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The Evolving Role of Microbial Biofilms in Infections in the Critical Care

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ABSTRACT

Increasing mortality due to infections caused by multi-drug resistant pathogens constitutes a major reason for hospitalization and admission in the intensive care unit. A deeper understanding of microbial pathogenesis along with in-depth knowledge of virulence factors is thus the need of the hour. Microbial biofilm is one such critical virulence factor that warrants our attention. A biofilm is a complex microbial architectural entity that is shown to resist human immune defenses as well as the inhibitory effect of antibiotics. Biofilms are associated with various infections such as device-related infections, diabetic foot infections, bone, and joint infections, and burns. This review focuses on staphylococcal biofilms and the currently available management options along with a special mention about a novel chemical entity, levonadifloxacin, and its potential as a therapeutic agent for difficult to treat MRSA infection complicated by the formation of biofilm.

INTRODUCTION

Until the late 1970s, the prevailing view of infectious diseases was based on the belief that acute infections are caused by microorganisms grown in the laboratory in a planktonic state. It is only in the last 40 years, that the role played by biofilms in microbial pathogenesis of disease has become ever more visible.[1]

The term bacterial biofilm was coined in 1978 by Costerton and colleagues, who described it "as a structured microbial community that is attached to a surface and encased by an extracellular matrix." Since the introduction of the biofilm model over 40 years ago, it has become clear that the majority of bacteria have the inherent capacity to grow in these self-generated ecosystems. Members of the staphylococcus genus along with members of pseudomonas, aspergillus, and candida genera are opportunistic pathogens that produce robust biofilms on both abiotic and biotic surfaces.[2]

Cells within the biofilm are physiologically different from the free-floating (planktonic) cells, with extensive differences in gene as well as protein expression patterns. Trademark of biofilm-related infections is their obstinacy to antimicrobial treatments, leading to tough-to-treat or recurring infections, or at times, leading to physical removal of the medical device or infected tissue.[1]

Biofilms can be surface-associated or untethered microbial aggregates that have been implicated in numerous subacute and chronic infections, such as diabetic foot infections, catheter-associated infections, burns, chronic osteomyelitis, and periodontal infections.

This review focuses on staphylococcal biofilms and the currently available management options along with a special mention about a novel chemical entity, levonadifloxacin, and its potential as a therapeutic agent for difficult to treat MRSA infection complicated by the formation of biofilm.

COMPOSITION

A biofilm has been credited with various functions, such as a pool of genetic material, source of nutrition for the member pathogens, matrix preservation, adhesion, and routes of bacterial communication. Most of the aforementioned functions depend on the particular constituents

present in the biofilm, which vary from species to species and even the particular strain of the bacteria.[3]

The extracellular polymeric substance (EPS) are natural polymers of high molecular weight that bind together biofilms formed by various distinctive groups of bacteria that are often with different genotypes, The EPS is responsible for forming and maintaining the functional and structural integrity of biofilms, determining its physiochemical properties.

1.1 Composition of the Extracellular polymeric substance

1.1.1 Polysaccharides

Polysaccharides constitute a major bulk and are responsible for the mechanical properties of the biofilm. For example, it has been demonstrated that Bap proteins can build amyloid-like scaffolds that promote S. aureus biofilm formation.[2]

1.1.2 Proteins

Biofilms are also home to a variety of enzymes, bound in a complex network and able to adapt. These enzymes break down polymers into low molecular mass products, help detachment by degrading the structural EPS, and act as virulence factors. For example, biofilm-associated surface protein (bap) from *S. aureus*.[3]

1.2 Extracellular DNA

eDNA is an important constituent of biofilms. Varying levels of eDNA perform varying functions within the biofilm. For example, it plays an essential role in the structural organization of the *S.aureus* biofilm and is even shown to have antimicrobial activity. It has further been shown to have the ability to chelate cations involved in lipopolysaccharide and the bacterial outer membrane stabilization, provoking cell lysis.[3]

1.3 Water and bio-surfactants

Water makes up the largest component of the biofilm matrix, and it is responsible for the flow of nutrients within the biofilm matrix.

STAPHYLOCOCCAL BIOFILM

S.aureus is an opportunistic pathogen that produces robust biofilms. While nasal and skin colonization with them widely occur in humans asymptomatically, the switch between planktonic state and a multicellular biofilm is a pivotal step for staphylococci to cause infections such as complicated skin wounds, diabetic foot infection, osteomyelitis.

2.1 STEPS OF FORMATION

Staphylococcal biofilm development can be divided into three main phases: (i) initial attachment, (ii) production of extracellular matrix and cell proliferation, and (iii) biofilm structuring and cell detachment.[2]

In the first phase, *S.aureus* attaches to surfaces using a range of different factors and mechanisms, including surface adhesins, wall teichoic acids, and cell surface hydrophobicity changes, etc. As part of the early biofilm maturation process, the bacteria also start to produce EPS, which eventually forms the biofilm matrix. Depending on the EPS composition, staphylococcal biofilms can be divided into two broad categories: biofilms consisting of a polysaccharide matrix and biofilms with a proteinaceous matrix. The attached bacteria then proliferate and build micro-colonies on the surface. In the next stage, those micro-colonies develop into distinct structures that establish the biofilm. Remodeling of the biofilm and cell detachment occurs through protease-driven disruptive factors, like nucleases and surfactants, which are thought to be crucial for the development of the three-dimensional structure of the mature staphylococcal biofilm with its distinctive towers and channels.[2]

A further refined model of biofilm development has been postulated recently. This model expands the traditional biofilm model by two distinct new phases, referred to as the multiplication and the exodus phases, which take place after the initial attachment and before the maturation phase.[4][5]

During the multiplication phase, the cells start to grow and divide and embed themselves in a matrix consisting of proteins and eDNA.

The exodus phase is then triggered by the expression and secretion of the major nuclease Nuc1, which leads to degradation of the eDNA, allowing the release of a subpopulation of cells from the biofilm.[4][5]

In more detail, the biofilm environment is thought to be acidic, due to low oxygen levels and the release of fermentation products. The positive charge of alkaline virulence factors and ribosomal proteins in the acidic environment is then thought to mediate electrostatic interactions with surface components and eDNA, leading to biofilm stabilization.[2]

BIOFILM FORMATION AND ASSOCIATED FACTORS

The formation of a biofilm on a medical device that is infected with pathogens is controlled by several variables.

Adherence to the exposed surface for a long enough duration is crucial for an irreversible attachment. The rate of attachment depends on the nature of the cells in the liquid, along with its rate of flow through the device and the physiochemical nature of the surface.

Following the irreversible binding and production of EPS, rate of biofilm growth depends on the flow rate, nutrient composition of the medium, antimicrobial-drug concentration, and circumambient temperature.[6]

MECHANISMS OF ANTIBIOTIC RESISTANCE

Biofilms are extremely resistant to antimicrobials, making them vexing to treat. Various mechanisms contribute to the highly resistant nature of biofilm. These mechanisms are (a) limited diffusion, (b) enzyme causing neutralization, (c) heterogeneous functions, (d) slow growth rate, (e) presence of persistent (non-dividing) cells, and (f) biofilm phenotype such adaptive mechanisms e.g. efflux pump and membrane alteration.[7]

The bacteria within the biofilm microenvironment make it hypoxic and nutrient-poor, thus slowing the rate of bacterial division. This sedated growth results in blunting the effect of the managing antibiotic.[3] It has been postulated that cells within a biofilm are 10 to 1,000 times more tolerant to antibiotics than are planktonic cells.[8]

High bacterial cell density within a biofilm community has been suggested to encourage the resistance genes transfer between bacterial cells. Besides the genetic diversity in biofilms, these communities are often a pool for persister cells, which represent a sub-population of cells that can endure antibiotic treatment without becoming resistant.[2]

In general, cells within a biofilm often show reduced proliferative and metabolic activity, leading to an increased tolerance to antibiotics targeting the bacterial cell wall, as well as DNA or protein synthesis inhibitors.

It is also generally assumed that bacterial cells within a biofilm are likely to encounter sub-inhibitory concentrations of antibiotics, which were shown to potentially stimulate biofilm production and alter the composition of the biofilm matrix. For example, the MIC for vancomycin is 10 times higher for biofilm-bound cells than for planktonic cells (2 µg/ml, v/s 20 µg/ml).[9]

ROLE OF ACIDIC pH ON BIOFILMS AND ANTIBIOTIC EFFICACY

Most of the bacteria which normally can grow only in a narrow pH range, when present in the biofilm community, possess the ability to survive within a pH range that would be inhibitory to their division under planktonic conditions. Furthermore, researchers believe that an acidic pH strongly enhances in vitro biofilm formation.

It has been postulated that antibiotic efficacy is affected by the pH. It does so by tempering the binding and/or target sites for few antibiotics. Particularly ciprofloxacin demonstrates a loss of activity at acid pH. However, it is also believed that an increased threshold of bacteria to antimicrobials at certain pH ranges might be because of changes in their metabolic state, more specifically, the generation of small colony variants.[10]

Furthermore, a study aimed to determine whether bacterial and fungal biofilms were able to resist the antimicrobial activity of chlorhexidine, which is a powerful antiseptic widely used in the hospital environment. The study concluded that chlorhexidine demonstrated excellent antimicrobial activity for most pathogens when tested in their planktonic state, but not so effective against biofilms of *Acinetobacter baumannii, Escherichia coli, MRSA,* and *Pseudomonas aeruginosa.*[11]

Silver has also been reported to establish efficacy on microbial biofilms, both within the *in vitro* and *in vivo* environments. However, when incorporated into a wound dressing, its antimicrobial efficacy on biofilms within the *in vivo* environment remains controversial due to a lack of adequate clinical evidence.

Lamp and colleagues have postulated that MIC of certain antimicrobials tends to increase at higher pH as compared to neutral or low pH. Such a pH effect on antibiotic performance has been demonstrated in Staphylococci.[12] Contemplation of the effect of pH on drug efficacy would therefore be needed when choosing an antibiotic for clinical use.

BIOFILM RELATED INFECTIONS

Biofilms are the source of more than 65% of healthcare-associated infections, which, according to the WHO, affect 1.4 million people annually.[13] The clinical significance of biofilms relates to an extensive range of medical disciplines, such as surgery, orthopedics (osteomyelitis), cardiology (infective endocarditis), vascular surgery, dental infections, and other chronic infections such as cystic fibrosis.

3.1 CATHETER RELATED BLOOD-STREAM INFECTION

Central venous catheters (CVCs) are vital in modern medicine practices, especially for intensive care unit (ICU) patients. Approximately 78% of critically ill patients require some type of CVC and 90% of catheter-related bloodstream infections are CVC related. Two-thirds of these infections are caused by Gram-positive bacteria, predominantly Gram-positive cocci, which are equally responsible for infections in ICU and non-ICU patients.[14] The pathogens that are most often responsible for causing biofilm-related infections are Staphylococci. Furthermore, increasing the involvement of MRSA in catheter-related infections poses a challenge for the healthcare community.[15] They could gain access to the catheter either externally or internally. Externally, they might wander from the skin, along the exterior surface of the catheter, and internally from the catheter hub or port.

Owing to the presence of a wide range of surface proteins that contain binding domains for the host, *S.aureus* can bind to the catheter surface. Upon 24hrs after the placement of the catheter, *S.aureus* is believed to colonize and form a biofilm, following which it flows into the systemic circulation resulting in bloodstream infection.

It has been observed that biofilm-producing bacteria are responsible for the causation of up to 60% of catheter-related infections. [16]

In infections associated with a biofilm, the host response to antimicrobials is severely compromised, making biofilms a threat for the nosocomial settings.

"Raad et al. found that biofilm formation on CVCs was universal, but the extent and location of biofilm formation largely depended on the duration of catheterization. The authors reported that short-term (<10 days) catheters had greater biofilm formation on the external surface, whereas the long-term catheters (>30 days) had more biofilm formation on the catheter inner lumen".[17]

A recent study attempted to isolate and identify the pathogens causing CVC-related infections and determine their biofilm potential, recognition of adhesion genes, and the antibiogram. The authors reported that out of the total samples tested, *S. aureus* was responsible for infection in 39% of CVCs. Furthermore, the antibiotic sensitivity revealed that out of the S.aureus samples isolated, 59% were MRSA. Interestingly, all MRSA isolated from CVC were biofilm producers. Half of MRSA (50%) were weak biofilm producers followed by moderate (27%) and 23% were strong biofilm producers.[18]

It could be concluded from the aforementioned study that most of the MRSA isolate consisted of adhesion genes. The presence of these genes corresponded with the formation of biofilm and displayed resistance to the treating antimicrobial agent.[18]

3.2 DIABETIC FOOT INFECTION

India is home to the highest number of diabetics in the world. Diabetic foot ulcers (DFU) and diabetic foot infections (DFI) leading to non-traumatic lower limb amputation is the most common distressing complication of diabetes. [19]

The presence of non-viable tissue in a diabetic foot wound proves to be an obstacle against efficient wound healing. Slough is one such barrier that supports the attachment and development of biofilms. Thus, its removal allows healing and reduces areas where pathogens can form biofilms, effectively reducing the risk of infection.

DFIs are usually polymicrobial with the ability to form biofilms. A prospective study reported that out of the total samples of DFUs tested, biofilm formation could be seen in 46.34%. Furthermore, these biofilms were predominantly formed by *S.aureus*.

This study concluded that to formulate an effective treatment plan for the well-being of chronic diabetics, in addition to the routine diagnostic procedures, screening for biofilm formation would add to the rapid diagnosis of chronic ulcers.[19]

Another prospective study aimed to detect the prevalence of biofilm producers and non-producer in the isolates from DFU patients. The authors reported among the 255 bacterial isolates tested, 179 (70%) were biofilm producers. A total of 68.1% of gram-positive isolates were biofilm producers and 71.2% of gram-negative isolates were biofilm producers.

Additionally, risk factors for biofilm-related infections were determined. On analyzing, significant risk factors that were associated with biofilm-related infection were "male sex, duration of diabetes, duration of ulcer >1 month, size of ulcer >4 cm2, Grade II ulcer, necrotic ulcer, previous antibiotic use, subcutaneous infection, HbA1c >7% and polymicrobial infection." The knowledge of these risk factors offered an enhanced understanding of the disease, leading to a more efficient treatment plan.[20]

To summarize, it has been recommended that a better understanding of the biofilm model of microbiology would aid in improved management strategies of DFUs.

3.3 BURNS

Infections of burn wounds represent one of the most commonly occurring and devastating challenges to today's global health scenario. To minimize the morbidity burden in the global health setting, increased and more efficient care is required for the management of these wounds. Loss or impaired action of defensins and acidic secretions from the sweat and sebaceous glands that constitute the normal protective defense mechanisms of the skin, following a burn results in the colonization and invasion of the wound by the micro-organisms.[21]

Initially, after the burn, there is predominant colonization of the wound by gram-positive bacteria such as *S. aureus*, following which there is an invasion by the gram-negative pathogens, namely *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. These micro-organisms with their ability to communicate and coordinate with one another, resulting in the expression of multiple virulence factors and the formation of biofilms in *A. baumannii*, *P. aeruginosa*, and *S. aureus.*

Mahmoudi H et al investigated the relationship between antibiotic resistance patterns and biofilm production amongst *S.aureus* samples isolated from burns. Amongst all the clinical isolates of S. aureus, 94% effectively formed a biofilm. Furthermore, the results demonstrated that MRSA strains were associated with strong biofilm production.[22]

3.4 BONE AND JOINT INFECTIONS

With the increase in the proportion of the aged population sub-group, the requirement of total joint replacement surgeries is also increasing. It has been anticipated that the annual number of primary total hip arthroplasties is expected to increase to 635,000 and knee arthroplasties to 935,000 by the year 2030.[23]

Despite practicing strict infection control methods, including debridement of the surgical site, complete exchange of the hardware, prescription of a robust long-term antimicrobial therapy; infections fail to subside. Certain reports suggest that the cost for the management of implant-associated osteomyelitis is projected to exceed $1.62 billion in the year 2020.[24]

Studies report that as high as 75% of the total osteomyelitis cases are caused by gram-positive pathogens, more specifically the pathogens of the Staphylococcus genus. *Sloan M et al* demonstrated that*S.aureus* was the most common pathogen that was isolated from implant-associated osteomyelitis samples. Furthermore, hard-to-treat MRSA strains were isolated from over 50% of those samples.[24]

Based on the species being studied, three main types of physio-pathological mechanisms can be involved in staphylococcal chronic Prosthetic Joint Infection, namely: formation of biofilm, bacterial internalization in osteoblasts, and formation of small colony variants.

Normally, in response to the maturing and fully matured *S. aureus* biofilm, the polymorphonuclear neutrophils of the host produce cytokines that are involved in bacterial lysis. However, these efforts remain inadequate, and ineffective efforts to engage in phagocytosis result in the release of cytotoxic and proteolytic substances contributing to tissue injury and ultimately to peri-prosthetic osteolysis. Biofilms are also resistant to phagocytosis, and the phagocytes that attempt an assault on the biofilm may do more harm to surrounding tissues than to the biofilm itself.[25]

With help from its coagulase or von Willebrand factor-binding protein, *S. aureus* possesses an innate property of converting soluble fibrinogen into a fibrin shield, making it tolerant to antimicrobial agents.[25]

Additionally, resorption of bone directly by the bacterial proteases presents within the biofilm has been reported in vitro recently. The authors state that the pathogens within the biofilms

by their skill of directly resorbing bone tissue combined with the capacity to drift into canaliculi and further form biofilm in osteocyte lacunae can explain their ability to induce peri-prosthetic osteolysis.[24]

DETECTION OF BIOFILM PRODUCING PATHOGENS

Presently, the diagnosis of wound infections relies majorly on causative pathogen identification. The host site of the infection is often ignored and kept in a back seat. Furthermore, since biofilms are only an observed mode of growth for the bacteria seen in the living hosts, it is extremely challenging to establish the same biofilm on culture in the laboratory.

These factors come in the way of an efficient diagnosis of biofilm-related infections.

Biofilm diagnostic methods can also be broadly categorized by their level of inspection into morphology assay, microbiology assay, and molecular assay.[26] Currently, there are no clear gold-standard tests available for the diagnosis of biofilm in chronic wounds.

Table No. 1:

DIAGNOSTIC METHOD	**AIM**
MORPHOLOGY ASSAYS	
Tissue sampling for histology	Pathogen identification Biofilm localization
Scanning electron microscopy and confocal laser scanning microscopy	Biofilm localization
MICROBIOLOGICAL ASSAYS	
Standard clinical microbiology culturing methods	Pathogen identification
Dithiothreitol treatment of prosthesis	Pathogen identification
Sonication of prosthesis	Pathogen identification
MOLECULAR ASSAYS	
16S rRNA PCR	Pathogen identification
Bacterial tag-encoded FLX amplicon pyrosequencing	Pathogen identification
FRACS; PRADS; PRAPS	Pathogen identification
Peptide nucleic acid fluorescence in situ hybridization	Pathogen identification

MANAGEMENT OF BIOFILM AND RELATED INFECTIONS

4.1 Treatment guidelines for biofilm infections

In the year 2014, ESCMID published guidelines on the diagnosis and treatment of biofilm-related infections. They postulated the administration of prophylactic perioperative antibiotics. Can help in the prevention and control of biofilm infections related to surgery.

For patients with indwelling urinary catheters or urethral stents, short course systemic antibiotic therapy can defer biofilm infections for up to 1–2 weeks, however, it is also crucial to mention the use of antibiotic prophylaxis because of concern about superinfection by multi-resistant strains is not recommended.

Also, in situations wherein, even after repeated positive blood culture with microbes from CVC along with the absence of clinical signs of infection, prevention of biofilm infections can be possible. This can be done by early antibiotic treatment of colonization, thereby eradicating signs of early infection.

Furthermore, the ESCMISD also points out the urgent need for new anti-biofilm effective antibiotics.[27]

4.2 CURRENT ANTI-BIOFILM STRATEGIES

It is now clear that biofilm-related infections are challenging to treat, and it is further exacerbated by the fact that microorganisms residing in the biofilms can develop antimicrobial resistance.

Initially, removal of the infected device/material was considered to be the conventional treatment option for biofilm infection. However, in few cases of implants and joint prosthesis or a compromised host, surgical removal is not considered the most recommended management method. Moreover, an exorbitant cost of implant removal adds to the economic burden of the patient.

Today, most of the anti-biofilm strategies that are in research activities on the following target methods:

(1) Inhibition of bacterial adhesion and colonization on the surface

(2) Meddling with the signal molecules responsible for modulation of biofilm development

(3) Disaggregation of the biofilm matrix

The first anti-biofilm strategy warrants the incorporation of anti-adhesive materials onto the surface, thereby altering its mechanical as well as chemical properties. Also, the addition of antimicrobial agents is done on the surfaces, thereby preventing chances of bacterial colonization. The second anti-biofilm strategy targets the ability of pathogens to communicate and signal one another, i.e Quorum Sensing. However, it is associated with toxicities, because of which its use is not preferred. Lastly, the third strategy uses substances such as dispersion B, Dnase I, and proteinase K to destroy the physical integrity of the EPS matrix of the biofilm. However, practical usage is restricted due to the high cost and limited commercial accessibility of different enzymes.[28]

Recently, new anti-biofilm agents have been developed as adjuncts or alternatives to classical antibiotic treatment. Some of them are garlic, cranberries, chlorogenic acids (from coffee, cinnamon, etc), Boswellic acids (from plants of Boswellia genus), the leaf extract of *Pongamia pinna***,** wheat bran extract. However, these could be promising agents but currently lack clinical evidence.

Another point worth mentioning is that oxidative stress in microorganisms plays an essential role in the production of EPS matrix and biofilm heterogeneity. Thus, antioxidants also have the potential to act as an alternative source for biofilm control by scavenging the free radicals and terminate the ROS chain reaction.[28]

One must keep in mind that treatment cannot solely consist of the above-mentioned strategies as bacteria always have the option of surviving and multiplying in the planktonic form.

4.3 LEVONADIFLOXACIN AND ITS ROLE IN ELIMINATING BIOFILMS

An ideal antimicrobial agent that would result in complete elimination of biofilm and related infection does not exist. However, for any antimicrobial agent to be effective against biofilm, it would need to possess certain desirable traits. These qualities could be summarized as 1. good penetration within the polymatrix, 2. bactericidal action against slow-growing bacteria, 3. Potent activity in acidic environments and 4. the ability to tackle high bacterial density.

Levonadifloxacin and its prodrug ala-levonadifloxacin are novel broad-spectrum agents belonging to the benzoquinolizine sub-class of fluoroquinolone that has been recently launched in India indicated for use in Acute Bacterial Skin and Skin Structure Infections, including DFI and concurrent bacteremia. This novel chemical entity possesses a unique structural trait of a tricyclic core with the absence of conventional amine at the C-8 position of the side chain, allowing better target permeation and entry into the biofilm matrix. It has a multi-spectrum coverage that includes multi-drug resistant gram-positive pathogens along with anaerobes as well as atypicals. It also has partial gram-negative coverage of quinolone-sensitive strains as well as respiratory gram-negative pathogens. A global surveillance program showed that levonadifloxacin had potent activity (MIC90 0.5–1 μg ml−1) against MRSA and quinolone-resistant *S. aureus* isolates.[29]

Furthermore, owing to its non-basic side chain, levonadifloxacin remains in an un-ionized form even in low pH. This unique attribute allows its easy entry into the bacterial cell. As a result, there is a significant enhancement in the potency of levonadifloxacin in acidic environments which could be a beneficial feature for antibacterial action in infections with an acidic environment such as complicated wounds, bone and joint infections, infections of the lower respiratory tract, etc.[29]

An *in vitro* study assessed the bactericidal activity of levonadifloxacin, along with vancomycin, linezolid, and daptomycin as comparators, in planktonic and biofilm-encapsulated MRSA and QRSA isolates. Levonadifloxacin displayed a potent killing of 90% in the biofilm encapsulated isolates, whereas the comparator agents showed limited or no cidal activity. This can be deciphered from the scanning electron microscope images wherein daptomycin showed negligible killing and vancomycin and linezolid displayed variable activities. Extensive disruption of biofilm can also be appreciated from the images confirmed by a decrease in the viable bacterial count by levonadifloxacin.[30]

This establishes the activity of levonadifloxacin against biofilm-forming MDR *S. aureus* infections. Additionally, the excellent bioavailability of oral formulation is helpful in the smooth switch from parenteral to oral therapy. Thus, levonadifloxacin shows potential for use against biofilm-forming *S. aureus* strains.

CONCLUSION

With advancing age, there will be an increase in the number of people experiencing hospitalization and receiving short- or long-term biomedical implants/devices.

The increasing rate of antibiotic resistance is a well-documented threat to the global health setting. However, another threat that has not gained enough appreciation but is equally important nonetheless is the ability of drug-resistant pathogens to form biofilms.

The biofilm formed by S.aureus specifically is a major virulence factor that protects it from the host defenses, as well as antibiotics. This increased resistance against the treating antimicrobial agents can be attributed to the fact that the biofilm matrix acts as a physical barrier between the bacteria and antibiotic. Also, biofilm embedded S.aureus undergoes phenotypic changes resulting in treatment failure. Thus, such factors make the eradication of biofilm-associated infections extremely hard and challenging.

Since most of the therapeutic agents, as well as procedures, aim at treating bacteria in the planktonic state, there is an urgent unmet need to develop new therapeutic strategies capable of targeting *S. aureus* in the biofilm state. The new agent, levonadifloxacin shows great promise in the scenario and has the potential to become the drug of choice for tough to treat MRSA biofilm-related infections.

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