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Mechanism of biofilm formation in ESKAPE organism and their treatment

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ABSTRACT

One of the major global issues is the urgency of nosocomial infections, biofilm development, and antibiotic resistance. The ESKAPE pathogens are a significant factor in these problems (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter species*). These pathogens, often known as hospital-acquired infections (HAIs), are common in hospital settings and present one of the most significant difficulties in treatment. The development of biofilms, in which the microbial cells adapt to a multicellular lifestyle by getting trapped inside the extracellular polymeric matrix, is a key biological concept in clinical contexts. For the creation of novel antimicrobial agents, as well as for the repurposing of currently accessible medications or pre-clinical substances and the broader use of combination therapies, it is essential to comprehend the process by which these bacteria build biofilms. The Pathomechanisms of these bacterial biofilm formations and alternate strategies to prevent biofilm formation in hospital management are highlighted in this review.

Keywords: ESKAPE, Biofilm formation, HAIs, extracellular matrix, therapies

INTRODUCTION

Biofilms are an aggregation of bacterial communities covered by a protective exopolysaccharide layer. Inside this layer, the bacterial colonies communicate through a quorum-sensing mechanism. Biofilms aid infectious condition persistence. Because of its protection against germs from host defense mechanisms, toxins, and antibiotics, biofilm formation during infection is hazardous. Exopolysaccharides in the biofilm are essential because they determine its architecture and safeguard the interior colonies from degradation. Gene transfers also occur within the biofilm environment via conjugation, forming F conjugative pilus. About 15% of biofilm consists of different microorganisms; the other 85% comprises exopolysaccharides, proteins, DNA, RNA, and some ions [1,2].

The formation of biofilm involves several stages; Attachment of the cell to the surface, production of extracellular exopolysaccharide matrix, bacterial growth and attachment, biofilm maturation, and dispersion to form new biofilms. The phenotype of the biofilm is mainly described by the genes expressed by the cells associated with biofilm formation [3]. The rise in the antimicrobial resistance by the ESKAPE pathogens, a subset of bacteria linked to nosocomial infections, places a considerable strain on the healthcare system and has significant global economic repercussions. As a result, there is high-rate morbidity in the human population, rising healthcare expenses, and imprecise diagnostic outcomes [4]. One of the most considerable difficulties in combating infectious diseases worldwide is the development of antibiotic resistance in pathologically deadly microorganisms. Nosocomial infections caused by antibiotic resistant organisms raise the possibility of patients in post-operative wards, burn units, and critical care units developing life-threat-

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ening diseases. Hospitals are the primary multidrug resistance organisms (MDROs) colonization sites, although hospital settings are not the only ones where these occur [5]. Community locations, including animal farms, biohazard waste disposal sites, freshwater habitats, etc., serve as substantial breeding grounds for MDROs. The risk of MDROs spreading in communal settings is rising due to a lack of aseptic techniques used in patient care, such as the use of one non-sterilized stethoscope or thermometer, the use of ungloved or single-gloved hands in multiple patients in hospital wards, unethical and improper use of antibiotics in animal farms, and the disposal of unsterilized hospital waste in dumping sites and freshwater sources. It also hastens the horizontal transfer of resistance genes into nearby bacteria. Various processes can be used to develop antibiotic resistance, depending on the nature of the microbes [6,7].

There are multiple broad categories in which antibiotic resistance is classified, such as drug inactivation, drug binding site alteration, cell permeability change that reduces intracellular drug accumulation, and the formation of biofilms [8]. Low O2, low pH, high CO2, and little water availability, for instance, are situations where medications have less effect due to the mechanical and biochemical protection provided by the biofilm matrix [9]. The use of antibiotics alone, in combination, or with adjuvants, bacteriophage therapy, antimicrobial peptides, photodynamic therapy, antibacterial antibodies, phytochemicals, and nanoparticles as antibacterial agents are all part of the general treatment for biofilm infections [10]. An antibiotic's high spectrum coverage makes it helpful in treating severe symptoms brought on by various bacteria [11,12].

Some compounds increase the uptake of antibiotics across bacterial membranes, obstruct efflux pumps, and alter the physiology of resistant cells to make antibiotics more effective when paired with adjuvants. These ineffectual medications have moderate antibacterial activity of their own [13]. The most popularly known adjuvants are β lactamase inhibitors (vaborbactam, avibactam, nacubactam, and tazobactam). In combination with antibiotics, metal chelators like EDTA, deferasirox, and deferoxamine also inhibit β lactamase and quorum quenchers (inhibit biofilm formation by inhibiting quorum sensing) [14,15]. In both planktonic and biofilm 1-[2,4-Dichlorophenethyl) amino]-3-Phenoxypropan-2-ol is the most potent antibacterial agent against ESKAPE infections [16].

Enterococcus faecium

The typical gut flora of humans and animals comprises *enterococci*, Gram-positive facultative bacteria. Endocarditis and other nosocomial infections, including bacteremia, meningitis, and urinary tract infections, are known to be brought on by these [17]. *Enterococci* are the usual residents of the GI tract, present in the microbiota of humans and other animals in small portions along with other beneficial bacteria. These bacteria tend to form colonies, which is how biofilm is created [18].

Patients' exposure to antibiotics alters the microbiota of the human gut forming colonization of vancomycin resistance *enterococci* (VRE). As there is a surplus of VRE in the microbiota, the lipopolysaccharide and flagellin of Gram-Negative bacteria and anaerobes induce the generation of protein Regenerating islet derived protein III gamma (REGIIIγ) by paneth cells via stimulation of toll-like receptors (TLRs) by pathogen-associated molecular patterns (PAMPs). A C-type lectin called REGIIIγ has antibacterial action against VRE and other Gram-positive bacteria. Reductions in the production of REGIIIγ, a C-type lectin with antibacterial activity against Gram-positive bacteria, including VRE, are caused by antibiotics' drops in the Gram-Negative microbiota. As a result of this decrease in REGIIIγ production, *enterococci* acquire control of the gut microbiota. Forming biofilm has been recognized as an essential element in developing antimicrobial tolerance and resistance. The gene mainly involved are *fsrA, fsrB,* and *frsC*, which function as quorum sensing and autolysis (release of eDNA), esp as an attachment, and epa (orfde5) as an attachment of biofilm accumulation. [19].

Treating *E. faecium* is very difficult as the strain develops multiple antibiotic resistance capacities. Following are some of the antibiotics showing resistance against this strain. Hospital-associated infections causing *E. faecium* show high resistance to ampicillin. The difference between penicillin-binding protein 5 (PBP5) of *E. faecium* and PBP5R, which has a lower affinity for β-lactams than PBP5S of other community strains, is the mechanism of ampicillin resistance [20]. The hospital-associated infection strains show resistance to ampicillin with MICs >64mg/L, with ampicillin concentration MIC <64mg/L; this strain has high resistance if the ampicillin concentration is higher in combination with aminoglycosides. Vancomycin was the substitute if ampicillin developed resistance to *E. faecium* in the past days. For instance, the recently isolated *E. faecium* of hospital-acquired infections showed resistance to vancomycin antibiotics, making it inadequate to treat infective endocarditis [17].

Vancomycin-resistant *E. faecium* can also be treated with the combination drug quinupristin-dalfopristin (Q/D), with 30% quinupristin (streptogramin B) and 70% dalfopristin (streptogramin) [21]. A bacteriostatic substance, linezolid, prevents the production of proteins by interacting with the A

TABLE 1. The genes/proteins responsible for the formation of biofilm

Microorganism	Genes/proteins responsible for biofilm formation	References
Enterococci faecium	*fsrA, fsrB, fsrC,* esp, epa	Arias *et al.* [19]
Klebsiella pneumoniae	*allS, iutA, fimA, fimH, mrkA, mrkD, pgaA, pgaB, pgaC, bcsA, wzc,* cpsD, *treC, wcaG, wabG,* rmpA,magA,k2a, *wzyk2, luxS, wcaJ, mrkABCDEF* cluster	Meng *et al.* [29] Bethny *et al.* [31]
Acinetobacter baumannii	CsuA/B, CsuA, CsuB, CsuE	Tomaras *et al.* [42]
Pseudomonas aeruginosa	Psl, Pel	Cynthia *et al.* [52]
Staphylococcus aureus	Bap, SasG, FnBPA, FnBPB, AtlA, *icaADBC, nuc, Sae*	Cucarella *et al.* [75] Matthias *et al.* [79] Derek *et al.* [94] Michael *et al.* [95]
Enterobacter spp.	csgBA(C), csgDEFG	Hammar *et al.* [107]

site of bacterial ribosomes. It has been FDA-approved for treating VRE infections and exhibits activity against various Gram-positive bacteria [22].

A cyclic lipopeptide known as daptomycin exhibits a bactericidal effect against *enterococci* resistant to vancomycin. Daptomycin was recently licensed by the US Food and Drug Administration to treat complex skin and skin-structure infections [23]. Antibiotic rifampicin shows resistance against many species. Rifampicin is effective against *S. aureus*, but it could act against *E. faecium* only when in combination with ciprofloxacin, linezolid, daptomycin, or tigecycline [24]. Numerous terpenoid derivatives can eradicate bacteria and prevent the formation of biofilms. Rhodethrin and rubrivivaxin, two new terpenoid derivatives, were discovered to be efficient antibiofilm agents against *E. faecalis*. Vancomycin-resistant *Enterococcus faecium* is resistant to the anti-biofilm effects of phage S2 [25,26].

Klebsiella pneumonia

Klebsiella pneumoniae is a Gram-negative, opportunistic, encapsulated bacterium that causes many diseases, including pneumonia, bacteremia, meningitis, and liver abscesses. Newborns, the elderly, and those with impaired immune systems are at risk for contracting *K. pneumoniae* infections. However, bacteria are also a growing source of community-acquired diseases. The bacterium can be found in the environment (soil and shallow waterways) and on abiotic surfaces like medical equipment. It invades human mucosal surfaces, particularly those of the gastrointestinal system and oropharynx, from which it can spread to other tissues. The prevalence of *K. pneumoniae* that is multidrug-resistant has significantly increased over the past ten years, underscoring the significance of better understanding *K. pneumoniae* pathophysiology [27,28].

K. pneumoniae biofilm formation methods are mediated by several genetic elements, including allantoin (*allS)*, aerobactin (*iutA*), type I (*fimA and fimH*), and type III *(mrkA and mrkD*) fimbriae, polysaccharides, and adhesins (*pgaA, pgaB, pgaC, bcsA*), capsular polysaccharide (CPS) (*wzc*, cpsD, *treC, wcaG, wabG*, rmpA/A2, magA, k2a, *wzyk2*), quorum sensing (QS) (*luxS*) and colonic acid (*wcaJ*) [29].

Duguid and coworkers were the first to identify and characterize the genetic element Type 3 fimbriae in the biofilm formation of Pneumoniae [30]. *K. pneumonia* fimbriae structures measure 0.5-2 nm in length and 2-4 nm in width. The *mrk* gene cluster (*mrkABCDF*), linked to five genes that code for the structural and assembly elements of the fimbriae, is responsible for encoding the fimbriae [31]. At least six *mrk* genes in *Klebsiella* mediate the production of type 3 fimbriae. *MrkD* promotes the production of

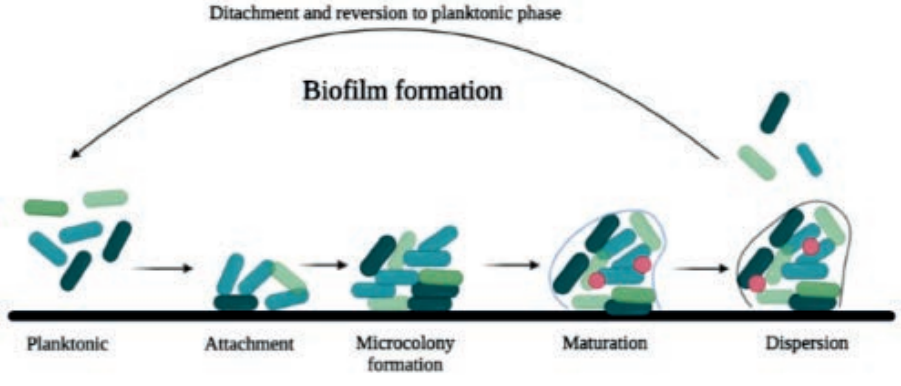

FIGURE 1. The life cycle of biofilm formation involves: 1. reversible attachment of cells to the surface.
2. irreversible attachment of the biofilm
3. colony formation
4. Maturation of the biofilm
5. cell death and dispersal of single cell that return to planktonic phase

TABLE 2. The characteristics and treatment options for pathogens involved in biofilm formation.

Pathogen	Characteristics	Infection Caused	Treatment
Enterococci faecium	Gram-positive, facultative anaerobic bacteria, resident of the human intestinal tract **Muita et al. [17]**	Endocarditis, Urinary tract infections, Bacteremia, Meningitis. **Muita et al. [17]**	Antibiotics- Ampicillin, Vancomycin, Quinupristin Dalfopristin, Linezolid, Daptomycin, Rifampicin, Rhodethrin, and Rubrivivaxin, **Carpenter *et al.* [23]** **Anna *et al.* [24]** **Eswara Rao *et al.* [25]**
			Phage S2 **Forough *et al.* [26]**
Staphylococcus aureus	Gram-positive, commensal bacteria, resident of the human nasal mucosa **Christian *et al.* [71]**	Endocarditis, Osteomyelitis, Septicemia, cystic fibrosis, abscesses of different organs, skin, and soft tissues infections, central nervous system infections, lung infections, infections caused by medical devices **Christian *et al.* [71]**	Antibiotics- Rifampicin, Linezolid, Vancomycin, a combination of oxacillin, linezolid, and tigecycline **Mohini *et al.* [97]**
			Antimicrobial peptide Ba49, LL-37, Pleurocidin **Ramita *et al.* [98]** **Su *et al.* [99]** **Mehmet *et al.* [102]**
			Phage SB-1 alone and in combination with Fosfomycin, Rifamycin, Vancomycin, Daptomycin, or Ciprofloxacin **Tamta *et al.* [100]**
			Chimeolysin (ClyF). **Hang *et al.* [101]**
Klebsiella Pneumoniae	Gram-negative, opportunistic bacteria, resident of the gastrointestinal tract and oropharynx of humans. **Paczosa et al. [27]**	Pneumonia, Meningitis, liver abscesses, bacteremia. **Guerra et al. [28]**	ZCKP1 phage, Siphoviridae phage Z, vBKpnS_Kp13 phage **Veronica *et al.* [38]**
			Antimicrobial peptides like WLBU2 with amoxicillin clavulanate or ciprofloxacin. **Samer *et al.* [40]**
Acinetobacter baumannii	Gram-negative, non-motile, non-fastidious, aerobic, opportunistic pathogen, resident of the urinary tract and lungs. **Gedefie et al. [39]**	Pneumonia, Meningitis, Blood stream infection, Surgical site infection **Gedefie et al. [39]**	Antibiotics- Ampicillin/Subactam, Ceftazidime/Avibactam **Emmanuel *et al.* [49]**
			Phage vBAbaM ISTD, phage vB_AbaM-IME-AB2 **Veronica *et al.* [38]**
			Antimicrobial peptide - cecropin A(CA)-melittin (ME), magainin2(MA), and HP (2-20) peptides **Ramamourthy *et al.* [50]**
Pseudomonas aeruginosa	Gram-negative, opportunistic pathogen resident of the urinary tract and lungs. **Denissen et al. [51]**	Urinary tract infections, Septicemia, Respiratory infection **Denissen et al. [51]**	Antibiotics- Azithromycin, Gentamycin, tobramycin
			Antimicrobial peptide Defensin, IDR-1018,6K-F17 **Daniel *et al.* [66]**
			Phage M1, phiKZ, LUZ24, AZ1 **Adanan *et al.* [67]** **Tigabu *et al.* [68]**
			Photodynamic therapy with RLP068/C1 photosensitiser **Zahra *et al.* [70]**
Enterobacter spp.	Gram-negative, encapsulated bacteria, resident of blood, urinary tract, and respiratory tract. **Vivas et al. [103]**	Urinary tract infection, Endocarditis, Osteomyelitis **Vivas et al. [103]**	Phage N5822 **Veronica *et al.* [109]**

type 3 fimbria-mediated biofilms by *K. pneumoniae* on surfaces covered with extracellular matrices from humans. In these circumstances, compared to bacteria-producing fimbriae containing the *MrkD* adhesin molecule, the capacity of fimbriae but non-adhesive mutants of *K. pneumoniae* to produce mature biofilms were dramatically reduced. Type 3 fimbrial productions are a vital factor in the development of biofilms on both biotic and abiotic surfaces for strains of *K. pneumoniae*. The primary fimbrial subunit (*MrkA*) of *K. pneumoniae*, separate from the *MrkD* adhesin that gives binding charac-

teristics and permits biofilm formation on biotic surfaces, is the immune-dominant structural protein [32,33].

MrkD, related to adhesion to the basement membranes of tissues and the basolateral surfaces of renal and pulmonary epithelia, is involved in the biofilm development in *K. pneumoniae* mediated by fimbriae. The *MrkD* adhesion in *K. pneumoniae* facilitates binding to collagen types IV and V and is housed within a chromosomally transmitted gene cluster. The chromosomally borne gene *mrkD* and the plasmid-borne determinant *mrkD* are not genetically related. Type 3 fimbriae are produced by numerous members of the Enterobacteriaceae, including *Klebsiella, Enterobacter*, *Proteus*, *Providencia*, and *Serratia* species. Some strains of enterobacteria have the *amrkD* allele, which is linked to hemagglutinating activity. This fimbrial type can be identified by the in-vitro agglutination of erythrocytes exposed to tannic acid; hemagglutination can occur with or without D-mannose. The accompanying adherence phenotype is sometimes called the mannose-resistant Klebsiella-like hemagglutination (MR/KHA) reaction. This distinctive trait was first identified in *Klebsiella*. The *MrkD* adhesin polypeptide of the type 3 fimbrial gene cluster mediates the activity of MR/KHA, and the adhesin enhances adherence to the basement membranes of human tissues [34,35].

Tannic acid-treated erythrocytes with and without D-mannose are agglutinated by type 3 fimbriae, also known as mannose-resistant Klebsiella-like hemagglutinins. Tracheal epithelial cells and elements of the basement membrane are attached to these surfaces through type 3 fimbriae. From a clinical isolate of *K. pneumoniae*, the genes encoding to produce type 3 fimbriae have been cloned. The phenotypic expression of these organelles on the surface of *E. coli* transformants was revealed to require at least four gene products. Physical mapping and mini-cell investigations were used to identify the size of these four proteins and the relationship between each gene and its corresponding protein. The nucleotide sequence of the *mrkA gene*, encoding the major fimbrial subunit, has been determined, and the transcription initiation site has been identified. It was demonstrated that a specific *mrkD* gene product mediates the mannose-resistant *Klebsiella*-like hemagglutinin-specific adhesin activity, and the nucleotide sequence of this gene has also been established [36,37].

Type 3 and type 1 fimbriae are two morphologically and functionally distinct filaments expressed by various *K. pneumoniae* strains. Trans-complementation investigation with the pap fimbrial gene cluster of *E. coli* allowed the identification of the gene (*mrkD*) encoding the adhesion of *K. pneumoniae* type 3 fimbriae. It was discovered that the *mrkD* gene's nucleotide sequence was also discovered, and its sequence determined was the determinant coding for the *K. pneumoniae* type 1 fimbrial adhesion. Comparing the projected amino acid sequences of the *K. pneumoniae* adhesion proteins, similarities with the two main structural proteins of the fimbria (Mrk A and Fim A) are found [34]. The type 1 fimbriae, which are closely linked to the type 1 fimbriae of *E. coli*, and the type 3 fimbriae, which are morphologically comparable to the K88 and K99 fimbriae, are the two fimbrial types that are most frequently expressed by strains of *K. pneumoniae*. Fresh guinea pig erythrocytes without mannose and tannic acid-treated erythrocytes in both the presence and absence of mannose are attached by type 1 and type 3 fimbriae [36].

Bacteriophage treatment: The exopolysaccharide depolymerase ZCKP1 phage that can disrupt *Klebsiella* reduces biofilm biomass rendering it more susceptible to antibacterial agents. Another phage, *Siphoviridae* phage Z and Phage vBKpnS_Kp13, also reduce the biofilm biomass of *Klebsiella* [38]. The treatment with an antibiotic is effective against planktonic culture, but it is inefficient against the biofilm of *Klebsiella*. When biofilm was treated with bacteriophage with antibiotic amoxicillin, there was a significant reduction in the biofilm formation by *Klebsiella spp* [39]. When combined with ciprofloxacin or amoxicillin-clavulanate, the cationic antimicrobial peptide WLBU2 inhibits the growth of multidrug-resistant (MDR) *K. pneumoniae* biofilms [40].

Acinetobacter baumannii

Acinetobacter baumannii is gram-negative, non-motile, non-fastidious, non-fermentative, catalase-positive, and oxidative-negative bacteria. It is an aerobic opportunistic pathogen that invades the urinary tract and lungs. A. baumannii is a low-grade pathogen that can be found in a range of habitats, including soil, water, and food. It is frequently isolated from medical equipment. It causes severe infections in individuals with impaired immune systems due to colonization and surviving on numerous pieces of medical equipment. It causes pneumonia, meningitis, and bloodstream infections in patients. A. baumannii account for 2–10% of overall nosocomial infections. It is a significant nosocomial pathogen due to invasive procedures, regular antibiotic usage, and immunocompromised hosts [41].

Csu pili is a mediator of *Acinetobacter baumannii* biofilm development on abiotic surfaces. CsuA/B, CsuA, CsuB, and CsuE are the four protein components that comprise the Csu pilus. Fibrous adhesive organelles are necessary for Gram-negative bacteria to adhere to their targets and spread infection. The primary class of these sticky pili (or fimbriae) is

assembled by the traditional chaperone-usher (CU) pathways as well as alternate and antiquated ones [42]. The linear polymers known as CU pili are formed of subunits that can either self-polymerize or assemble with other subunits. A periplasmic chaperone and an outer membrane assembly platform known as the usher are required to synthesize CU fibers [43].

The crystal structure of the CsuC-CsuA/B chaperone-subunit complex involved in preassembly gave researchers their first high-resolution understanding of how archaic pili are put together. The chaperone-bound CsuA/B possesses an extremely flexible partial Ig-like fold in a six-stranded beta-sandwich, where the missing seventh strand (G) leaves a significant hydrophobic cleft. Donor strand complementation is used in the polymer to link the CsuA/B subunits (DSC) [42-46]. One subunit's N-terminal sequence is inserted into the hydrophobic cleft of a different subunit next to it. Unlike CsuA/B, CsuE is not able to assemble itself. This subunit is projected to have a different domain in place of the donor sequence; CsuE is found at the tip of the pilus. Since numerous two-domain tip subunits have been demonstrated in classical systems to serve as host cell-binding adhesins (TDAs) [47].

Treatment of biofilm by *A. baumannii* Phage vBAbaM_ISTD reduces the biofilm-associated viable bacteria in a time-dependent manner; vB_AbaM-IME-AB2 phage disrupts the biofilm [38]. Antimicrobial peptide Cec4 is also effective against the *A. baumannii* biofilm [48]. According to the British infection, the association recommended ampicillin/sulbactam has potent activity in treating MDR and biofilm-associated infection of *A. baumanni*. Similarly, ceftazidime/avibactam has shown inhibitory activity against isolated from ICUs MDR *A. baumanni* [49]. A chimeric AMPS, cecropin A (CA)-melittin (ME) peptides, magainin 2 (MA), and HP (2-20) has potent activity against the elucidation of biofilm of MDR *Acinetobacter* [50].

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative opportunistic human pathogen in urinary tract infections associated with respiratory ailments in immunocompromised patients. P. aeruginosa is responsible for 10% of all nosocomial infections. It causes urinary tract infection, septicemia respiratory infection. *Pseudomonas aeruginosa* grows in soil and water settings and often colonizes the surfaces of plants, humans, and animals. P. aeruginosa has managed to persist in both hospital and community settings due to its capacity to live in habitats with limited nutrients and a range of physical conditions and its resistance to several medical disinfection techniques [51].

An opportunistic human pathogen called *Pseudomonas aeruginosa* comprises a minimum of three exopolysaccharides: alginate, Psl, and Pel. These exopolysaccharides are all implicated in the development of biofilms [52]. Alginates are linear polyanionic exopolysaccharides made of β-D-mannuronic acid and α-L-glucuronic acid and have essential structural stability and protection against biofilm. *P. aeruginosa* overproduces alginates following patient infection [53,54]. In contrast to wild-type strains, mutant organisms that manufacture excessive amounts of alginate form enormous finger-like microcolonies. A key indicator of immunological resistance is the elaboration of the extracellular, O-acetylated mucoid exopolysaccharide or alginate. The resistance of mucoid *P. aeruginosa* to immune defense is increased by O acetylation of alginate [55]. The production of mature biofilms and initial adhesion are both facilitated by the mannose- and galactose-rich Psl polysaccharide [56]. A cellulose- and glucose-rich pel is necessary to develop a pellicle at the air-liquid interface [57].

Extracellular DNA (eDNA) and exopolysaccharides have been demonstrated to be a crucial part of the biofilm matrix. eDNA mediates cell-cell interactions in biofilms. Most eDNA was discovered in the microcolonies stalk region [58]. According to a study, Psl and eDNA are spatially apart, with Psl at the biofilm's edge and eDNA predominantly present in the Psl-free matrixi [59]. The secondary messenger guanosine-5'-monophosphate phosphate is an essential regulator of the biofilm lifecycle in pseudomonas (c-di-GMP) [60].

Contrary to low c-di-GMP levels, which downregulate the synthesis of adhesins and extracellular matrix components and cause biofilm dispersal, high cellular levels of c-di-GMP promote the development of adhesins and extracellular matrix components, which result in the formation of biofilm. Diguanylate cyclases (DGCs) and c-di-GMP phosphodiesterase, which function in opposition to one another, are responsible for the synthesis and breakdown of c-di-GMP in bacteria (PDEs). The presence of sensory domains in many DGCs and PDEs is hypothesized to allow bacteria to react to environmental stimuli and modify the production of biofilm matrix components [61,62].

P. aeruginosa's two-component signaling systems control the synthesis of extracellular matrix constituents. It has been demonstrated that the two components GacA/GacS interact with c-di-GMP signaling to regulate the expression of several genes and the genes responsible for Pel and Psl exopolysaccharide synthesis [63]. Additionally, *P. aeruginosa* biofilm development is influenced by quorum sensing (QS) [64].

Treatment modality for *P. aeruginosa* biofilm includes an insect-derived peptide defensin from rab-

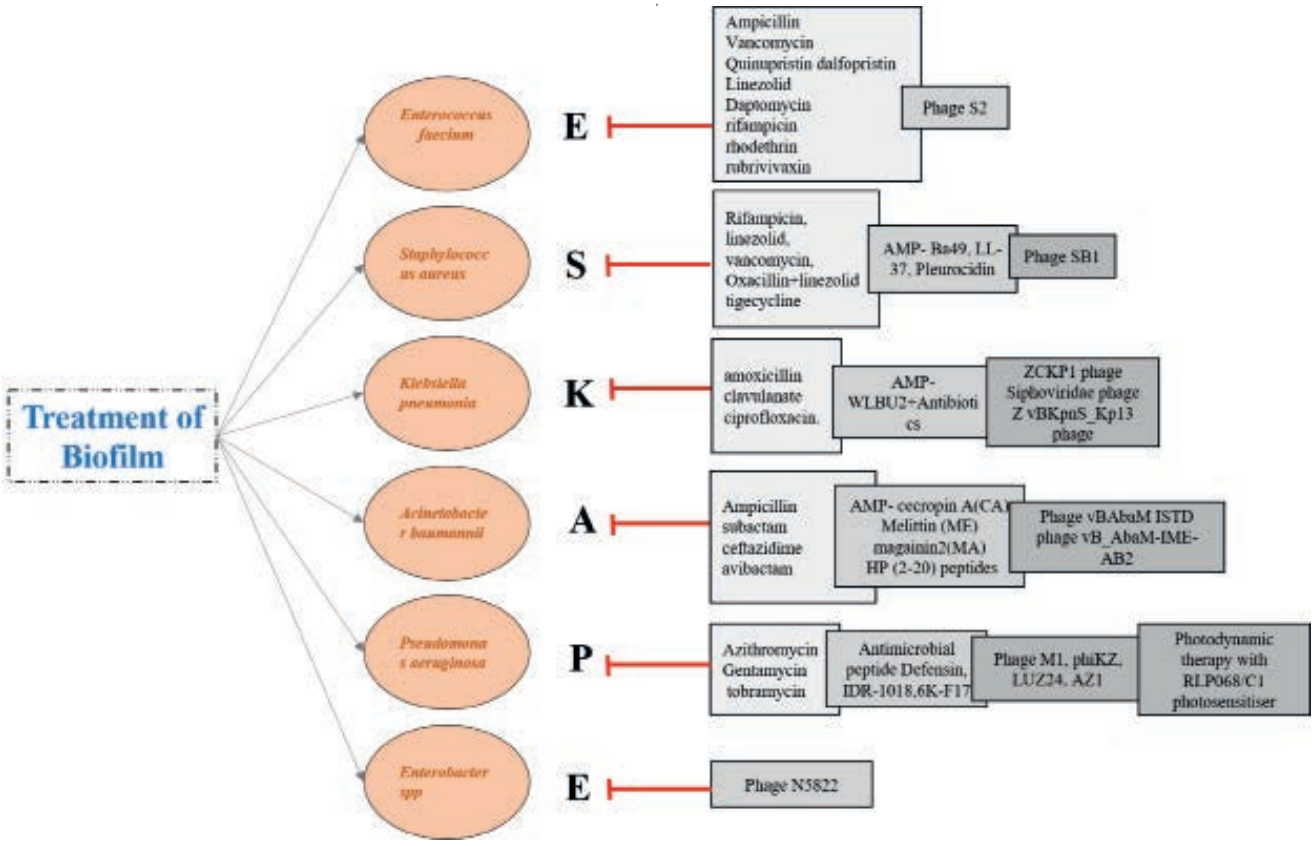

FIGURE 2. Treatment of Biofilm in ESKAPE organism

bit neutrophils exerting bactericidal activity against *P. aeruginosa* [65]. The antimicrobial peptide IDR-1018 and 6K-F17 have shown efficacy against MDR *P. aeruginosa* biofilm-producing isolates [66]**.** Phage M-1 has shown inhibiting biofilms caused by MDR isolates of *P. aeruginosa*. [Adana *et al.*]. PB-like, phiKZ-like, and LUZ24- -like phage have an effect against MDR *P. aeruginosa* under variable growth conditions; the results indicated that each phage alone was able to suppress planktonic and biofilm from MDR isolates [67]. Both planktonic and biofilm cells are susceptible to the anti-biofilm effects of bacteriophage AZ1 against *P. aeruginosa* [68]. Azithromycin effectively inhibited the development of biofilms, the synthesis of quorum-sensing signaling molecules, and the motility of clinical isolates of *P. aeruginosa*. Tobramycin and the antibiotic gentamycin both promoted the growth of P. aeruginosa isolates biofilms [69]. Antimicrobial photodynamic therapy (APDT) with RLP068/Cl, a novel photosensitizer, has shown a valuable approach to treating prosthetic joint infections (PJIs) associated with biofilm formed by *P. aeruginosa* [70].

Staphylococcus aureus

Staphylococcus aureus is an extracellular growing, gram-positive, commensal bacteria present in the nasal mucosa of humans. It is a significant cause of mortality in hospitals. It can cause a variety of infections, from skin infections to life-threatening conditions like chronic lung infections linked to cystic fibrosis, abscesses of different organs, infections of the skin and soft tissues, infections of the central nervous system, infections of the lungs, infections caused by medical devices, pneumonia, osteomyelitis, endocarditis, arthritis, and sepsis. *Staphylococcus aureus* can develop resistance to most antimicrobial agent types, including penicillins, macrolides, aminoglycosides, chloramphenicol, and tetracycline. Methicillin-resistant *Staphylococcus aureus* (MRSA) originated due to the widespread usage of methicillin and other semisynthetic penicillins in the late 1960s. MRSA is still present in both healthcare and community settings [71].

Biofilm formation is initiated by surface-attached cells that may be biotic or abiotic [72]. Surface proteins of *S. aureus* are attached to their cell wall by sortase, an enzyme that cleaves polypeptides at a conserved LPXTG motif [73]. The early phases of attachment and biofilm formation are influenced by the surface elements that recognize the adhesive molecules and microbial surface components identifying adhesive matrix molecules (MSCRAMMs) [74]. *S. aureus* expresses the proteins Bap, SasC, FnBPA, and FnBPB during the development of biofilms [75–78]. Teichoic acids have been proven to impact

staphylococcal biofilm development in studies [63]. Hydrolases such as AtlA were initially engaged in the attachment of *S. aureus* [79]. AtlA sticks to the wall and breaks down the cell wall, releasing DNA that aids in producing sticky EPS [80-82].

The extracellular polysaccharide intercellular adhesin (PIA)-1,6-N-acetyl glycosaminoglycan (PNAG) in the polysaccharide-dependent pathway was the first molecule shown to be in charge of intercellular adhesion [83]. The four gene locus icaADBC (intercellular adhesion) in S. aureus, under transcriptional control of IcaR, was initially identified in S. epidermis and later found present in other staphylococcus spp. [63, 84–86]. It is responsible for managing the synthesis of PIA/PNAG in the polysaccharide-dependent EPS. The genes icaA and icaD encode to produce the polysaccharide PIA/PNAG [80]. For polysaccharides to remain on the bacterial cell surface, the PIA/PNAG must be exported by *IcaC* and deacetylated by the *icaB* gene product [83,87,88]. The pathway mediators on which the protein-mediated intercellular adhesion of the independent polysaccharide pathway is dependent are *S. aureus* surface protein G (SasG), biofilm-associated protein (Bap), and fibronectin-binding protein A (FnBPA) [89-91]. Extracellular DNA (eDNA) can also be found in *S. aureus* biofilms in addition to polysaccharide PIA and EPS. eDNA is an electrostatic polymer that binds cells to surfaces, host factors, and other cells because it has a negative electric charge [92].

Recent research has revealed that *S. aureus* contains the extra biofilm cycle Exodus. When sessile bacterial cells proliferate and accumulate, the subset of biofilm-associated cells enters this exodus stage and begins to migrate. The enzyme produced by the nuc gene, a nuclease, which is governed by the Sae regulatory system, controls the exodus stage. Some cells are liberated from the biofilm due to the *staphylococcal* nuclease being secreted from the bacteria. Because only a small portion of the population has the nuc promoter activated, nuc expression is controlled stochastically [93- 96].

The treatment of biofilm formation for *S. aureus* infections has been demonstrated using in combination. *S. aureus* shows resistance to vancomycin, so there developed the strategy to use antibiotics rifampicin and linezolid, and vancomycin combined with oxacillin, linezolid, and tigecycline is also effective [97]. The antimicrobial peptide Ba49 isolated from *B. substitutes* subsp, *Spizizenii*, is also effective against *S. aureus* [98]. Peptide LL-37 inhibits biofilm-forming *S. aureus* strains isolated from chronic wound infections [99]. Pleurocidin derived from winter flounder has inhibited and eradicated biofilm-related infection caused by *S. aureus* [100]. When used alone or in conjunction with several antibiotic classes, the bacteriophage SB-1 destroys the extracellular matrix and targets persistent cells, eradicating the biofilm. Methicillin-resistant *S. aureus* (*MRSA)* ATCC 43300 biofilms were successfully treated with Sb-1 alone or in conjunction with fosfomycin, rifamycin, vancomycin, daptomycin, or ciprofloxacin (simultaneously or staggered) [101]. In-vitro and in vivo testing of a new chimeolysin (ClyF) against planktonic and biofilm MRSA showed good bactericidal activity [102].

Enterobacter spp

The intestinal system of humans and animals is the natural habitat of the diverse family of Gram-negative *Enterobacteriaceae.* Urinary tract infections (UTIs), lower respiratory tract infections, and bloodstream infections are the fatal *Enterobacteriaceae* infections acquired in hospitals. UTIs are the most prevalent. 8–10. Different adhesins, hemolysin production, serum resistance, and biofilm formation are virulence factors in the *Enterobacteriaceae* family. These elements, particularly the capacity of the human intestine to produce biofilms, may promote gut colonization and significantly affect the operation of the intestinal microbiome and its interactions with the host [103].

More often than not, isolates of *E. aerogenes, E. cloacae*, and *E. hormaechei* are found in clinical infections, particularly in immune-compromised patients and those admitted to an intensive care unit (ICU). These viruses are linked to a multidrug resistance phenotype due to their capacity to adapt to the hospital environment and their ease in acquiring multiple genetic mobile elements encoding resistant and virulence genes. Two divergently distinct operons are necessary for the expression of the curli gene, which codes for the highly proteinaceous, aggregated extracellular fibers known as curli fimbriae (csgBA(C) and csgDEFG) [104-106].

The primary subunit protein of the fiber, CsgA, is encoded by the csgBA operon. CsgB is related to csgA in sequence, and the csgDEFG operon encodes [107]. CsgE, CsgF, and CsgG are three potential curli assembly factors, while CsgD is a positive transcriptional activator of the csgBA operon [108]. It has been hypothesized that the curli fimbriae are an essential part of the extracellular matrix of E. cloacae biofilms [106].

The treatment of *Enterobacter* spp. Biofilm includes the treatment by bacteriophage N5822 isolated from a highly virulent environment that reduces the preformed static host biofilm and inhibits the formation of new biofilm up to 90% [109].

CONCLUSIONS

Nosocomial infections are the most common infections affecting hospitalized patients associated

with prolonged hospitalization having health issues leading to death. The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*) are the major pathogens causing these infections. Biofilms are an aggregation of bacterial communities covered by a protective layer that protects inside colonies from deterioration. The antimicrobial agents introduced to combat these bacteria would not act against them effectively. This increases the difficulty of treatment and slows the treatment process, making it urgent to develop novel antibacterial drugs. By studying and understanding the mechanism of biofilm formation, we can formulate antimicrobial agents or other alternative tools like using antibiotics singly or in combination or with adjuvants, bacteriophage therapy, antimicrobial peptides, photodynamic therapy, antibacterial antibodies, phytochemicals, and nanoparticles as antibacterial agents. The advanced discovery has put potential interest in drug repurposing for biofilm control. Where an already existing drug is applied in a new, previously unknown way, in this review, we have highlighted the Pathomechanisms involved in the biofilm formation and different treatments available for individual ESKAPE Pathogens to combat this biofilm formation.

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REFERENCES

1. Zubair M, Ashraf M, Arshad M, Raza A, Mustafa B, Ahsan A. Formation and Significance of Bacterial biofilms. *Int J Curr Microbiol Appl Sci.* 2014;(12):917-923.
2. Rodney MD. Biofilms: Microbial life on surfaces. *Emerg Infect Dis* 2022;8(9):881-90.
3. Netsanet B, Yalew T, Tarekegn T. Review on biofilm formation and its control options. *Int. J. Biol. Sci* 2017;4(8):122-33.
4. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J. Infect. Dis.* 2008;197(8):1079–81.
5. Chia PY, Sengupta S, Kukreja A, Ponnampalavanar SS, Ng OT, Marimuthu K. The role of hospital environment in transmissions of multidrug-resistant gram-negative organisms. A*ntimicrob Resist Infect Control.* 2020;9:1–11.
6. Nnadozie CF, Odume ON. Freshwater environments as reservoirs of antibiotic resistant bacteria and their role in the dissemination of antibiotic resistance genes. *Environ Pollut.* 2019;254:1-12.
7. Salyers A, Shoemaker, NB Reservoirs of Antibiotic Resistance Genes. *Anim Biotechnol.* 2006;17:137–46.
8. Sirijan S, Nitaya I. Mechanisms of Antimicrobial Resistance in ESKAPE pathogens. *Biomed Res Int.* 2016;1-8.
9. Del Pozo JL, Patel R. The challenge of treating biofilm associated bacterial infections. *Clin Pharmacol Ther.* 2007;82(2):204–209.
10. Mansura SM, Ekta EK, Shital NK, Madhumita ST, Karishma RP. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A Review. *Front Microbiol.* 2019;10(539):1-24.
11. Gloria VG, Anand K. Optimizing antimicrobial therapy of sepsis and septic shock: focus on antibiotic combination therapy. *Semin Respir Crit Care Med.* 2015;36(1):154–66.
12. Armin A, Afzal A, Mohan G, Arvind KB. Current concepts in combination antibiotic therapy for critically ill patients. *Indian J Crit Care Med.* 2014;18(5):310–4.
13. Patricia B, Carlos MS, Abdelali D, Maria AL. Antibiotic adjuvants: identification and clinical use. *Microb Biotechnol.* 2013;6(5):445–9.
14. Santos A, Sodre CL, Valle RS, Silva BA, Abi-Chacra EA, Silva LV et al. Antimicrobial action of chelating agents: repercussions on the microorganism development, virulence, and pathogenesis. *Curr Med Chem.* 2012;19(17):2715–37.
15. Ayumi Y, Yoshikazu I, Soichiro K, Tomoo S, Sohei HK, Yamaguchi et al. Efficacies of calcium-EDTA in combination with imipenem in a murine model of sepsis caused by *Escherichia coli* with NDM-1 β-lactamase. *J Infect Chemother.* 2013;19(5):992–5.
16. Valerie D, Laure V, Françoise VB, Ahalieyah A, Eleanor MT, Gordon R et al. Antibacterial activity of 1 - [(2,4- Dichlorophenethyl) amino]-3-Phenoxypropan-2-ol against antibiotic-resistant strains of diverse bacterial pathogens, biofilms and in pre-clinical infection models. *Front Microbiol.* 2017;8:2585
17. Munita JM, Arias CA, Murray BE. Enterococcal endocarditis: can we win the war? *Curr Infect Dis Rep.* 2012;14(4):339-49.
18. Barnes AT, Dale JL, Chen Y, Manias D, Greenwood Quaintance KE, Karau MK et al. Enterococcus faecalis readily colonizes the entire gastrointestinal tract and forms biofilms in a germfree mouse model. *Virulence.* 2017;8(3):282-96.
19. Arias CA, Murray BE. The rise of the *Enterococcus:* beyond vancomycin resistance. *Nat Rev Microbiol.* 2012;10(4):266-78.
20. Louis BR, Samuel B, Lenore LC, Rebecca HT, Robert AB, Patrick C et al. Impact of specific pbp5 mutations on expression of beta-lactam resistance in *Enterococcus faecium*. Antimicrob Agents Chemother. 2004;48(8):3028-32.
21. Bryson HM, Spencer CM. Quinupristin-dalfopristin. *Drugs* 1996;52(3):406-15.
22. Arias CA, Murray BE. Emergence and management of drug-resistant enterococcal infections. *Expert Rev Anti Infect Ther.* 2008;6(5):637-55.
23. Carpenter CF, Chambers HF. Daptomycin: another novel agent for treating infections due to drug-resistant gram-positive pathogens. *Clin Infect Dis.* 2004;38(7):994-1000.
24. Anna H, Magnus R. Antibiotic regimens with rifampicin for the treatment of *Enterococcus faecium* in biofilm. *Int J Antimicrob Agents.* 2014;44(1):78-80.
25. EswaraRao T, Ranjith K. Rhodethrin and Rubrivivaxin as potential sources of anti-biofilm agents against vancomycin resistant *Enterococcus faecalis* (ATCC 19443). *Microb Pathog.* 2020;148:1-7.
26. Forough G, Masoumeh H, Mohammad S, Malihe T, Vahid PM, NOur A. Biological characteristics and anti-biofilm activity of a lytic phage against vancomycin resistant *Enterococcus faecium*. *Iran J Microbiol.* 2021;13(5):691-702.
27. Paczosa MK, Mecsas J. *Klebsiella Pneumoniae*: Going on the Offense with a Strong Defense. *Microbiol Mol Biol Rev.* 2016;80 (3), 629–61.
28. Guerra MS, Destro G, Vieira B, Lima AS, Ferraz LF, Hakansson AP et al. *Klebsiella pneumoniae* Biofilms and their role in disease pathogenesis. *Front Cell Infect Microbiol.* 2022;12:1-13.
29. Meng-C Wu, Tzu-Lung L, Pei-Fang H, Hui-Ching Y, Jin-Town W. Isolation of genes involved in biofilm formation of a *Klebsiella pneumoniae* strain causing pyogenic liver abscess. *PLOS One.* 2011;6(8):1-11.
30. Murphy CN, Clegg S. *Klebsiella pneumoniae* and type 3 fimbriae: nosocomial infection, regulation, and biofilm formation. *Future Microbiol.* 2012;7(8):991-1002.
31. Bethny M, Aneika CL, Ana TR, Gilles P, William JA, Denis V, Gabriel W, Alison E. A, Sheena ER. The role of chaperone-subunit usher domain interactions in the mechanism of bacterial pilus biogenesis revealed by ESI-MS. *Mol Cell Proteom.* 2012;11(7):1365-2958.
32. Allen BL, Gerlach GF, Clegg S. Nucleotide sequence and functions of mrk determinants necessary for expression of type 3 fimbriae in *Klebsiella pneumoniae*. J Bacteriol 1991;173(2):916-20.

33. Elbrolosy AM, Eissa NA, Al-Rajhy NA, El-Sayed E, El-Mahdy, Mostafa RG. MrkD gene as a regulator of biofilm formation with correlation to antibiotic resistance among clinical *Klebsiella pneumoniae* isolates from Menoufia University Hospitals. *Egypt J Med Microbiol.* 2020;29(3):137-44.
34. Sebghati TA, Korhonen TK, Hornick DB, Clegg S. Characterization of the type 3 fimbrial adhesins of *Klebsiella* strains. *Infection Immunology* 1998;66(6):2887-94.
35. Allen BL, Gerlach GF, Clegg S. Nucleotide sequence and functions of mrk determinants necessary for expression of type 3 fimbriae in *Klebsiella pneumoniae*. *J Bacteriol* 1991;173:916–20.
36. Gerlach GF, Clegg S, Allen BL. Identification and characterization of the genes encoding the type 3 and type 1 fimbrial adhesins of *Klebsiella pneumoniae*. *J Bacteriol* 1989;171(3):1262-70.
37. Gerlach GF, Clegg S. Characterization of two genes encoding antigenically distinct type-1 fimbriae of *Klebsiella pneumoniae*. *Gene* 1988;64(2):231–40.
38. Veronica L, Alina MH, Carmen C, Mariana CC. Modulation of Quorum Sensing and biofilms in less investigated gram negative ESKAPE pathogens. *Front Microbiol.* 2021;12:1-18.
39. Manmeet SB, Vivek V, Sanjay C. Amoxicillin and specific bacteriophage can be used together for eradication of biofilm of *Klebsiella pneumoniae* B5055. *World J Microbiol Biotechnol.* 2009;25(7):1145-51.
40. Samer S, Zaina S, Ammar A. Synergism of cationic antimicrobial peptide WLBU2 with antibacterial agents against biofilms of multidrug resistant *Acinetobacter baumannii* and *Klebsiella pneumonia.* I*nfect Drug Resist.* 2019;12:2019-30.
41. Gedefie A, Demis W, Ashagrie M, Kassa Y, Tesfaye M, Tilahun M. *Acinetobacter baumannii* biofilm formation and its role in disease pathogenesis: *A review. Infect Drug Resist.* 2021;14:3711-9.
42. Tomaras AP, Dorsey CW, Edelmann RE, Actis LA. Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. *Microbiology (Reading).* 2003;149(12):3473-84.
43. Natalia P, James AG, Minna T, Sari P, Mamou D, Yingqi Xu, *et.al.* Structural insight into archaic and alternative chaperone-usher pathways reveals a novel mechanism of pilus biogenesis. *PLOS Pathology.* 2015;11(11):1-22.
44. Busch A, Waksman G. Chaperone-usher pathways: diversity and pilus assembly mechanism. *Philos Trans R Soc Lond B Biol Sci.* 2012 367(1592):1112-22.
45. Choudhury D, Thompson A, Stojanoff V, Langermann S, Pinkner J, Hultgren SJ, Knight SD. X-ray structure of the FimC-FimH chaperone-adhesin complex from uropathogenic Escherichia coli. *Science,* 1999;285(5430):1061-6.
46. Sauer FG, Pinkner JS, Waksman G, Hultgren SJ. Chaperone priming of pilus subunits facilitates a topological transition that drives fiber formation. *Cell.* 2002;1(4):543-51.
47. Le Trong I, Aprikian P, Kidd BA, Forero-Shelton M, Tchesnokova V, Rajagopal P, *et.al.* Structural basis for mechanical force regulation of the adhesin FimH via finger trap-like beta sheet twisting. *Cell.* 2010;141(4):645-55.
48. Weiwei Liu, Zhaoying Wu, Chengju Mao, Guo Guo, Zhu Zeng, Ying Fei, *et.al.* Antimicrobial peptide Cec4 eradicates the bacteria of clinical Carbapenem resistant *Acinetobacter baumannii* biofilm. *Front Microbiol.* 2020;11(1532):1-13.
49. Emmanuel CE, Hafizah YC, Mohamed EEZ. *Acinetobacter baumannii* biofilms:effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation and future antimicrobial treatments. *Infect Drug Resist.* 2018;11:2277-99.
50. Ramamourthy G, Young GK, Jun HL, Seog KL, Jeong DC, Byoung KS, *et.al.* Synergistic effects and antibiofilm properties of chimeric peptides against multidrug-resistant *Acinetobacter baumanni* strains. *Antimicrob Agents Chemother.* 2014;58(3):1622-9.
51. Denissen J, Reyneke B, Waso Reyneke M, Havenga B, Barnard T, Khan S, Khan W. Prevalence of ESKAPE pathogens in the environment:Antibiotic resistance status, community acquired infection and risk to human health. *Int J Hyg Environ Health.* 2022;244:1-15.
52. Cynthia R, Matthew B, Daniel JW. Role of polysaccharides in *Pseudomonas aeruginosa* biofilm development. Curr Opin Microbiol. 2007;10(6):644-8.
53. Iain DH, Kali G, Andrea CJ, ZoeJordens, Bernd HA, Rehm. Impact of Alginate overproduction on attachment and biofilm architecture of a supermucoid *Pseudomonas aeruginosa* strain. *Appl Environ Microbiol.* 2009;5(18):6022-5.
54. Kamali E, Jamali A, Ardebili A, Ezadi F, Mohebbi A. Evaluation of antimicrobial resistance, biofilm forming potential and presence of biofilm related genes among clinical isolates of *Pseudomonas aeruginosa*. *BMC Res Notes.* 2020;13(27):1-6.
55. Gerald BP, Fadie C, Martha G, Michael F, Dennis E, Ohman. Role of alginate O acetylation in resistance of mucoid *Pseudomonas aeruginosa* to opsonic phagocytosis. *Infect Immun.* 2001;69(3):1895-901.
56. Luyan M, Mathew C, Heiping Lu, Matthew RP, Kenneth B, Daniel JW. Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix. *PLOS pathogens.* 2009;5(3):1-11.
57. Aamir G, Iain DH, Bernd HA, Rehm. Role of exopolysaccharides in *Pseudomonas aeruginosa* biofilm formation and architecture. *Appl Environ Microbiol*. 2011;77(15):5238-46.
58. Allesen-Holm M, Barken KB, Yang L, Klausen M, Webb JS, Kjelleberg S, *et.al.* A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms. *Mol Microbiol.* 2006;59(4):1114-28.
59. Luyan Ma, Matthew C, Haiping Lu, Matthew RP, Kenneth B, Daniel JW. Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix. *PLOS pathogens.* 2009;5(3):1-11.
60. Ciofu O, Tim Tolker-Nielsen. Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents-how *P. aeruginosa* can escape antibiotics. *Front Microbiol.* 2019;10:1-15.
61. Shuwen A, Jien wu, Lian-Hui, Zhang. Modulation of *Pseudomonas aeruginosa* biofilm dispersal by a cyclic-Di-GMP phosphodiesterase with a putative hypoxia-sensing domain. *Appl Environ Microbiol.* 2010;76(24):8160-8173.
62. Goodman AL, Kulasekara B, Rietsch A, Boyd D, Smith RS, Lory S. A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in *Pseudomonas aeruginosa*. *Dev Cell.* 2004;7(5):745-54.
63. Li Y, Heine S, Entian M, Sauer K, Frankenberg-Dinkel N. NO-induced biofilm dispersion in *Pseudomonas aeruginosa* is mediated by an MHYT domain-coupled phosphodiesterase. *J Bacteriol.* 2013;195(16):3531-42.
64. Goodman AL, Kulasekara B, Rietsch A, Boyd D, Smith RS, Lory S. A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in *Pseudomonas aeruginosa*. *Dev Cell.* 2004;7(5):745-54.
65. Qinghua Wu, Jiri Patocka and Kamil Kuca. Insect Antimicrobial peptides, A- mini review. *Toxins (Basel).* 2018;10(11):461.
66. Daniel P, Robert EW, Hancock. Antibiofilm peptides: Potential as broad-spectrum agents. *J Bacteriol.* 2016;98(19):2572-8.
67. Adanan M, Shah MRA, Jamal M, Jalil F, Andleeb S, Nawaz MA, *et.al.* Isolation and characterization of bacteriophage to control multidrug-resistant *Pseudomonas aeruginosa* planktonic cells and biofilm. *Biologicals.* 2020;63:89-96.
68. Tigabu A, Getaneh A. *Staphylococcus aureus*, ESKAPE bacteria challenging current health care and community settings: A literature review. *Clinical lab*. 2021;1-11.
69. Jamal M, Andleeb S, Jalil F, Imran M, Nawaz MA, Hussian T, *et.al.* Isolation and characterization of a bacteriophage and its utilization against multidrug resistant *Pseudomonas aeruginosa*-2995. *Life Sci.* 2017;190:21-8.
70. Zahra Y, Fateme D, Parviz O. Evaluating the effects of sub-MIC Concentration of Gentamicin on biofilm formation in clinical isolates of *Pseudomonas aeruginosa*. *Iranian J Pathol.* 2021;6(4):403-40.
71. Christian V, Simone F, Francesco G, Lia F, Gabrio R, Giulio S, *et.al.* Photodynamic antibacterial and antibiofilm activity of RLP068/Cl against *Staphylococcus aureus* and *Pseudomonas aeruginosa* forming biofilms on prosthetic material. *Int J Antimicrob Agents.* 2014;44:47-55.
72. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 1998;280(5361):295-8.
73. Christian K, Danae MA, Marita TM, Lucia F, Pilar G, Morten K, Dzung BD. *Staphylococcal* biofilms: challenges and novel therapeutic perspectives. Antibiotics (Basel). 2021;10(2):131.

74. Mazmanian SK, Liu G, Jensen ER, Lenoy E, Schneewind O. *Staphylococcus aureus* sortase mutants defective in the display of surface proteins and in the pathogenesis of animal infections. *Proc Natl Acad Sci U S A.* 2000;97(10):5505-10.
75. Cucarella C, Solano C, Valle J, Amorena B, Lasa I, Penadés JR. Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. *J Bacteriol.* 2001;183(9):2888-96.
76. Schroeder K, Jularic M, Horsburgh SM, Hirschhausen N, Neumann C, Bertling A, *et.al.* Molecular characterization of a novel *Staphylococcus aureus* surface protein (SasC) involved in cell aggregation and biofilm accumulation. *PLoS One.* 2009;4(10):7567.
77. Greene C, McDevitt D, Francois P, Vaudaux PE, Lew DP, Foster TJ. Adhesion properties of mutants of *Staphylococcus aureus* defective in fibronectin-binding proteins and studies on the expression of fnb genes. *Mol Microbiol* 1995;17(6):1143-52.
78. Barian PC, Joan AG, Elain MW, Hannah McCarthy, Sarah ER, Julia RD, *et.al.* Role for the domain of unprocessed accumulation-associated protein (Aap) in the attachment phase of the *Staphylococcus epidermidis* biofilm phenotype. *J Bacteriol.* 2014;96(24):4268-75.
79. Matthias G, Sarah EC, Friedrich G, Andreas P. Key role of teichoic acid net charge in *Staphylococcus aureus* colonization of artificial surfaces. Infect Immun. 2001;69(5):3423-6.
80. Raja B, Lalitha V, Uwe KS, Petra H, Gunther T, Friedrich G. Activity of the major *staphylococcal* autolysin Atl. *FEMS Microbiol Lett.* 2006;259(2):260-8.
81. Hirschhausen N, Schlesier T, Schmidt MA, Götz F, Peters G, Heilmann C. A novel staphylococcal internalization mechanism involves the major autolysin Atl and heat shock cognate protein Hsc70 as host cell receptor. *Cell Microbiol.* 2010;12(12), 1746-64.
82. Kelly CR, Ethan EM, Jennifer LE, Elizabeth CW, James EC, Mark SS, Kenneth WB. The cidA murein hydrolase regulator contributes to DNA release and biofilm development in *Staphylococcus aureus. Proc Natl Acad Sci USA.* 2007;104(19):8113-8.
83. Cramton SE, Gerke C, Schnell NF, Nichols WW, Gotz F. The intercellular adhesion (ica) locus is present in *Staphylococcus aureus* and is required for biofilm formation. *Infection Immunology* 1999;67(10):5427-33.
84. Ameya AM, Casey MG, Tyler DS, Tammy K, Jeffrey MB. SaeRS is responsive to cellular respiratory status and regulates fermentative biofilm formation in *Staphylococcus aureus*. *Infection Immunology.* 2017;85(8):157-17.
85. Mack D, Haeder M, Siemssen N, Laufs R. Association of biofilm production of coagulase-negative *staphylococci* with expression of a specific polysaccharide intercellular adhesin. *J Infect Dis* 1996;174(4):881-884.
86. Heilmann C, Hussain M, Peters G, Gotz F. Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. *Mol Microbiol* 1997;24(5):1013-1024.
87. Gerke C, Kraft A, Sussmuth R, Schweitzer O, Gotz F. Characterization of the N-acetylglucosaminyl transferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. *J Biol Chem* 1998;273(29):18586-93.
88. CuongV, Stanislava K, Jovanka MV, Yufeng Y, Elizabeth RF, Frank RD, Michael O. A Crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence. *J Biol Chem.* 2004;279(52):54881-6.
89. Varvara P, Joanna P, Dustin JL, Heather G, Howell PL, Mark N. Functional characterization of *Staphylococcus epidermidis* IcaB, a de-N-acetylase important for biofilm formation. *Biochemistry.* 2013;52(32):5463-71.
90. Nuno C, Kimberly KJ, Tomas ML, Danielle BP, Casie K, Donald AG, Joana A, Gerald BP. Molecular basis for preferential protective efficacy of antibodies directed to the poorly acetylated form of *staphylococcal* poly-N-acetyl-beta-(1-6)-glucosamine. *Infect Immun.* 2007;75(7):3406-13.
91. Rebecca MC, David R, Pauline H, Timothy JF. The role of *Staphylococcus aureus* surface protein SasG in adherence and biofilm formation. *Microbiology.* 2007;153(8):2435-2446.
92. Cucarella C, Solano C, Valle J, Amorena B, Lasa I, Penades JR. Bap A. *Staphylococcus aureus* surface protein involved in biofilm formation. *J Bacteriol.* 2001;183(9):2888-96.
93. Eoghan O' Neill, Clarissa P, Patrick H, Hilary H, Ashley R, Anthony L, Timothy JF, O'Gara JP. A novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. *J Bacteriol.* 2008;190(11):3835-50.
94. Derek EM, Jefferey LB, Alexander RH, Bayles KW. Temporal and stochastic control of *Staphylococcus aureus* biofilm development. *mBio*. 2014;5(5):341-14.
95. Michael EO, Tyler KN, Laynez A, Robert LW, Oliwia WZ, Kyler BP *et.al. Staphylococcus aureus* nuclease is an SaeRS-dependent virulence factor. *Infect Immun.* 2013;81(4):1316-24.
96. Elizabeth AD, Derek EM, Jennifer LE, Rebecca EH, Marat RS, Alexander RH, Kenneth WB. Stochastic expression of Sae-Dependent virulence genes during *Staphylococcus aureus* biofilm development is dependent on SaeS. *mBio*. 2020;11(1):3081-19.
97. Mohini B, Daniel W, Paul S, Luanne H. Prevention and treatment of *Staphylococus aureus* biofilm. *Anti Infect Ther.* 2015;13(2):1499-1516.
98. Ramita T, Sanpreet S, Vijayender B, Mani SB, Debendra KS. Deciphering the Antibacterial role of peptide from subsp *Bacillus subtilis*. Spizizenii Ba49 against *Staphylococcus aureus*. *Front Microbiol.* 202112,1-14.
99. Su JK, Na HK, Min KK, Jonggwan P, Eunji P, Ga HP *et.al.* Antibacterial and anti-biofilm activity, and mechanism of action of pleurocidin against drug resistant *Staphylococcus aureus. Microb Pathog.* 2019;127:70-78.
100. Tamta T, Lisa L, Ann Bk, Andrej T, Mariagrazia DL. Bacteriophage Sb-1 enhances antibiotic activity against biofilm, degrades exopolysaccharide matrix and targets persisters of *Staphylococcus aureus. Int J Antimicrob Agents.* 2018;52(6):842-853.
101. Hang Y, Huaidong Z, Jing W, Junping Y, Hongping W. A novel chimeric lysine with robust antibacterial activity against planktonic and biofilm Methicillin-resistant *Staphylococcus aureus. Sci Rep.* 2017;7:40182.
102. Mehmet D, Akin Y, Cemil D. Efficacy of antimicrobial peptide LL-37 against biofilm forming *Staphylococcus aureus* strains obtained from chronic wound infections. *Microb Pathog.* 2022;162:105368.
103. Vivas JR, Gonzalez IC, Martinez MF, Rico CG, Fortun J, Escudero R. Biofilm formation by multidrug resistant Enterobacteriaceae strains isolated from solid organ transplant recipients. *Sci Rep.* 2019;9:1-10.
104. Anne DR, Jean PL, Jean MP. *Enterobacter sps*: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. *ASM.* 2019;32(4):2-22.
105. Anne DR, Jean M, Jean MP. *Enterobacter aerogenes* and *Enterobacter cloacae*;versatile bacterial pathogens confronting antibiotic treatment. *Front Microbiol* . 2015;392(6):1-10.
106. Sung-Min Kim, Hee-Woo Lee, Yeh-Wan Choi, Shuk-Ho Kim, Je-Chul Lee, Yoo-Chul Lee *et.al.* Involvement of Curli Fimbriae in the biofilm formation of *Enterobacter cloacae*. *J Microbiol.* 2012;50(1):175-178.
107. Hammar M, Arnqvist A, Bian Z, Olsen A, Normark, S. Expression of two csg operons is required for production of fibronectin and Congo red-binding curli polymers in *Escherichia coli* K-12. *Mol Microbiol* 1995;18:661-670.
108. Eva B, Andrea B, Corinne D, Paolo L. Gene expression regulation by the curli activator CsgD protein: Modulation of cellulose biosynthesis and control of negative determinants for microbial adhesion. *J Bacteriol.* 2006;188(6):2027-2037.
109. Veronica L, Alina MH, Carmen C, Mariana C. Modulation of Quorum Sensing, and biofilms in less investigated Gram negative ESKAPE pathogens. *Front Microbiol.* 2021;12:1-18.