We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

New Approaches for Competing Microbial Resistance and Virulence

*Mohammed El-Mowafy, Abdelaziz Elgaml
and Mona Shaaban*

Abstract

The spread of multidrug-resistant pathogens together with the development of fatal cases of infectious microorganisms is on the rise. Therefore, there must be new approaches for combating pathogenic microorganisms, either by overcoming antibiotic resistance or via inhibiting their virulence factors. Several virulence factors extremely increase the antimicrobial resistance of various species of pathogens; as a result, the screening of antivirulence agents has gained more and more attention recently. In this aspect, non-traditional strategies that are considered promising in overcoming virulence and pathogenicity of microorganisms will be discussed including; quorum sensing inhibition, antibiofilm, control of the global regulators, bacteriocins and bacteriophages. Applying these methods could provide innovative approaches for competing microbial resistance and virulence.

Keywords: bacterial virulence, resistance, quorum sensing inhibition, global regulators, phage therapy, inhibition of biofilm formation, bacteriocins

1. Introduction

The high incidence of microbial resistance and the spread of multidrug-resistant and pan drug-resistant pathogens have been developed to threaten human mankind. Fortunately, there are upcoming alternative therapeutic approach for eliminating bacterial virulence and host-pathogen interaction [1, 2]. Quorum sensing signals [3, 4] and global regulators represent the main players to control virulence circuits and coordinate host-pathogen interaction [5]. Thus, targeting these regulators provide a promising trend to overcome microbial pathogenicity. Bacterial cells have the ability to grow in matrices of polysaccharides, proteins and DNA forming biofilm [6]. The cell communities inside the biofilm matrices are highly resistant to antibiotics [7]. In this chapter, we will focus on the agents that are known to exhibit antibiofilm assembly including bacteriocins.

Moreover, bacteriophages have specific ability to infect and lyse bacteria [8]. Hence, phage therapy has many potential applications in the treatment of infectious diseases, with high therapeutic index and diminished adverse effects [9, 10]. Inhibitors of quorum sensing signaling, control of the global regulators, and the development of antibiofilm agents will be discussed in detail in this chapter. Additionally, the use of bacteriophages either for eradication of bacterial infections or as an efficient delivery system for antimicrobial agents will be described in this part.

2. Control of microbial virulence and resistance

2.1 Quorum sensing inhibition

Quorum sensing (QS) is a cellular signaling system, which is developed in response to population cell density [3, 4]. QS cascade relays on the release of signaling molecules called QS autoinducers/signals. The QS signals are produced at low levels with the start of microbial growth and accumulate upon increase in the cell density. Quorum sensing signals coordinate the microbial virulence behaviors such as secretion of toxins, secretion of exoenzymes, microbial motility, adhesion and biofilm assembly [11]. Furthermore, microbial communication systems have been assigned in fungi [12] and viruses [13]. Studies of QS provide significant insights into different mechanisms that control the interactions in microbial communities and how these interactions affect microbial pathogenesis. Several QS systems are well understood including Gram-negative bacteria that produce acyl-homoserine lactone (AHL) signals, including *Pseudomonas aeruginosa*, *Vibrio* sp., *Acinetobacter baumannii* and *Serratia marcescens* [5, 14, 15]. Alternatively, Gram-positive species such as *Staphylococcus aureus* utilize autoinducer peptide (AIP)-based QS systems [16].

Various strategies for quorum sensing inhibition have been explored. The quorum sensing inhibition approaches could be accomplished via interference with the synthesis of QS signals, elimination of the signal accumulation and disruption of signal-receptor interaction [17–19].

2.1.1 Interference with the synthesis of the autoinducing signals

One of the main quorum sensing inhibiting approaches is the interference with the synthesis of the autoinducing signals [20]. AI-2 compounds are considered as “universal” signal molecules of Gram-negative and Gram-positive bacteria [14, 21]. Moreover, they are encountered in species communications. The biosynthesis of AI-2 requires two main enzymes: methylthioadenosine/S-adenosylhomocysteine nucleosidase (MTA/SAH nucleosidase) and LuxS. AI-2 molecules contribute in various virulence behaviors, biofilm formation and host-pathogen interaction. Therefore, targeting AI-2 elaborates broad spectrum quorum sensing inhibition [22, 23]. In this instance, Gutierrez group have identified the transition analogs, 5'-methylthio- (MT-), 5'-ethylthio- (EtT-) and 5'-butylthio- (BuT) DADMe-immucillin, which specifically bind and inhibit MTA enzymes in *Escherichia coli* O157:H7. Also, 4,5-dihydroxy-2,3-pentanedioneS-ribosyl-homocysteine analogs have been developed as competitive inhibitor of LuxS [24–26].

On other instance, inhibiting AHL-synthesis has been extensively studied, for instance, triclosan inhibited both N-3-oxo-dodecanoyl-L-homoserine lactone and N-butyryl-L-homoserine lactone [27, 28], anthranilate derivatives are a *Pseudomonas* quinolone signal inhibitors [28], and proanthocyanidins have been approved as inhibitor of LasI/RhlI AHL synthases expression [29]. Furthermore, precursors of *Pseudomonas* quinolone signals (PQS) such anthranilic acid derivatives reduced the pathogenicity of *P. aeruginosa* in lung-infected mice [15].

2.1.2 Elimination of the QS signals accumulation

Other common strategy is eliminating the accumulation of the QS signals, which have been attained by degrading the QS signal using enzymes or through sequestering the signal by synthetic polymers [30, 31] or utilizing antibodies that bind with the signals. Synthesized monoclonal antibodies (AP4-24H11) by Park group provoke high binding affinity for sequestering AIP-IV and decrease α -hemolysin

production in *S. aureus* with relief of abscess formation in the infected murine model [32]. Kaufmann and coauthors inhibited the *P. aeruginosa* QS cascade via development of AHL-specific monoclonal antibodies. Synthetic polymers such as itaconic acid sequester the signaling molecules AHL and attenuate QS in *V. fischeri* [31, 33].

Moreover, disturbing enzymes responsible for biosynthesis of QS signals is a chief method, which affects both production and accumulation of different signals and perturb quorum sensing circuit [30]. Acylases, lactonases and oxidoreductases are the widely identified enzymes that target AHLs. AHL lactonases are broad AHL degrading enzymes, which produce its effect via hydrolyzing the ester bond of the AHL ring [34]. Lactonases have been isolated from various *Bacillus* sp., which harbor *aiiA* (autoinducer inactivation gene) [35, 36]. Ulrich study showed that, the heterologous expression of *aiiA* in *Burkholderia thailandensis* and *P. aeruginosa* lowered the levels of AHL and QS-related virulence factors [37]. Other important AHL lactonases are AttM and AiiB, which have been isolated from *Agrobacterium* sp. [38], AhlD from *Arthrobacterium*, AhlK from *Klebsiella* [39] and AidC from *Chryseobacterium* [40], QsdA from *Rhodococcus erythropolis* strain W2 [41], AiiM of *Microbacterium testaceum* [42], AidH of *Ochrobactrum* sp. T63 [43] and QsdH of *Pseudoalteromonas yunnanensis* [44]. Furthermore, paraoxonases 1, 2 and 3 (PON1 to –3) are mammalian lactonases were identified in the airway epithelia and mammalian sera [45].

AHL acylases enzymes (*aiiD*) and homologs were found in *Ralstonia* [46], *Actinoplanes utahensis* and *Pseudomonas* sp. The purified AiiD protein has the ability to degrade 3OC10HSL into HSL and 3-oxodecanoic acid. In addition, PvdQ, QuiP and HacB are specific AHL acylases of *P. aeruginosa*, in addition, HacA and HacB acylases of *Pseudomonas syringae* [47, 48]. Furthermore, the broader substrate specificity of AHL acylase (AhlM) was detected in *Streptomyces* sp. strain M664 with activity towards medium- and long-chain AHLs [49].

Oxidoreductases from *Rhodococcus erythropolis* inactivates AHLs (oxidation or reduction) with subsequent elimination of bacterial virulence *in vivo*. *Rhizobium* strain NGR234 possess diverse AHL-inactivation loci: *dhlR*, *qsdR1* and *qsdR2*, with lactonases activity, *aldR*, and *hydR-hitR* [50]. Enzymatic degradation of other QS autoinducers have been described: *carA* and *carB* from *Bacillus*, *E. coli* DH10B, *Staphylococcus* and *Pseudomonas* as the genes responsible for inhibition of DSF signaling [51]. Hod (3-hydroxy-2-methyl-4(1H)-quinolone 2,4-dioxygenase) stimulates the cleavage of PQS and attenuates PQS-regulated virulence factors. Roy and coauthors elicit the AI-2 activation activity of endogenous LsrK in *E. coli*, however, exogenously phosphorylation of AI-2 by LsrK eliminates its intracellular transport and hinders subsequent activation of AI-2 [52].

2.1.3 Elimination of the QS signal-receptor interaction

Interference with signal detection through eliminating the QS signal-receptor binding represents a successful approach [53, 54]. Various synthetic and natural AHL analogs have been reported to block the binding of the signal with specific receptors in *P. aeruginosa* and *Vibrio* sp. The prototype signal inhibitors, halogenated furanones, which are produced from *Delisea pulchra* represent a good example [55, 56]. Natural analogs have been also isolated with signal-receptor interference including ajoene [57], eugenol [58], flavonoids [59], iberin [60], furocoumarins [61], ellagic acid, penicillanic acid and patulin [62], phenethyl amide [63] and 1H-pyrrole-2-carboxylic acid [64].

The synthetic furanone derivative C-30 interferes and hinders the interaction of AHLs with the receptors [65]. Other furanone analogs have been developed

including S-phenyl-L-cysteine sulfoxide and diphenyl disulfide [66] and tetrazole derivatives [67]. Furthermore, synthetic LasR derivatives have been developed such as indole derivatives, non-AHL-like antagonists [68], the synthesized azines derivatives, 4-(alkyloxy)-6-methyl-2H-pyran-2-one [69] and aspirin [70]. Triphenyl hybrid- γ -butyrolactones and cyclopentanones derivatives are potent inhibitors of LuxR [71]. Putative LasI inhibitors have been identified using molecular docking methods including the trans-cinnamaldehyde [72], (z)-5-octylidene-thiazolidine-2, 4-dione [73] and fatty acyl purified from marine *Streptomyces* sp. [74]. Additionally, meta-bromo-thiolactone is a potent inhibitor of RhlI and subsequent PQS cascade [11].

In *S. aureus*, the interference with agr system has been accomplished using solonamide A and B that are cyclodepsipeptides derivatives, which purified from marine *Photobacterium* and reduced the expression of *hla* and RNAPIII. Solonamide can act through competitive inhibition of agr system such as *S. aureus* agr system via structure similarity to the AIPs [75]. Other *S. aureus* quorum-sensing inhibitors have been identified including linear peptidomimetics as competitive inhibitors to AgrC [76], savirin as potent inhibitor of AgrA [77] and the polyhydroxy anthraquinone ω -hydroxyemodin as inhibitor of AgrA [78].

2.2 Control of the global regulators

Beside the QS regulons, other global regulators exhibit crucial functions in dominating the expression of various genes in assortment style as a response to environmental stimuli and changes, most notably the temperature change [5]. These so-called global regulators enable the bacterial communities to survive different environmental stresses including starvations, pH changes and temperature fluctuations, through the quick conformation of bacterial physiology and structure [79].

Among many regulators that coordinate gene expression in bacteria, in Gram-negative bacteria, the global regulator termed histone-like nucleoid-structuring (H-NS) protein is relatively significant and of paramount importance [80]. H-NS has been considered as the main model of studying how global regulators can affect bacterial structure and physiology. The H-NS protein is incorporated in the regulation of many genes responsible for controlling the physiological functions of Gram-negative bacterial cells involving cellular functions, survival under different environmental conditions and production of various virulence factors [81, 82]. Moreover, in Gram-positive bacteria, there are several global regulatory loci [83]. Among them in the *S. aureus*, SarA, a regulatory DNA binding protein involved in controlling the virulence genes expression, is well documented [84]. During regulation of the expression of various genes, these regulators have been demonstrated to act either as a positive regulators through enhancing the stability of the mRNA of expressed genes, resulting in excessive translation, or as a silencer protein that alter and decrease the gene expression by hindering binding of RNA polymerases to the promoters of target genes [85, 86].

This would open up novel approaches for the treatment and eradication of pathogenic bacteria utilizing inhibitors or modulators of these global loci to vanquish the global concerns of antimicrobial resistance and immune evasion of microbial pathogens. Among these approaches, the interesting inhibitor of SarA (SarABI), 4-[(2,4-difluorobenzyl)amino] cyclohexanol, was confirmed as SarA-based new curative medicament against *S. aureus*-related infections [87]. This might encourage research groups for screening other compounds that might affect global regulators in bacteria to give a new therapy for multi-drug resistant (MDR) bacterial strains.

2.3 Biofilm inhibition and eradication

Biofilm is a sessile community of microbial cells that is found to be attached to animate or inanimate surface, and usually surrounded by a matrix of polysaccharides, proteins and DNA [6]. The cells in these sessile communities differs phenotypically from those present in planktonic communities [88]. Bacterial cells in planktonic forms are almost one thousand times more sensitive to antibiotics than their biofilm counterparts [7]. Additionally, biofilms act as a defense mechanism against different stress conditions or immune cells attack [89].

In this part, we will focus on the agents that are known to exhibit antibiofilm activity.

2.3.1 Antimicrobial peptides

Antimicrobial peptides (AMPs) that are crucial players of innate immunity are reported to prevent biofilm formation in different pathogens. AMPs with anti-biofilm activity are either natural or synthetic. The human cathelicidin peptide, LL-37, has been demonstrated to have antibiofilm activity in case of *P. aeruginosa* (at a concentration of 0.5 µg/mL), while the minimum inhibitory concentration for planktonic cells was 64 µg/mL [90]. In this study, it was reported that LL-37 was able to interfere with the adherence of microbial cells, enhancing twitching motility and downregulation of genes required for biofilm formation via affecting quorum sensing systems (Las and RhI) [90]. Furthermore, such peptide was shown to prevent biofilm formation in *E. coli* and *S. aureus* [91]. The mouse cathelicidin-derived peptide AS10 was reported to exhibit antibiofilm activity in *Candida albicans* [92]. The synthetic cathelicidin-derived peptides; peptide 1018, DJK5 and DJK6, were reported to prevent biofilm formation in addition to enhancement of biofilm dispersion via prompting the hydrolysis of nucleotide signaling systems, and therefore, leads to its depletion in bacteria [93].

Another synthetic peptide, S4(1–16) M4Ka, has been found to inhibit biofilm formation and detach bacterial cells in *P. aeruginosa* [94]. The human β -defensin 3 (hBD-3) was found to inhibit the expression of *icaA*, *icaD* and *icaR* genes of *Staphylococcus epidermidis*, thus interfering with biofilm formation, where biofilm formation in *Staphylococci* is dependent on the synthesis of the polysaccharide inter-cellular adhesin PIA encoded by *icaADBC* locus [95]. Another example of human AMP with antibiofilm activity in *S. epidermidis*, is the liver-derived hepcidin 20. This peptide can inhibit extracellular matrix formation of biofilms via targeting PIA [95].

The natural AMP piscidin-3, obtained from fish, exhibits nucleosidase activity and can degrade extracellular DNA of *P. aeruginosa* [96]. Another example of natural AMP, that possesses antibiofilm activity, is esculentin, which is obtained from frog's skin. It acts by permeabilization of the cellular membrane of *P. aeruginosa* PAO1 cells in the biofilm [97]. A synthetic peptide P1, derived from a tick antifreeze protein, significantly inhibited biofilm formation in *Streptococcus mutans*. Such peptide reduced biofilm biomass by about 75% in microtiter plates and *in vitro* tooth models [98].

2.3.2 Surfactants

The anionic surfactant, sodium dodecyl sulfate, has been reported to destruct biofilm via enhancing the formation of central cavity within biofilm [99]. Cetyltrimethylammonium bromide (Catanionic surfactant), together with application of high shear stress, increased the detachment of *Pseudomonas fluorescens* biofilms [100]. The non-ionic surfactants, polyoxy ethylene sorbitan monolaurate

(Tween-20) and ethoxylated p-tert-octyl phenol (Triton X-100), were demonstrated to cause biofilm detachment [100]. Certain biosurfactants, which are surface active molecules formed by microorganisms, were reported to have antibiofilm activity. For example, surfactin, obtained from *Bacillus subtilis*, was found to have antibiofilm activity in case of *Salmonella enterica* in polyvinyl chloride microtiter wells and urethral catheters [101]. Another example is Rhamnolipids, that are produced principally, by *P. aeruginosa*, were found to promote the dispersal of bacterial biofilm [99]. Additionally, biosurfactants from *P. fluorescens* prevent the attachment of *Listeria monocytogenes* to stainless steel surfaces [102].

2.3.3 Free fatty acids

Free fatty acids obtained via hydrolysis of lipids by enzymes [103]. Certain members of free fatty acids are reported to exhibit antibiofilm activity [104]. For example, cis-2-decenoic acid from *P. aeruginosa* enhanced the dispersal of biofilms and inhibited its formation in different pathogens, such as *Klebsiella pneumoniae*, *E. coli*, *Proteus mirabilis*, *Streptococcus pyogenes*, *B. subtilis* and *S. aureus*, in addition to *C. albicans* [105]. Another example is cis-9-octadecenoic acid (oleic acid) that was reported to repress biofilm formation in *S. aureus* by interference with the initial attachment of bacterial cells [106]. The diffusible signal factor; cis-11-methyl-2-dodecenoic acid, from *Xanthomonas campestris* inhibits biofilm formation in case of *Bacillus cereus* [107]. This study showed also that diffusible signal factor or its structural analogs increased the antibiotic susceptibility of numerous bacterial pathogens, by inhibition of biofilm formation [107].

2.3.4 Metal chelators

Removal of metals from the microbial environment via metal chelators renders bacteria more susceptible to antimicrobial agents, as metals are essential for different cellular processes [108]. Ethylenediaminetetraacetic acid (EDTA), the most-known metal chelator, has been reported to exhibit antibiofilm activity against *S. aureus*, and to eradicate the *in vivo* biofilm models on catheters [109]. Combination of EDTA with minocycline has effectively reduced the colonization of *S. epidermidis*, *S. aureus* and *C. albicans* on catheters [110]. Similarly, the combination of EDTA and fluconazole remarkably inhibited biofilm assembly in *C. albicans* [111].

2.3.5 Enzymes

Based on their target, the antibiofilm enzymes are classified into three types: polysaccharide-degrading enzymes, nucleases and proteases.

2.3.5.1 Polysaccharide-degrading enzymes

Alpha amylase enzyme was found to inhibit biofilm formation by *S. aureus* through the detachment of biofilm and interfering with aggregation of cells [112]. Dispersion B, a bacterial glycoside hydrolase, degrades poly-N-acetylglucosamine (PNAG), a main matrix exopolysaccharide of *S. aureus* and *E. coli* [113]. Such polysaccharide is produced by many bacteria and fungi and plays an important role in surface adhesion, and biofilm formation. Furthermore, PNAG was reported to successfully disrupt the biofilm matrix of *S. epidermidis* [114]. Moreover, the combination of dispersion B and triclosan was reported to significantly reduce biofilm formation of *E. coli*, *S. aureus* and *S. epidermidis* [115].

2.3.5.2 Nucleases enzymes

Deoxyribonuclease I (DNase I) degrades DNA in biofilm matrix [104]. Moreover, it was shown to have antibiofilm activity and to detach the biofilms produced by different bacterial species [116]. Such nuclease can prevent the initial adherence of microbial cells to surfaces via the degradation of cell surface-associated nucleic acids that act as surface adhesins [117]. Furthermore, DNase I has been found to increase the sensitivity of bacterial cells in biofilm matrix to antibiotics, resulting in reduction of biofilm mass [118].

2.3.5.3 Proteases

Proteases act as antibiofilm agents because they are able to inhibit cell-cell communication, in biofilms, via hydrolysis of extracellular protein fibers and surface adhesins [104]. Subtilisins, a class of serine proteases produced by *Bacillus* species, were reported to prevent the adherence of microorganisms to surfaces [119]. The coating of silicone surfaces with multiple layers of amylase or acylase has been found to inhibit biofilm formation in case of *P. aeruginosa* and *S. aureus* [120]. Another example is lysostaphin, a metalloprotease produced by *Staphylococcus simulans*, was shown to prevent the adherence of *S. aureus* to lysostaphin-coated catheters [121].

2.3.6 Amino acids

D-Amino acids have been shown to inhibit biofilm formation in *B. subtilis*, via activating the release of amyloid fibers [122]. Such inhibitory effect was reversed by their cognate L-amino acids [123]. Furthermore, D-amino acids were shown to have antibiofilm activity in case of *P. aeruginosa* and *S. aureus* [122].

2.3.7 Nitric oxide generators

Exogenous generation of nitric oxide (NO) by agents, for example, sodium nitroprusside has been shown to trigger the bacterial growth from the biofilm form to the planktonic form via the reduction of the level of cyclic di-GMP inside the bacterial cells [104]. Further NO-generators, for example, S-nitroso-N-acetyl penicillamine and S-nitroso-L-glutathione were found also to induce the dispersion of *P. aeruginosa* biofilm [124]. The dispersion of biofilm by NO-generators was also demonstrated in *B. subtilis* [125]. Recently, it has been reported that catheters charged with NO prevented the adherence and the colonization of *P. aeruginosa*, *E. coli* and *C. albicans* on their surfaces [126].

2.3.8 Natural agents

Alkaloids are a group of natural organic compounds that contain a nitrogen atom and are present in different species of plants. The alkaloid berberine has been reported to inhibit biofilm formation in *S. epidermidis* biofilm at a concentration of 30 µg/mL, possibly via binding to the amyloid proteins in the biofilm matrix [127]. Reserpine has been shown to effectively prevent biofilm formation in *K. pneumoniae* at a concentration of 0.0156 mg/mL, which was 64-fold lower than its minimum inhibitory concentration [128]. Tetrandrine inhibited biofilm formation of *C. albicans* at a concentration of 32 mg/L, which is the MIC₅₀ of that alkaloid against *C. albicans* SC5314 [129].

Guaijaverin, a flavonoid obtained from the leaves of *Psidium guajava*, has been shown to prevent the attachment of *S. mutans* to smooth surfaces by 83.7% at a concentration of 500 µg/mL. Eembelin, which is isolated from *Embelia ribes*, has been shown to inhibit biofilm formation in *S. mutans* [130]. Macelignan, isolated from the nutmegs of *Myristica fragrans*, was shown to reduce more than 50% of *S. mutans* biofilm at a concentration of 10 µg/mL [131].

Terpenes are a large class of natural hydrocarbons that are synthesized in microorganisms, plants and animals. Bakuchiol, isolated from the seeds of *Psoralea corylifolia*, has been shown to inhibit the adherence of *S. mutans* [132]. Other examples for terpenes that inhibit biofilm formation in *S. mutans*, are Xanthorrhizol (in combination with chlorhexidine gluconate) and casbane diterpene [133, 134].

2.4 Bacteriocins

Bacteriocins are proteins or peptides that are produced by bacteria or archaea, and are usually active against strains of bacteria that are related or unrelated to the producer strain [135]. Several bacteriocins are reported to exhibit antibiofilm activity and/or antimicrobial activity. The results of some these reports are summarized in Table 1.

2.5 Phage therapy

Phage therapy, which is also termed viral phage therapy, is the utilization of bacteriophages as medicaments for controlling and treating diseases brought by pathogenic bacterial infections [145]. Bacteriophages, like other viruses, are obligate intracellular parasites that utilize the enzymatic machinery of their hosts for establishing their physiological functions and replication [131]. The hosts for bacteriophages are bacteria, and phages have unique ability to specifically infect bacterial hosts resulting in their lysis [8].

Bacteriocin	Source	Antimicrobial activity	Antibiofilm activity
Mutacin 1140	<i>Streptococcus mutans</i>		Oral biofilm-associated with <i>Streptococcus sobrinus</i> , <i>Streptococcus oralis</i> [136]
Nisin A	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	<i>Enterococcus faecalis</i> and <i>Streptococcus gordonii</i> [137]	<i>Listeria monocytogenes</i> [138]
Gallidermin	<i>Staphylococcus gallinarum</i>		<i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i> [139, 140]
Sonorensin	<i>Bacillus sonorensis</i> MT93	<i>Listeria monocytogenes</i> and <i>Staphylococcus aureus</i> [141]	<i>Staphylococcus aureus</i> [141]
Epidermicin NIO	<i>Staphylococcus epidermidis</i>	MRSA, <i>Enterococci</i> [142]	<i>Staphylococcus epidermidis</i> [142]
Amylolysin	<i>Bacillus amyloliquefaciens</i> GA1	<i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i> [143]	
Philipimycin	<i>Actinoplanes philippinensis</i> MA7347	MRSA [144]	

Table 1. Bacteriocins produced from different sources and exhibit antimicrobial and antibiofilm activity.

There are many conceivable usages for phage therapy in the treatment of crucial diseases in plants, animals as well as human [8, 145]. An outstanding advantage of utilizing bacteriophages over commonly used antibiotics, during treating infectious diseases, is their selectivity and specificity to infect and lyse infectious bacteria only without harming the host [9]. Besides, bacteriophages cause no harm to other organisms that live in a commensalism within hosts, such as the normal flora in human, which decreases significantly the incidence of superinfections or other opportunistic infections [10]. Moreover, due to their mode of action that phages replicate *in vivo* within their bacterial hosts, they can be used in modicum concentrations, which results in decreasing any side effects may rise during therapy and giving them a high therapeutic index [9, 10]. In addition, the capability of bacteriophages to penetrate bacterial biofilms that act as shields during the conventional antibiotic therapy, gives phages a superiority in controlling and treating diseases brought by pathogenic bacterial infections [146]. As living organisms, the capability of bacteriophages of continuous evolution, gives them the ability to overcome any resistance that can be developed by the evolution of pathogenic bacteria [146, 147]. All these tremendous advantages put the bacteriophage treatment as a superior alternative for treating diseases brought about multidrug resistant MDR bacterial pathogens [132]. On the other hand, the high bacterial host specificity of bacteriophages is encountered as a disadvantage during therapy, where, a phage can kill only its specific bacterial strain. However, this drawback can be solved by utilizing mixtures of bacteriophages, which is termed phage cocktails that have different pathogenic specific bacterial hosts as targets, to enhance the opportunities of unguis complete treatment [148]. Attention must be given, during the preparation of these cocktails, to the fact of continuous evolution of new MDR strains, so the cocktails must be updated periodically to be sufficient enough to treat infections brought by these strains [148, 149].

Historically, the first trials for the utilization of bacteriophages as medicaments for treating bacterial pathogens was reported in the Eastern world before the discovery of marvelous medicaments so-called antibiotics; however, there was any report of their usage in the Western world [150, 151]. The ability of bacteriophages to infect and lyse pathogenic bacteria was discovered by the scientists Frederick Twort and Felix D'Hérelle, who worked on *Shigella dysenteriae* [152]. They found that the cultures of stool specimens recovered from convalescent patients who were suffering from *Shigella* dysentery always depicting a high titer of phages [153]. Subsequently, they recorded that phages are the most abundant organisms in the environment and there are many sources where they can be found combined with their bacterial hosts; including gut and feces of convalescent patients as well as sewages [153]. Thereafter, due to their ubiquity especially in sewages, bacteriophages were widely utilized as medicaments for controlling and eradication of diseases brought by pathogenic bacteria [8].

It has been estimated that there are more than 100 different phage species and at least 10 phages for each bacterium. The International Committee for the Taxonomy of Viruses (ICTV) was affirmed at 1971 with the objective to always bring to date the taxonomic guidelines of viruses. The ICTV classified tailed bacteriophages (bacterial infecting phages) under the order of viruses which is termed *Caudovirales*. In this respect, three main families are involved within this order named *Siphoviridae*, *Myoviridae* and *Podoviridae*. The main difference between bacteriophages belonging to each of these families is the characteristics of the tail. Phages under the *Siphoviridae* family have long and non-contractile tails, and those belong to *Myoviridae* family have long and contractile tails, while those belong to the *Podoviridae* family have short, stubbed tails and a striking lack of features. Each of these three families can also be divided into different genera [8].

Compared with antibiotics and other therapeutic regimens, the steps and cost of production of bacteriophages are much easier and cheaper, respectively [10]. The easiest process for capturing of bacteriophages is done through collecting samples that seem to involve high titers of phages like sewage water samples. The collected samples are inoculated with the host bacterium, which seems to be infected by phages, on suitable growth medium. The successful isolation of certain lytic phage is depicted by the presence of clear inhibition zones in which bacteria cannot grow termed plaques; which indicates the lytic power of the isolated phage. Thereafter, the titer of isolated phage is increased by passing the phage in its specific bacterial strain several times to increase its concentration. Then, the pure supernatants containing phages are gained by centrifugation of bacterial/phage mixture, filtered through bacterial filters to remove any bacterial debris and pure phages are participated using special solutions containing NaCl and polyethylene glycol 8000 (PEG8000) [154].

Caution must be given during isolation of phages as a type called lysogenic bacteriophage may be isolated rather than the required bacterial pathogen killing type, which is called lytic bacteriophage. Lysogenic bacteriophages do not lyse bacterial cells, but they perform as tools for transfer of genetic elements of the nucleic acid between bacteria; including the genes responsible for antibiotic resistance. Fortunately, the most abundant phages are of the lytic type not the lysogenic [8, 145, 150].

Practically, bacteriophages can be dispensed and used through many routes including; less commonly oral or systemic route and most commonly topical route as sprays, liquid solutions or their application on surgical dressings for the treatment of wound infections [154]. The possibility of their clearance during the presence in blood stream by immune system or presence of any trace hazards of chemicals or parts of the bacterial host used during their production, made bacteriophage usage as intravenous injections uncommon and very rare [148, 149]. Lyophilization of bacteriophages and their production as solid dosage forms as pills or tablets do not decrease their potency and increase their shelf life as oral dosage forms [155, 156]. The supplementation of oral forms of phages, either solid or liquid, with antacid increases its stability, as it protect them from the high acidity during their bypassing in the stomach [155, 156].

The application of bacteriophages as therapeutic medicaments has been extensively reported. For example, in the field of human health promotion and food protection, different bacteriophages have been employed to eradicate common bacterial pathogens that may cause food spoilage as *Listeria* sp. and *Campylobacter* sp. [157, 158]. In the fields of veterinary medicine and agriculture different bacteriophages were employed to control and eradicate bacterial pathogens like *Xanthomonas*, *Escherichia*, *Campylobacter* and *Salmonella* [159]. Moreover, in the field of fish production and aquacultures, different bacteriophages were employed to control and eradicate bacterial pathogens like *Vibrio* sp. [160]. In the field of human medicine, different bacteriophages were employed to control and eradicate bacterial pathogens including *P. aeruginosa*, *Staphylococci*, *Streptococci*, *E. coli*, *Vibrio* and *Shigella* and *Mycobacterium* sp. [161, 162]. Most recent application of bacteriophages in human medicine is their utilization as drug delivery system, which is very interesting as they can be used for the delivery of common antibiotics [163, 164] or antitumor agents [165].

A more recent policy, termed enzybiotic, for using phages as therapeutic agents is the utilization of their enzymes only, which are produced by recombinant technology, combined with other antibacterial agents or as a separate antibacterial agents [166].

As other therapeutic regimens for controlling bacterial pathogens, the patients may develop extensive fever and shock, when the bacteria are lysed due to the release of what is called pyrogens or endotoxins within the patient [167]. This

problem can be coped during phage therapy through the utilization of genetically modified phages that harbor enzymes having the ability to lyse these endotoxins and the other bacterial structures into harmless products [168].

Examples of therapeutic approaches of bacteriophages and their enzymes are illustrated in **Table 2**.

Infection/ disease	Model	Causative agent	Route of administration of phages/enzymes	Treatment outcomes	Reference
Chronic otitis	Human	<i>Pseudomonas aeruginosa</i>	Oral administration of phages	Successful treatment	[169]
Typhoid	Human	<i>Salmonella typhi</i>	Oral administration of phages	Successful treatment	[170]
Diabetic foot ulcer	Human	<i>Staphylococcus aureus</i>	Topical application of phages	Successful treatment	[171]
Sepsis	Murine	<i>Vibrio parahaemolyticus</i>	Intraperitoneal and oral administration of phages	Successful treatment	[172]
Pneumonia	Murine	<i>Pseudomonas aeruginosa</i>	Intranasal administration of phages	Successful treatment	[154]
Ulcers and wounds	Human	<i>Proteus vulgaris</i>	Topical application of phages	Successful treatment	[173]
Meningitis	Murine	<i>Escherichia coli</i>	Intraperitoneal or subcutaneous administration of phages.	Successful treatment	[174]
Sepsis	Murine	<i>Acinetobacter baumannii</i>	Intraperitoneal administration of phages	Successful treatment	[175]
Bacteremia	Murine	<i>Enterococcus faecium</i>	Intraperitoneal administration of phages	Successful treatment	[176]
Ileocectitis	Hamster	<i>Clostridium difficile</i>	Oral administration of phages	Successful treatment	[177]
Dysentery	Human	<i>Shigella dysenteriae</i>	Oral administration of phages	Successful treatment	[178]
Cholera	Human	<i>Vibrio cholerae</i>	Oral administration of phages	Successful treatment	[178]
Pneumonia	Murine	<i>Streptococcus pneumoniae</i>	Intraperitoneal administration of Cpl-1 lysin enzyme	Successful treatment	[179]
Bacteremia	Murine	<i>Streptococcus pyogenes</i>	Intraperitoneal administration of PlySs2 lysin enzyme	Successful treatment	[179]
<i>In vitro</i>	<i>In vitro</i>	<i>Bacillus anthracis</i>	Application of PlyG lysin enzyme	Significant reduction in bacterial density	[180]
Endophthalmitis	Murine	<i>Staphylococcus aureus</i>	Application of Ply187 lysin as eye drops	Successful treatment	[181]
Bacteremia	Murine	<i>Acinetobacter baumannii</i>	Administration of PlyF307 lysin enzyme	Successful treatment	[182]
<i>In vitro</i>	<i>In vitro</i>	<i>Pseudomonas aeruginosa</i> and <i>Salmonella typhimurium</i>	Application of ABgp46 lysin enzyme	Significant reduction in bacterial density	[183]

Table 2.
Therapeutic approaches of bacteriophages and their enzymes.

3. Conclusion

Various approaches have been developed for competing microbial virulence and resistance. Quorum sensing signals and global regulators play an essential role in controlling the gene expression of virulence factors, and the expression of proteins required for adaptation to environmental and stress condition. Therefore, control of these regulators will stop the microbial pathogenicity. In addition, biofilms act as a defense mechanism against host immunity and antimicrobial therapy. Natural and synthetic compounds have approved activities in eradication of biofilm formation. Besides, phage therapy, which is currently successful in destruction of bacterial pathogens that do not respond to conventional antimicrobials. These methods would open up new perspectives for management the up growing problem of microbial resistance. Further, *in vivo* studies are required for real applications of these trends in eradication of microbial infections.

Author details

Mohammed El-Mowafy, Abdelaziz Elgaml and Mona Shaaban*
Department of Microbiology and Immunology, Faculty of Pharmacy, Mansoura
University, Mansoura, Egypt

*Address all correspondence to: mona_ibrahem@mans.edu.eg

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Reuter K, Steinbach A, Helms V. Interfering with bacterial quorum sensing. *Perspectives in Medicinal Chemistry*. 2016;**8**:1-15
- [2] Finch RG, Pritchard DI, Bycroft BW, Williams P, Stewart GS. Quorum sensing: A novel target for anti-infective therapy. *The Journal of Antimicrobial Chemotherapy*. 1998;**42**(5):569-571
- [3] Miller MB, Bassler BL. Quorum sensing in bacteria. *Annual Review of Microbiology*. 2001;**55**:165-199
- [4] Geske GD, O'Neill JC, Miller DM, Mattmann ME, Blackwell HE. Modulation of bacterial quorum sensing with synthetic ligands: Systematic evaluation of N-acylated homoserine lactones in multiple species and new insights into their mechanisms of action. *Journal of the American Chemical Society*. 2007;**129**(44):13613-13625
- [5] Elgaml A, Miyoshi SI. Regulation systems of protease and hemolysin production in *Vibrio vulnificus*. *Microbiology and Immunology*. 2017;**61**(1):1-11
- [6] Bogino PC, Oliva Mde L, Sorroche FG, Giordano W. The role of bacterial biofilms and surface components in plant-bacterial associations. *International Journal of Molecular Sciences*. 2013;**14**(8):15838-15859
- [7] Hengzhuang W, Wu H, Ciofu O, Song Z, Høiby N. Pharmacokinetics/ pharmacodynamics of colistin and imipenem on mucoid and nonmucoid *Pseudomonas aeruginosa* biofilms. *Antimicrobial Agents and Chemotherapy*. 2011;**55**(9):4469-4474
- [8] Abedon ST. Phage therapy: Various perspectives on how to improve the art. *Methods in Molecular Biology*. 1734;**2018**:113-127
- [9] Grasis JA. Host-associated bacteriophage isolation and preparation for viral metagenomics. *Methods in Molecular Biology*. 1746;**2018**:1-25
- [10] Nobrega FL, Costa AR, Kluskens LD, Azeredo J. Revisiting phage therapy: New applications for old resources. *Trends in Microbiology*. 2015;**23**(4):185-191
- [11] O'Loughlin CT, Miller LC, Siryaporn A, Drescher K, Semmelhack MF, Bassler BL. A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(44):17981-17986
- [12] Hornby JM, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, et al. Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. *Applied and Environmental Microbiology*. 2001;**67**(7):2982-2992
- [13] Erez Z, Steinberger-Levy I, Shamir M, Doron S, Stokar-Avihail A, Peleg Y, et al. Communication between viruses guides lysis-lysogeny decisions. *Nature*. 2017;**541**(7638):488-493
- [14] Schaefer AL, Val DL, Hanzelka BL, Cronan JE Jr, Greenberg EP. Generation of cell-to-cell signals in quorum sensing: Acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. *Proceedings of the National Academy of Sciences of the United States of America*. 1996;**93**(18):9505-9509
- [15] Calfee MW, Coleman JP, Pesci EC. Interference with pseudomonas quinolone signal synthesis inhibits virulence factor expression by *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;**98**(20):11633-11637

- [16] Xiong YQ, Willard J, Yeaman MR, Cheung AL, Bayer AS. Regulation of *Staphylococcus aureus* alpha-toxin gene (hla) expression by agr, sarA, and sae in vitro and in experimental infective endocarditis. The Journal of Infectious Diseases. 2006;**194**(9):1267-1275
- [17] Kong C, Neoh HM, Nathan S. Targeting *Staphylococcus aureus* toxins: A potential form of anti-virulence therapy. Toxins. 2016;**8**(3): pii: E72; 1-21
- [18] Remy B, Mion S, Plener L, Elias M, Chabriere E, Daude D. Interference in bacterial quorum sensing: A biopharmaceutical perspective. Frontiers in Pharmacology. 2018;**9**:203
- [19] Shaaban M, Elgaml A, Habib EE. Biotechnological applications of quorum sensing inhibition as novel therapeutic strategies for multidrug resistant pathogens. Microbial Pathogenesis. 2019;**127**:138-143
- [20] Sedlmayer F, Hell D, Muller M, Auslander D, Fussenegger M. Designer cells programming quorum-sensing interference with microbes. Nature Communications. 2018;**9**(1):1822
- [21] Xavier KB, Bassler BL. LuxS quorum sensing: More than just a numbers game. Current Opinion in Microbiology. 2003;**6**(2):191-197
- [22] Guo M, Gamby S, Nakayama S, Smith J, Sintim HO. A pro-drug approach for selective modulation of AI-2-mediated bacterial cell-to-cell communication. Sensors. 2012;**12**(3):3762-3772
- [23] Ren D, Li C, Qin Y, Yin R, Li X, Tian M, et al. Inhibition of *Staphylococcus aureus* adherence to Caco-2 cells by lactobacilli and cell surface properties that influence attachment. Anaerobe. 2012;**18**(5):508-515
- [24] Gutierrez JA, Crowder T, Rinaldo-Matthis A, Ho MC, Almo SC, Schramm VL. Transition state analogs of 5'-methylthioadenosine nucleosidase disrupt quorum sensing. Nature Chemical Biology. 2009;**5**(4):251-257
- [25] Malladi VL, Sobczak AJ, Meyer TM, Pei D, Wnuk SF. Inhibition of LuxS by S-ribosylhomocysteine analogues containing a [4-aza]ribose ring. Bioorganic & Medicinal Chemistry. 2011;**19**(18):5507-5519
- [26] Wnuk SF, Lalama J, Garmendia CA, Robert J, Zhu J, Pei D. S-Ribosylhomocysteine analogues with the carbon-5 and sulfur atoms replaced by a vinyl or (fluoro)vinyl unit. Bioorganic & Medicinal Chemistry. 2008;**16**(9):5090-5102
- [27] Hoang TT, Schweizer HP. Characterization of *Pseudomonas aeruginosa* enoyl-acyl carrier protein reductase (FabI): A target for the antimicrobial triclosan and its role in acylated homoserine lactone synthesis. Journal of Bacteriology. 1999;**181**(17):5489-5497
- [28] Coleman JP, Hudson LL, McKnight SL, Farrow JM 3rd, Calfee MW, Lindsey CA, et al. *Pseudomonas aeruginosa* PqsA is an anthranilate-coenzyme A ligase. Journal of Bacteriology. 2008;**190**(4):1247-1255
- [29] Maisuria VB, Los Santos YL, Tufenkji N, Deziel E. Cranberry-derived proanthocyanidins impair virulence and inhibit quorum sensing of *Pseudomonas aeruginosa*. Scientific Reports. 2016;**6**:30169
- [30] LaSarre B, Federle MJ. Exploiting quorum sensing to confuse bacterial pathogens. Microbiology and Molecular Biology Reviews. 2013;**77**(1):73-111
- [31] Piletska EV, Stavroulakis G, Karim K, Whitcombe MJ, Chianella I, Sharma A, et al. Attenuation of *Vibrio fischeri* quorum sensing using rationally

- p>designed polymers.
- Biomacromolecules*
- . 2010;
- 11**
- (4):975-980
- [32] Park J, Jagasia R, Kaufmann GF, Mathison JC, Ruiz DI, Moss JA, et al. Infection control by antibody disruption of bacterial quorum sensing signaling. *Chemistry & Biology*. 2007;**14**(10):1119-1127
- [33] Cavaleiro E, Duarte AS, Esteves AC, Correia A, Whitcombe MJ, Piletska EV, et al. Novel linear polymers able to inhibit bacterial quorum sensing. *Macromolecular Bioscience*. 2015;**15**(5):647-656
- [34] Dong YH, Xu JL, Li XZ, Zhang LH. AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;**97**(7):3526-3531
- [35] Dong YH, Gusti AR, Zhang Q, Xu JL, Zhang LH. Identification of quorum-quenching N-acyl homoserine lactonases from *Bacillus* species. *Applied and Environmental Microbiology*. 2002;**68**(4):1754-1759
- [36] Liu D, Momb J, Thomas PW, Moulin A, Petsko GA, Fast W, et al. Mechanism of the quorum-quenching lactonase (AiiA) from *Bacillus thuringiensis*. 1. Product-bound structures. *Biochemistry*. 2008;**47**(29):7706-7714
- [37] Ulrich RL. Quorum quenching: Enzymatic disruption of N-acylhomoserine lactone-mediated bacterial communication in *Burkholderia thailandensis*. *Applied and Environmental Microbiology*. 2004;**70**(10):6173-6180
- [38] Zhang HB, Wang LH, Zhang LH. Genetic control of quorum-sensing signal turnover in *Agrobacterium tumefaciens*. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;**99**(7):4638-4643
- [39] Park SY, Lee SJ, Oh TK, Oh JW, Koo BT, Yum DY, et al. AhlD, an N-acylhomoserine lactonase in *Arthrobacter* sp., and predicted homologues in other bacteria. *Microbiology*. 2003;**149**(Pt 6):1541-1550
- [40] Wang WZ, Morohoshi T, Someya N, Ikeda T. AidC, a novel N-acylhomoserine lactonase from the potato root-associated cytophaga-flavobacteria-bacteroides (CFB) group bacterium *Chryseobacterium* sp. strain StRB126. *Applied and Environmental Microbiology*. 2012;**78**(22):7985-7992
- [41] Uroz S, Oger PM, Chapelle E, Adeline MT, Faure D, Dessaux Y. A *Rhodococcus* qsdA-encoded enzyme defines a novel class of large-spectrum quorum-quenching lactonases. *Applied and Environmental Microbiology*. 2008;**74**(5):1357-1366
- [42] Wang WZ, Morohoshi T, Ikenoya M, Someya N, Ikeda T. AiiM, a novel class of N-acylhomoserine lactonase from the leaf-associated bacterium *Microbacterium testaceum*. *Applied and Environmental Microbiology*. 2010;**76**(8):2524-2530
- [43] Mei GY, Yan XX, Turak A, Luo ZQ, Zhang LQ. AidH, an alpha/beta-hydrolase fold family member from an *Ochrobactrum* sp. strain, is a novel N-acylhomoserine lactonase. *Applied and Environmental Microbiology*. 2010;**76**(15):4933-4942
- [44] Huang W, Lin Y, Yi S, Liu P, Shen J, Shao Z, et al. QsdH, a novel AHL lactonase in the RND-type inner membrane of marine *Pseudoalteromonas byunsanensis* strain 1A01261. *PLoS One*. 2012;**7**(10):e46587
- [45] Yang F, Wang LH, Wang J, Dong YH, Hu JY, Zhang LH. Quorum

quenching enzyme activity is widely conserved in the sera of mammalian species. *FEBS Letters*. 2005;**579**(17):3713-3717

[46] Lin YH, Xu JL, Hu J, Wang LH, Ong SL, Leadbetter JR, et al. Acyl-homoserine lactone acylase from *Ralstonia* strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Molecular Microbiology*. 2003;**47**(3):849-860

[47] Bokhove M, Nadal Jimenez P, Quax WJ, Dijkstra BW. The quorum-quenching N-acyl homoserine lactone acylase PvdQ is an Ntn-hydrolase with an unusual substrate-binding pocket. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;**107**(2):686-691

[48] Huang JJ, Petersen A, Whiteley M, Leadbetter JR. Identification of QuiP, the product of gene PA1032, as the second acyl-homoserine lactone acylase of *Pseudomonas aeruginosa* PAO1. *Applied and Environmental Microbiology*. 2006;**72**(2):1190-1197

[49] Park SY, Kang HO, Jang HS, Lee JK, Koo BT, Yum DY. Identification of extracellular N-acylhomoserine lactone acylase from a *Streptomyces* sp. and its application to quorum quenching. *Applied and Environmental Microbiology*. 2005;**71**(5):2632-2641

[50] Krysciak D, Schmeisser C, Preuss S, Riethausen J, Quitschau M, Grond S, et al. Involvement of multiple loci in quorum quenching of autoinducer I molecules in the nitrogen-fixing symbiont *Rhizobium* (*Sinorhizobium*) sp. strain NGR234. *Applied and Environmental Microbiology*. 2011;**77**(15):5089-5099

[51] Llamas I, Suarez A, Quesada E, Bejar V, del Moral A. Identification and characterization of the carAB genes responsible for encoding carbamoylphosphate synthetase in

Halomonas eurihalina. *Extremophiles*. 2003;**7**(3):205-211

[52] Roy V, Fernandes R, Tsao CY, Bentley WE. Cross species quorum quenching using a native AI-2 processing enzyme. *ACS Chemical Biology*. 2010;**5**(2):223-232

[53] Koch B, Liljefors T, Persson T, Nielsen J, Kjelleberg S, Givskov M. The LuxR receptor: The sites of interaction with quorum-sensing signals and inhibitors. *Microbiology*. 2005;**151**(Pt 11):3589-3602

[54] Singh RP, Desouky SE, Nakayama J. Quorum quenching strategy targeting Gram-positive pathogenic bacteria. *Advances in Experimental Medicine and Biology*. 2016;**901**:109-130

[55] Manefield M, de Nys R, Kumar N, Read R, Givskov M, Steinberg P, et al. Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein. *Microbiology*. 1999;**145** (Pt 2):283-291

[56] Givskov M, de Nys R, Manefield M, Gram L, Maximilien R, Eberl L, et al. Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *Journal of Bacteriology*. 1996;**178**(22):6618-6622

[57] Fong J, Yuan M, Jakobsen TH, Mortensen KT, Delos Santos MM, Chua SL, et al. Disulfide bond-containing Ajoene analogues As novel quorum sensing inhibitors of *Pseudomonas aeruginosa*. *Journal of Medicinal Chemistry*. 2017;**60**(1):215-227

[58] Zhou L, Zheng H, Tang Y, Yu W, Gong Q. Eugenol inhibits quorum sensing at sub-inhibitory concentrations. *Biotechnology Letters*. 2013;**35**(4):631-637

- [59] Paczkowski JE, Mukherjee S, McCready AR, Cong JP, Aquino CJ, Kim H, et al. Flavonoids suppress *Pseudomonas aeruginosa* virulence through allosteric inhibition of quorum-sensing receptors. *The Journal of Biological Chemistry*. 2017;**292**(10):4064-4076
- [60] Jakobsen TH, Bragason SK, Phipps RK, Christensen LD, van Gennip M, Alhede M, et al. Food as a source for quorum sensing inhibitors: Iberin from horseradish revealed as a quorum sensing inhibitor of *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology*. 2012;**78**(7):2410-2421
- [61] Girenavar B, Cepeda ML, Soni KA, Vikram A, Jesudhasan P, Jayaprakasha GK, et al. Grapefruit juice and its furocoumarins inhibits autoinducer signaling and biofilm formation in bacteria. *International Journal of Food Microbiology*. 2008;**125**(2):204-208
- [62] Rasmussen TB, Skindersoe ME, Bjarnsholt T, Phipps RK, Christensen KB, Jensen PO, et al. Identity and effects of quorum-sensing inhibitors produced by *Penicillium* species. *Microbiology*. 2005;**151** (Pt 5):1325-1340
- [63] Teasdale ME, Liu J, Wallace J, Akhlaghi F, Rowley DC. Secondary metabolites produced by the marine bacterium *Halobacillus salinus* that inhibit quorum sensing-controlled phenotypes in gram-negative bacteria. *Applied and Environmental Microbiology*. 2009;**75**(3):567-572
- [64] Hassan R, Shaaban MI, Abdel Bar FM, El-Mahdy AM, Shokralla S. Quorum sensing inhibiting activity of *Streptomyces coelicoflavus* isolated from soil. *Frontiers in Microbiology*. 2016;**7**:659
- [65] Wu H, Song Z, Hentzer M, Andersen JB, Molin S, Givskov M, et al. Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice. *The Journal of Antimicrobial Chemotherapy*. 2004;**53**(6):1054-1061
- [66] Cady NC, McKean KA, Behnke J, Kubec R, Mosier AP, Kasper SH, et al. Inhibition of biofilm formation, quorum sensing and infection in *Pseudomonas aeruginosa* by natural products-inspired organosulfur compounds. *PLoS One*. 2012;**7**(6):e38492
- [67] Muh U, Schuster M, Heim R, Singh A, Olson ER, Greenberg EP. Novel *Pseudomonas aeruginosa* quorum-sensing inhibitors identified in an ultra-high-throughput screen. *Antimicrobial Agents and Chemotherapy*. 2006;**50**(11):3674-3679
- [68] Biswas NN, Kutty SK, Barraud N, Iskander GM, Griffith R, Rice SA, et al. Indole-based novel small molecules for the modulation of bacterial signalling pathways. *Organic & Biomolecular Chemistry*. 2015;**13**(3):925-937
- [69] Park S, Kim HS, Ok K, Kim Y, Park HD, Byun Y. Design, synthesis and biological evaluation of 4-(alkyloxy)-6-methyl-2H-pyran-2-one derivatives as quorum sensing inhibitors. *Bioorganic & Medicinal Chemistry Letters*. 2015;**25**(15):2913-2917
- [70] El-Mowafy SA, Abd El Galil KH, El-Messery SM, Shaaban MI. Aspirin is an efficient inhibitor of quorum sensing, virulence and toxins in *Pseudomonas aeruginosa*. *Microbial Pathogenesis*. 2014;**74**:25-32
- [71] O'Reilly MC, Blackwell HE. Structure-based design and biological evaluation of triphenyl scaffold-based hybrid compounds as hydrolytically stable modulators of a LuxR-type quorum sensing receptor. *ACS Infectious Diseases*. 2016;**2**(1):32-38
- [72] Chang CY, Krishnan T, Wang H, Chen Y, Yin WF, Chong YM, et al.

Non-antibiotic quorum sensing inhibitors acting against N-acyl homoserine lactone synthase as druggable target. *Scientific Reports*. 2014;**4**:7245

[73] Lidor O, Al-Quntar A, Pesci EC, Steinberg D. Mechanistic analysis of a synthetic inhibitor of the *Pseudomonas aeruginosa* LasI quorum-sensing signal synthase. *Scientific Reports*. 2015;**5**:16569

[74] Kamarudheen N, Rao KVB. Fatty acyl compounds from marine *Streptomyces griseoincarnatus* strain HK12 against two major bio-film forming nosocomial pathogens; an in vitro and in silico approach. *Microbial Pathogenesis*. 2019;**127**:121-130

[75] Mansson M, Nielsen A, Kjaerulff L, Gotfredsen CH, Wietz M, Ingmer H, et al. Inhibition of virulence gene expression in *Staphylococcus aureus* by novel depsipeptides from a marine photobacterium. *Marine Drugs*. 2011;**9**(12):2537-2552

[76] Karathanasi G, Bojer MS, Baldry M, Johannessen BA, Wolff S, Greco I, et al. Linear peptidomimetics as potent antagonists of *Staphylococcus aureus* agr quorum sensing. *Scientific Reports*. 2018;**8**(1):3562

[77] Sully EK, Malachowa N, Elmore BO, Alexander SM, Femling JK, Gray BM, et al. Selective chemical inhibition of agr quorum sensing in *Staphylococcus aureus* promotes host defense with minimal impact on resistance. *PLoS Pathogens*. 2014;**10**(6):e1004174

[78] Daly SM, Elmore BO, Kavanaugh JS, Triplett KD, Figueroa M, Raja HA, et al. Omega-hydroxyemodin limits *Staphylococcus aureus* quorum sensing-mediated pathogenesis and inflammation. *Antimicrobial Agents and Chemotherapy*. 2015;**59**(4):2223-2235

[79] Lee SE, Kim SY, Kim CM, Kim MK, Kim YR, Jeong K, et al. The pyrH gene of *Vibrio vulnificus* is an essential in vivo survival factor. *Infection and Immunity*. 2007;**75**(6):2795-2801

[80] Elgaml A, Miyoshi S. Role of the histone-like nucleoid structuring protein (H-NS) in the regulation of virulence factor expression and stress response in *Vibrio vulnificus*. *Biocontrol Science*. 2015; **20**(4):263-274

[81] Dorman CJ. H-NS: A universal regulator for a dynamic genome. *Nature Reviews. Microbiology*. 2004;**2**(5):391-400

[82] Dorman CJ, Deighan P. Regulation of gene expression by histone-like proteins in bacteria. *Current Opinion in Genetics & Development*. 2003;**13**(2):179-184

[83] Zheng W, Liang Y, Zhao H, Zhang J, Li Z. 5,5'-methylenedisalicylic acid (MDSA) modulates SarA/MgrA phosphorylation by targeting Ser/Thr phosphatase Stp1. *Chembiochem*. 2015;**16**(7):1035-1040

[84] Cheung AL, Nishina KA, Trottonda MP, Tamber S. The SarA protein family of *Staphylococcus aureus*. *The International Journal of Biochemistry & Cell Biology*. 2008;**40**(3):355-361

[85] Ono S, Goldberg MD, Olsson T, Esposito D, Hinton JC, Ladbury JE. H-NS is a part of a thermally controlled mechanism for bacterial gene regulation. *The Biochemical Journal*. 2005;**391**(Pt 2):203-213

[86] Brescia CC, Kaw MK, Sledjeski DD. The DNA binding protein H-NS binds to and alters the stability of RNA in vitro and in vivo. *Journal of Molecular Biology*. 2004;**339**(3): 505-514

- [87] Arya R, Ravikumar R, Santhosh RS, Princy SA. SarA based novel therapeutic candidate against *Staphylococcus aureus* associated with vascular graft infections. *Frontiers in Microbiology*. 2015;**6**:416
- [88] Donlan RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. *Clinical Microbiology Reviews*. 2002;**15**(2):167-193
- [89] Roy R, Tiwari M, Donelli G, Tiwari V. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. *Virulence*. 2018;**9**(1):522-554
- [90] Overhage J, Campisano A, Bains M, Torfs EC, Rehm BH, Hancock RE. Human host defense peptide LL-37 prevents bacterial biofilm formation. *Infection and Immunity*. 2008;**76**(9):4176-4182
- [91] Aka ST. Killing efficacy and anti-biofilm activity of synthetic human cationic antimicrobial peptide cathelicidin hCAP-18/LL37 against urinary tract pathogens. *Journal of Microbiology and Infectious Diseases*. 2015;**5**:15-20
- [92] De Brucker K, Delattin N, Robijns S, Steenackers H, Verstraeten N, Landuyt B, et al. Derivatives of the mouse cathelicidin-related antimicrobial peptide (CRAMP) inhibit fungal and bacterial biofilm formation. *Antimicrobial Agents and Chemotherapy*. 2014;**58**(9):5395-5404
- [93] De la Fuente-Núñez C, Reffuveille F, Haney EF, Straus SK, Hancock RE. Broad-spectrum anti-biofilm peptide that targets a cellular stress response. *PLoS Pathogens*. 2014;**10**(5):e1004152
- [94] Quiles F, Saadi S, Francius G, Bacharouche J, Humbert F. 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*. 2016;**1858**(1):75-84
- [95] Rohde H, Frankenberger S, Zahringer U, Mack D. 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*. 2010;**89**(1):103-111
- [96] Libardo MDJ, Bahar AA, Ma B, Fu R, McCormick LE, Zhao J, et al. Nuclease activity gives an edge to host-defense peptide piscidin 3 over piscidin 1, rendering it more effective against persister and biofilms. *The FEBS Journal*. 2017;**284**(21):3662-3683
- [97] Luca V, Stringaro A, Colone M, Pini A, Mangoni ML. Esculentin(1-21), an amphibian skin membrane-active peptide with potent activity on both planktonic and biofilm cells of the bacterial pathogen *Pseudomonas aeruginosa*. *Cellular and Molecular Life Sciences: CMLS*. 2013;**70**(15):2773-2786
- [98] Ansari JM, Abraham NM, Massaro J, Murphy K, Smith-Carpenter J, Fikrig E. Anti-biofilm activity of a self-aggregating peptide against *Streptococcus mutans*. *Frontiers in Microbiology*. 2017;**8**:488
- [99] Boles BR, Thoendel M, Singh PK. Rhamnolipids mediate detachment of *Pseudomonas aeruginosa* from biofilms. *Molecular Microbiology*. 2005;**57**(5):1210-1223
- [100] Simoes M, Pereira MO, Vieira MJ. Action of a cationic surfactant on the activity and removal of bacterial biofilms formed under different flow regimes. *Water Research*. 2005;**39**(2-3):478-486

- [101] Mireles JR 2nd, Toguchi A, Harshey RM. *Salmonella enterica* serovar typhimurium swarming mutants with altered biofilm-forming abilities: Surfactin inhibits biofilm formation. *Journal of Bacteriology*. 2001;**183**(20):5848-5854
- [102] Meylheuc T, van Oss CJ, Bellon-Fontaine MN. Adsorption of biosurfactant on solid surfaces and consequences regarding the bioadhesion of *Listeria monocytogenes* LO28. *Journal of Applied Microbiology*. 2001;**91**(5):822-832
- [103] Desbois AP, Smith VJ. Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*. 2010;**85**(6):1629-1642
- [104] Li X-H, Lee J-H. Antibiofilm agents: A new perspective for antimicrobial strategy. *Journal of Microbiology*. 2017;**55**(10):753-766
- [105] Davies DG, Marques CN. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. *Journal of Bacteriology*. 2009;**191**(5):1393-1403
- [106] Stenz L, Francois P, Fischer A, Huyghe A, Tangomo M, Hernandez D, et al. Impact of oleic acid (cis-9-octadecenoic acid) on bacterial viability and biofilm production in *Staphylococcus aureus*. *FEMS Microbiology Letters*. 2008;**287**(2):149-155
- [107] Deng Y, Lim A, Lee J, Chen S, An S, Dong YH, et al. Diffusible signal factor (DSF) quorum sensing signal and structurally related molecules enhance the antimicrobial efficacy of antibiotics against some bacterial pathogens. *BMC Microbiology*. 2014;**14**:51
- [108] Gadd GM. Metals, minerals and microbes: Geomicrobiology and bioremediation. *Microbiology (Reading, England)*. 2010;**156**(Pt 3):609-643
- [109] Kite P, Eastwood K, Sugden S, Percival SL. Use of in vivo-generated biofilms from hemodialysis catheters to test the efficacy of a novel antimicrobial catheter lock for biofilm eradication in vitro. *Journal of Clinical Microbiology*. 2004;**42**(7):3073-3076
- [110] Raad I, Chatzinikolaou I, Chaiban G, Hanna H, Hachem R, Dvorak T, et al. In vitro and ex vivo activities of minocycline and EDTA against microorganisms embedded in biofilm on catheter surfaces. *Antimicrobial Agents and Chemotherapy*. 2003;**47**(11):3580-3585
- [111] Casalnuovo IA, Sorge R, Bonelli G, Di Francesco P. Evaluation of the antifungal effect of EDTA, a metal chelator agent, on *Candida albicans* biofilm. *European Review for Medical and Pharmacological Sciences*. 2017;**21**(6):1413-1420
- [112] Craigen B, Dashiff A, Kadouri DE. The use of commercially available alpha-amylase compounds to inhibit and remove *Staphylococcus aureus* biofilms. *Open Microbiology Journal*. 2011;**5**:21-31
- [113] Ramasubbu N, Thomas LM, Ragunath C, Kaplan JB. Structural analysis of dispersin B, a biofilm-releasing glycoside hydrolase from the periodontopathogen *Actinobacillus actinomycetemcomitans*. *Journal of Molecular Biology*. 2005;**349**(3):475-486
- [114] Chaignon P, Sadovskaya I, Ragunah C, Ramasubbu N, Kaplan JB, Jabbouri S. Susceptibility of staphylococcal biofilms to enzymatic treatments depends on their chemical composition. *Applied Microbiology and Biotechnology*. 2007;**75**(1):125-132
- [115] Darouiche RO, Mansouri MD, Gawande PV, Madhyastha S. Antimicrobial and antibiofilm efficacy of triclosan and DispersinB

- p>combination. Journal of Antimicrobial Chemotherapy. 2009;
- 64**
- (1):88-93
- [116] Kaplan JB. Therapeutic potential of biofilm-dispersing enzymes. The International Journal of Artificial Organs. 2009;**32**(9):545-554
- [117] Qin Z, Ou Y, Yang L, Zhu Y, Tolker-Nielsen T, Molin S, et al. Role of autolysin-mediated DNA release in biofilm formation of *Staphylococcus epidermidis*. Microbiology (Reading, England). 2007;**153**(Pt 7):2083-2092
- [118] Tetz GV, Artemenko NK, Tetz VV. Effect of DNase and antibiotics on biofilm characteristics. Antimicrobial Agents and Chemotherapy. 2009;**53**(3):1204-1209
- [119] Leroy C, Delbarre C, Ghillebaert F, Compere C, Combes D. Effects of commercial enzymes on the adhesion of a marine biofilm-forming bacterium. Biofouling. 2008;**24**(1):11-22
- [120] Ivanova K, Fernandes MM, Francesko A, Mendoza E, Guezguez J, Burnet M, et al. Quorum-quenching and matrix-degrading enzymes in multilayer coatings synergistically prevent bacterial biofilm formation on urinary catheters. ACS Applied Materials & Interfaces. 2015;**7**(49):27066-27077
- [121] Shah A, Mond J, Walsh S. Lysostaphin-coated catheters eradicate *Staphylococcus aureus* challenge and block surface colonization. Antimicrobial Agents and Chemotherapy. 2004;**48**(7):2704-2707
- [122] Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R, Losick R. D-amino acids trigger biofilm disassembly. Science. 2010;**328**(5978):627-629
- [123] Leiman SA, May JM, Lebar MD, Kahne D, Kolter R, Losick R. D-amino acids indirectly inhibit biofilm formation in *Bacillus subtilis* by interfering with protein synthesis. Journal of Bacteriology. 2013;**195**(23):5391-5395
- [124] Barraud N, Schleheck D, Klebensberger J, Webb JS, Hassett DJ, Rice SA, et al. Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic di-GMP levels, and enhanced dispersal. Journal of Bacteriology. 2009;**191**(23):7333-7342
- [125] Schreiber F, Beutler M, Enning D, Lamprecht-Grandio M, Zafra O, Gonzalez-Pastor JE, et al. The role of nitric-oxide-synthase-derived nitric oxide in multicellular traits of *Bacillus subtilis* 3610: Biofilm formation, swarming, and dispersal. BMC Microbiology. 2011;**11**:111
- [126] Margel D, Mizrahi M, Regev-Shoshani G, Ko M, Moshe M, Ozalvo R, et al. Nitric oxide charged catheters as a potential strategy for prevention of hospital acquired infections. PLoS One. 2017;**12**(4):e0174443
- [127] Wang X, Yao X, Zhu Z, Tang T, Dai K, Sadovskaya I, et al. Effect of berberine on *Staphylococcus epidermidis* biofilm formation. International Journal of Antimicrobial Agents. 2009;**34**(1):60-66
- [128] Magesh H, Kumar A, Alam A, Priyam SU, Sumantran VN, et al. Identification of natural compounds which inhibit biofilm formation in clinical isolates of *Klebsiella pneumoniae*. Indian Journal of Experimental Biology. 2013;**51**(9):764-772
- [129] Zhao L-X, Li D-D, Hu D-D, Hu G-H, Yan L, Wang Y, et al. Effect of tetrandrine against *Candida albicans* biofilms. PLoS One. 2013;**8**(11):e79671
- [130] Dwivedi D, Singh V. Effects of the natural compounds embelin and piperine on the biofilm-producing property of *Streptococcus mutans*. Journal

of Traditional and Complementary Medicine. 2015;**6**(1):57-61

[131] Rukayadi Y, Kim KH, Hwang JK. In vitro anti-biofilm activity of macelignan isolated from *Myristica fragrans* Hoult. against oral primary colonizer bacteria. *Phytotherapy Research*. 2008;**22**(3):308-312

[132] Katsura H, Tsukiyama RI, Suzuki A, Kobayashi M. In vitro antimicrobial activities of bakuchiol against oral microorganisms. *Antimicrobial Agents and Chemotherapy*. 2001;**45**(11):3009-3013

[133] Rukayadi Y, Hwang JK. In vitro activity of xanthorrhizol against *Streptococcus mutans* biofilms. *Letters in Applied Microbiology*. 2006;**42**(4):400-404

[134] Sá NC, Cavalcante TTA, Araújo AX, Santos HS, Albuquerque MRJR, Bandeira PN, et al. Antimicrobial and antibiofilm action of Casbane Diterpene from *Croton nepetaefolius* against oral bacteria. *Archives of Oral Biology*. 2012;**57**(5):550-555

[135] Santos V, Nardi R, Dias-Souza M. Bacteriocins as antimicrobial and antibiofilm agents. In: *Current Developments in Biotechnology and Bioengineering: Human and Animal Health Applications*. 2017. pp. 403-436

[136] Hillman JD. Genetically modified *Streptococcus mutans* for the prevention of dental caries. *Antonie Van Leeuwenhoek*. 2002;**82**(1-4):361-366

[137] Turner SR, Love RM, Lyons KM. An in-vitro investigation of the antibacterial effect of nisin in root canals and canal wall radicular dentine. *International Endodontic Journal*. 2004;**37**(10):664-671

[138] Gonzalez-Toledo SY, Dominguez-Dominguez J, Garcia-Almendarez BE,

Prado-Barragan LA, Regalado-Gonzalez C. Optimization of nisin production by *Lactococcus lactis* UQ2 using supplemented whey as alternative culture medium. *Journal of Food Science*. 2010;**75**(6):M347-M353

[139] Gerke C, Kraft A, Sussmuth R, Schweitzer O, Gotz F. Characterization of the N-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. *The Journal of Biological Chemistry*. 1998;**273**(29):18586-18593

[140] Zoll S, Schlag M, Shkumatov AV, Rautenberg M, Svergun DI, Gotz F, et al. Ligand-binding properties and conformational dynamics of autolysin repeat domains in staphylococcal cell wall recognition. *Journal of Bacteriology*. 2012;**194**(15):3789-3802

[141] Chopra L, Singh G, Choudhary V, Sahoo DK. Sonorensin: An antimicrobial peptide, belonging to the heterocycloanthracin subfamily of bacteriocins, from a new marine isolate, *Bacillus sonorensis* MT93. *Applied and Environmental Microbiology*. 2014;**80**(10):2981-2990

[142] Sandiford S, Upton M. Identification, characterization, and recombinant expression of epidermicin NI01, a novel unmodified bacteriocin produced by *Staphylococcus epidermidis* that displays potent activity against *Staphylococci*. *Antimicrobial Agents and Chemotherapy*. 2012;**56**(3):1539-1547

[143] Arguelles Arias A, Ongena M, Devreese B, Terrak M, Joris B, Fickers P. Characterization of amylolysin, a novel lantibiotic from *Bacillus amyloliquefaciens* GA1. *PLoS One*. 2013;**8**(12):e83037

[144] Zhang C, Occi J, Masurekar P, Barrett JF, Zink DL, Smith S, et al. Isolation, structure, and antibacterial activity of philipimycin, a thiazolyl

peptide discovered from *Actinoplanes philippinensis* MA7347. Journal of the American Chemical Society. 2008;**130**(36):12102-12110

[145] Gu Y, Xu Y, Xu J, Yu X, Huang X, Liu G, et al. Identification of novel bacteriophage vB_EcoP-EG1 with lytic activity against planktonic and biofilm forms of uropathogenic *Escherichia coli*. Applied Microbiology and Biotechnology. 2019;**103**(1):315-326

[146] Pires DP, Melo L, Vilas Boas D, Sillankorva S, Azeredo J. Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections. Current Opinion in Microbiology. 2017;**39**:48-56

[147] Colavecchio A, Goodridge LD. Phage therapy approaches to reducing pathogen persistence and transmission in animal production environments: Opportunities and challenges. Microbiology Spectrum. 2017;**5**(3):1-14

[148] Villarroel J, Larsen MV, Kilstrup M, Nielsen M. Metagenomic analysis of therapeutic PYO phage cocktails from 1997 to 2014. Viruses. 2017;**9**(11): pii: E328; 1-22

[149] McCallin S, Alam Sarker S, Barretto C, Sultana S, Berger B, Huq S, et al. Safety analysis of a Russian phage cocktail: From metagenomic analysis to oral application in healthy human subjects. Virology. 2013;**443**(2):187-196

[150] Pallavali RR, Degati VL, Lomada D, Reddy MC, Durbaka VRP. Isolation and in vitro evaluation of bacteriophages against MDR-bacterial isolates from septic wound infections. PLoS One. 2017;**12**(7):e0179245

[151] Lin DM, Koskella B, Lin HC. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World Journal of Gastrointestinal Pharmacology and Therapeutics. 2017;**8**(3):162-173

[152] Twort FW. An investigation on the nature of ultra-microscopic viruses. The Lancet. 1915;**186**(4814):1241-1243

[153] Schofield DA, Wray DJ, Molineux IJ. Isolation and development of bioluminescent reporter phages for bacterial dysentery. European Journal of Clinical Microbiology & Infectious Diseases. 2015;**34**(2):395-403

[154] Abd, El-Aziz AM, Elgamal A, Ali YM. Bacteriophage therapy increases complement-mediated lysis of bacteria and enhances bacterial clearance after acute lung infection with multidrug-resistant *Pseudomonas aeruginosa*. The Journal of Infectious Diseases. 2019;**219**(9):1439-1447

[155] Orlova ZN, Garnova NA. Use of tablet-form polyvalent bacteriophage with acid resistant coating in the treatment of dysentery in children. Voprosy Okhrany Materinstva i Detstva. 1970;**15**(3):25-29

[156] Vinner GK, Rezaie-Yazdi Z, Leppanen M. Microencapsulation of salmonella-specific bacteriophage Felix O1 using spray-drying in a pH-responsive formulation and direct compression tableting of powders into a solid oral dosage form. 2019;**12**(1): pii: E43; 1-14

[157] Endersen L, O'Mahony J, Hill C, Ross RP, McAuliffe O, Coffey A. Phage therapy in the food industry. Annual Review of Food Science and Technology. 2014;**5**:327-349

[158] Janez N, Loc-Carrillo C. Use of phages to control *Campylobacter* spp. Journal of Microbiological Methods. 2013;**95**(1):68-75

[159] Svircev A, Roach D, Castle A. Framing the future with bacteriophages in agriculture. Viruses. 2018;**10**(5): pii: E218; 1-13

[160] Doss J, Culbertson K, Hahn D, Camacho J, Barekzi N. A review of

phage therapy against bacterial pathogens of aquatic and terrestrial organisms. *Viruses*. 2017;**9**(3): pii: E50; 1-10

[161] Eyer L, Pantucek R, Ruzickova V, Doskar J. New perspectives of the phage therapy. *Klinická Mikrobiologie a Infekční Lékařství*. 2007;**13**(6):231-235

[162] Kutateladze M, Adamia R. Bacteriophages as potential new therapeutics to replace or supplement antibiotics. *Trends in Biotechnology*. 2010;**28**(12):591-595

[163] Yacoby I, Bar H, Benhar I. Targeted drug-carrying bacteriophages as antibacterial nanomedicines. *Antimicrobial Agents and Chemotherapy*. 2007;**51**(6):2156-2163

[164] Yacoby I, Shamis M, Bar H, Shabat D, Benhar I. Targeting antibacterial agents by using drug-carrying filamentous bacteriophages. *Antimicrobial Agents and Chemotherapy*. 2006;**50**(6):2087-2097

[165] Bar H, Yacoby I, Benhar I. Killing cancer cells by targeted drug-carrying phage nanomedicines. *BMC Biotechnology*. 2008;**8**:37

[166] Borysowski J, Weber-Dabrowska B, Gorski A. Bacteriophage endolysins as a novel class of antibacterial agents. *Experimental Biology and Medicine* (Maywood, NJ). 2006;**231**(4):366-377

[167] Pohane AA, Jain V. Insights into the regulation of bacteriophage endolysin: Multiple means to the same end. *Microbiology* (Reading, England). 2015;**161**(12):2269-2276

[168] Schmelcher M, Loessner MJ. Bacteriophage endolysins: Applications for food safety. *Current Opinion in Biotechnology*. 2016;**37**:76-87

[169] Wright A, Hawkins CH, Anggard EE, Harper DR. A controlled

clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clinical Otolaryngology*. 2009;**34**(4):349-357

[170] Kutateladze Á, Adamia R. Phage therapy experience at the Eliava Institute. *Médecine et Maladies Infectieuses*. 2008;**38**(8):426-430

[171] Fish R, Kutter E, Wheat G, Blasdel B, Kutateladze M, Kuhl S. Bacteriophage treatment of intransigent diabetic toe ulcers: A case series. *Journal of Wound Care*. 2016;**25**(Sup7):S27-S33

[172] Jun JW, Shin TH, Kim JH, Shin SP, Han JE, Heo GJ, et al. Bacteriophage therapy of a *Vibrio parahaemolyticus* infection caused by a multiple-antibiotic-resistant O3: K6 pandemic clinical strain. *The Journal of Infectious Diseases*. 2014;**210**(1):72-78

[173] Markoishvili K, Tsitlanadze G, Katsarava R, Glenn J, Morris M Jr, Sulakvelidze A. A novel sustained-release matrix based on biodegradable poly (ester amide) s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds. *International Journal of Dermatology*. 2002;**41**(7):453-458

[174] Pouillot F, Chomton M, Blois H, Courroux C, Noelig J, Bidet P, et al. Efficacy of bacteriophage therapy in experimental sepsis and meningitis caused by a clone O25b: H4-ST131 *Escherichia coli* strain producing CTX-M-15. *Antimicrobial Agents and Chemotherapy*. 2012;**56**(7):3568-3575

[175] Soothill J. Treatment of experimental infections of mice with bacteriophages. *Journal of Medical Microbiology*. 1992;**37**(4):258-261

- [176] Biswas B, Adhya S, Washart P, Paul B, Trostel AN, Powell B, et al. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. *Infection and Immunity*. 2002;**70**(1):204-210
- [177] Ramesh V, Fralick JA, Rolfe RD. Prevention of *Clostridium difficile*-induced ileocectitis with bacteriophage. *Anaerobe*. 1999;**5**(2):69-78
- [178] Chanishvili N. Phage therapy—History from Twort and d’Herelle through Soviet experience to current approaches. *Advances in Virus Research*. 2012;**83**:3-40
- [179] Gilmer DB, Schmitz JE, Euler CW, Fischetti VA. Novel bacteriophage lysin with broad lytic activity protects against mixed infection by *Streptococcus pyogenes* and methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*. 2013;**57**(6):2743-2750
- [180] Yang H, Wang D-B, Dong Q, Zhang Z, Cui Z, Deng J, et al. Existence of separate domains in lysin PlyG for recognizing *Bacillus anthracis* spores and vegetative cells. *Antimicrobial Agents and Chemotherapy*. 2012;**56**(10):5031-5039
- [181] Singh PK, Donovan DM, Kumar A. Intravitreal injection of the chimeric phage endolysin Ply187 protects mice from *Staphylococcus aureus* endophthalmitis. *Antimicrobial Agents and Chemotherapy*. 2014;**58**(8):4621-4629
- [182] Lood R, Winer BY, Pelzek AJ, Diez-Martinez R, Thandar M, Euler CW, et al. Novel phage lysin capable of killing the multidrug-resistant gram-negative bacterium *Acinetobacter baumannii* in a mouse bacteremia model. *Antimicrobial Agents and Chemotherapy*. 2015;**59**(4):1983-1991
- [183] Oliveira H, Vilas Boas D, Mesnage S, Kluskens LD, Lavigne R, Sillankorva S, et al. Structural and enzymatic characterization of ABgp46, a novel phage endolysin with broad anti-gram-negative bacterial activity. *Frontiers in Microbiology*. 2016;**7**:208