Antimicrobial peptides properties beyond growth inhibition and bacterial killing

Israel Castillo-Juárez¹, Blanca Esther Blancas-Luciano², Rodolfo García-Contreras² and Ana María Fernández-Presas²

¹ Laboratorio de Fitoquímica, Posgrado de Botánica, Colegio de Postgraduados, Texcoco, Estado de México, Mexico
² Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico

ABSTRACT

Antimicrobial peptides (AMPs) are versatile molecules with broad antimicrobial activity produced by representatives of the three domains of life. Also, there are derivatives of AMPs and artificial short peptides that can inhibit microbial growth. Beyond killing microbes, AMPs at grow sub-inhibitory concentrations also exhibit anti-virulence activity against critical pathogenic bacteria, including ESKAPE pathogens. Anti-virulence therapies are an alternative to antibiotics since they do not directly affect viability and growth, and they are considered less likely to generate resistance. Bacterial biofilms significantly increase antibiotic resistance and are linked to establishing chronic infections. Various AMPs can kill biofilm cells and eradicate infections in animal models. However, some can inhibit biofilm formation and promote dispersal at sub-growth inhibitory concentrations. These examples are discussed here, along with those of peptides that inhibit the expression of traits controlled by quorum sensing, such as the production of exoproteases, phenazines, surfactants, toxins, among others. In addition, specific targets that are determinants of virulence include secretion systems (type II, III, and VI) responsible for releasing effector proteins toxic to eukaryotic cells. This review summarizes the current knowledge on the anti-virulence properties of AMPs and the future directions of their research.

INTRODUCTION

The discovery of antibiotics is one of the most important events in modern medicine. The scientific community interest and the pharmaceutical industry for their commercialization in the mid-20th century favored the so-called golden age of these molecules (Díaz-Nuñez, García-Contreras & Castillo-Juárez, 2021). However, the generation of resistance of microorganisms to bactericides is a global public health problem and represents one of the critical challenges to be solved by humanity (Muñoz Cazares et al., 2017). Therefore, new targets or mechanisms of action are being investigated, in which antimicrobial peptides (AMPs) are an option to combat drug-resistant infections (Boparai & Sharma, 2019; Lei et al., 2019; Magana et al., 2020).
Most living organisms produce antimicrobial peptides as a defense mechanism in eukaryotes or as a microenvironmental competition strategy in prokaryotes (Moretta et al., 2021). Around 17,363 AMPs have been described, in which 82.7% are synthetic, and the rest are produced naturally in the three domains of life (Bulet, Stöcklin & Menin, 2004; Boparai & Sharma, 2019; Zasloff, 2019). They are classified according to their source of origin, activity, structure, and amino acid composition (Huan et al., 2020). Most AMPs are monomers of 4 to 50 amino acids that can acquire an amphipathic secondary structure of α-helix, β-hairpin-like β-sheet, β-sheet, or α-helix/β-sheet mixed structures (Bulet, Stöcklin & Menin, 2004).

In mammals, AMPs are a fundamental part of the innate immune system to counteract microbial infections (Boman, 2000). Some, such as defensins, are produced by epithelial cells to prevent the establishment of pathogens and are generally found in phagocytic cells to help eliminate microorganisms when ingested (Bulet, Stöcklin & Menin, 2004; de la Fuente-Núñez et al., 2017). In plants, AMPs are produced in different tissues to protect against pathogens; specifically, thionins and snakins are the best known (Tang et al., 2018). Bacteriocins are AMPs produced by bacteria, which have been identified as having a high antimicrobial activity (Soltani et al., 2021), while in archaea, halocins and sulfolobicins are the two main classes of archaeocins, which meet several ecological functions of competition in the environment with extreme conditions (Besse et al., 2015).

Classical antimicrobial properties are the main characteristic described for AMPs, and they are active against a broad spectrum of microorganisms, including viruses and parasites (Harris, Dennison & Phoenix, 2009; Huan et al., 2020). The primary mechanism reported for AMPs is related to their ability to lyse microbial cells (Pasupuleti, Schmidtchen & Malmsten, 2012; Mankoci et al., 2019) since the cationic properties (net positive charge) of most of them allows them to interact with the membranes of microorganisms (Alghalayini et al., 2019) (Fig. 1). However, other action mechanisms have also been described in which AMPs interact directly with specific target molecules (Brogden, 2005; Le, Fang & Sekaran, 2017; Graf & Wilson, 2019). Some of them have similar action mechanisms to antibiotics, including the inhibition of protein synthesis (pleurocidin and indolein) (Subbalakshmi & Sitaram, 1998; Patrzykat et al., 2002), or cell wall synthesis (mersacidin) (Brötz et al., 1998). Others, such as temporin L and the synthetic peptide 35409 (RYRRKKMKKALQYIKLLKE), inhibit Escherichia coli divisome machinery (Barreto-Santamaría et al., 2016; Di Somma et al., 2020). Unfortunately, because AMPs affect the viability of microorganisms, resistance mechanisms towards them are also reported (Cassone et al., 2009; Haney, Straus & Hancock, 2019).

In addition, AMPs influence several other biological processes (Haney, Straus & Hancock, 2019); for example, they interfere with the regulation of the microbiota, wound healing, induction of adaptive immunity, as well as possess anti-inflammatory, pro-inflammatory, anti-cancer, and cytotoxic properties, among others (Beisswenger & Bals, 2005; Haney, Straus & Hancock, 2019; Huan et al., 2020). Thus, due to its multifunctional nature, some authors have begun to use the broader term “host defense peptide” (HDP) (Haney, Straus & Hancock, 2019).
Figure 1  Antibacterial properties of antimicrobial peptides (AMP). The bactericidal properties are one of the main characteristics of AMP, its lytic capacity being one of the best-studied mechanisms of action. However, other targets have been identified in which they act as nucleic acids, proteins, or the divisome machinery. Unfortunately, as with other bactericidal agents, they also induce resistance. When AMPs work at sub-inhibitory concentrations, they exhibit anti-virulence properties, reducing the production of various factors that cause damage, but without affecting the viability of the bacteria. One of the targets is the inhibition of quorum sensing (QS), a general regulator of virulence. Furthermore, AMPs inhibit bacterial secretion systems, inactivate toxins, and exhibit adjuvant properties, restoring the activity of antibiotics on resistant strains. In the anti-biofilm activity, AMPs can act by bactericidal mechanisms or anti-virulence by inhibiting QS. An ideal property for anti-virulence therapies is that they do not generate resistance or are expected to do so to a lesser degree. MIC = minimum inhibitory concentration.

Full-size DOI: 10.7717/peerj.12667/fig-1
Anti-virulence activity is the antimicrobial property that has been discovered in various molecules when used at growth sub-inhibitory concentrations, in which they block the ability of bacteria to cause damage without interfering with their viability (Castillo-Juarez et al., 2017). There are different anti-virulence targets, but the most studied are the quorum sensing (QS) systems (Jiang et al., 2019) and the type 3 secretion systems (T3SS) (Hotinger & May, 2019).

QS is a phenomenon of gene regulation at the population level dependent on bacterial density that allows bacteria to exhibit collective or multicellular behaviors (Díaz-Nuñez, García-Contreras & Castillo-Juárez, 2021). It is one of the best-studied anti-virulence targets because it regulates the expression of various virulence factors, including the formation of biofilms, which is a multicellular behavior that gives them high resistance to antimicrobials (FleitasMartínez et al., 2018; Jiang et al., 2019).

In this regard, it is reported that some AMPs also exhibit anti-virulence properties at sub-inhibitory concentrations. In which the inhibition of biofilms (Di Somma et al., 2020), QS systems (Overhage et al., 2008), and secretion systems (McShan & De Guzman, 2015) stand out. They also neutralize enzymes, such as exoproteases and toxins (Kudryashova, Seveau & Kudryashov, 2017; Gusman, Malonneet & Atassi, 2001). In addition, they are reported to have adjuvant properties, which help restore the bactericidal effect of antibiotics on resistant strains (Fig. 1) (Geitani et al., 2019).

This review focuses on describing and analyzing the anti-virulence properties of AMPs exhibited in sub-inhibitory concentrations described so far, highlighting the evidence of their possible application.

**Survey methodology**

To ensure an inclusive and unbiased analysis of literature and to accomplish the review's objectives, a comprehensive analysis of published articles on the activity of antimicrobial peptides using the following online databases: Medline (PubMed), Science Direct (http://sciencedirect.com) database, Web of Science, Scopus, and Google Scholar system. Additionally, the following keywords were used: antimicrobial peptides, anti-virulence properties, quorum sensing, biofilms, targets together with Boolean operators such as “AND” and “OR”.

**Anti-biofilm and anti-quorum sensing activity of AMPs**

Biofilms are the preferred lifestyle of bacteria and are structured microbial aggregates, surrounded by a self-produced extracellular matrix, and attached to biotic or abiotic surfaces. Biofilms are involved in most chronic bacterial infections (Bjarnsholt, 2013). Moreover, they are crucial determinants of bacterial virulence. The biofilm matrix is formed by diverse components present in the extracellular polymeric substances: mainly proteins, polysaccharides, extracellular nucleic acids, and ions (Donlan, 2002). Biofilm formation is an ordered process, beginning with the initial contact and attachment to surfaces, mainly mediated by structures such as flagellum and fimbria, followed by micro-colony formation, maturation, and formation of the complex biofilm architecture, finally, detachment and dispersal of some cells from the biofilm occur (Sutherland, 2001).
Biofilms are pivotal for bacterial survival as they protect against adverse environmental conditions. They increase drug resistance by various mechanisms such as the decrease in the permeability of antibiotics, the promotion of dormancy and induction of bacterial persistence, the expression of the efflux pumps of antibiotics, and the synthesis of periplasmic glucans (aminoglycosides) that inactivate antibiotics (Hall & Mah, 2017). Biofilms also allow bacteria to evade the human defense mechanisms (Mirzaei et al., 2020) since several biofilm matrix proteins protect biofilms against human innate immune cells, opsonization, and phagocytosis (Lewis, 2008). Moreover, it has been demonstrated that some bacterial species, previously known as extracellular pathogens, can reside inside various host cells by adapting to intracellular life through the formation of microbial aggregates similar to bacterial biofilms, leading to their long-term survival inside the cells (Mirzaei et al., 2020).

Unlike antibiotics, AMPs are suitable for slowing growth and killing cells in the biofilm. Several examples of effective AMPs with this activity have been described that correlate with the ability of AMPs to resolve bacterial infections in vivo. For a recent full review of these activities and the translation potential of such peptides, see the work of Gislaine and coworkers (Silveira et al., 2021).

Since the aim of this work is to discuss the anti-virulence potential of AMPs, and one of the premises of anti-virulence therapies is not to affect directly bacterial growth and survival, most of the examples of AMPs with anti-biofilm activity discussed here will be peptides that inhibit biofilm formation at growth sub-inhibitory concentrations (Table 1).

AMP activity against biofilms is mediated by the degradation or destabilization of the extracellular matrix (Yasir, Willcox & Dutta, 2018). The PI peptide (derived from polyphemusin I) induces the degradation of the exopolysaccharides produced by Streptococcus mutans, causing the biofilm formation to be attenuated (Zhang et al., 2019). Also, an AMP complex produced by the insect Calliphora vicina promotes the degradation of the matrix of the biofilm produced by E. coli, Staphylococcus aureus, and Acinetobacter baumannii (Gordya et al., 2017). Hepcidin 20 from the human liver decreases the extracellular matrix and disrupts the architecture of S. epidermidis biofilms (Brancaisano et al., 2014). S4 (1-16) M4Ka (dermaseptin S4 derivative), which inhibits immature biofilms of Pseudomonas fluorescens (Quilès et al., 2016). Piscidin-3 is derived from fish, which degrades the extracellular DNA of P. aeruginosa biofilms (Libardo et al., 2017).

Biofilm inhibition by AMPs is also mediated by the downregulation of genes responsible for biofilm formation and transport of binding proteins; for example, in Staphylococcal biofilms, the β-defensin 3 from humans decreases the expression of the icaA, icaD, and icaR genes that codify enzymes responsible for the biosynthesis of the adhesin PIA, essential for biofilm formation (Rohde et al., 2010; Zhu et al., 2013). In addition, AMPs also inhibit genes that control the transport and binding proteins, such as ABC transporters that are involved in biofilm formation since they promote cell-to-surface and cell-to-cell interactions (Zhu et al., 2013; Wang et al., 2017).
| Name | Source | Activity | Effect | References |
|------|--------|----------|--------|------------|
| PI peptide (Derived from polyphemusin I) | Horseshoe crab | Anti-biofilm | Inhibits the development of biofilm of *S. mutans* in the dental plaque of rabbit incisors. | Zhang et al. (2019) |
| Hepcidin 20 | Derived from human liver | Anti-biofilm | Inhibit the production and accumulation of extracellular matrix in the biofilm of *S. epidermidis*. | Brancatisano et al. (2014) |
| AMP complex (defensin, cecropin, diptericin and proline rich peptide families) | Calliphora vicina worms | Anti-biofilm | Destroys the matrix and cells of the biofilm of *E. coli*, *S. aureus*, and *A. baumannii*. | Gordya et al., 2017 |
| S4 (1-16) M4Ka (Dermaseptin S4 derivative) | Amphibian skin | Anti-biofilm | Destroys immature *P. fluorescens* biofilms. | Quilès et al., 2016 |
| Piscidin-3/(Cu^{2+}) | Fish | Anti-biofilm | Damages *E. coli* DNA in a copper-dependent manner. | Libardo et al., 2017 |
| β-defensin 3 | Humans | Decreases the formation of biofilms in *Staphylococcus*, as well as the expression of genes responsible for its production. | Zhu et al. (2013) |
| LL-37 (Derived from cathelicidin) | Humans | Anti-biofilm, anti-QS | Reduces the expression of the Las and Rhl genes. Inhibits the biofilm formation in *P. aeruginosa*, *F. novicida*, *S. epidermidis*, and *S. aureus*. | Hancock & Sahl (2006); Overhage et al. (2008); Chengsupat et al. (2009); Amer, Bishop & van Hoek, 2010; Hell et al., 2010; Kang, Dietz & Li, 2019. |
| LIVRHK and LIVRRK | Synthetics | Anti-QS, anti-biofilm | They inhibit biofilm formation and the production of virulence factors (pyocyanin, protease, and rhamnolipids) in *P. aeruginosa*. Also, they reduce the expression of lasI, lasR, rhlI, and rhlR. | Taha et al., 2019 |
| Peptide 1037 | Synthetic | Anti-biofilm | Inhibits the formation of biofilms of *P. aeruginosa*, *B. cenocepacia*, and *L. monocytogenes*. Also, it reduces the expression of a variety of genes involved in its formation. | De La Fuente-Núñez et al. (2012) |
| D-Bac8c^{2},5Leu | Synthetic | Anti-biofilm | Prevents the formation of *S. aureus* biofilms on catheters. | Zapotoczna et al. (2017) |
| Bovicin HC5 | *Streptococcus bovis* HC5 | Anti-biofilm, anti-QS | Reduces the formation of biofilms in *S. aureus*. | Pimentel-Filho Nde et al., 2014 |
| Nisin | *Lactococcus lactis* | Anti-biofilm, anti-QS | Reduces the production of violacein in *C. violaceum*. Also, biofilm formation and AI-2 production in *G. vaginalis*. | Algburi et al., 2017 |

(continued on next page)
Table 1 (continued)

| Name | Source | Activity | Effect | References |
|------|--------|----------|--------|------------|
| RBP15 | Synthetic | Anti-QS | Inhibits the phosphorylation of the RNAIII activator protein (TRAP) in *S. aureus*. | Yang et al. (2003) |
| P1(ELWESDNLNEEQ) and P2 (TKLTRTWRQ) | Synthetic | Anti-T2SS | They disrupt the XcpVW pseudopilin nucleus complex and the tip of the pseudopilus. Inhibit T2SS and reduce the virulence of *P. aeruginosa* in the *Caenorhabditis elegans* model. | Zhang et al. (2018) |
| Lactoferrin | Mammals | Anti-T3SS | Inhibit T3SS in *Salmonella, Shigella*, and *E. coli* through the degradation of translocon proteins. | McShan & De Guzman (2015) |
| CoilA, Coil B and CesA2 | Synthetic | Anti-T3SS | Inhibit the formation of the T3SS needle in EPEC and reduce hemolysis. | Larzábal et al. (2019) |
| HNP, HD5 | Human | Anti-toxin | Inhibit the Lethal Factor of *B. anthracis*, diphtheria toxin, exotoxin A of *P. aeruginosa* and cytotoxin B of *C. difficile*. | Kim et al. 2005, 2006; Giesemann, Guttenberg & Aktories (2008); |
| hBD | Human | Anti-toxin | Inhibit the gonococcal toxin NarE of *N. gonorrhoeae* and the Lethal Factor of *B. anthracis*. | Rodas et al., 2016; Wei et al., 2009 |
| Retrocyclins | Human | Anti-toxin | Inhibit the Lethal factor of *B. anthracis* and the vaginolysin of *G. vaginalis*. | Wang et al. (2006); Hooven et al. (2012) |
| Bacitracin | *Bacillus subtilis* | Anti-toxin | They inhibit various toxins such as Lethal Factor (*B. anthracis*), C2 toxin (*C. botulinum*), CDT transferase (*C. difficile*), and epsilon toxin (*C. perfringens*). | Schnell et al. (2019) |
| Histatin 5 | Human | Anti-toxin | Inhibit the exoproteases of *P. gingivalis* involved in the generation of damage in periodontal disease and the cysteine proteinases of *C. histolyticum*. | Gusman et al., 2001; Le, Fang & Sekaran (2017) |
| Unarmycin A and C | Marine bacteria | Adjuvants | Inhibit theazoleantifungal efflux pumps and restore antifungal sensitivity in *C. albicans*. | Tanabe et al. (2007) |
| Plantaricin PLNC8 αβ | *Lactobacillus plantarum* | Adjuvants | Enhance the activity of conventional antibiotics against *Staphylococcus* strains. | Bengtsson et al. (2020) |

Notes.
- T3SS, type 3 secretion system; T2SS, type 2 secretion system; QS, quorum sensing; EPEC, enteropathogenic *E. coli*. 

---

**Notes.**
- T3SS, type 3 secretion system; T2SS, type 2 secretion system; QS, quorum sensing; EPEC, enteropathogenic *E. coli*.
Beyond inhibiting biofilm maturation, AMPs can also inhibit initial attachment and increase cell dispersal. One of the first discovered AMPs with the ability to eradicate biofilms was LL-37 (Overhage et al., 2008), derived from human cathelicidin, an amphipathic peptide widely distributed in body fluids (Burton & Steel, 2009). At low concentrations, LL-37 inhibits the adhesion of P. aeruginosa cells to surfaces, and at higher concentrations, it reduces the thickness of the biofilms (Hancock & Sahl, 2006). Moreover, LL-37 also eradicates P. aeruginosa biofilms in vivo (Chennupati et al., 2009). The anti-biofilm effects of LL-37 in P. aeruginosa at concentrations that do not affect viability and growth are related to the upregulation of the expression of type IV pili genes that lead to the promotion of twitching motility which is linked to biofilm dispersal and to the decrease in the expression of flagellar genes which leads to lower attachment to surfaces (Overhage et al., 2008). In addition, LL-37 treatment induces a strong down-regulation of the core genes of the Las and Rhl QS systems and the repression of genes that encode QS-dependent virulence factors such as LasB elastase and those responsible for the biosynthesis of rhamnolipids (Overhage et al., 2008). In addition, it inhibits the biofilm formation of other pathogens such as Francisella novicida and S. epidermidis (Amer, Bishop & van Hoek, 2010; Hell et al., 2010).

Other AMPs can prevent biofilm formation by inhibiting quorum sensing (Overhage et al., 2008). For example, Trp-containing peptides inhibit QS-regulated virulence and biofilm growth of multidrug-resistant P. aeruginosa. Significantly, peptides containing tryptophan at low concentrations reduced the production of virulence factors that regulate the gene expression of the Las and Rhl systems. Biofilm formation was inhibited in a concentration-dependent manner, which was associated with inhibiting extracellular polysaccharide production by negatively regulating the transcription of pelA, algD, and pslA. These changes were correlated with alterations in the extracellular production of virulence and motility.

Also, two novel synthetic peptides (LIVRHK and LIVRRK) can inhibit biofilm formation of P. aeruginosa PA01, and QS-dependent phenotypes such as pyocyanin exoprotease, and rhamnolipid production were identified. In addition, a down-regulation of the expression of the core QS genes lasRI and rhlRI were observed, corroborating the inhibition of QS (Taha et al., 2019).

The discovery of the anti-biofilm and anti-QS properties of LL-37 led to the search for other natural and synthetic peptides with similar properties. De la Fuente and his colleagues in 2012 selected 50 small synthetic peptides and identified 16 with anti-biofilm activity against P. aeruginosa, with HH15 being one of the best. According to their sequence, 15 small peptides were designed, including peptide 1037 of only nine amino acids, reducing the biofilm formation of Burkholderia cenocepacia and Listeria monocytogenes (De La Fuente-Núñez et al., 2012).

The peptide 1037, like LL-37, stimulates twitching motility and decreases the expression of flagellar genes, leading to potent inhibition of swimming and swarming motilities (De La Fuente-Núñez et al., 2012). Comparison between the effect in gene expression of peptides (LL-37 vs. 1037) allowed the identification of ten common downregulated genes and four upregulated ones. The role of those genes in biofilm formation was confirmed using
transposon mutants of each one, being nine of the ten mutants in the downregulated genes lower biofilm producers than the parental strain. Although the involved genes mainly were hypothetical proteins, the flagellar gene flgB, rhlB, involved in rhamnolipid biosynthesis and nirS encoding a nitrite reductase were identified. In addition, two of the four mutants in the upregulated genes (a hypothetical protein and actP, encoding an acetate permease have) higher biofilm producer than the parental strain (De La Fuente-Núñez et al., 2012).

In another study, it was shown that LL-37 exhibits anti-biofilm activity against S. epidermidis, where at low concentrations they prevent cell attachment, while at high concentrations, they prevent the maturation and establishment of biofilms. Also, LL-37 has a potent S. aureus biofilm eradication activity (Kang, Dietz & Li, 2019).

Other synthetic peptides such as D-Bac8c2,5Leu, D-HB43, and D-ranalexin have effectively killed S. aureus biofilms. For example, the synthetic peptide D-Bac8c2,5Leu, when applied as a catheter lock solution, has inhibitory activity on early and mature S. aureus biofilms in a rat venous catheter infection model (Zapotoczna et al., 2017).

Although some classic antibiotics at sub-MIC concentrations have shown anti-virulence and QS system regulation behaviors (Skindersoe et al., 2008; Zhang & Li, 2016), there is still little research on peptide antibiotics. However, bovicin HC5 (broad-spectrum lantibiotic) and nisin (polycyclic peptide antibiotic) have been reported to have anti-biofilm activity through QS interference from S. aureus (Pimentel-Filho et al., 2014). Similarly, subtilosin (cyclic lantibiotic) reduces violacein production in Chromobacterium violaceum (indicative of QS inhibition), as well as biofilm formation and autoinducer-2 (AI-2) production in Gardnerella vaginalis (Algburi et al. al., 2017) (Table 1).

Other anti-virulence targets of AMPs

Although biofilms and other QS-controlled phenotypes (exoproteases, phenazines, rhamnolipids, swarm motility) are essential for bacterial virulence, some important virulence factors are not positively regulated by QS. Eight secretion systems have been found in Gram-negative and Gram-positive bacteria. However, these systems are sometimes unregulated by QS or may even be downregulated, as in some vibrio species. Therefore, in cases where QS negatively regulates them, QS inhibition can promote virulence through secretion systems (Pena et al., 2019). Therefore, specific inhibitors of these systems in combination with QS inhibitors may be necessary to develop more robust antibacterial therapies (García-Contreras, 2016).

Accordingly, Zhang and coworkers elucidated the structural and functional details of the pseudopilus tip complex of the type II secretion system of P. aeruginosa, which is an essential component of the system that functions as a piston, allowing the export of multiple effectors (Zhang et al., 2018). Based on the structural details of the complex, two mimicking peptides [P1(EWESDNRLNEEQ) and P2 (TKLTRTWRQ)] that were able to compete with the binding of the XcpV and XcpW pseudolipins were designed, retaining the specific amino acids that allow the interaction between those pseudolipins and introducing other hydrophilic amino acids to enhance solubility. The utilization of those peptides precluded the formation of the core complex essential for the pseudopilus tip formation and strongly attenuated secretion through the type II secretion system (Zhang et al., 2018).
Interestingly, natural mammalian peptides, such as iron-binding lactoferrin, are potent inhibitors of T3SS in enteric bacteria (Salmonella, Shigella, and E. coli) by inducing translocon protein degradation. This activity is mediated by its binding to the lipopolysaccharide on the bacterial surface, destabilizing the protein-protein interactions essential for the system. Furthermore, lactoferrins have serine protease activity that can affect T3SS cleavage proteins (McShan & De Guzman, 2015).

Beyond the anti-T3SS of natural peptides, the strategy of using polypeptides that mimic some components of the systems and that compete with the binding of the natural bacterial components was effective to inhibit the system in Chlamydia, Salmonella, and Shigella, blocking their entrance to eukaryotic cells in cultures (McShan & De Guzman, 2015). Similarly, in enteropathogenic E. coli (EPEC), coiled-coil peptide mimetics, analogs of the EspA, EscF, and CesA proteins (CoilA, Coil B and CesA2) of its T3SS, inhibit the T3SS mediated hemolysis (Larzábal et al., 2019).

Another critical virulence determinant is the type VI secretion system, which delivers multiple effectors to prokaryotic and eukaryotic cells. Those effectors target cell walls, cell membranes, DNA, and to avoid self-poisoning, bacteria that produce them also produce neutralizing proteins that bid the effectors. Recently, in P. aeruginosa, the effector TplE, a lipolytic toxin effective against other bacteria and able to disrupt the endoplasmic reticulum in eukaryotic cells, had been characterized; this protein is neutralized by TplEi (Jiang et al., 2016).

Based on this interaction, Gao and coworkers generated a small peptide capable of competing with the TplEi-TplE interaction, for this TplE was hydrolyzed, generating a 26 amino acid fragment that strongly binds with TplEi, releasing the TplE toxin, and thus inducing the autointoxication of P. aeruginosa (Gao et al., 2017). This approach is attractive and represents a new concept for generating new inhibitors of secretory systems and other potential targets. A similar approach was recently used for the identification of small peptides that inhibit antitoxins that belong to the toxin-antitoxin systems (Lee et al., 2015; Sundar, Rajan & Piramanayagam, 2019), which are related to latency, persistence (Page & Peti, 2016) and bacterial virulence (Fernández-García et al., 2016). These systems are abundant in intracellular bacterial pathogens such as Mycobacterium tuberculosis (Sala, Bordes & Genevaux, 2014).

Additional anti-virulence activities of some AMPs, such as defensins are the capacity to bind and inhibit the activity of several bacterial toxins and related virulence factors (Table 1). Defensins are components of the innate immunity of mammals and are also found in invertebrates, plants, and fungi. Although these peptides had low sequence similarity, they share common structural features and display broad antibacterial and antiviral activity at high concentrations; in addition, they modulate inflammation and promote angiogenesis and wound healing. Moreover, they can neutralize several bacterial toxins, among them cytolysin, listeryolysin that promote pore formation, ribosyltransferase toxins, glycosylation promoting toxins, the MARTX toxins from Vibrio and Aeromonas, the Panton-Valentine leucocidin, staphylokinase from S. aureus, SIC which is the Streptococcal inhibitor of complement (Kudryashova, Seveau & Kudryashov, 2017). Upon binding to the toxins, defensins promote their unfolding, disrupting their secondary and tertiary structure,
making them more susceptible to proteolysis and promoting their precipitation. Although the physicochemical properties that allow defensins to bind and neutralize a broad range of structurally diverse toxins are not completely understood, recent studies demonstrate that defensins act by recognizing regions of proteins showing structural plasticity and thermodynamic instability, features that are shared by a wide range of bacterial toxins (Kudryashova, Seveau & Kudryashov, 2017). In general, of the alpha-class such as HNP and HD5, they inhibit the lethal factor of Bacillus anthracis, the diphtheria toxin, the exotoxin A of P. aeruginosa, the cytotoxin B of Clostridioides difficile, among others (Kim et al. 2005, 2006; Giesemann, Guttenberg & Aktories, 2008). While those in the beta-class, such as hBD, inhibit the gonococcal toxin NarE from Neisseria gonorrhoeae and the lethal factor from B. anthracis (Rodas et al., 2016; Wei et al., 2009). In the case of those of the theta-class, the retrocyclins inhibit the lethal factor of B. anthracis and the vaginolysin of G. vaginalis (Wang et al., 2006; Hooven et al., 2012).

Beyond defensins, there are other notable examples of toxin-neutralizing peptides, such as the artificial peptide Pep19−2.5 and related ones, capable of inactivating lipopolysaccharides (LPS or endotoxin) and lipoproteins in vitro and in vivo. They also decrease inflammation mediated by the activation of signaling cascades (Heinbockel et al., 2018), and their efficacy has been reported in several mouse infection models, including endotoxemia and bacteremia (Heinbockel et al., 2013). Several other peptides with the ability to neutralize a wide variety of bacterial toxins have been described, for which we recommend consulting the following reviews (Jerala & Porro, 2005; Kudryashova, Seveau & Kudryashov, 2017; Schnell et al., 2019). The effect was not always determined at sub-MIC concentrations; however, the inhibition of toxins is a strategy contemplated within the anti-virulence targets.

Bacitracin is an antibiotic that inhibits cell wall synthesis, but recently it has also been reported to neutralize type A/B protein exotoxins by inhibiting pore formation, preventing translocation of the A subunit to the host cell cytosol. These toxins are made up of an enzymatic component (A subunit) and a binding/transport component (B subunit), such as the lethal factor of Bacillus anthracis, the toxin C2 of Clostridium botulinum, the CDT transferase of C. difficile, and epsilon toxin of Clostridium perfringens (Schnell et al., 2019). In addition to neutralizing bacterial toxins, some AMPs can inhibit exoproteases implicated in the generation of host damage during periodontal disease. For example, the salivary peptide histatin 5 inhibits the host metalloproteases and exoproteases from bacterial pathogens such as the gingipains produced by Porphyromonas gingivalis attenuating damage and inflammation (Gusman, Malonnet & Atassi, 2001). Moreover, histatin 5 also inhibits cysteine proteinases such as clostripain, which is produced by Clostridium histolyticum during gangrene, while other AMPs inhibit exoproteases such as subtilisin A, proteinase K, elastase, and chymotrypsin (Le, Fang & Sekaran, 2017).

Finally, a characteristic of some anti-virulence molecules is their adjuvant properties, which enable them to restore the activity of antibiotics on resistant strains (Díaz-Nuñez, García-Contreras & Castillo-Juárez, 2021). This strategy is very promising, and although it does not prevent the generation of resistance, it allows the reactivation of antimicrobials that are in danger of falling into disuse (González-Bello, 2017). In the case of AMPs at
sub-MIC concentrations, some reports of adjuvant properties have been made in the literature, such as unarmycin A and C (Tanabe et al., 2007). These cyclopeptides isolated from marine bacteria are azole antifungal ejection pump inhibitors and restore fluconazole sensitivity of resistant strains and clinical isolates of Candida albicans (Tanabe et al., 2007).

Also, plantaricin PLNC8 α β showed an adjuvant effect by potentiating the activity of conventional antibiotics (vancomycin, rifampicin, and gentamicin) against S. epidermidis, although the mechanism of action involved is unknown (Bengtsson et al., 2020).

**CONCLUSIONS**

There is currently enough evidence to support the participation of the QS, T3S, two-component regulatory systems, and other virulence determinants in the generation of bacterial pathogenicity and damage (Marshall & Brett Finlay, 2014; Totsika, 2016; Tiwari et al., 2017; Tsai et al., 2020). It is reported that the interruption of genes that code for these systems reduces virulence and bacterial pathogenicity in vivo models of animals and plants (Castillo-Juárez et al., 2015; Jiang et al., 2019). Also, similar results are obtained with the administration of small molecules that inhibit these systems (Marshall & Brett Finlay, 2014; Jiang et al., 2016; Hotinger & May, 2019). Similarly, there are reports of the anti-virulence properties of synthetic peptides analogous to the autoinducers of Gram-positive bacteria, such as the so-called RIP and its derivatives (RBP15), which reduce pathogenicity at the preclinical level (Yang et al., 2003; Singh, Desouky & Nakayama, 2016). However, to achieve the implementation of anti-virulence therapies in the clinical practice, there are some challenges to overcome, such as determining their toxicity, their possible side effects, including their effects on the microbiota, the generation of resistance, and verifying their efficacy at the clinical level (Díaz-Nuñez, García-Contreras & Castillo-Juárez, 2021).

The anti-virulence effects of substances at low concentrations has generated significant interest due to the possibility of controlling microbial infections and probably avoiding the appearance of resistance (Totsika, 2016; Díaz-Nuñez, García-Contreras & Castillo-Juárez, 2021). Various substances, including natural products, antibiotics, and drugs of mass consumption such as ibuprofen and aspirin have been identified to reduce virulence at sub-MIC concentrations (Bernardo et al., 2004; Skindersoe et al., 2008; El-Mowafy et al., 2014; Soo et al., 2017; Dai et al., 2019). The information related to the effect of low-dose AMPs is scarce and highly debatable. Recently, evidence that indicates adverse effects of the use of AMP at sub-inhibitory doses was compiled. The possible adverse effects include the induction of resistance (strong stress generators) and directly or indirectly stimulating virulence through different signaling pathways (Vasilchenko & Rogozhin, 2019). It should be noted that the main characteristic of the ideal anti-virulence molecule is that it does not interfere directly with bacterial viability, lowering the selection pressure for the generation of resistance (Díaz-Nuñez, García-Contreras & Castillo-Juárez, 2021). Most peptides stress bacterial cells at growth inhibitory concentrations; however, there is evidence to suggest that in their native environment, peptides are in relatively low concentrations that do not kill microorganisms, and hence the high growth inhibitory concentrations are hardly reached (Dorschner et al., 2001; Monnet, Juillard & Gardan, 2016; Vasilchenko & Rogozhin,
Therefore, the ubiquitous microbicidal activity of AMPs may not be their primary natural or ecological function.

Also, it has been pointed out that AMPs exhibit anti-virulence properties, but the effect is unpredictable and possibly uncontrollable since AMPs also may stimulate virulence at specific concentrations (Vasilchenko & Rogozhin, 2019). In this regard, hormesis is a widely studied phenomenon that occurs at low doses, in which the same compound can exhibit antagonistic effects depending on the dose (Mattson, 2008). This phenomenon has been described in some small and synthetic molecules, but it is not a generality for all the anti-virulence molecules described. In the case of AMPs, a possible case of hormesis of the LL-37 peptide is pointed out, which at sub-MIC concentrations reduces the gene expression of QS and the production of virulence factors, but at the same time stimulates others (Overhage et al., 2008; Strempe et al., 2013). In hormesis, concentration is essential to obtain the desired effect; however, identifying peptides that can stimulate QS systems could be helpful if applied to bacteria that regulate the expression of beneficial phenotypes. As in the case of beneficial microorganisms in agriculture, for the treatment of wastewater or the intestinal microbiota (Schikora, Schenk & Hartmann, 2016; Zhang & Li, 2016; Bivar Xavier, 2018). In this sense, nanotechnological techniques will be essential to help to maintain their bioavailability efficiently (Boparai & Sharma, 2019).

With the information available to date, some behaviors, or effects of peptides at sub-MIC concentrations can be classified as autoinducer peptides, inducer peptides, signal peptides, and anti-virulence peptides (Fig. 2). Autoinducer peptides are produced by Gram-positive bacteria [AIP (autoinducing peptide), CSP (competence stimulating peptide), ComX, and CSF (competence and sporulation factor)] and participate in bacterial communication through QS systems (Monnet, Juillard & Gardan, 2016). In comparison, the inducer peptides are those that are produced by other microorganisms (antibiotics, bacteriocins) or the host (defensins) and that modify the gene expression of the QS systems or virulence (Baishya et al., 2021). The signaling peptides are produced by host cells for specific functions (such as promoting the establishment of beneficial microorganisms of the intestinal microbiota), but bacteria also capture them as environmental signals for regulating virulence systems. Finally, anti-virulence peptides are produced by the hosts (or by competing microorganisms) as a strategy to reduce pathogenicity and avoid the establishment and damage of bacteria (Fig. 2). It should be noted that one and two-component environmental signaling systems can participate in all these effects (Tiwari et al., 2017).

Some authors mention the term “pheromone” to refer to the induction of gene expression “at a distance” (distances are challenging to define in the microscopic world) by specific peptides (Monnet, Juillard & Gardan, 2016; Yajima, 2016; Vasilchenko & Rogozhin, 2019). However, we consider it a confusing term and suggest that it should be avoided in this study topic as it is based on an analogy of the functioning of pheromones in macroscopic organisms with sexual reproduction. Likewise, in the classification by activity of anti-virulence peptides, their possible effects on host cells should be considered (Tornesello et al., 2018) as well as their immunogenic activity, which some authors point out is the
main responsible for eliminating bacteria in vivo (Hancock & Sahl, 2013; Mansour, Pena & Hancock, 2014).

On the other hand, although the U.S. Food and Drug Administration (FDA) has approved several AMPs used at growth inhibitory concentrations, most are restricted to the topical application due to limitations found with other routes of administration, such as a short half-life, low stability, and low bioavailability (Lei et al., 2019). Likewise, its use at inhibitory concentrations for prolonged periods is reported to generate toxic effects, like hemolysis, and to induce resistance (Rathinakumar, Walkenhorst & Wimley, 2009; Starr et al., 2018; Lei et al., 2019), coupled with the high cost of producing them commercially (Moretta et al., 2021). Investigating the activities of AMPs at low or growth sub-inhibitory concentrations could help resolve some of these difficulties and favor their clinical application. Therefore, we can conclude that the action of AMPs and the response they elicit at sub-MIC concentrations is a fertile and promising area of knowledge that requires further research to develop safe and effective anti-virulence therapies.

Finally, regardless of the anti-biofilm and anti-virulence properties of the peptides discussed here, some aspects should be further tested, including their utilization to...
attenuate infections produced by clinical strains in vivo, and more significant efforts for their implementation in clinical trials should be encouraged (Silveira et al., 2021).

Another aspect that needs to be studied further is the mechanistic details of the action of peptides that inhibit biofilm formation (without affecting bacterial growth), virulence factors controlled by QS, and secretion systems. It is essential to study the possibility of selecting resistance in vivo, its effects, and the mechanisms involved. Although it is proposed that the peptides are more robust and less likely to induce resistance compared to the usual antibiotics, some possible mechanisms are proposed, such as modifications of the membrane and the composition of the cell wall, expulsion by efflux pumps, AMP sequestration, and protease inactivation (Assoni et al., 2020). In this regard, it is expected that similar mechanisms will eventually evolve to attenuate the effects of AMP on biofilm and inhibit virulence, even if these peptides do not affect viability and growth in vitro in principle.

It was especially considering that although scarce, resistance mechanisms against anti-virulence therapies, mediated by QS inhibition (García-Contreras, 2016) and biofilm inhibition, had been described (Travier et al., 2013).

Funding
Ana María Fernández-Presas is funded by PAPITT, DGAPA, UNAM, Mexico City, grant #IN218419, Rodolfo García-Contreras is funded by CONACYT grant CB 2017-2018 number A1-S-8530 and by PAPITT UNAM grant number IN214218. Israel Castillo Júarez is funded by Cátedras-CONACYT program. Blanca Esther Blancas-Luciano is supported by CONACYT grant # 424031 for her doctoral studies. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
PAPITT, DGAPA, UNAM, Mexico City: #IN218419.
CONACYT grant: CB 2017-2018 number A1-S-8530.
PAPITT UNAM: IN214218.
Cátedras-CONACYT program.
CONACYT: # 424031.

Competing Interests
The authors declare there are no competing interests.

Author Contributions
• Israel Castillo-Juárez analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Blanca Esther Blancas-Luciano, Rodolfo García-Contreras and Ana María Fernández-Presas analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
Data Availability
The following information was supplied regarding data availability:
This is a literature review.

REFERENCES

Alghalayini A, Garcia A, Berry T, Cranfield CG. 2019. The use of tethered bilayer lipid membranes to identify the mechanisms of antimicrobial peptide interactions with lipid bilayers. *Antibiotics* 8(1):12 DOI 10.3390/antibiotics8010012.

Algburi A, Comito N, Kashtanov D, Dicks LMT, Chikindas ML. 2017. Control of Biofilm Formation: Antibiotics and Beyond. *Applied and Environmental Microbiology* 83(6):e00165–17 DOI 10.1128/AEM.00165-17.

Amer LS, Bishop BM, van Hoek ML. 2010. Antimicrobial and antibiofilm activity of cathelicidins and short, synthetic peptides against Francisella. *Biochemical and Biophysical Research Communications* 396(2):246–251 DOI 10.1016/j.bbrc.2010.04.073.

Baishya J, Bisht K, Rimbey JN, Yihunie KD, Islam S, Mahmoud H Al, Waller JE, Wakenman CA. 2021. The impact of intraspecies and interspecies bacterial interactions on disease outcome. *Pathogens* 10:96 DOI 10.3390/pathogens10020096.

Barreto-Santamaría A, Curtidor H, Arévalo-Pinzón G, Herrera C, Suárez D, Pérez WH, Patarroyo ME. 2016. A new synthetic peptide having two target of antibacterial action in E. coli ML35. *Frontiers in Microbiology* 7:2006 DOI 10.3389/fmicb.2016.02006.

Beisswenger C, Bals R. 2005. Functions of antimicrobial peptides in host defense and immunity. *Current Protein & Peptide Science* 6:255–264 DOI 10.2174/1389203054065428.

Bengtsson T, Selegård R, Musa A, Hultenby K, Utterström J, Sivlér P, Skog M, Nayeri F, Hellmark B, Söderquist B, Aili D, Khalaf H. 2020. Plantaricin NC8 αβ exerts potent antimicrobial activity against Staphylococcus spp. and enhances the effects of antibiotics. *Scientific Reports* 10:3580 DOI 10.1038/s41598-020-60570-w.

Bernardo K, Pakulat N, Fleer S, Schnaith A, Utermöhlen O, Krut O, Müller S, Krönke M. 2004. Subinhibitory concentrations of linezolid reduce staphylococcus aureus virulence factor expression. *Antimicrobial Agents and Chemotherapy* 48:546–555 DOI 10.1128/AAC.48.2.546-555.2004.

Besse A, Peduzzi J, Rebuffat S, Carré-Mlouka A. 2015. Antimicrobial peptides and proteins in the face of extremes: lessons from archaeocins. *Biochimie* 118:344–355 DOI 10.1016/j.biochi.2015.06.004.

Bivar Xavier K. 2018. Bacterial interspecies quorum sensing in the mammalian gut microbiota. *Comptes Rendus - Biologies* 341 DOI 10.1016/j.crvi.2018.03.006.

Bjarnsholt T. 2013. The role of bacterial biofilms in chronic infections. *APMIS* 121:1–51 DOI 10.1111/apm.12099.

Boman HG. 2000. Innate immunity and the normal microflora. *Immunological Reviews* 173:5–16 DOI 10.1034/j.1600-065X.2000.917301.x.
Boparai JK, Sharma PK. 2019. Mini review on antimicrobial peptides, sources, mechanism and recent applications. *Protein & Peptide Letters* **27**:4–16 DOI 10.2174/0929866526666190822165812.

Brancatisano FL, Maisetta G, Di Luca M, Esin S, Bottai D, Bizzarri R, Campa M, Batoni G. 2014. Inhibitory effect of the human liver-derived antimicrobial peptide hepcidin 20 on biofilms of polysaccharide intercellular adhesin (PIA)-positive and PIA-negative strains of Staphylococcus epidermidis. *Biofouling* **30**:435–446 DOI 10.1080/08927014.2014.888062.

Brogden KA. 2005. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nature Reviews Microbiology* **3**:238–250 DOI 10.1038/nrmicro1098.

Brötz H, Bierbaum G, Leopold K, Reynolds PE, Sahl HG. 1998. The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II. *Antimicrobial Agents and Chemotherapy* **42**:154–160 DOI 10.1128/aac.42.1.154.

Bulet P, Stöcklin R, Menin L. 2004. Anti-microbial peptides: from invertebrates to vertebrates. *Immunological Reviews* **198**:169–184 DOI 10.1111/j0105-28962004.0124.x.

Burton MF, Steel PG. 2009. The chemistry and biology of LL-37. *Natural Product Reports* **26**:1572–1584 DOI 10.1039/b912533g.

Cassone M, Frith N, Vogiatzi P, Wade JD, Otvos L. 2009. Induced resistance to the designer proline-rich antimicrobial peptide A3-APO does not involve changes in the intracellular target DnaK. *International Journal of Peptide Research and Therapeutics* **15**:121–128 DOI 10.1007/s10989-009-9176-1.

Castillo-Juárez I, López-Jácome LE, Soberón-Chávez G, Tomás M, Lee J, Castañeda Tamez P, Hernández-Bárragan IA, Cruz-Muñiz MY, Maeda T, Wood TK, García-Contreras R. 2017. Exploiting quorum sensing inhibition for the control of pseudomonas aeruginosa and acinetobacter baumannii biofilms. *Current Topics in Medicinal Chemistry* **17** DOI 10.2174/1568026617666170105144104.

Castillo-Juárez I, Maeda T, Mandujano-Tinoco EA, Tomás M, Pérez-Eretza B, García-Contreras SJ, Wood TK, García-Contreras R. 2015. Role of quorum sensing in bacterial infections. *World Journal of Clinical Cases* **3**(7):575–598 DOI 10.12998/wjcc.v3.i7.575.

Chennupati SK, Chiu AG, Tamashiro E, Banks CA, Cohen MB, Bleier BS, Konofow JM, Tam E, Cohen NA. 2009. Effects of an LL-37-derived antimicrobial peptide in an animal model of biofilm Pseudomonas sinusitis. *American Journal of Rhinology and Allergy* **23**:46–51 DOI 10.2500/ajra.2009.23.3261.

Dai L, Wu TQ, Xiong YS, Ni HB, Ding Y, Zhang WC, Chu SP, Ju SQ, Yu J. 2019. Ibuprofen-mediated potential inhibition of biofilm development and quorum sensing in Pseudomonas aeruginosa. *Life Sciences* **237**:116947 DOI 10.1016/j.lfs.2019.116947.

Di Somma A, Avitabile C, Cirillo A, Moretta A, Merlino A, Paduano L, Dulio A, Romanelli A. 2020. The antimicrobial peptide Temporin L impairs E. coli cell division by interacting with FtsZ and the divisome complex. *Biochimica Et Biophysica Acta - General Subjects* **1864**:129606 DOI 10.1016/j.bbagen.2020.129606.
Díaz-Núñez JL, García-Contreras R, Castillo-Juárez I. 2021. The new antibacterial properties of the plants: quo vadis studies of anti-virulence phytochemicals? *Frontiers in Microbiology* 12:667126 DOI 10.3389/fmicb.2021.667126.

Donlan RM. 2002. Biofilms: microbial life on surfaces. *Emerging Infectious Diseases* 8:881–890 DOI 10.3201/eid0809.020063.

Dorschner RA, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, Agerberth B, Gudmundsson GH, Gallo RL. 2001. Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A streptococcus. *Journal of Investigative Dermatology* 117:91–97 DOI 10.1046/j1523-17472001.01340.x.

De La Fuente-Núñez C, Korolik V, Bains M, Nguyen U, Breidenstein EBM, Horsman S, Lewenza S, Burrows L, Hancock REW. 2012. Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. *Antimicrobial Agents and Chemotherapy* 56:2696–2704 DOI 10.1128/AAC00064-12.

De la Fuente-Núñez C, Silva ON, Lu TK, Franco OL. 2017. Antimicrobial peptides: role in human disease and potential as immunotherapies. *Pharmacology and Therapeutics* 178:132–140 DOI 10.1016/j.pharmthera.2017.04.002.

El-Mowafy SA, Abd El Galil KH, El-Messery SM, Shaaban MI. 2014. Aspirin is an efficient inhibitor of quorum sensing, virulence and toxins in *Pseudomonas aeruginosa*. *Microbial Pathogenesis* 74:25–32 DOI 10.1016/j.micpath.2014.07.008.

Fleitas Martínez O, Rigueiras PO, Pires Á da S, Porto WF, Silva ON, De la Fuente-Nunez C, Franco OL. 2018. Interference with quorum-sensing signal biosynthesis as a promising therapeutic strategy against multidrug-resistant pathogens. *Frontiers in Cellular and Infection Microbiology* 8:444 DOI 10.3389/fcimb.2018.00444.

Fernández-García L, Blasco L, Lopez M, Bou G, GarcÃa-Contreras R, Wood T, Tomas M. 2016. Toxin-antitoxin systems in clinical pathogens. *Toxins* 8: DOI 10.3390/toxins8070227.

Gao X, Mu Z, Qin B, Sun Y, Cui S. 2017. Structure-based prototype peptides targeting the *Pseudomonas aeruginosa* type VI secretion system effector as a novel antibacterial strategy. *Frontiers in Cellular and Infection Microbiology* 7:411 DOI 10.3389/fcimb.2017.00411.

García-Contreras R. 2016. Is quorum sensing interference a viable alternative to treat *Pseudomonas aeruginosa* infections? *Frontiers in Microbiology* 7:1454 DOI 10.3389/fmicb.2016.01454.

Geitani R, Ayoub Moubareck C, Touqui L, Karam Sarkis D. 2019. Cationic antimicrobial peptides: alternatives and/or adjuvants to antibiotics active against methicillin-resistant Staphylococcus aureus and multidrug-resistant *Pseudomonas aeruginosa*. *BMC Microbiology* 19(1):54 DOI 10.1186/s12866-019-1416-8.

Giesemann T, Guttenberg G, Aktories K. 2008. Human α-Defensins Inhibit Clostridium difficile Toxin B. *Gastroenterology* 134(7):2049–58 DOI 10.1053/j.gastro.2008.03.008.

González-Bello C. 2017. Antibiotic adjuvants –A strategy to unlock bacterial resistance to antibiotics. *Bioorganic and Medicinal Chemistry Letters* 27:4221–4228 DOI 10.1016/j.bmcl.2017.08.027.
Gordya N, Yakovlev A, Kruglikova A, Tulin D, Potolitsina E, Suborova T, Bordo D, Rosano C, Chernysh S. 2017. Natural antimicrobial peptide complexes in the fighting of antibiotic resistant biofilms: Calliphora vicina medicinal maggots. *PLOS ONE* 12(3):e0173559 DOI 10.1371/journal.pone.0173559.

Graf M, Wilson DN. 2019. Intracellular antimicrobial peptides targeting the protein synthesis machinery. *Advances in Experimental Medicine and Biology* 1117:73–89 DOI 10.1007/978-981-13-3588-4_6.

Gusman J, Malonneet H, Atassi G. 2001. A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. *Carcinogenesis* 22(8):1111–1117 DOI 10.1093/carcin/22.8.1111.

Gusman H, Travis J, Helmerhorst EJ, Potempa J, Troxler RF, Oppenheim FG. 2001. Salivary histatin 5 is an inhibitor of both host and bacterial enzymes implicated in periodontal disease. *Infection and Immunity* 69(3):1402–1408 DOI 10.1128/IAI.69.3.1402-1408.2001.

Hall CW, Mah TF. 2017. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEBS Letters* 581(23):4385–4400 DOI 10.1007/s00012-017-0492-8.

Hancock REW, Sahl HG. 2006. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology* 24:1551–1557 DOI 10.1038/nbt1267.

Hancock REW, Sahl HG. 2013. New strategies and compounds for anti-infective treatment. *Current Opinion in Microbiology* 16:519–521 DOI 10.1016/j.mib.2013.08.004.

Haney EF, Straus SK, Hancock REW. 2019. Reassessing the host defense peptide landscape. *Frontiers in Chemistry* 7:43 DOI 10.3389/fchem.2019.00043.

Harris F, Dennison S, Phoenix D. 2009. Anionic antimicrobial peptides from eukaryotic organisms. *Current Protein & Peptide Science* 10:585–606 DOI 10.2174/138920309789630589.

Heinbockel L, Sánchez-Gómez S, De Tejada GM, Dömming S, Brandenburg J, Kaconis Y, Hornef M, Dupont A, Marwitz S, Goldmann T, Ernst M, Gutsmann T, Schürholz T, Brandenburg K. 2013. Preclinical investigations reveal the broad-spectrum neutralizing activity of peptide pep19-2.5 on bacterial pathogenicity factors. *Antimicrobial Agents and Chemotherapy* 57(3):1480–1487 DOI 10.1128/AAC.02066-12.

Heinbockel L, Weindl G, Martinez-de-Tejada G, Correa W, Sanchez-Gomez S, Bārcena-Varela S, Goldmann T, Garidel P, Gutsmann T, Brandenburg K. 2018. Inhibition of lipopolysaccharide-and lipoprotein-induced inflammation by antitoxin peptide Pep19-2.5. *Frontiers in Immunology* 9:1704 DOI 10.3389/fimmu.2018.01704.

Hell E, Giske CG, Nelson A, Römling U, Marchini G. 2010. Human cathelicidin peptide LL37 inhibits both attachment capability and biofilm formation of Staphylococcus epidermidis. *Letters in Applied Microbiology* 50(2):211–215 DOI 10.1111/j.1472-765X.2009.02778.x.

Hooven TA, Randis TM, Hymes SR, Rampersaud R, Ratner AJ. 2012. Retrocyclin inhibits Gardnerella vaginalis biofilm formation and toxin activity. *Journal of Antimicrobial Chemotherapy* 67:2870–2872 DOI 10.1093/jac/dks305.
Hotinger JA, May AE. 2019. Animal models of type III secretion system-mediated pathogenesis. *Pathogens* 8:257 DOI 10.3390/pathogens8040257.

Huan Y, Kong Q, Mou H, Yi H. 2020. Antimicrobial peptides: classification, design, application and research progress in multiple fields. *Frontiers in Microbiology* 11:582779 DOI 10.3389/fmicb.2020.582779.

Jerala R, Porro M. 2005. Endotoxin neutralizing peptides. *Current Topics in Medicinal Chemistry* 4:1173–1184 DOI 10.2174/1568026043388079.

Jiang F, Wang X, Wang B, Chen L, Zhao Z, Waterfield NR, Yang G, Jin Q. 2016. The pseudomonas aeruginosa type VI secretion PGAP1-like effector induces host autophagy by activating endoplasmic reticulum stress. *Cell Reports* 16:1502–1509 DOI 10.1016/j.celrep.2016.07.012.

Jiang Q, Chen J, Yang C, Yin Y, Yao K, Song D. 2019. Quorum sensing: a prospective therapeutic target for bacterial diseases. *BioMed Research International* 2019:2015978 DOI 10.1155/2019/2015978.

Kang J, Dietz MJ, Li B. 2019. Antimicrobial peptide LL-37 is bactericidal against *Staphylococcus aureus* biofilms. *PLOS ONE* 14(6):e0216676 DOI 10.1371/journal.pone.0216676.

Kim C, Gajendran N, Mittrücker HW, Weiwad M, Song YH, Hurwitz R, Wilmanns M, Fischer G, Kaufmann SHE. 2005. Human Îś-defensins neutralize anthrax lethal toxin and protect against its fatal consequences. *Proceedings of the National Academy of Sciences of the United States of America* 102(13):4830–4835 DOI 10.1073/pnas.0500508102.

Kim C, Slavinskaya Z, Merrill AR, Kaufmann SHE. 2006. Human Îś-defensins neutralize toxins of the mono-ADP-ribosyltransferase family. *Biochemical Journal* 399(2):225–229 DOI 10.1042/BJ20060425.

Kudryashova E, Seveau SM, Kudryashov DS. 2017. Targeting and inactivation of bacterial toxins by human defensins. *Biological Chemistry* 398:1069–1085 DOI 10.1515/hsz-2017-0106.

Larzábal M, Baldoni HA, Suvire FD, Curto LM, Gomez GE, Da Silva WM, Giudicesi SL, Camperi SA, Delfino JM, Cataldi AA, Enriz D. 2019. An inhibitory mechanism of action of coiled-coil peptides against type three secretion system from enteropathogenic Escherichia coli. *Journal of Peptide Science* 25:e3149 DOI 10.1002/psc.3149.

Le CF, Fang CM, Sekaran SD. 2017. Intracellular targeting mechanisms by antimicrobial peptides. *Antimicrobial Agents and Chemotherapy* 61:AAC.02340-16 DOI 10.1128/AAC.02340-16.

Lee IG, Lee SJ, Chae S, Lee KY, Kim JH, Lee BJ. 2015. Structural and functional studies of the Mycobacterium tuberculosis VapBC30 toxin-antitoxin system: Implications for the design of novel antimicrobial peptides. *Nucleic Acids Research* 43(15):7624–7637 DOI 10.1093/nar/gkv689.

Lei J, Sun LC, Huang S, Zhu C, Li P, He J, Mackey V, Coy DH, He QY. 2019. The antimicrobial peptides and their potential clinical applications. *American Journal of Translational Research* 11:3919–3931.
Lewis K. 2008. Multidrug tolerance of biofilms and persister cells. *Current Topics in Microbiology and Immunology* 322:107–131 DOI 10.1007/978-3-540-75418-3_6.

Libardo MDJ, Bahar AA, Ma B, Fu R, McCormick LE, Zhao J, McCallum SA, Nussinov R, Ren D, Angeles-Boza AM, Cotten M. 2017. Nuclease activity gives an edge to host-defense peptide piscidin 3 over piscidin 1, rendering it more effective against persisters and biofilms. *FEBS Journal* 284(21):3662–3683 DOI 10.1111/febs.14263.

Magana M, Pushpanathan M, Santos AL, Leanse I, Fernandez M, Ioannidis A, Giulianotti MA, Apidianakis Y, Bradfute S, Ferguson AL, Cherkasov A, Seleem MN, Pinilla C, De la Fuente-Nunez C, Lazaridis T, Dai T, Houghten RA, Hancock REW, Tegos GP. 2020. The value of antimicrobial peptides in the age of resistance. *The Lancet Infectious Diseases* 20:e216–e230 DOI 10.1016/S1473-30992030327-3.

Mankoci S, Ewing J, Dalai P, Sahai N, Barton HA, Joy A. 2019. Bacterial membrane selective antimicrobial peptide-mimetic polyurethanes: structure-property correlations and mechanisms of action. *Biomacromolecules* 20:4096–4106 DOI 10.1021/acs.biomac.9b00939.

Mansour SC, Pena OM, Hancock REW. 2014. Host defense peptides: front-line immunomodulators. *Trends in Immunology* 35:443–450 DOI 10.1016/j.it.2014.07.004.

Marshall NC, Brett Finlay B. 2014. Targeting the type III secretion system to treat bacterial infections. *Expert Opinion on Therapeutic Targets* 18:137–152 DOI 10.1517/14728222.2014.855199.

Mattson MP. 2008. Hormesis defined. *Ageing Research Reviews* 7 DOI 10.1016/j.arr.2007.08.007.

McShan AC, De Guzman RN. 2015. The bacterial type III secretion system as a target for developing new antibiotics. *Chemical Biology and Drug Design* 85:30–42 DOI 10.1111/cbdd.12422.

Mirzaei R, Mohammadzadeh R, Alikhani MY, Shokri Moghadam M, Karampoor S, Kazemi S, Barfipoursalar A, Yousefimashouf R. 2020. The biofilm-associated bacterial infections unrelated to indwelling devices. *IUBMB Life* 72:1271–1285 DOI 10.1002/iub.2266.

Monnet V, Juillard V, Gardan R. 2016. Peptide conversations in Gram-positive bacteria. *Critical Reviews in Microbiology* 42:339–351 DOI 10.3109/1040841X.2014.948804.

Moreta A, Scieuzo C, Petrone AM, Salvia R, Manniello MD, Franco A, Lucchetti D, Vassallo A, Vogel H, Sgambato A, Falabella P. 2021. Antimicrobial peptides: a new hope in biomedical and pharmaceutical fields. *Frontiers in Cellular and Infection Microbiology* 11:668632 DOI 10.3389/fcimb.2021.668632.

Muñoz Cazares N, García-Contreras R, Pérez-López M, Castillo-Juárez I. 2017. Phenolic compounds with anti-virulence properties. In: *Phenolic compounds - biological activity*. London, UK: IntechOpen DOI 10.5772/66367.

Overhage J, Campisano A, Bains M, Torfs ECW, Rehm BHA, Hancock REW. 2008. Human host defense peptide LL-37 prevents bacterial biofilm formation. *Infection and Immunity* 76:4176–4182 DOI 10.1128/IAI00318-08.

Page R, Peti W. 2016. Toxin-antitoxin systems in bacterial growth arrest and persistence. *Nature Chemical Biology* 12(4):208–214 DOI 10.1038/nchembio.2044.
Pasupuleti M, Schmidtchen A, Malmsten M. 2012. Antimicrobial peptides: key components of the innate immune system. *Critical Reviews in Biotechnology* **32**:143–171 DOI 10.3109/07388551.2011.594423.

Patrzykat A, Friedrich CL, Zhang L, Mendoza V, Hancock REW. 2002. Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **46**:605–614 DOI 10.1128/AAC.46.3.605-614.2002.

Pena RT, Blasco I, Ambroa A, González-Pedrajo B, Fernández-García L, López M, Bleriot I, Bou G, García-Contreras R, Wood TK, Tomás M. 2019. Relationship between quorum sensing and secretion systems. *Frontiers in Microbiology* **10**:1100 DOI 10.3389/fmicb.2019.01100.

Quilés F, Saadi S, Franciú G, Bacharouche J, Humbert F. 2016. In situ and real time investigation of the evolution of a *Pseudomonas* fluorescens nascent biofilm in the presence of an antimicrobial peptide. *Biochimica et Biophysica Acta* **1858**(1):75–84 DOI 10.1016/j.bbamem.2015.10.015.

Rathinakumar R, Walkenhorst WF, Wimley WC. 2009. Broad-spectrum antimicrobial peptides by rational combinatorial design and high-throughput screening: the importance of interfacial activity. *Journal of the American Chemical Society* **131**:7609–7617 DOI 10.1021/ja8093247.

Rodas PI, ÁAlamos-Musre AS, Álvarez FP, Escobar A, Tapia CV, Osorio E, Otero C, Calderón IL, Fuentes JA, Gil F, Paredes-Sabja D, Christodoulides M. 2016. The NarE protein of *Neisseria gonorrhoeae* catalyzes ADP-ribosylation of several ADP-ribose acceptors despite an N-terminal deletion. *FEMS Microbiology Letters* **363**(17):fnw181.

Rohde H, Frankenberger S, Zähringer U, Mack D. 2010. Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to *Staphylococcus epidermidis* biofilm formation and pathogenesis of biomaterial-associated infections. *European Journal of Cell Biology* **89**:103–111 DOI 10.1016/j.ejcb.2009.10.005.

Sala A, Bordes P, Genevaux P. 2014. Multiple toxin-antitoxin systems in *Mycobacterium tuberculosis*. *Toxins* **6**(3):1002–1020 DOI 10.3390/toxins6031002.

Schikora A, Schenk ST, Hartmann A. 2016. Beneficial effects of bacteria-plant communication based on quorum sensing molecules of the N-acyl homoserine lactone group. *Plant Molecular Biology* **90**:605–612 DOI 10.1007/s11103-016-0457-8.

Schnell L, Felix I, Müller B, Sadi M, Von Bank F, Papatheodorou P, Popoff MR, Aktories K, Waltenberger E, Benz R, Weichbrodt C, Fauler M, Frick M, Barth H. 2019. Revisiting an old antibiotic: bacitracin neutralizes binary bacterial toxins and protects cells from intoxication. *FASEB Journal* **33**(fj):201802453R DOI 10.1096/fj.201802453R.

Silveira GGOs, Torres MDT, Ribeiro CFA, Meneguetti BT, Carvalho CME, De La Fuente-Nunez C, Franco OL, Cardoso MH. 2021. Antibiofilm peptides: relevant preclinical animal infection models and translational potential. *ACS Pharmacology and Translational Science* **4**:55–73 DOI 10.1021/acsptsci.0c00191.
Singh RP, Desouky SE, Nakayama J. 2016. Quorum quenching strategy targeting gram-positive pathogenic bacteria. In: Advances in Experimental Medicine and Biology. 901. 109–130 DOI 10.1007/5584_2016_1.

Skindersoe ME, Alhede M, Phipps R, Yang L, Jensen PO, Rasmussen TB, Bjarnsholt T, Tolker-Nielsen T, Høiby N, Givskov M. 2008. Effects of antibiotics on quorum sensing in Pseudomonas aeruginosa. Antimicrobial Agents and Chemotherapy 52:3648–3663 DOI 10.1128/AAC01230-07.

Soltani S, Hammami R, Cotter PD, Rebuffat S, Ben SL, Gaudreau H, Bédard F, Biron E, Drider D, Fliss I. 2021. Bacteriocins as a new generation of antimicrobials: Toxicity aspects and regulations. FEMS Microbiology Reviews 45:fuaa039 DOI 10.1093/femsre/fuaa039.

Soo VWC, Kwan BW, Quezada H, Castillo-Juárez I, Pérez-Eretza B, García-Contreras SJ, Martínez-Vázquez M, Wood TK, García-Contreras R. 2017. Repurposing of anticancer drugs for the treatment of bacterial infections. Current Topics in Medicinal Chemistry 17:1157–1176 DOI 10.2174/156802661666160930131737.

Starr CG, Maderdrut JL, He J, Coy DH, Wimley WC. 2018. Pituitary adenylate cyclase-activating polypeptide is a potent broad-spectrum antimicrobial peptide: structure–activity relationships. Peptides 104:35–40 DOI 10.1016/j.peptides.2018.04.006.

Strempe N, Neidig A, Nusser M, Geffers R, Vieillard J, Lesouhaitier O, Brenner-Weiss G, Overhage J. 2013. Human host defense peptide LL-37 Stimulates virulence factor production and adaptive resistance in Pseudomonas aeruginosa. PLOS ONE 8:e82240 DOI 10.1371/journal.pone.0082240.

Subbalakshmi C, Sitaram N. 1998. Mechanism of antimicrobial action of indolicidin. FEMS Microbiology Letters 160:91–96 DOI 10.1016/S0378-1097(98)00008-1.

Sundar S, Rajan MP, Piramanayagam S. 2019. In Silico Derived Peptides for Inhibiting the Toxin–Antitoxin Systems of Mycobacterium tuberculosis: Basis for Developing Peptide-Based Therapeutics. International Journal of Peptide Research and Therapeutics 25:1467–1475 DOI 10.1007/s10989-018-9792-8.

Sutherland IW. 2001. The biofilm matrix - An immobilized but dynamic microbial environment. Trends in Microbiology 9:222–227 DOI 10.1016/S0966-842X(01)02012-1.

Taha MN, Saafan AE, Ahmedy A, El Gebaly E, Khairalla AS. 2019. Two novel synthetic peptides inhibit quorum sensing-dependent biofilm formation and some virulence factors in Pseudomonas aeruginosa PAO1. Journal of Microbiology 57(7):618–625 DOI 10.1007/s12275-019-8548-2.

Tanabe K, Lamping E, Adachi K, Takano Y, Kawabata K, Shizuri Y, Niimi M, Uehara Y. 2007. Inhibition of fungal ABC transporters by unnarmicin A and unnarmicin C, novel cyclic peptides from marine bacterium. Biochemical and Biophysical Research Communications 364:990–995 DOI 10.1016/j.bbrc.2007.10.110.

Tang SS, Prodhan ZH, Biswas SK, Le CF, Sekaran SD. 2018. Antimicrobial peptides from different plant sources: Isolation, characterisation, and purification. Phytochemistry 154:94–105 DOI 10.1016/j.phytochem.2018.07.002.

Tiwari S, Jamal SB, Hassan SS, Carvalho PVSD, Almeida S, Barh D, Ghosh P, Silva A, Castro TLP, Azevedo V. 2017. Two-component signal transduction systems of
pathogenic bacteria as targets for antimicrobial therapy: an overview. *Frontiers in Microbiology* 8:1878 DOI 10.3389/fmicb.2017.01878.

**Tornesello AL, Buonaguro L, Tornesello ML, Buonaguro FM. 2018.** The role of sensing peptides in the cross-talk between microbiota and human cancer cells. *Mini-Reviews in Medicinal Chemistry* 18:1567–1571 DOI 10.2174/1389557518666180713112119.

**Totsika M. 2016.** Benefits and challenges of antivirulence antimicrobials at the dawn of the post-antibiotic era. *Drug Delivery Letters* 6:30–37 DOI 10.2174/2210303106666180713112119.

**Travier L, Rendueles O, Ferrières L, Herry JM, Ghigo JM. 2016.** Escherichia coli resistance to nonbiocidal antibiofilm polysaccharides is rare and mediated by multiple mutations leading to surface physicochemical modifications. *Antimicrobial Agents and Chemotherapy* 57(8):3960–3968 DOI 10.1128/AAC.02606-12.

**Tsai CN, MacNair CR, Cao MPT, Perry JN, Brown ED, Coombes BK. 2020.** Targeting two-component systems uncovers a small-molecule inhibitor of salmonella virulence. *Cell Chemical Biology* 27:793–805.e7 DOI 10.1016/j.chembiol.2020.04.005.

**Vasilchenko AS, Rogozhin EA. 2019.** Sub-inhibitory effects of antimicrobial peptides. *Frontiers in Microbiology* 10:1160 DOI 10.3389/fmicb.2019.01160.

**Wang Hyan, Lin L, Tan LS, Yu HY, Cheng JW, Pan YP. 2017.** Molecular pathways underlying inhibitory effect of antimicrobial peptide Nal-P-113 on bacteria biofilms formation of Porphyromonas gingivalis W83 by DNA microarray. *BMC Microbiology* 17:37 DOI 10.1186/s12866-017-0948-z.

**Wang W, Mulakala C, Ward SC, Jung G, Luong H, Pham D, Waring AJ, Kaznessis Y, Lu W, Bradley KA, Lehrer RI. 2006.** Retrocyclins kill bacilli and germinating spores of Bacillus anthracis and inactivate anthrax lethal toxin. *Journal of Biological Chemistry* 281:32755–32764 DOI 10.1074/jbc.M603614200.

**Wei G, de Leeuw E, Pazgier M, Yuan W, Zou G, Wang J, Ericksen B, Lu WY, Lehrer RI, Lu W.** Through the looking glass, mechanistic insights from enantiomeric human defensins. *Journal of Biological Chemistry* 284(42):29180–29192 DOI 10.1074/jbc.M109.018085.

**Yajima A. 2016.** Recent advances in the chemistry and chemical biology of quorum-sensing pheromones and microbial hormones. *Studies in Natural Products Chemistry* 47:331–355 DOI 10.1016/B978-0-444-63603-4.00010-3.

**Yang G, Cheng H, Liu C, Xue Y, Gao Y, Liu N, Gao B, Wang D, Li S, Shen B, Shao N. 2003.** Inhibition of Staphylococcus aureus pathogenesis in vitro and in vivo by RAP-binding peptides. *Peptides* 24:1823–1828 DOI 10.1016/j.peptides.2003.09.017.

**Yasir M, Willcox MDP, Dutta D. 2018.** Action of Antimicrobial Peptides against Bacterial Biofilms. *Materials* 11(12):2468 DOI 10.3390/ma11122468.

**Zapotoczna M, Forde É, Hogan S, Humphreys H, O’gara JP, Fitzgerald-Hughes D, Devoceille M, O’Neill E. 2017.** Eradication of staphylococcus aureus biofilm infections using synthetic antimicrobial peptides. *Journal of Infectious Diseases* 215:975–983 DOI 10.1093/infdis/jix062.
Zasloff M. 2019. Antimicrobial peptides of multicellular organisms: my perspective. *Advances in experimental medicine and biology* 1117:3–6 DOI 10.1007/978-981-13-3588-4_1.

Zhang L yu, Fang Z hui, Li Q li, Cao CY. 2019. A tooth-binding antimicrobial peptide to prevent the formation of dental biofilm. *Journal of Materials Science: Materials in Medicine* 30:45 DOI 10.1007/s10856-019-6246-6.

Zhang W, Li C. 2016. Exploiting quorum sensing interfering strategies in gram-negative bacteria for the enhancement of environmental applications. *Frontiers in Microbiology* 6:1535 DOI 10.3389/fmicb.2015.01535.

Zhang Y, Faucher F, Zhang W, Wang S, Neville N, Poole K, Zheng J, Jia Z. 2018. Structure-guided disruption of the pseudopilus tip complex inhibits the Type II secretion in Pseudomonas aeruginosa. *PLOS Pathogens* 14:e1007343 DOI 10.1371/journal.ppat.1007343.

Zhu C, Tan H, Cheng T, Shen H, Shao J, Guo Y, Shi S, Zhang X. 2013. Human β-defensin 3 inhibits antibiotic-resistant Staphylococcus biofilm formation. *Journal of Surgical Research* 183 DOI 10.1016/j.jss.2012.11.048.