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Review Article

A review on the potential of antibacterial peptides to control biofilm resistance

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Abstract
Biofilms are comprised of communities of bacteria that are encased in the matrix. In biofilm, microbes become dormant and less sensitive to ordinary antibiotics. It is difficult for the antibiotics to penetrate and reach the depth of biofilm. These characteristics of biofilm make it 1000 times more resistant than planktonic microbes. Antibacterial peptides are used as an alternative of antibiotics to deal with the infections that are related to biofilm. Antibacterial peptides are small peptides that have broad action against Gram-positive and Gram-negative bacteria. Significant mechanisms of antibacterial peptides are: 1) degradation or disruption of the membrane potential of biofilms' embedded cell, 2) interruption of bacterial cells' signaling system, 3) degradation of polysaccharides and matrix of biofilm, 4) inhibition of the bacterial alarmone to avoid the stringent responses and 5) down-regulation of the genes that are responsible for biofilm formation and transportation of the binding proteins. The main purpose of this review article is to summarize the fresh material related to antibacterial peptides, their activity, and mechanism of action against the biofilm. Furthermore, health community and society can get new information regarding the suitable alternatives of ordinary antibiotics to control resistance in biofilms.

Keywords: Antibacterial peptides; Anti-biofilm activity; Antibiotic alternatives; Biofilm; Biofilm degradation; Enhanced resistance

Introduction

Mostly, bacterial microbes like to live in the accumulated form at the interfaces, thus forming the poly microbial aggregates (biofilms). These biofilms may exist both in nature and in clinical devices. The lifestyle of biofilm is different from planktonic state bacteria. They have approximately 1000-fold more resistance against antibiotics as compared to freely existing bacteria **[1]**. A biofilm has 10% microorganisms and a 90% matrix. The matrix is also called extracellular polymer substance (EPS) that is produced by bacterial cells of biofilm. EPS shows a significant character in the cell-to-cell communication in biofilms. The matrix of biofilm is made up of protein (fibrin), polysaccharides, and extracellular DNA. The matrix protects microbes from the immune system of the host **[2]**.

Later on, it was observed that biofilms are involved in many clinical infections. Many reproductive, respiratory, and urinary diseases such as bacterial vaginosis, chronic rhinosinusitis, and the infections of urinary tract are initiated by biofilms. Furthermore, biofilms may form on the various implanted devices and they may be implicated in the diseases such as pneumonia, cystic fibrosis, wounds, and osteomyelitis **[3]**.

Biofilm is adapted to the environmental anoxia and limitations in nutrients by exhibiting altered metabolism, expression

of genes, and addition of protein. These types of alterations make the bacteria extra resistant against antimicrobial therapies by inactivation of the antimicrobial targets and reduction in the necessities for the cellular functions with which anti-microbial agents impede [4].

Due to their effectiveness, low cost, and broad availability, antibiotics are being used extensively [5]. The overuse of antibiotics has led to resistance in microorganisms against antibiotics. The treatment of infections that are biofilm-related is very challenging. Now scientific attention has turned toward the development of agents that can treat biofilm-related infections. Antibacterial peptides are considered as suitable alternatives to control biofilm resistance [6]. These peptides are small molecules that have amino acids in range 10 to 100 [7]. Antibacterial peptides have broad action against Gram negative bacteria and Gram positive bacteria. Antibacterial peptides are chains of amino acids with both hydrophilic and hydrophobic domains. It is also has primary and secondary structures [8]. The secondary structure is complex and based on the primary structure. Antibacterial peptides are divided into extended indalicidin, α helical, β sheeted, loop structure, and mixed structure [9]. They bind with the cell wall of organisms present in biofilm. These peptides initiate the lysis of the outer membrane. As a result of this lysis, the essential material of bacterial cells comes out and ultimately results in the death of cells [10].

Bacterial microbes

Bacterial microbes are microscopic organisms that exist either in single-celled or `colony forms [11]. Doubt about the presence of microbes was started in the 6th century B.C. However, the scientific study of microbes started with the invention of the microscope by Anton Van Leeuwenhoek in the 1670s. Later on, it was observed by Louis Pasteur that microbes are the causative agents of food spoilage [12]. In the 1880s, it was observed by Robert Koch that microbes can cause diseases such as anthrax, diphtheria, tuberculosis, and cholera [13]. Bacteria have different habitats and they live on poles to the equator, rocks, geysers, deserts, and the deep sea. They live in different regions according to their adaptations [14].

Biofilm

Bacterial communities that live in the extracellular matrix are called biofilm. Biofilms are developed both on biotic and abiotic surfaces [15]. Stepwise process is involved in bio-film formation that includes adhesion, growth, extracellular polysaccharides' formation and motility. The nature of bio-film and physiological state of the bacterial cells confer the resistance at a significant level against antibacterial agents [16].

Stages of biofilm development

Biofilm formation is a gradual process. There exist a five-stage growth cycle of biofilm. Stage 1 is called the attachment phase which takes seconds for activation and this activation is induced by environmental signals. These types of signals may vary by the organisms and affected by pH, oxygen concentration, temperature, nutrient concentration and osmolality. Microbes prefer rough surfaces for biofilm formation because these surfaces have a large surface area and also have reduced shear forces [17]. Microorganisms like to make biofilms on hydrophobic materials such as teflon and other plastics. In stage 1 the initial binding is a reversible process. During this stage the growth rate of bacterial cells is logarithmic [18].

Stage 2 is started in few minutes after stage 1 and in this stage, irreversible binding happens. After the adhesion of bacterial cells with an epithelial surface, these cells multiply and emit chemical signals for communication. Once, the intensity of signals exceeds the threshold level, the genetic mechanism for the production of EPS is activated that is able for trapping the planktonic bacteria and nutrients. In stage 2 the aggregates of cells are formed and these

aggregates decrease the motility of cells. The aggregates of cells make a layer and when this layer reaches the thickness of 10 micrometers then biofilm enters into phase 3. This phase is named as the maturation 1. When thickness of the bio-film becomes greater than 100 mm then the stage will be stage 4 or maturation 2. Dispersion of cells was observed during stage 5 [19]. Some bacteria start to leave biofilm by developing the planktonic phenotype.

Physical location of biofilm and immunologic components of the host have a countless influence upon structure of the biofilm [20]. Stages of biofilm development are shown in (Fig. 1).

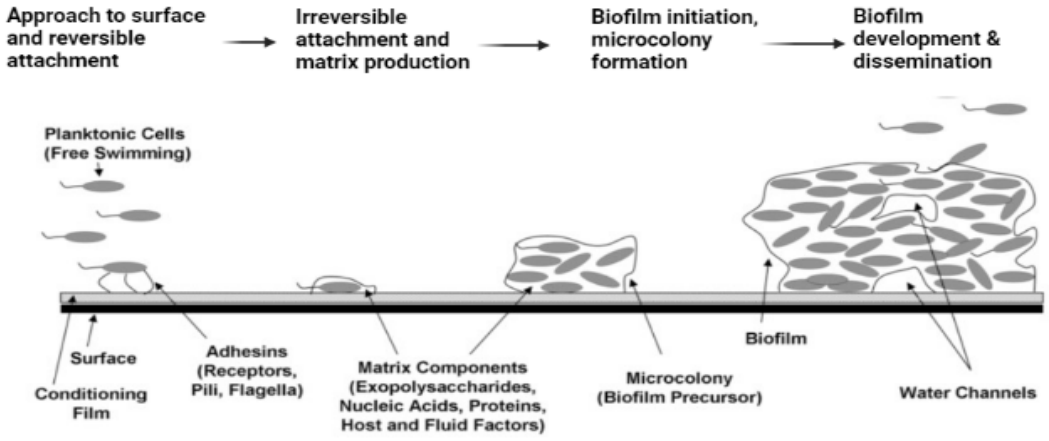

Figure 1: Various stages of biofilm development

Antibiotics and biofilms

The only available armament that can treat bacterial infections is antibiotics. These antibiotics can stop bacterial growth or kill the fee living bacteria. The formation of these antibiotics does not involve the biology of biofilms that is unique from free-living bacteria. Antibiotics can reverse the symptoms that are produced by the bacterial cells that are released from a biofilm. But bio-film can't be killed by these antibiotics [21]. After the treatment of biofilm-related infections with antibiotics, the recurring symptoms continue to appear until the biofilm is removed surgically. Biofilm avoids antimicrobial agents by various mechanisms.

- Antibiotics fail to penetrate and can't reach the full depth of biofilm [22].
- Some cells in biofilm experience nutrients shortage and adopt the starved state. These types of cells are not susceptible to antimicrobial agents.
- A protected and distinct phenotype is adopted by some cells of biofilm. The response is biologically programmed and is not initiated due to limitations of nutrients [23].

Resistance in the microbes and biofilms

Antibiotic resistance issues are of recent origin. The antibiotics are being used for human infectious diseases' treatment and also for other applications such as aquaculture, animal husbandry and aquaculture field to get enhanced yield. Antibiotics are playing a significant role in controlling infectious diseases for the last half-century. Now the situation is a major threat to increased antibiotic resistance in microbes. This increase in resistance has made it difficult to treat various infectious diseases [24].

Antibiotic resistance development

With few exceptions, the resistance to antibiotics in bacteria was observed after antibiotic introduction into practice. Certainly, the mutation was a major cause of this resistance development such as in the case of *Mycobacterium tuberculosis*. This pathogen developed resistance against

streptomycin. The occurrence of resistance at a low level was frequent and this low-level resistance was an initial step in developing resistance at a high level. Mostly, acquisition from the exogenous sources (still unidentified) was the main mechanism with which the bacteria get the gene that encodes resistance to antibiotics. The susceptibility of antibiotics in the overall population of microbes can't be analyzed but infection-related strains were observed and analyzed frequently [25]. Today, the demographics of the populations that are under the stress of antibiotics are not understood clearly due to the lack of pieces of information about the nature of the microbial populations. Horizontal transfer of genes showed an important role in development and dissemination of antibiotic resistance gene [26].

Causes of antibiotic resistance

Overuse

Alexander Flemming proposed the alarm about the overuse of antibiotics in 1945. Resistance evolution was driven by the overuse of antibiotics [27]. Epidemiological studies exposed the affiliation between consumption of the antibiotics and emergence of the resistance in strains of bacteria. In the bacteria, the genes responsible for resistance are delivered from relatives or they may be acquired from non-relatives by the use of mobile genetic elements [28]. Among different bacterial species, horizontal genes transfer (HGT) allows the transfer of resistance. The mutation is a way by which resistance occurs spontaneously. The drug-sensitive competitors are removed by antibiotics and only resistant bacterial strains are left as the result of natural selection [29].

Inappropriate prescription

Incorrect prescription of antibiotics promotes resistance in bacterial species. Studies have exposed that indication of treatment, choice of anti-microbial agent and duration of antibiotic therapy is not appropriate in 30-50% of cases. In the intensive care unit (ICU), 30-60% of unnecessary suboptimal antibiotics are being prescribed. Concentrations of sub-inhibitory antibiotics develop antibiotic resistance by changing gene expressions, mutagenesis and HGT. Changes in gene expression make pathogens more virulent. An increase in HGT and mutagenesis support spreads and increases antibiotic resistance [30].

Extensive agriculture use

In livestock, antibiotics are being used as growth supplements in both developing and developed countries. 80% of the sold antibiotics in the U.S are used in animals to avoid infections and promote growth [31]. The environmental microbiome is affected by antibiotics that are used in agricultural fields. 90% of antibiotics that are used in livestock treatment are excreted in stool and urine, then dispersed through the fertilizers, surface runoff and groundwater [32].

Antibacterial peptides

The antibacterial peptides are considered as the utmost studied peptide. The main feature of antibacterial peptides is that they have both hydrophilic and hydrophobic domains [33]. Mostly these peptides are cationic and they have a net positive charge on them. This net positive charge permits these types of peptides to intermingle with negatively charged membrane of the bacteria. Mechanism of action of antibacterial peptides has been deliberated in detail. These peptides can cause the death of bacterial cells both by non-membranolytic and membranolytic mechanisms by interacting with the intracellular RNA, DNA and proteins [34]. Gram-positive and the Gram-negative bacteria have molecules in their membrane. These molecules in outer membrane produce the negative charge and this negative charge attracts the cationic peptides [35].

Structural classification of antibacterial peptides

The secondary structure of antibacterial peptides plays a significant role in their classification. Antibacterial peptides are classified into β sheets, α helical, loop and

extended peptides. β sheet and α helical peptides are common [36].

α helical antibacterial peptides

In aqueous solution, linear structure of α helical antibacterial peptides was observed. LL-37 and Magainin-2 are common examples of these types of peptides. In the conformation of α helix, the space among the two neighboring amino acids is approximately 0.15nm and angle between them is 100 degrees from top view [37].

β-sheet antibacterial peptides

Two disulphide bridges play a significant role in organizing and stabilizing the β sheet antibacterial peptides and these di-sulphide bridges create the amphipathic structure. Defensins, protegrins and tachyplesins are included in this class of antibacterial peptides. Lactoferricin and Thanatin are peptides that have a loop structure and this structure is stabilized by the di-sulphide and amide bonds [38].

Extended antibacterial peptides

Regular secondary structure is absent in this type of antibacterial peptide. These peptides have tryptophan, proline, arginine, glycine and residues of histidine. Simple substitution can bring changes in functional and structural properties because this class of peptides have a very short length. For an instance, if we replace Pro residue with Ala in the tritpticin then the peptide structure will be transformed into α helical conformation that has higher cytotoxicity and improved activity against antimicrobial agents [39].

Biofilm and antibacterial peptides

Antibacterial peptides' activity against biofilm is less studied as compared to the study of these peptides against microorganisms. Apart from the antibacterial activity, valuation of the particular capability to impair the formation of biofilm is hard to achieve. The antibacterial peptide will be called anti-biofilm peptides if the minimum inhibitory concentration (MIC) is above the minimum biofilm inhibitory concentration (MBIC). Preformed biofilm eradication is a very difficult process as compared to the inhibition process. Minimum biofilm eradication concentration (MBEC) is greater as compared to MBIC [40]. Straus and Raheem in 2019 demonstrated biophysical and biological methods to define the functions of anti-biofilm and antibacterial peptides. Famous antibacterial peptides that are active against biofilm are shown in (Table 1).

Table 1: **Antibacterial peptides as anti-biofilm agents** [41].

Peptides	Source	Sequence	Mode of action	Micro-organisms
DJK-5	Denovo	VQWRAAIRVR IR	Suppresses the spoT promoter activities	*P. aeruginosa*
Nal-P-113	Denovo	AKR-Nal-Nal-GYKKF-Nai-	Down regulate the genes associated with binding proteins.	*P. gingivalis*
1018	Denovo	VRLVAVRIWR	Reduces intracellular (p) PpGpp	*P. aeruginosa*
Esculitin-la (1-21)	Denovo	GIFSLGAKINK NLISGLKG	Disturbs cell membrane	*P. aeruginosa*
Pl	Tick	PARKARATAA TAATAAT	Destroys the EPS matrix.	*S. mutans*
Nisin A	Denovo	MSTKDFNLDV SVSKDSGASPR	Depolarizes the cell membrane	*S. aureus*
Pisccidin-3	Fish	FIHHIFRGIHAG RSGRFLG	Degrades the eDNA	*P. aeruginosa*

Antibacterial peptides can disturb formation of the biofilm. They attack the different stages of biofilm development by numerous mechanisms such as inhibition of the biofilm formation, adhesion, quorum sensing, down-regulation and killing of the

preformed biofilms. Antibacterial peptides also have the ability to degrade the biofilms' extracellular polymer matrix. Hepcidin 20 reduces the mass of the extra-cellular matrix of *Staphylococcus epidermidis*. This peptide can target polysaccharide intracellular adhesion and alter the architecture of biofilm. Antibacterial peptides target stringent stress responses in the Gram-positive and in Gram-negative bacteria. These peptides can downregulate genes that are involved in formation of the biofilm and transportation of the binding protein. The formation of biofilm in the *Staphylococci* depends upon polysaccharide intracellular adhesion synthesis that is encoded by locus icaADBC [42].

Mechanism of action of antibacterial peptides

Pre-established *P. aeruginosa* biofilm was degraded by specific synthetic antibacterial peptides rapidly but mechanism of biofilm degrading is not understood clearly. Abrupt degradation of biofilm indicates that these peptides disrupted the membrane of bacterial cells [43]. Various mechanisms of antibacterial peptides are discussed in the literature by careful considerations. To elaborate multifaceted antibacterial peptides, the term host defence peptides (HDP) was devised [44]. Due to their adaptability HDPs are part of biological processes [45]. They have ubiquitous nature and play essential role in innate immune system [46]. They have the ability to modulate immune response, affect cancerous cells and destroy the biofilm. HDP as anti-biofilm agents work by disturbing the EPS, membrane, cell signaling and stringent responses [47]. Furthermore, the action of host defence peptides against biofilm is shown in (Fig. 2).

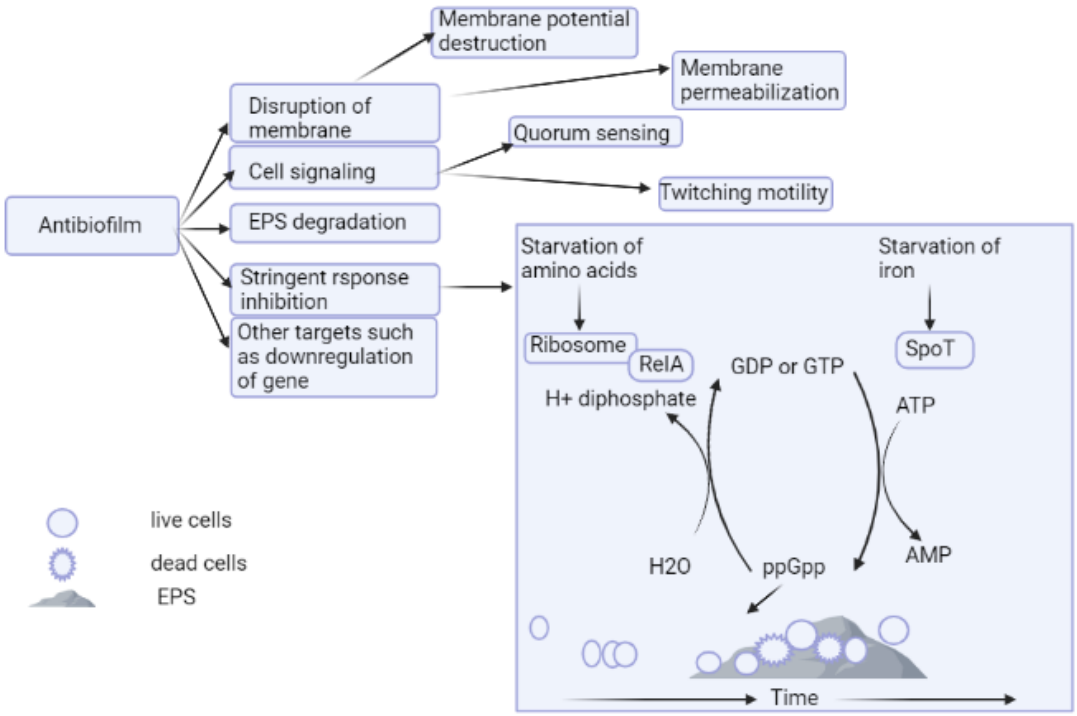

Figure 2: **Mechanism of action of host defense peptides as an anti-biofilm agent**

Degradation or disruption of membrane potential of biofilms' embedded cell

Nisin A, Nukacin ISK-1 and lacticin Q are the three bacteriocins that can abolish membrane potentials of the entrenched cells of the *S. aureus* biofilm that cause release of ATP from the cells. RN3(5-17P22-36) is an engineered peptide that is derived from eosinophile granules cationic proteins. This engineered peptide can kill the bacteria by the process of membrane disruption [48]. At the same concentration, the

depolarization of membrane in the biofilm was 2 to 3 times less as compared to the planktonic bacteria. Antibacterial peptide esculetin was derived from the skin of frogs and this peptide can permeabilize membrane of the *P. aeruginosa* (PAO1) in the biofilm that causes the release of the beta-galactosidase. In the case of biofilm, this effect did not show any significant result as compared to its effect on the planktonic bacteria **[49]**. Antibacterial peptide CSA-13 can penetrate rapidly into the biofilm and permeabilizes cell membrane of *P. aeruginosa* biofilm.

Interruption of bacterial cells' signal system

Indolicidin and Cathelicidic (LL-37) play a major role in preventing the development of *P. aeruginosa* biofilm. This process is done by down-regulation of transcription of the two main quorum sensing systems such as Rh 1 and Las **[50]**. Another mechanism that is used by antibacterial peptides to stop the creation of the biofilm is increasing the twitching motility of *P. aeruginosa*. The twitching motility is increased by the stimulation of gene expression that is essential for the biosynthesis of pili of type 4. Type 4 pili increase movement on the surfaces that may promote the cells' removal.

Degradation of polysaccharides and matrix of the biofilm

Antibacterial peptides target the extracellular matrix of the biofilms. As an instance, peptide P1 act upon the extracellular polymer matrix (EPS) that is produced by the *Streptococcus mutans* leading to the biofilm reduction formed upon the polystyrene **[51]**. Antibacterial peptide, obtained from maggots of the blowfly (*Calliphora Vicina*), can damage matrix of the biofilm formed by the drug-resistant *Staphylococcus aureus*, *Acinetobacter baumannii* and *Escherichia coli* but mechanism of the degradation is not explored. Antibacterial peptide (Hepcidin 20) derived from liver of human and this peptide can decrease mass of the EPS and change architecture of the *S. epidermitis* biofilm by targeting the polysaccharide intracellular adhesion (PIA). S4(1-16) M4ka is a peptide that is a derivative of the S4. This peptide has activity against biofilms of immature *P. aeruginosa*. This activity is performed by the integrations and release of the membrane lipid, bacterial detachment and hang up the formation of biofilm. Piscidin-3 is an antibacterial peptide, derived from the fish, exhibiting nucleosidase activity. This peptide can terminate extracellular DNA of the *P. aeruginosa*. This destruction is done by coordination with Cu2+ by its N-terminus **[52]**.

Inhibition of the bacterial alarmone to dodge the stringent responses

Antibacterial peptides target the stringent stress responses of Gram-positive and the Gram-negative bacteria. In numerous cases, bacteria yield guanosine 5-di-phosphate 3-di-phosphate (ppGpp) and the (p) ppGpp that are signaling nucleotides and regulate the gene expression. The nucleotides of the signal system show a major role in the development of biofilm. Other antibacterial peptides such as 1018 and DJK-6 target stringent response of the *P. aeruginosa*. They act by defeating the spot promoters' activity. In *P. aeruginosa* biofilms, DJK-6 and DJK-5 degrade the (p) ppGpp at a higher level as compared to 1018 **[53]**.

Down-regulation of the genes responsible for biofilm formation and transportation of the binding proteins

The development of *Staphylococci* biofilm is an acquisitive process that is dependent on synthesis of the polysaccharide's intracellular adhesion that is programmed by locus icaADBC in the *Staphylococci*. Human β defensins that are abbreviated as hBD-3 have the ability to lessen the manifestation of icaD, icaR and icaA genes of the *Staphylococcus epidermis* and in this way the formation of biofilm is reduced **[54]**. Antibacterial peptide stop the genes that control mobility of the extra chromosomal element, transport and binding proteins. NAI-P-113 is a peptide that hinders the biofilm of *Porphyromonas*

gingivalis by down-regulation of the genes such as PG1663 and PG0282 that encode the ATP binding protein and ABC transporter. ABC transporter plays a significant part in cell to cell and cell-to-surface connections in the formation of biofilm [55]. Various anti-bacterial peptides and their way of targeting the biofilm is shown in (Fig. 3).

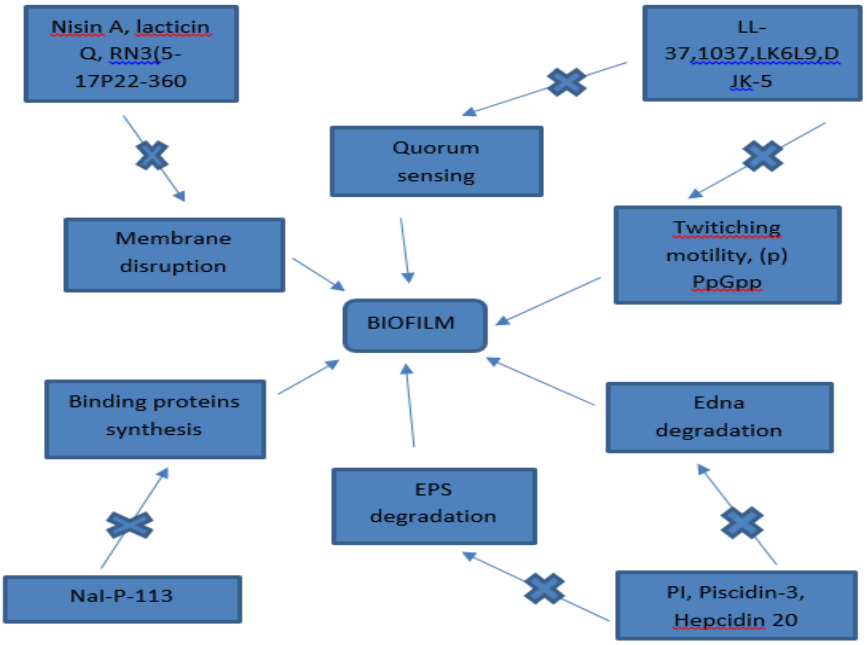

Figure 3: **Various targets of anti-biofilm peptides and cross sign (x) indicate inhibition of action**

Future directions and concluding remarks

In the current situation, infections that are related to biofilm are considered recalcitrant diseases. Treatment of these diseases with ordinary antibiotics is a hard challenge. Consequently, the need of research for growth of a novel therapeutic strategy increased at a significant level and antibacterial peptides emerge as an encouraging approach to deal with the biofilm. Administration of solo antibiotic is not sufficient to deal with biofilm infections, a high concentration of antibiotics creates toxicity and multiple strains are emerging with high level of resistance. Antibacterial peptides kill the various species of microbes in biofilm and these peptides can inhibit the developed biofilm. This strategy is promising because it allows the administration of antibacterial peptides concentration at a low level and represents a hopeful alternative of ordinary antibiotics for treatment of the infectious diseases. Antibacterial peptides control the signal in the system of Gram-negative and Gram-positive bacteria. It indicates that antibacterial peptides are broad in action. Interfering with the complex regulatory system of biofilm will help to control the emergence of the drug-resistant population. Future considerations will help to understand the downstream process of the antibacterial peptides. This will also assist to optimize the peptides and make them able to be developed as antibiotic adjuvants.

Author's contributions

Collected the relevant material and wrote up this review article: M Mahd, Supervised and proofread this review article: I Liaqat.

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