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Review Article

Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms

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ABSTRACT

Background: Biofilms are multicellular communities of microorganisms held together by a self-produced extracellular matrix. The ability of microbes to form biofilm is a universal, ubiquitous, and dynamic process. This dynamic process of biofilms establishes an important strategy to withstand and survive harsh environmental conditions and antimicrobial agents.

Objective: This review paper aims to give an overview of antibiotic resistance, intervention, and treatment of infections caused by biofilm-forming organisms. Moreover, it can also help to motivate scholars to search for new anti-biofilm strategies and most appropriate methods to tackle the effect of biofilm infections on healthcare services.

Methods: This paper was written by reviewing recent research and review articles which are reporting about the antibiotic resistance, prevention, and treatment of biofilm-producing organisms.

Conclusion: Bioprospecting for quorum quenching compounds can be an appropriate solution for controlling biofilm infections.

1. Introduction

Although the first existence of biofilms was reported by Anton van Leeuwenhoek, after the analysis of plaque scraped from his own teeth, research about biofilms was basically begun since the 1970s [1]. Subsequently, different scholars were able to define biofilms in many ways: (i) "Biofilms consist of communities of bacteria attached to surfaces encased in a glycocalyx matrix." [2]; (ii) "Biofilms are consisted of single cells and microcolonies of sister cells all embedded in a hydrated, predominantly anionic matrix of bacterial exopolymers and trapped extraneous macromolecules." [2]; (iii) "A microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription." [3]; (iv) "A community of microbial cells permeated by water channels allowing efficient biomass exchange between the population and the environment" but only aqueous environment [1,4]; (v) "City of microbes" with 85% total biomass of extracellular polymeric substances (EPS) as "house of the biofilm cells" [5]; (vi) "Microbial communities comprise a large number of different bacterial cells living together encased in a self-produced matrix of extracellular polymeric substances" [6].

In biofilms, extracellular polymeric substances (EPS) plays a vital role in the formation of physical and social interactions, an enhanced rate of gene exchange, and antimicrobials tolerance [7]. EPS consists of cellulose, alginates, poly-N-acetyl glucosamine, extracellular teichoic acid, proteins, lipids, nucleic acids, phospholipids, polysaccharides, extracellular DNA, and other organic compounds [8]. About 90% of biofilms biomass is comprised of EPS that contributes to the resemblance of the mushroom-like structure [9]. Besides, the mechanical firmness of biofilms is attributed to the viscoelastic features of the EPS matrix [10].

Biofilms show numerous characteristics, which are very important to their survival strategy. Some of the general characteristics of biofilms include three-dimensional structure, presence of one or more microbial species, adherence to each other, adherence to surfaces, and adherence to

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interfaces of either solid/liquid, liquid/air, liquid/liquid or solid/air, and decrease antimicrobial susceptibility and host defense systems [1]. Although the extracellular matrix produced by the constituent cells of biofilms is a critically significant factor for their structural integrity, the chemistry and physiology of the biofilms can be fluctuated depending on resident microbes and their surrounding environment [1,11]. For example, biofilms formed on submerged rocks in acid mine drainage are extremely different from the plaque formed on our teeth and differ from their planktonic counterparts [11].

On the basis of their growth characteristics, microorganisms have primarily characterized as planktonic [3]. It is now widely accepted that about 99% of all microorganisms attached to a surface and grow as a biofilm which is a universal microbial strategy for their survival [1]. Biofilms have high cell densities ranging from 10^8 to 10^{11}cells per gram of wet weight. They are persistently attached to both biotic and abiotic surfaces ranging from the human tooth or lung and the intestine of a cow to a rock submerged in a fast-moving stream. Medical devices that can be colonized by biofilms include intrauterine contraceptive devices, implants, prosthetic medical devices, catheters, dental materials, cardiac valves, and contact lenses [12].

In contrast to the planktonic cells, biofilms constitute a distinct growth phase [13]. Most microbes form biofilms as a means of response to their unfavorable environmental conditions. Biofilms are formed as a result of coordinated gene expression of the individual cells via quorum sensing [14]. Most microbial biofilms encompass and grow together not only as a single microbial species but also as many as microbial communities of pathogens and non-pathogens [15]. Due to the elastic nature of the biofilms, they can challenge and withstand environmental stresses such as starvation and desiccation, which is an imperative advantage for their survival [16].

2. Main text

Depending on the interaction between the surface and the constituent cells, biofilms could be either monolayer or multilayered [17]. The monolayer biofilm has prominent interactions between the cell and the surface rather than the interaction between the constituent cells. Different classes of adhesive structures such as flagellum and pilus are helpful to accelerate and increase the formation of the monolayer biofilm. On the other hand, microbes often develop multilayer biofilms when they adhere to a surface as well as to each other [18]. In many cases, it has been well-known that the nature of the outer surface of bacteria leads to repulsion. For instance, the chemical properties of the cell wall of Gram-negative bacteria are generally negatively charged due to the presence of the O antigen. The multilayer biofilms are formed by masking and neutralizing the repulsive force of negatively charge organisms by the process of mutation, down-regulation of the O antigen synthesizing genes, the addition of divalent cations, or synthesis of extracellular polymeric substances [17].

Numerous techniques have been developed for the detection of biofilms. These include tube culture, Congo red agar, microtiter plate assay, and confocal scanning laser microscopy, to name a few [6]. The tube culture method is considered the gold-standard method to detect and quantify biofilm formation. Congo red agar medium is one of the simplest methods to detect biofilm production qualitatively [19].

Biofilm architecture has been extensively studied using optical sectioning, confocal laser scanning microscopy, scanning electron microscopy, and three-dimensional imaging [1]. The combination of flow-cell technology and fluorescence *in situ* hybridization (FISH) with confocal scanning laser microscopy is the most favored tools to get quantifiable evidence on both the overall biomass and the individual strains. Identification of a particular strain from a given multispecies of biofilms by the means of the FISH method is extremely labor-intensive and needs skillful personnel to avoid drawbacks. Microtiter plate assay is suitable to quantify biofilms based on crystal violet retention [20, 21, 22].

Microorganisms are often found as organized communities growing on different surfaces, rather than as free-swimming planktonic cells [23]. Many scholars have been studied the development of bacterial biofilm and they have found that all microorganisms have a similar process [24, 25]. For example, the biofilm formation in *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* consist of initiation, microcolony formation, maturation along with EPS production, and finally dispersal of individual cells [25].

Biofilm formation is a cyclical and dynamic process that contains diffusion, transportation, and chemical reaction with numerous biological and ecological mechanisms. However, it can be influenced by numerous processes such as adhesion, mass transport, quorum sensing, detachment, cell death, and active dispersal [1,26]. Although there are a number of differences among the bacterial species during the process of biofilm formation, it has been observed in almost all bacterial species [27]. In the early stages of biofilm formation, planktonic bacterial cells adhere to the surface by the help of bacterial appendages such as flagella, pili, and fimbriae [17] and with the aid of physical forces, including van der Waals forces, steric and electrostatic interactions [28]. After surface attachment, the process continues to the development of biofilm formation and maturation [29].

Biofilm formation encompasses three basic stages; namely, attachment, maturation, and dispersal. For example, in *P. aeruginosa*, these three stages have been sub-categorized into five phases [29,30]. These five phases are: (i) development of a surface adjusting film (layer) on which the biofilm grows (ii) movement of microbial cells into a nearby surface (iii) adhesion (reversible or irreversible) of microbes to a conditioned surface (iv) division and growth of microbes, microcolony formation, and phenotype and genotype changes (v) lastly, dispersal of cells using swarming (twitching motility), clumping (rolling motility), and surface/clumping (sliding motility) manner [31,32].

E. coli is the most prevalent Gram-negative biofilm-forming bacterium with genetically diverse microbial strains [34,35]. *E. coli* strains show different structural phenotypes of biofilm, such as simple flat, compact, loose biofilm structures can be observed [36,37]. *E. coli* biofilm formation passes through a series of developmental stages, including adhesion, proliferation, structural maturation, and dispersal of cells. But, depending on the environmental conditions and the particular strain of the bacterium, biofilm formation can be facilitated by certain contributing factors, such as appendages (flagella, pili, and fimbriae), receptor proteins, autotransporter proteins, extracellular polysaccharides, and different genes [38,39]. Of the contributing factors, for example, (i) surface contact and reversible attachment is assisted by flagellar motility; (ii) irreversible attachment is taking place by fimbriae and unbranched β-1,6-N-acetyl-D-glucosamine polysaccharide; (iii) microcolony formation and early development of biofilm architecture coordinated by the help of motility, curli, antigen 43 (autotransporter protein), colanic acid, and extracellular polysaccharides; (iv) maturation process takes place by colanic acid, curli conjugative pili; finally, dispersal by flagella and motility [40].

S. aureus is one of the most common nosocomial Gram-positive bacterium [42,43]. Biofilm formation provides several survival advantages to the bacterial cells, such as quorum sensing, increased protection against external stresses of host immune system and antimicrobials agents due to the shielding effect by an extracellular polymeric matrix [44,45].

S. aureus biofilm development has five-stages: (i) attachment, (ii) multiplication, (iii) exodus, (iv) maturation and (v) dispersal. Initial surface attachment is dependent on the bacterial surface molecules such as murein hydrolase AtlA (required for cell division, cell wall turnover, and bacterial lysis), fibronectin-binding proteins, and teichoic acids [45]. *S. aureus* cells could attach to abiotic and biotic surfaces via hydrophobic interactions and microbial surface adhesive matrix molecules, respectively. After attachment, it develops into a mat-like structure of cells composed of an extracellular DNA and proteinaceous matrix. Then cells released from the biofilm via nuclease-mediated extracellular DNA

degradation to allow for the formation of three-dimensional microcolonies. There is also a rapid cell division for maturation. Finally, the dispersal of cells is taking place via protease activation.

Biofilms are highly tolerant of antimicrobials and host defense mechanisms because of their harboring mechanism under multiple physiological states, such as growing, stress-adapted, and dormant nature. For instance, *Staphylococcus epidermidis* protects itself against the innate human immune system using EPS intercellular adhesion [47, 48, 49]. Bacteria grown in biofilms have higher horizontal gene transmission than planktonic bacteria [50]. In comparison to planktonic cells, the significantly frequent rate of mutation is taking place in biofilms [51]. Biofilms increase the chance of gene transfer by the help of virulence factors and antibiotic-resistant genes from resistant to susceptible bacterial species, which leads to antibiotic resistance [52,53]. If patients are infected by biofilm-forming pathogens, it is difficult to treat even with high doses of antibiotics and can lead to their death [14]. For example, to treat and eradicate pathogenic biofilms from patients, it requires 10–1000 times higher doses of antibiotics than identical strain living in planktonic form. Because embedded bacterial cells are getting an optimal defense mechanism against the adverse effects of antibiotics and the immune system of the host [27,54].

The nature of biofilm structure and the physiological attributes of biofilm organisms confer an inherent resistance to antimicrobial agents [3]. Even though the defensive mechanisms of biofilms against antimicrobials are not yet clearly understood, some possible reasons are suggested by many scholars [1,55]. Some of the reasons include an altered gene expression in biofilm-specific resistance genes (e.g., efflux pumps or exclusion of antibiotics) compared to planktonic cells, less sensitivity of most antibiotics against slower growth rate and reduced metabolic activity of cells, reduced biofilm-specific phenotype to decline the efficacy of antibiotics, degradation of antibiotics, impaired penetration of antibiotics into the biofilm matrix, stress response to hostile environmental conditions (e.g., leading to an overexpression of antimicrobial agent-destroying enzymes), altered microenvironment inside the biofilm matrix (pH, oxygen content), antimicrobial agents may be trapped and destroyed by enzymes in the biofilm matrix, altered growth rate of cells inside the biofilm, nature of antibiotics, and modification of targets [12, 56].

Biofilms have shown important phenotypic changes, which lead them to genotypic alterations. Regardless of the composition and types of the biofilms, the cells sheltered within the matrix of EPS from environmental factors, but can communicate through quorum sensing molecules called autoinducers. Quorum sensing regulation leads to an overall change in gene expression, increasing virulence, and accelerating the gaining of antibiotic resistance [57]. Co-existence of diverse bacterial species within biofilms is likely to catalyze complex substrates. For example, a biofilm formation between the bacterial species of *P. aeruginosa* PAO1 and *Burkholderia* sp.NK8 directly benefits the bioremediation of chlorobenzoates in drinking water systems [58].

Biofilm forming organisms are supposed to cause 65–80% of human infections [29]. Biofilm-forming bacteria are causing chronic illnesses despite antibiotic therapy and innate and adaptive immune responses of the host [59]. Some of the human diseases caused by bacterial biofilms-associated infections are wound infection, osteomyelitis, chronic sinusitis, central nervous system shunt infection, contact lens-associated keratitis, chronic otitis media, cochlear implant infection, burn-related infection, intravascular catheter infection, prosthetic valve endocarditis, pacemaker infection, electrophysiological wire endocarditis, biliary stent infection, peritoneal dialysis, catheter infection, prosthetic joint infection, urinary stent infection, intravascular stent infection, pulmonary infection in cystic fibrosis patient, ventilator-associated pneumonia, and breast implant infection [60]. High incidences of biofilms formation are also occurring on medical devices, for instance, catheters, orthopedic implants, contact lenses, and implantable electronic devices [61]. Some of the biofilm-forming pathogenic microbes include *P. aeruginosa, Burkholderia cepacia*, *Pseudomonas pseudomallei, Haemophilus influenza*, *E. coli*, *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumonia, Streptococcus pyogenes*, other *Streptococcus* species, and *Candida albicans* [56,61,62].

There are few pieces of evidence provided on biofilm-forming fungal species, in recent years, some genera of pathogenic fungi have been gaining attention and correlated with the biofilm formation [63,64]. Concerning yeasts, *C. albicans* is the most widely studied model of a biofilm formation with respect to morphological and molecular perspectives. The *C. albicans* ability to become an opportunistic pathogen and biofilm formation is the major health problem in humans [65]. A multifaceted role of an extracellular matrix of *C. albicans* is considered a highly attractive target to combat biofilm-related infectious diseases [66]. Furthermore, extracellular DNA has critical roles in biofilm formation of *C. albicans* to induce the morphological alteration from yeast to the hyphal growth [67]. Subsequently, a mature *C. albicans* biofilm with higher cell density displays more antifungal resistance than an early biofilm with lower cell density [68].

Biofilm-forming pathogens are very challenging to treat with conventional antibiotics because of their greater resistance behavior. Hence, new and effective approaches are urgently needed. Searching for microbial biofilms inhibiting compounds from fungi mainly mushroom species is reasonable [69]. For example, a coprinuslactone compound isolated from *Coprinus comatus* fruiting bodies has shown in the reduction of the formation of pathogenicity factors like pyocyanin and a rhamnolipid B from *P. aeruginosa*. Coprinuslactone was also applied to *S. aureus* cells inside biofilms and it has shown significant damage to the cells. It also disrupts the cell membranes and the synthesis of the extracellular polysaccharide and consequently reduces the biofilm formation. Likewise, the coprinuslactone was inhibited by UDP-N-acetyl glucosamine enolpyruvyl transferase (MurA), which is essential for the synthesis of the bacterial cell wall [70].

Though biofilm matrices do not inhibit diffusion of antibiotics, antibiotics could bind to the components of the biofilm matrix or the bacterial membranes [71, 72, 73]. Restricted penetration of antimicrobials may occur as negatively charged exopolysaccharide restrict permeation of positively charged antibiotics [25]. However, some of the antimicrobials can penetrate the biofilm matrix. For example, tetracycline reached all biofilm cells in uropathogenic *E. coli* biofilms within 10 min of exposure; ciprofloxacin effectively permeated into *Klebsiella pneumoniae* biofilms; rifampin, daptomycin, amikacin, and ciprofloxacin penetrated biofilms formed by staphylococci without having a significant impact on cellular viability [74].

The presence of bacteria in various physiological states as a result of nutrient gradients in the biofilm is another underlying cause of biofilm-associated antimicrobial tolerance. When there is a scarcity of nutrients and oxygen, biofilm cells altered their metabolic activity [75]. For example, in *P. aeruginosa,* unlike conventional planktonic cells, biofilm cells are heterogeneous with respect to the physiological state of the cells that they harbor. Key nutrients and electron acceptors may be locally depleted inside a biofilm cell cluster [76].

Differential expression of specific genes in biofilms is another factor for the antimicrobial resistance which is characterized by depending on bacterial responses to the local environmental conditions. Because many antibiotics target processes that occur in actively growing bacteria. Biofilm-forming bacteria with low metabolic activity display increased antimicrobial tolerance to high doses of antibiotics [72]. For instance, *E. coli* biofilm cells could bring physiological changes that led to contribute to antibiotic resistance due to *rpoS*- mediated stress response [76]. A better understanding of these genes which are differentially expressed under biofilm and planktonic growth conditions could help to find new and effective treatments for biofilm-associated infections [75].

Bacterial biofilms are also comprised of persister cells, which are neither growing nor dying when they are exposed to antimicrobials. Consequently, persister cells lead to multidrug resistance. For example, though *P. aeruginosa* biofilm was exposed to high doses of ofloxacin, the persister cells were not killed. The persister cells remained safe compared

to their fairly sensitive *P. aeruginosa* biofilm counterparts [77]. The persister cells are tolerant to the antibiotics by preventing their bactericidal binding sites and by deterring the lethal action of the antibiotics. The reason behind is that they produce multidrug resistance proteins to halt the antibiotic targets [78].

Persister cells are metabolically inactive, a subset of dormant, phenotypic variants of regular bacteria and highly tolerant to antibiotics without undergoing genetic change. Persister cells form in response to several environmental factors, such as nutrients and oxygen deprivation, oxidative stress, DNA damage and antibiotics [79]. Persister cells remain viable and regrow in the biofilms when the level of antibiotics drops. Unlike resistant cells that grow in the presence of antibiotics, persister cells do not grow in the presence of antibiotics [80,81]. Persister cells comprise 1% of the total bacterial population. Persister cells are specialized survivors which are distinct from both growing and stationary cells, and they are the only cells to survive treatment with high doses of antimicrobials.

Biofilms are the most widely distributed and successful modes of life on earth [7]. Biofilms are consisting of either single or several microbial species with complex and dynamic structures. The complex nature of biofilms is very important to the microbial cells to withstand harsh environments and gives a multitude of strategies not to be affected by antimicrobial agents [14]. Nowadays effective approaches like quorum sensing inhibitions are required to interfere, treat, and prevent biofilm-related infections. As a result, many scholars are looking for compounds that can inhibit quorum-sensing systems [27]. Although numerous strategies have been investigated by many researchers to control the process of bacterial quorum sensing, this remains an area of continuous and intensive research in the quest to control biofilm development [1,82,83].

These days, screening of quorum sensing inhibitory compounds is highly acclaimed in tackling biofilm-related infections. Quorum sensing inhibition can be done through high-throughput techniques either to inhibit the production of autoinducers or their receptors [84]. To date, the quorum-sensing process and its mechanism are studied only in a few numbers of bacterial pathogens. As a result, researchers have very limited information about the communication process among microbial interspecies. Anti-quorum sensing compounds are neither lethal nor lead to the drug resistance of microbial pathogens [85]. Moreover, the advantages of applying the anti-quorum sensing compounds are not only active against the pathogens, but also have minimal adverse effects to a host in comparison to standard drugs. However, during the evaluation of novel anti-biofilm compounds, assessment of their cytotoxicity is mandatory because either they do have a variety of targets or their targets are even not yet well known [14,86]. Therefore, some of the interference mechanisms are discussed as follows.

Hindering of the quorum sensing process is a very critical approach to interrupt and control biofilm-forming pathogens. It can be accomplished in several ways, such as enzymatic degradation of signaling molecules, blocking of signal transduction, and/or blocking of signal receptors. A study has confirmed that preventing the production of autoinducers or hindering their receptor proteins has led to thinner and lesser structured biofilms [14]. As a result, the biofilm is much easier to be demolished via a host immune system. Exploration of biofilm curbing compounds has enormous importance for the interruption of quorum sensing systems [14,87,88].

Although the communication process of individual bacterial cells is an essential element during the process of biofilm formation, it can be blocked by different compounds [14]. For example, halogenated furanones, which are naturally produced by the red macroalga *Delisea pulchra,* have structural similarity and antagonistic effect to acyl-homoserine lactones [89, 90, 91]. As several Gram-negative bacteria use acyl-homoserine lactones as signaling molecules, the halogenated furanones have the ability to interfere with the quorum sensing performance of several bacterial pathogens [92]. A study conducted on *E. coli* indicated that furanones have shown a significant interference between the interaction of acyl-homoserine lactone and LuxR. Although furanones bind LuxR, the complex appears to be unstable and accelerates its turnover rate [93]. This results in the rapid disruption of the quorum sensing mediated gene regulation [94].

Quorum sensing inhibitors are important either to prevent the process of biofilms formation or to disperse already established biofilms [14]. It has been reported that many organisms can produce cyclic dipeptides to perform their communication [95]. In a *P. aeruginosa*, acyl-homoserine lactones (AHLs) can be activated by a cyclo (L-Pro-L-Val) which is a diketopiperazine formed by the fusion of valine and proline amino acids. The cyclo (L-Pro-L-Val) can also either agonize (activate) or antagonize (suppress) other LuxR-based quorum-sensing activities. Whereas the mechanism of action of the cyclo (L-Pro-L-Val) in the quorum sensing process is not yet well known; it might be due to the presence of cross-communication among bacterial signaling systems [96,97]. The other synthetic signal inhibitors, namely, cyclo (L-4-iodo-Phe-L-Pro) and cyclo (L-4-chloro-Phe-L-Pro) showed a moderate quorum sensing mediated luminescence inhibition in *Vibrio fischeri* [14,98].

Quorum sensing inhibitors are produced and identified not only from bacterial species, but also from fungal species. For instance, farnesol, farnesoic acid, tyrosol, tryptophol, phenylethyl alcohol, and other quorum quenching molecules have been identified from *C. albicans* [99]. In *C. albicans,* farnesol has stopped germ tube formation and hyphal-inducing conditions in the later stages of biofilm development. Therefore, farnesol acts as a repressor of the switch from yeast to hyphal growth, but unable to block the elongation of pre-existing hyphae [100]. Farnesol is not produced under anaerobic conditions, but other alcohol-based auto-regulating substances can be produced to inhibit biofilm development and to stimulate the distribution of yeast cells from the biofilm [101]. Another quorum quenching molecule called sesquiterpene alcohol farnesol is also capable of blocking the yeast to hyphal switch and biofilm formation. Moreover, it has been suggested as an outlook for an anti-infective strategy in *C. albicans* pathogenesis [102].

Microbial biofilms are formed when conditions are unfavorable. The external matrix of biofilms gives protection to the microbial cells and allows them for the production of virulence factors. Biofilms dissolving strategies would be given the highest priority to combat biofilm infections. For example, nitric oxide was formerly identified as a signal for biofilm dispersal in *P. aeruginosa* [103]. Furthermore, nitric oxide has also been observed in the dispersal of pathogenic biofilm-forming microbes, including *E. coli, V. cholera, N. gonorrhoeae*, *S. aureus*, and other bacterial multispecies and fungal species [103, 104, 105].

A study conducted on 12 biofilm-forming *P. aeruginosa* clinical isolates indicates that the addition of nitric oxide consistently caused dispersal of the bacterial cells after 5 h as determined by fluorometric measurements and confocal microscopy [106]. Although it is appealing to use nitric oxide, the increased nitric oxide concentration has a number of side effects such as immunosuppression, inhibition of angiogenesis or even cytotoxicity via nitrosylation of proteins [107].

The combination of conventional antibiotics with biofilm controlling compounds could disperse and treat biofilm infections. Dissolving an existing biofilm is an important aspect of the host immune system to the clearance of microbial pathogens [108]. Since most of the biofilm dispersing medicines do not kill the pathogenic cells; the mixture with antibiotics can bring a promising result. For example, in *P. aeruginosa,* patulin was tested to antagonize the purpose of acyl-homoserine lactone, but it had no effect on the presence of *P. aeruginosa* cells in a given biofilm. However, the mixture of patulin with antibiotic tobramycin was more powerful and leads to severe killings of the bacterial cells [109]. Another report had also shown that the combination of the quorum-quenching compound with the antibiotic tigecycline increased the sensitivity of *S. aureus* four-fold related to tigecycline alone [110]. Moreover, the treatment of *S. aureus* with the combination of cis-2-decenoic acid and ciprofloxacin was augmented from 11% for the antibiotic alone to 87% [111].

Similar observations have been made for other bacterial species such

as *Mycobacterium smegmatis, Neisseria subflava* or *Bacillus thuringiensis* [112] or in other mixed biofilms of *Klebsiella pneumonia* [113]. A 4, 5-disubstituted-2-aminoimidazole quorum quenching compound conjugates with triazole had shown biofilm formation inhibition against methicillin-resistant *S. aureus* or *Acinetobacter baumannii* [114].

3. Conclusion

Biofilm infections are highly resistant to the current antimicrobial agents and remain a serious concern in healthcare services. To discover promising treatment strategies against biofilm-associated infections are an urgent task due to biofilm resistance to already used antimicrobial agents. Few innovative and effective antibiofilm strategies, such as isolations of quorum quenching compounds, dispersal of formed biofilms, combinations of antibiotics with quorum quenching compounds, and a combination of all these novel techniques were tried. Even though the aforementioned antibiofilm strategies are important fields of investigation, still they are in the infancy stage and they did not pass through clinical trials and entered into the commercial market but we hope in the near future. We forward our suggestions and recommendations to researchers to find sustainable approaches to prevent and control biofilm infections.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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