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Alternative Approaches to Combat Medicinally Important Biofilm-Forming Pathogens

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Abstract

Bacteria have developed the capability to produce structured communities (or cluster of cells) via adherence to surface to form biofilms that facilitate or prolong their survival under extreme environmental condition. Bacterial biomass adheres to inanimate and biotic surfaces in the hospital setting as well as in the environment. In the healthcare system, the biofilm formation on medical devices allows bacteria to sustain as a reservoir and becomes more resistant to antimicrobial agents. However, biofilm formation facilitates pathogens to sabotage the host defenses that are linked to long-term retention within the host cell. Therefore, in this review, we provide some steps leading to the formation of biofilm within the host and on inanimate surfaces, also emphasizing various medically significant pathogens and debate current developments on novel approaches that aimed to prevent biofilm formations and its dispersion to patients.

Keywords: biofilm, antibiotic resistant, distribution, control, therapeutic strategy

1. Introduction

Biofilm formation is structured accumulation of fastidious microorganisms attached on inanimate objects or compact surfaces that extensively have been examined in the past decades because they particularly cause infections and more often responsible for chronic infections [1–3]. They are predominantly problematic due to their antimicrobial resistant properties and their ability to evade host defense mechanisms, which substantially hinders disease treatment in the hospital [1–4]. Bacterial biofilms are ubiquitous in nature and harbor phenotypic adaptations in the environment with respect to broader perspective [1]. The nature of single cell organisms enables them to adhere to each other and form a "complex structure," which assists to survive under adverse environmental condition. The biofilm formation occurs from planktonic bacteria due to environmental changes and involves in conjugation gene transfer "multiple regulatory network" from one bacterium to another in response to environmental stress [5–9]. This type of cell-to-cell adhesion and gene transformation changes the expression of surface molecules, virulence factors, and nutrient utilization that enables their survival under unfavorable environmental condition [8, 10–17].

Bacteria are cocooned within the biofilm and form extracellular matrix, which represents 90% of the biomass [18]. The matrix as a stabilizing scaffold for the three-dimensional structure is composed of extracellular polymeric substance (EPS) along with extracellular DNS and carbohydrate binding protein [19–21]. Nutrients are trapped by the resident bacteria in the matrix and water is retained efficiently via H-bond interaction with hydrophilic polysaccharides [18, 22]. The composition of extracellular polymeric substance (EPS) is modified in response to alterations in nutrient availability [23, 24] by certain enzyme secretion of bacteria, thus tailoring biofilm formation to the more specific environment [23, 25]. Therefore, the skeletal components of the extracellular matrix are highly hydrated and provide high tensile strength that enables bacteria to exchange their DNA by conjugation and promote cell-to-cell interaction while defending the biomass from predation, radiation, desiccation, oxidizing molecules, and other dangerous agents [18, 26–28].

The multifaceted nature of biofilms that allow the bacteria to form a community, i.e., division of labor and express their virulence factors in response to local oxygen and nutrient availability, makes them resistant against different antimicrobial agents [29, 30]. Some studies have shown that there are presence of nondividing metabolically inactive recalcitrant bacteria within the biomass [29, 31], which play very crucial role to cause tolerance against broad-spectrum antimicrobial drugs. The matrix protein inside the host cell protects bacterial biofilm against innate immune defenses, i.e., phagocytosis and opsonization [32]. The spread of other virulence factors inside the host cell and drug resistance marker is due to the cell-to-cell interaction [15]. Thus, biofilm-forming pathogens retained and adhere to the infected surface and cause recalcitrant and chronic infection, i.e., upper respiratory tract infection (particularly, *Pseudomonas aeruginosa*) [33, 34], dental decay (mixed culture of *Streptococcus mutans,* and other pathogens) [35], ventilated-induced and other device-associated infections (*Escherichia coli*, *Klebsiella* spp., *Enterococcus faecalis*, *Staphylococcus aureus,* etc.) [36, 37], urinary tract infections [*Proteus* spp., uropathogenic *E. coli* (UPEC)] [38]. In particular, immunocompromised patients are the most common target to all these biofilm-forming pathogens, causing a devastating impact on patients, and in many cases, leading to death. Here, we analyze the formation of intracellular and extracellular biofilm which is the underlying factor for various medically important microorganisms. Given the recalcitrance and prevalence of infections caused by biofilm-forming pathogens, we discuss knowledge about the most current progresses in the advancement of novel strategies of biofilm.

2. Extracellular formation of biofilm

2.1 Bacterial attachment on surfaces and what does make it adhere to object surface?

Bacterial biofilm growth, subsequent maturation, and aggregation consist of irreversible and reversible stages, which involve various conserved and species-specific aspects. At the first stage, the bacteria are introduced on the surface; a process of at least a part of stochastic that is driven by gravitational forces and Brownian motion, and usually influenced by nearby hydrodynamic forces [39, 40]. Microorganisms encounter with repelling or attractive forces—within the niche that alter depending on ionic strength, pH, nutrient levels, and temperature. Bacterial cell wall composition, along with medium properties, affects direction and velocity toward or away by the contact surface of pathogens [39]. Motile bacteria utilize flagella in order to overcome repulsive and hydrodynamic forces, by

having a competitive advantage. The main function of flagella is to provide motility and initial cell attachment to the surface for various pathogens, including *Listeria monocytogenes*, *E. coli*, *Vibrio cholerae*, and *P. aeruginosa* [41–45]. In some species of bacteria, chemotaxis plays very important role in direct attachment to nutrient composition, for instance, mutations arise in CheR1 methyltransferase, which have been observed to vary the response of amino acid of *P. aeruginosa* and impair maturation of bacterial biofilm and attachment [46]. Some earlier studies have been shown that chemotaxis in *E. coli* is dispensable [5]; moreover, current observations revealed that the disruption occurs in the chemotaxis methyl accepting protein II and informs biofilm defects particularly in uropathogenic *E. coli* cells [47]. With respect to intercepting surface, bacterial attachment is facilitated by additional secreted molecules such as adhesin protein and extracellular adhesive appendages.

Initially, the attachment is reversible and dynamic during which pathogens can separate and rejoin planktonic biomass if agitated through repulsive forces [48], hydrodynamic forces—detach bacteria off from the surface. Some bacteria attained irreversible attachment in order to maintain a firm grip on the cell surface. Serotypes of other *E. coli* and uropathogenic *E. coli* depend intensely on the type 1 pili [5, 40, 49–51]. Uropathogenic *E. coli* harbors several pili systems (means CUP system), which mediate adhering to a specific niche [38]. Attachment on the bacterial surface is facilitated by the adhesion protein (FimH), which identifies mannosylated moieties [50–52]. The adhesive protein (FimH) plays a critical role in the pathogenesis of uropathogenic *E. coli* because it facilitates adherence and causes invasion to epithelial cells of bladder in human, adheres to the human uroplakin and is also critical in preclinical murine cystitis model, which causes human disease [51, 53, 54]. FimH is much more consistent to play a critical role in the virulence of human disease under positive selection [52–56].

Furthermore, antigen 43, curli fibers, and type 1 pili have been observed to facilitate attachment and cell-to-cell interaction on inanimate surfaces [57]. Curli fiber also mediates attachment to the extracellular matrix components in eukaryotes such as plasminogen, fibronectin, and laminin [58]. *Pseudomonas aeruginosa,* for instance, uses various additional organelles, which assist in adherence to the surface, irreversibly. Contrary to UPEC and *P. aeruginosa*, Gram-positive bacteria (*Enterococci*) are lactose producing, nonmotile, and recently identified to contain nonadhesive (pili) that mediate attachment to the extracellular matrix components in eukaryotes. Examples of these include Ace (*E. faecalis*) and SagA (*E. faecium*), which attach to the collagen protein [59] and surface protein (Esp). This has been observed to stimulate abiotic formation of biofilm on the contact surface specifically in *E. faecalis* [60]. Current studies showed the existent of biofilm-associated pili (Ebp) and also confirmed their contribution toward urinary tract infections, endocarditis, and biofilm formation and attachment [61].

2.2 Maturation of biofilm

Cell-to-cell interaction triggers specific intrinsic responses that cause changes in the gene expression, upregulating factors favorable to sessility especially for those involved in extracellular matrix protein formation [40]. However, relatively very little information is obtained about the matrix constituents with respect to *E. coli* pathogen. Initially, cellulose was recognized as essential components in *E. coli* pellicle biofilms and later on expressed with curli fibers in gastrointestinal *E. coli* strains [62]. Curli fiber plays a critical role in pellicles, for instance, curli fiber (amyloid) that leads to the pellicle biofilm formation. It also acts as a curlicide to prevent pellicle formation, and some of them have deficient to form pellicles (known as curli mutants) [63]. Further studies revealed that colonic acid and polyglucosamine

(PGA) take part in biofilm architecture [64], while the PGA being predominant among the clinical strains, particularly in UPEC isolates. Thus, more detailed investigations are required for further characterization of extracellular matrix protein in *E. coli*. The composition of extracellular matrix protein has been extensively analyzed in *P. aeruginosa* and varies depending on external environmental conditions [65]. The primary components of EPS are Psl and Pel [25]. Psl enhances the attachment of *P. aeruginosa* to epithelial cells [66] and mucin, while the expression of Pel increased in small colony variants (SCV) isolated from the cystic fibrosis patients associated with *Pseudomonas* persistence in the airways of lung [67]. Moreover, intercellular interactions and biofilm stabilizations in *P. aeruginosa* are critical in response to environmental DNA (eDNA) [68].

Mature *P. aeruginosa* biofilm formations are more resistant to treatment with DNase as compared to young biofilms, demonstrating that eDNA remains stable because the components of EPS are not abundant during the initial stage of biofilm when the bacterial cells come to attach each other. In contrast, the concentration of eDNA increases during biofilm maturation stage due to the occurrence of bacterial cell lysis in response to quorum sensing mechanism of *Pseudomonas* quinolone signal (Pqs) [69]. In *Pseudomonas*, type IV pili play an essential role in the migrating pathogens to form aggregation in the area of high eDNA binding attraction [70]. The amount of eDNA to form biofilm structure has already been observed in *E. faecalis*. Some reports identified that biofilm formation in this organism is influenced by the affected autolysis of cells and intracellular release of DNA [71, 72]. Initial study reported that the mutant reduced the biofilm formation by 30% due to the lack of autolysin gene, Atn [59]. In another study investigated, it showed that specific stage of bacterial biofilm formation required temporal regulation by Atn for the release of DNA [73].

2.3 Matrix escape mechanisms

Bacterial mature biofilm provides a suitable living environment to the resident microorganisms for making compact surface adherence community, so as to share products and actively exchange their genetic materials by conjugation. Moreover, as biofilms mature, dispersal becomes a choice. In addition to passive dispersal caused by shear stress, the pathogen develops different ways to recognize environmental changes, which make it to stay within the biofilm. Bacterial biofilm dispersal occurs as a result of various clues such as oxygen fluctuations, modifications in nutrient availability, and increases in toxic products [74]. Biofilm dispersal is induced by the increase of extracellular iron in uropathogenic *E. coli* [75], while in *Pseudomonas* spp., it is due to the increased quantities of various nitrogen and carbon source [76]. The amounts of small molecules such as alterations in environment and changes in gene expression are monitored by various sensory systems [77]. Among various other signals, for instance, universal cyclic-di-GMP has been used in *P. aeruginosa* and *E. coli* causing implication in a shift between motility and sessility. Typically, an increase in the level of cyclic-di-GMP is favorable to sessility, while a reduction in cyclic-di-GMP induces upregulation of motility [78].

Recently, some results reported the factors responsible for such changes such as downregulation of extracellular polymeric substance, reduction of cyclic-di-GMP in bacterial biofilm communities, and upregulation of swarming and swimming motility [25]. Certain type of enzymes (such as alginate lyase) also participates in pathogen detachment from surface especially in *P. aeruginosa* [79], whereas in *E. coli,* the enzyme (CsrA) is responsible to repress the synthesis of PGA [80]. Along with that downregulation of EPS, certain molecules of surfactant are produced causing a reduction in cell-to-cell interaction. Moreover, studies identified that

flagellated populations within the biofilms of *P. aeruginosa* migrate to other void surface in order to make colonies [65]. Initially, these colonies loosely attach to compact surface, but after maturation process, they make a hard shell in the surrounding and use the infected surface as a source of nutrient. Sometimes, live cells use dead cells as a source of carbon. When bacteria become dead, then live cells accumulate on it, bind to each other by sharing their genetic materials and form a compact layer that is usually very hard to break. Dead cells are also responsible for creating cavity within the bacterial biomass. The bacteria within the biofilm can be scattered by applying dispersal mechanism.

Due to dispersing nature of bacteria, they may have the ability to restart the biofilm formation process after encountering a favorable environmental condition [81]. This is another sophisticated mechanism of dispersal revealed by using *B. subtilis,* which could be prevalent among the bacterial species. Researchers reported that the pathogen (*B. subtilis*) lost its cellular integrity within 5–8 days and also found that disassembly of biofilm is associated with a mixture of different amino acids (D-tyrosine, D-methionine, etc.) that are formed during bacterial stationary growth phase [82]. These D-types of amino acids interfere with bacterial attachment to cell surface and perturbation to fiber dissociation, without influencing matrix component expression or bacterial growth [83]. In *B. subtilis*, the performance of biofilm is disrupted by the addition of D-type amino acid mixture [83]. Further studies showed that another factor such as norspermidine, which is produced by *B. subtilis,* works together with D-type amino acid leading to biofilm disassembly [84]. So, this type of association—norspermidine/D-type amino acid—is essential for the eradication of bacterial biofilm and makes them vulnerable to antimicrobial agents used in the hospital.

3. Bacterial intracellular biofilms

Gathering evidence have showed that numerous bacterial pathogenic species formerly considered as extracellular can retain within the host cell by adapting intracellular bacterial lifestyle that includes the bacterial communities having biofilm-like properties. First, a murine model of infection was used to assess the bacterial communities for UPEC [85]. Type 1 pili in uropathogenic *E. coli* bind to the receptor on superficial bladder cells [86], triggering to induce bacterial internalization. Toll-like receptor-4 (TLR-4)-dependent process used to expel out from inside the UPEC [87], but certain bacteria elude exocytic procedure and leave out from the cytoplasm of host cell, where they duplicate into intracellular bacterial communities (IBCs) [85]. Several developmental stages lead to the process of IBCs that indicate distinct morphological features [85]. After passing first 6 h ensuing bladder inoculation, UPEC rapidly divides (replication time 30–35 min) causing small clusters associated with loosely attached rods (during early IBCs), having a coccoid shape and an average bacterial length of about 0.7 mm. The bacterial exponential growth rate dramatically drops between 6 and 8 h, exceeding replication time to 60 min. This is the second stage where bacteria accumulate and are tightly packed within the biofilm and organized a compact sphere-shaped structure (mature-stage IBCs) (**Figure 1**).

The amount of IBCs is found between 3 and 700 in an infected patient's bladder—IBCs are composed of 10^4–10^5 bacterial cells [88]. There are numerous fibers surrounded on IBC bacteria that originate from the surface of pathogen and enclose pathogens in individualized sections. One of the main components present on the surface of IBCs called polysaccharide (sialic acid) that provides protection from the attack of immune system and environmental stress. The heterogeneous nature
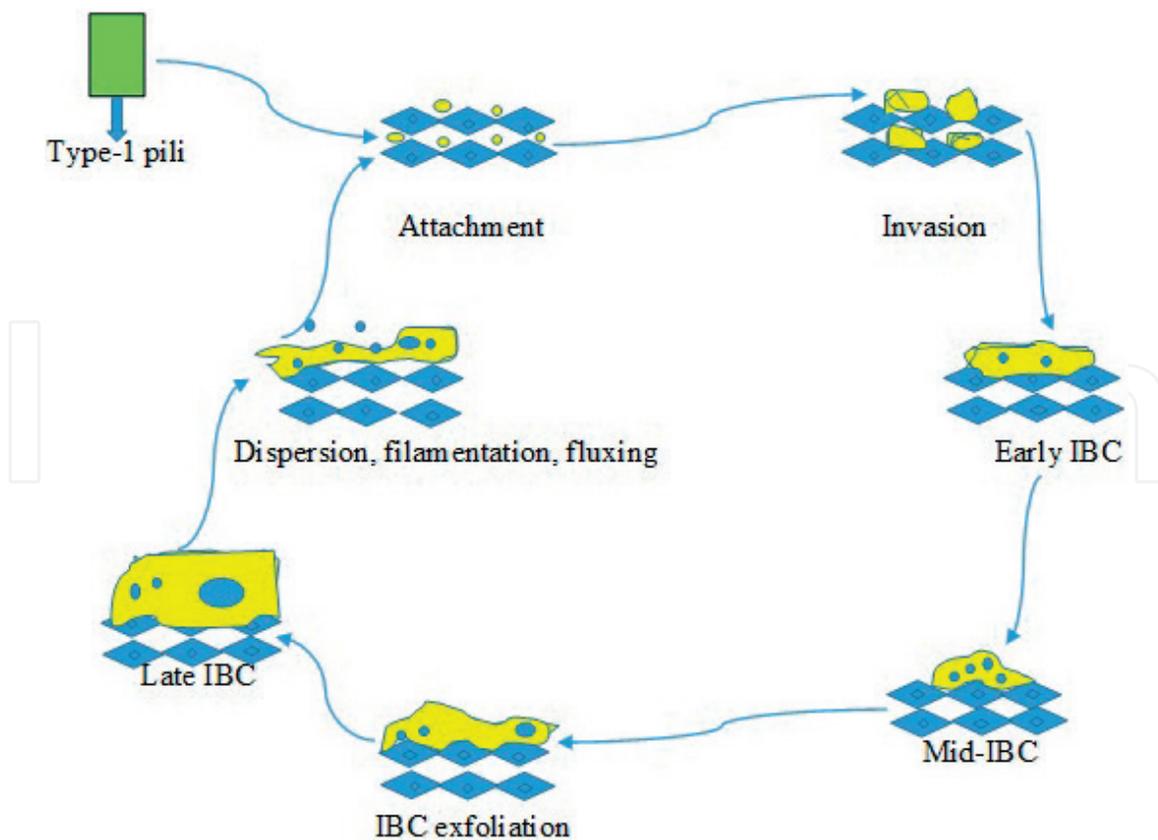
Figure 1.
Schematic diagram of the development of IBC cascade in uropathogenic E. coli (UPEC), taken by scanning electron microscope (SEM) images indicating different structural changes from attachment to dispersion and fluxing.

of IBCs, such as extracellular bacterial biofilm, composed of different subpopulation having distinct gene expression systems [89]. As IBCs expand, they induce the bacterial biofilm to cause interruption against cell membrane of host, producing a pod-like structure on the infected cell surface. Ultimately, UPEC detaches as filament or single rod at the IBC boundary and the infected cells are flux out into the lumen of bladder where can invade epithelial cells and restart the process through binding [85]. The inhibitor (SuIA) of cell division has been observed to be crucial for dispersal and filamentation of UPEC from the bacterial biofilm. The patients suffered from urinary tract infections (UTIs) are more likely observed with the UPEC filaments in their urine, but not in comparison with healthy controls [90].

The formation of IBC is prevented by intense molecular blockages and during acute infection—development of chronic cystitis—the IBC numbers are higher, representing the significance of intracellular pathways in the pathogenesis of UTIs [88]. The cycle of IBC is dependent on FimH, causing interruption in the expression of type-1 pili after invasion to host cell, and disrupts normal development of IBC due to attenuation of UPEC [54]. The two-component system (QseBC) is a key factor influencing curli expression, formation of IBC and type-1 pili. Some studies indicated that the intracellular pathway of UPEC is necessary for the TCA cycle completion [47]. The techniques such as qPCR and DNA microarray analyses interpreting the UPEC expression patterns within IBC pathogen exposed that acquisition of iron in bacteria is upregulated, representing the significance of system biomass formation [91]. While in clinical isolates of UPEC, the iron acquisition patterns are prevalent [92]. Moreover, the pathogen *Klebsiella pneumoniae* is more commonly seen in community- and hospital-acquired infection. About up to 5% forms intracellular communities and is more predominant in hospitalized diabetic patients [93]. Likewise to UPEC, the *Klebsiella pneumoniae* invasion is mediated by

type-1 pili and formation of IBC, although the differences occur in the expression kinetic of pili and filaments [90]. The ability to occupy an intracellular niche and persist within the host cell through transitioning from single microbial cell to the multicellular community is not confined to uropathogens. Researchers showed that by using different animal models and cell line of acute lung infection, the cluster formation occurs inside the lung airways due to *P. aeruginosa*, morphology similar to *Klebsiella* and UPEC (IBCs) [94]. The biofilm formation ability could be evolutionary adaptation of pathogens that enable the bacteria to persist within the host cell. All these findings represent the formation of IBC, a process that enables the bacteria to rapidly expand inside the host cell and take part in bacterial persistence.

4. Postantibiotic period: treatment strategy for biofilm

Broad-spectrum antibiotics are the drug of choice for the treatment of bacterial infections. Conventional antibiotics act as either killing the bacterial cell (bactericidal) or inhibiting the cell division (bacteriostatic). Numerous evidence shows that the use of antibiotics extensively causes damage to the host microbiota, producing a condition where invading bacteria can prevail and enhance the selective pressure against drug resistance [95]. Furthermore, surgery proceeded by administering antibiotics is highly successful in order to minimize the infection prophylactically. In certain cases, the perfect treatment of choice for foreign material associated with biofilm infections is the removal of infectious device. In some cases like pacemakers, cardiac implants and implantable prostheses, device removal is difficult [37]. Biofilm formation nature of bacteria that make them recalcitrant against different antimicrobial drugs is a result of prolonged treatment. There is a need for the irradiation or complete removal of these kinds of pathogens. Antibiotic resistance is not only due to increased resistance markers transmitted within the bacterial biofilm community, but also due to high metal ion concentration, low pH, and the presence of persistent cells that are metabolically inactive and inactivate the antibiotics
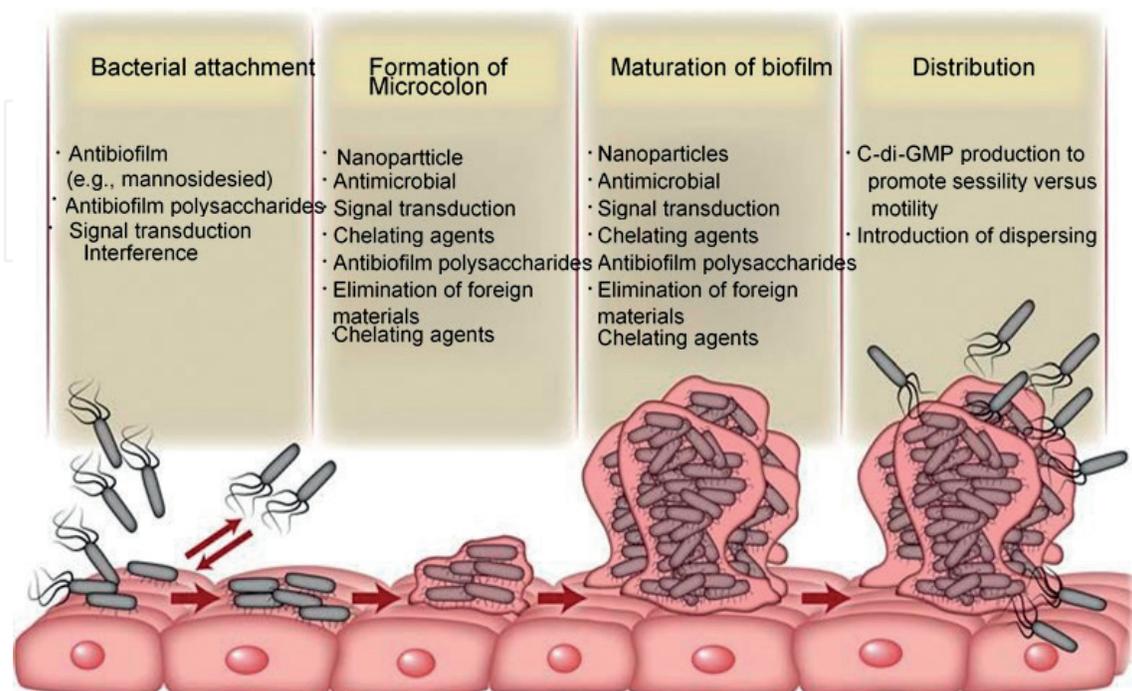

Figure 2.
Schematic diagram about the different stages in the development of biofilm and indicating the strategies to preventing and damaging the bacterial biofilm production at particular stages.

[31]. All these characteristics make bacterial biofilm more tolerant/resistant to antimicrobial drugs up to 1000-fold more when compared to planktonic bacterial cells [96]. Therefore, an alternative strategy must be investigated to combat the antibiotic resistant strains and make them vulnerable to antimicrobial drugs. Here below, we have mentioned some of the recent developments in strategies that are considered to prevent formation of biofilm by bactericidal mechanism or targeting distinct developmental stages of biofilm (**Figure 2**).

5. Bacterial killing strategies

5.1 Elimination of foreign material (indwelling devices) and abscess

There are studies that have reported that the presence of any foreign body (indwelling medical devices such as implants or prostheses or catheters) in low inoculums of *Staphylococcus aureus* (10^2 CFU/ml) in animal tissues was sufficient to form abscesses in the patients (95%) despite significant existence of leukocytes. In fact, this could be associated with the existence of any foreign material considerably intracellular bactericidal effects of body immune cells (leukocytes) and down-regulated the mechanism of phagocytosis [97]. The polymorphonuclear leukocytes cannot perform well in the presence of any foreign body because it provides a surface ideal for the bacterial attachment. Therefore, the existence of any foreign material considerably increases the chances of bacterial biofilm formation. This leads to the pathogen becoming more persistent and resistant against conventional antibiotics. Thus, potential therapeutic strategy is required for the elimination of such type of bacterial biofilm formations. Certain precautionary measures could be employed, for instance, to replace the infected devices used for medical purposes in the patients with a new one. Otherwise, it would be hard to overcome the problem regardless of applying various effective antimicrobial drugs in response to fastidious pathogens. Changing dialysis catheter if it is infected by the pathogens is another measure that could be taken. When pathogen forms biomass on the catheter, it could be the source of bacterial colonization leading to bacteremia which may be caused by a deadly bacterial strain. For the cure of catheter-associated infections caused by bacterial biofilm formation, it is important to change the catheter infected with pathogens along with administration of antibiotic intravenously during a short time in order to eradicate the pathogen before it invades into the bloodstream. However, in some cases, it is hard to change the catheter temporarily; therefore, antimicrobial drugs and other alternative therapy may be the best option for the minimal release of pathogens from the infected site.

5.2 Phage therapy

An alternative approach to antibiotic treatment is phage therapy [98]. Phages are present in a wide range in the environment. It can be isolated easily and ubiquitous in nature. Their host ranges from specific to narrow, they are able to self-replicate, and therefore, a small dosage may be sufficient to disturb the host microorganisms. Furthermore, high mutation rate of phage facilitates adaptation as conforming bacterial host aggregate mutations to fix in a specific environment. Phage therapy has various advantages during lytic cycle phage that does not enter prophage cycle and rarely transfers or contains a virulence gene, thus causing destruction of bacterial cell rapidly. Many phages are associated with EPS degrading protein [99] or spread during stationary growth phase; these features allow to persist inside the bacterial biofilm [100].

5.3 Antimicrobial peptides

This is another alternative approach used for the improvement of new type of antimicrobial drug, usually produced by innate immune response mechanism [101]. Contrary to that, their mechanism of action and antimicrobial spectrum activity must be defined more accurately before applying as a therapeutic strategy. Cathelicidin, for instance, possesses most essential type of antibacterial peptides. The biofilm formation of multidrug-resistant *Pseudomonas* strains, isolated from cystic fibrosis (CF) patients, is reduced considerably by BMAP-28, BMAP-27, and BMAP-29 [102]. According to a recent study by Pompilio et al. [102], antimicrobial activity of tobramycin against multidrug-resistant strains is less than cathelicidin peptides. This study indicates that the multidrug-resistant strains are vulnerable to cathelicidins due to antibiofilm agents. Another important group that can be used to assess the inhibitory effects is called lytic peptides. These peptides assist in attachment of lipopolysaccharides (LPS) to the cell membrane of pathogen and cause cell membrane disruption. The study on *Staphylococcus aureus* indicated that *in vitro* formation of biofilm is prohibited by the lytic peptide (PTP-7) and easily penetrates the bacterial biofilm causing death of the bacteria at a rate of 99.9%. This peptide has the capacity to bear extreme acidic environment and inhibit the biofilm formation of *Staphylococcus aureus* [103].

5.4 Silver nanoparticles

Many researchers have done research on the antimicrobial property of silver nanoparticles. Fey [37] found that the silver nanoparticles are the best alternative strategy to combat the bacterial biofilms. For example, antimicrobial agents (silver nanoparticles) have been incorporated with medical devices and have showed to inhibit the device-associated bacterial biofilms. Silver was frequently used as an antimicrobial agent for different pathogens over a 100 years; for instance, during World War 1, it was extensively used to sterilize the wound infections [104]. The antimicrobial activity of silver nanoparticles depends on the positively charged ions of metal and electrostatic interactions between negatively charged cell membrane of bacteria [105]. The thiol group in silver is the main cause of death in bacteria that play an important role in the inactivation of enzyme [106]. This is the reason why silver nanoparticles are increasingly used in response to various bacterial infections. The antimicrobial agents contain different properties such as high aspect ratios, nonimmunogenic, biocompatible, nonbiodegradable, ultralight weight, and easy cell membrane penetration. Due to such remarkable properties, we can apply silver nanoparticles in various applications such as infection therapy, gene therapy, and as antioxidants. The size of silver nanoparticles is typically smaller than 100 nm. The mechanism of action of silver nanoparticle is to interrupt the cell membrane of bacteria, generate the reactive oxygen species (ROS), interrupt the metabolic pathway, prevent the replication of DNA, disrupt the bacterial electron transport chain (ETC) [106], and release the toxic ions outside the bacterial cells that lead to the death of bacteria. There are large numbers of studies conducted regarding toxicity mechanism of silver nanoparticles in rabbits. There is a study that showed that silver nanoparticles inhibited bacterial biofilm formation against *Staphylococcus aureus,* without accumulating inside the host tissue [106, 107].

5.5 Polysaccharides

Bacterial cell-to-cell interaction mediated by the exopolysaccharides is a serious threat to the formation of biofilm and stabilization. Mutants incapable to export or

synthesize such exopolysaccharides are usually deficient in the formation of biofilm and adherence and hence are extremely sensitive to killing through host immune defenses and antimicrobial drugs [108]. Recent studies showed that certain bacterial exopolysaccharides destabilize or prevent biofilm formation by some pathogenic species. For instance, the existence of *Pseudomonas aeruginosa* prevented biofilm formation of *S. epidermidis* in in vitro experiments [109]. Polysaccharides along with nonbactericidal antibiofilm characteristics have been separated from acellular biofilm (or biomass) extracts of various species [108]. The antibiofilm properties of *Pseudomonas aeruginosa* have the ability to act as signaling molecules that effect the expression of genes in susceptible pathogens, change the physical features of isolated bacterial cells, and prevent the protein-carbohydrate interactions. Most polysaccharides with antibiofilm properties allow a broad-spectrum inhibition of biofilm, while some are proficient of scattering preformed biofilms. So far, there are evidence suggests that polysaccharide with antibiofilm features acts as a surfactant molecule that alters the physical properties of abiotic surfaces and bacterial cells. Some results also show that polysaccharides might modulate the expression of genes of the recipient pathogenic bacteria by acting as signaling molecules [110]. Another potential mode of action of polysaccharide is to prevent competitively the multivalent protein-carbohydrate interactions [66]. As a result, polysaccharides with antibiofilm properties might block tip adhesins of pili and fimbriae, or block sugar or lectin-binding proteins that are present on the outer surface of pathogens. In pathogen *P. aeruginosa*, for instance, lectin-dependent adhesion to human cell is proficiently repressed by galactomannans [111]. This kind of polysaccharides that inhibit the biofilm could be a prominent strategy appropriate for the prevention of bacterial infections. Some scientist showed that antibiofilm polysaccharides can be used as an adjuvant because of enhancing antibiotic drug functions [108].

5.6 Interference with signal transference

Many studies have been carried out on biofilm inhibition caused by interruption of the pathogen signaling cascades. This is possible provided that the two-component systems in bacteria establish a dominant means of translating and intercepting the environmental changes. Signal transduction inhibition system plays a critical role in response to antimicrobial therapy because of this type of signaling cascade interruption. Not only does it kill the pathogen, but it also interferes with the gene expression. Two-component system (QseBC) is the best alternative candidate for targeting the drugs, particularly in Gram-negative biofilm-forming pathogens [112]. QseC/QseB establishes a significant association between the bacterial environmental signaling and the host stress response. The pathogen (*E. coli*) responds to autoinducer-3 in the intestine that is formed by the human stress hormones (such as epinephrine and norepinephrine) and gut flora. The cascade of signaling transduction comprises chemotaxis by activation of QseC and by using the serine receptor Tsr. In the quest for novel antimicrobial drugs and therapeutic targets, two-component system (QseBC) can play an important role to inhibit biofilm formation by blocking the binding of epinephrine or norepinephrine to QseC, as a result to reduce QseB/QseC signaling and decrease virulence and motility [113]. Studies have also suggested that the removal of QseC in EHEC and UPEC causes an excessive activation of response regulator QseB, owing to particular QseC phosphatase activity required for deactivation of QseB. The optimal strategy behind targeting the phosphate activity is to interfere with common gene expression in QseC containing pathogens [47]. Some other studies focused on the FsrATC/FsrA inhibitors in *E. faecalis*. The expression of gelE-sprE and FsrBDC

control by the FsrC/FsrA leads to increase in the production of serine protease and gelatinase, both are crucial for the proper eDNA production [71].

5.7 Antimatrix agents

Apart from that, extracellular matrix with disrupting components is also very important to target the bacterial aggregates. Various observations exploited the inhibiting enzymes potentially involved in the modification or synthesis of cell wall-secreted or associated with EPS components. In these studies, use of engineered or naturally occurring enzyme and use of phage therapy as an enzyme delivery vehicle or to interrupt with matrix integrity by taking benefits from metal chelators have been recommended.

5.8 Chelating agents

Metal cations such as iron, magnesium, and calcium have been associated with stabilizing the matrix integrity [114]. Chelating agents indicated to cause interruption in the bacterial cell membrane stability besides disrupting the bacterial biomass structure [39]. *In vitro* study showed that biofilm formation was inhibited in various *Staphylococcus* species by sodium citrate [115]. Furthermore, eradication of bacterial biofilms in *in vitro* experiments is also facilitated by tetrasodium EDTA, while disodium EDTA only reduced the bacterial biofilm formations in *P. aeruginosa* and *Staphylococcus* species [116]. Current reports suggested that the solution of minocycline-EDTA was used to inhibit indwelling catheter-associated infections especially in children. There were no adverse side effects observed in patients treated with the solution of minocycline-EDTA but only a limited number (21%) of untreated group (control) developed infections [117]. Moreover, in hemodialysis patients, catheter-associated bloodstream infections were observed after applying minocycline-EDTA [118].

5.9 Enzyme

The main mechanism of active dispersal of bacterial biofilm is through the formation of extracellular enzymes (proteins) that act on several structural components (such as exopolysaccharides, surface proteins, and extracellular DNA) of the extracellular polymeric substances. These enzymes play an important role in the cell separation from the bacterial biofilm colonies and facilitate their planktonic discharge into the environment [119]. Through purifying and isolating these enzymes, therapist can apparently add them to preformed bacterial biofilms exogenously at raised concentrations, in order to make biofilm-associated bacteria more susceptible to antimicrobials/antibiotics and to achieve interventional dispersal of biofilms. For this purpose, several classes of enzymes (specifically proteases, glycoside hydrolases, and deoxyribonucleases) have been explored for the eradication of bacterial biofilms [119]. The enzymes dispersin-B and DNase-I have gained greater attention as possible antibiofilm agents, especially in response to Gram-positive bacteria. The DNase effect depends on its capability to interrupt the eDNA that is established within the bacterial biomass structure [73]. The treatment of DNase prevents biofilm formation in *Enterococcus* and *Staphylococcus* and dispersed bacterial biofilm [73]. For the treatment of patients with cystic fibrosis (CF), a recombinant enzyme (pulmozyme) is used in some cases [37]. However, treatment with dispersin-B represented to be more effective in response to *S. aureus* and *S. epidermidis* [77]. *In vitro* studies indicated that engineered dispersin-B used

bacteriophage machinery in order to replicate during the stationary phase of cell growth, hence causing disruption of complete *E. coli* biofilms [120].

6. Conclusion

Currently, the removal of bacterial biofilm is the most challenging task for the clinicians and microbiologists. Antibiotics are not the best choice for the treatment of infections caused by bacteria forming biofilm. Biofilm formation allows the pathogen to adhere to the host surface under extreme condition and is resistant against a wide range of antibiotics. The choice of drug depends on the characteristics of the biofilm such as composition, age, solidity, and type of pathogens. These are the major components influencing the microbial susceptibility. As the bacterial biofilm matures, it enhances the accumulation of exopolymeric substance (EPS), attaches with the oxygen and nutrient gradients that effect bacterial growth rates and metabolism of cells, becomes impermeable, and reduces the activity of antimicrobial agents. This leads to resistance to most antibiotic regime. Therefore, novel potential therapeutic strategies should be considered to curb bacterial biofilm formation at specific stage without harming the pathogen. Antiadhesion and antimatrix agents are exciting strategies that may be used pending further investigation.

List of abbreviations

EPS	extracellular polymeric substance
DNS	deoxyribonuclease
CBP	carbohydrate-binding protein
DNA	deoxyribonucleic acid
CUP	chaperone-usher pathway
UPEC	uropathogenic *E. coli*
PGA	polyglucosamine
SCV	small colony variants
eDNA	environmental deoxyribonucleic acid
PQS	*Pseudomonas* quinolone signal
c-di-GMP	cyclic di-GMP
AL	alginate lyase
BS	*Bacillus subtilis*
TLR-4	toll-like receptor-4
IBC	intracellular bacterial communities
UTIs	urinary tract infections
SEM	scanning electron microscope
TCA	tricarboxylic acid
qPCR	quantitative polymerase chain reaction
HAIs	hospital-acquired infections
CFU	colony forming unit
MDR	multidrug resistant
LPS	lipopolysaccharides
ETC	electron transport chain
ROS	reactive oxygen species
EHEC	enterohemorrhagic *E. coli*
EDTA	ethylene-diamine-tetra-acetic acid
CF	cystic fibrosis

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