* cells begins to divide and accumulate. Approximately six hours after the initiation of multiplication, these cells start to be released, a process called exodus, which is followed by maturation. At this step, a microcolony structure will form, which further increases contact with the underlying substrate. Finally, when threshold cell density is reached, the level of which varies with conditions, triggers autogenous induction of the production of dispersal cells [10]. This process is under the command of *S. aureus* accessory gene regulator quorum sensing, a cell-to-cell communication system that controls expression of many genes in response to bacterial population density [7,10].

CHALLENGES TO TARGETING BIOFILMS

Inasmuch as biofilms maintain a barrier to effectively shield bacteria from potentially hostile conditions, their formation provides an extra degree of complexity to the challenge of overcoming antimicrobial resistance [11]. Biofilm bacteria are phenotypically distinct from their isogenic, free-floating planktonic bacteria counterparts by, for instance, actively expressing extracellular proteins, virulence factors and surfactants [12]. Treatments that are developed using only planktonic *S. aureus* (such as antibiotic susceptibility testing, performed routinely in diagnostic laboratories) are thus unlikely to be effective against the biofilm phenotype. Moreover, treating *S. aureus* biofilms based on the minimum inhibitory concentration (MIC) that suppresses growth of planktonic phenotypes may actually promote the emergence of antibiotic-resistant subpopulations within the ECM [13]. Even methicillin (MET)-sensitive strains may tolerate exposure to considerably higher concentrations of antibiotic, often exceeding those dosages that are achievable clinically without cytotoxic side-effects, thereby making these infections difficult to eradicate [14].

It is now acknowledged that biofilms are responsible for most chronic staphylococcal infections, especially those that are associated with indwelling medical devices, a mainstay of modern surgical practice [15]. Since *S. aureus* can successfully colonize and establish biofilms in different host environments (notably, prosthesis and implant surfaces, and tissues such as lung, bone and skin), this is a primary consideration in the design of therapies against

infection. However, the majority of treatments that are currently approved for clinical administration are applicable only to planktonic or acute *S. aureus* infections [16]. Hence, there is a pressing, and as yet unfulfilled, need for new therapeutic strategies to target biofilm-dwelling *S. aureus*. Novel agents under consideration and progress made in their development to date are discussed below.

TREATMENT FOR *STAPHYLOCOCCUS AUREUS* BIOFILM

Clinical case reports of biofilms associated with *S. aureus* infections have increased in number over recent years, a disquieting trend that makes it imperative to find novel therapies. Overuse and misuse of frontline antibiotics to combat *S. aureus* has led to the global emergence of multi-drug resistance, notably to MET, which makes it a difficult-to-treat infection. MET-resistant *S. aureus* (MRSA) can form biofilms, a property that underpins its virulence. This is of considerable relevance to hospitals and other healthcare facilities, settings in which MRSA is particularly prevalent and where it is associated with reduced clinical outcomes [17]. Therefore, a range of efforts has been made to ameliorate this major public health concern [18].

Antibiotics

Several approaches both to prevent and to treat *S. aureus* biofilm infections have been adopted. Based on recent findings MIC, MBIC (the minimum concentration of a chemical, usually a drug, that prevents visible biofilm formation) and MBEC (the minimum concentration necessary to eradicate preformed biofilm) each vary dependent on the antibiotic tested [18]. Other factors such as the apparent age of the biofilm (young or mature) should also be considered. Although rapid diagnosis of biofilm-producing infections is challenging early detection has a substantial influence on the type and effectiveness of treatment [19]. In general, combination therapy of more than one antibiotic, each from a different class, is far more effective than administration of a single antibiotic [20]. It is also asserted that, dependent on concentrations used, azithromycin (AZM, a broad-based antibiotic) and vancomycin (VAN, an agent of last resort) can both exert not an inhibitory influence but instead an inducing effect on biofilm formation [21].

In antibiotic sensitivity tests using the agar dilution method, clinically isolated strains were totally resistant to erythromycin, 75% resistant to trimethoprim/sulfamethoxazole, clindamycin and ciprofloxacin (CIP), 50% resistant to gentamicin (GEN), but completely susceptible to amikacin and tetracycline [22]. In another study various combinations of antibiotics were shown to be bacteriostatic or bactericidal to *S. aureus* strains. AZM along with fusidic acid (FA) and oxacillin (OXA) are effective when administered together against MET-sensitive *S. aureus* (MSSA). Cefazolin (CFZ), FA, GEN, OXA and rifampicin (RIF) is another group that displays antibacterial synergism. Other combinations with a similar bacteriostatic or bactericidal effect towards MSSA are AZM, CFZ, CIP and FA; AZM, FA and VAN; CFZ, GEN, RIF and VAN; AZM, CIP and FA; FA and GEN; and of RIF and VAN. On the other hand, only the combinations of CIP, FA and RIF and of FA, RIF and VAN are bactericidal against MRSA biofilms [23].

Natural products

Several different human body products are known to be generated as an innate immune defence against *S. aureus* biofilm. RNAIII-inhibiting peptide and the human cathelicidin peptide LL-37 and its synthetic mimetic D-LL-37 are examples of anti-microbial peptides (AMPs) that disrupt biofilm through either disturbance of embedded cells or its matrix, interruption of its signalling system or alteration in gene regulation of bacteria [18,24]. Enzymes including proteases, deoxyribonucleases and the metalloendopeptidase lysostaphin exhibit anti-biofilm properties such as rapidly disconnecting *S. aureus* biofilm from its substratum and impeding its formation [25]. While each of these enzymes involves a distinct biochemical pathway, when used in harness the effect is synergistic, so the overall antimicrobial activity increases commensurately [24].

In addition, phytochemicals and ethanol extracts from medicinal plants such as *Kaempferia rotunda L., Caesalpinia sappan L. and Cinnamomum burmanii* Nees ex Bl show inhibitory properties towards *S. aureus* biofilm growth [18,26]. These Indonesian plants provide an unusual, but probably not exclusive, botanical source of putative anti-biofilm agents.

Although it may seem that of these approaches the use of AMPs is the most promising, for both this and a strategy based on phytochemicals extrapolating from *in vitro* studies may not be an entirely reliable indicator of the human condition. Therefore, testing *in vivo* on an experimental model is a prerequisite to undertaking trials in volunteers [27,28], a focus for future research.

Antibodies are produced by the humoral arm of the immune system in response to antigen-specific stimulation. Vaccine design can be predicated on artificially inducing generation of host-protective immunoglobulins. Hence, the development of novel vaccines that stimulate a rapid and enhanced antibody response to *S. aureus* antigens is considered a potentially effective tool to combat antibiotic-resistant infections [29]. Possible target antigens are key components of the bacterial cell structure or metabolism and include capsular polysaccharide, clumping factor-A and -B, fibronectin-binding protein and ATP-binding cassette transporter. While several vaccine prototypes have passed preclinical development, to date no phase III clinical trial has been completed successfully [30]. Thus, despite considerable research investment – and significant progress – there remains no commercially available vaccine against *S. aureus*.

Other treatments

As the extent of the prevalence of *S. aureus* infections and the resistance of biofilms to standard antibiotic regimens have become increasingly apparent, endeavors to attain a solution have expanded. The nano-packaging of materials, such as inert metals, that have demonstrable antimicrobial effects is viewed as a practical means to treat biofilms in parallel to the use of antibiotics [31]. Nanoparticles of various compositions have been synthesized and tested against *S. aureus* biofilm with qualified success. In one study, $Ag_3PW_{12}O_{40}$ nanoparticles were shown to cause damage to the peptidoglycan component of the Gram-positive cell membrane and to down-regulate biofilm-related gene expression [32].

The effectiveness towards *S. aureus* of an antibiotic, either in combination or monotherapy, is reported to improve if administered as a conjugate with nanoparticles made of materials that have antibacterial properties like elemental silver, zinc oxide and titanium dioxide [33,34]. The exciting potential of this mode of co-delivery requires further investigation.

Phage therapy has emerged as an effective means to treat biofilm infections because of its selective specificity. In recent times significant advances have been made in this area [35]. For example, LysCSA13, an endolysin from the *S. aureus*-virulent bacteriophage CSA_{13}, can effectively remove staphylococcal biofilms from various surfaces, raising the possibility that LysCSA13 may be utilised as a promising infection control agent [36]. A potential concern of this strategy is that in some patients it may provoke a host-versus-phage inflammatory immune response. This may result in phage neutralization and thereby impair therapeutic efficacy [37]. Thus, any clinical trial to test the suitability of this phage will be diligent to this possibility.

Conclusion

Biofilm is a thick extracellular polysaccharide matrix the formation of which is a property of several bacterial human opportunistic pathogens, notably members of the Gram-positive genus *Staphylococcus*. The resilience characteristics of cell adhesion and aggregation that are intrinsic to biofilm promote bacterial survival and growth, while its slime-like coating heightens resistance to antibiotics. Collectively, these factors contribute to persistence of infection. The adherence of biofilms, typically of *S. aureus*, to commonly used medical devices such as catheters constitutes a serious challenge to the control and prevention of hospital-acquired infections. For medically important bacteria, including MRSA, that show high levels of resistance to frontline antimicrobial therapies, it is an increasing imperative to identify and develop effective alternative ways to combat biofilm formation. An example of the public health relevance of this issue is that of an increasingly ageing, and therefore immunologically weakened, population in many industrialized nations worldwide that requires implants and prostheses. When undergoing surgery such patients are rendered particularly vulnerable to *S. aureus* biofilm infection, including MRSA.

New translational approaches under development include the judicious use of novel antibiotics in various combinations, as well as other agents with anti-biofilm properties such as phytochemicals, AMPs and enzymes. To date, most of these treatments have been tested under *in vitro* conditions only, and thus their suitability for patient application requires prior assessment *in vivo* in experimental models and clinical trials. This also applies to the utilisation of nanoparticles and phage therapy. These technologies may achieve optimal efficacy when used in synergy with another remedy, most probably antibiotics. In order to find the most efficacious antibiotic regimen in any given hospital setting it is crucial to evaluate antibiotic resistance and to use this local information to choose a suitable treatment regimen. In this regard, it is a fundamental issue to examine the effect on biofilm formation of antibiotics that are prescribed routinely by physicians for chronic staphylococcal infections such as MRSA.

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