Chapter

New Approaches for Competing Microbial Resistance and Virulence

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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 anthranilatic 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(1*H*)-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 hybridγ-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-octylidenethiazolidine-2, 4-dione [73] and fatty acyl purified from marine *Streptomyces* sp. [74]. Additionally, meta-bromo-thiolactone is a potent inhibitor of RhiI 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 RNAIII. 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-diflurobenzyl)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 form 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 Rhl) [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 intercellular 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 MIC_{50} 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	*Streptococcus mutans*		Oral biofilm-associated with *Streptococcus sobrinus*, *Streptococcus oralis* [136]
Nisin A	*Lactococcus lactis* subsp. lactis	*Enterococcus faecalis* and *Streptococcus gordonii* [137]	*Listeria monocytogenes* [138]
Gallidermin	*Staphylococcus gallinarum*		*Staphylococcus aureus* and *Staphylococcus epidermidis* [139, 140]
Sonorensin	*Bacillus sonorensis* MT93	*Listeria monocytogenes* and *Staphylococcus aureus* [141]	*Staphylococcus aureus* [141]
Epidermicin NI0	*Staphylococcus epidermidis*	MRSA, *Enterococci* [142]	*Staphylococcus epidermidis* [142]
Amylolysin	*Bacillus amyloliquefaciens* GA1	*Listeria monocytogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [143]	
Philipimycin	*Actinoplanes philippinensis* 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	*Pseudomonas aeruginosa*	Oral administration of phages	Successful treatment	[169]
Typhoid	Human	*Salmonella typhi*	Oral administration of phages	Successful treatment	[170]
Diabetic foot ulcer	Human	*Staphylococcus aureus*	Topical application of phages	Successful treatment	[171]
Sepsis	Murine	*Vibrio parahaemolyticus*	Intraperitoneal and oral administration of phages	Successful treatment	[172]
Pneumonia	Murine	*Pseudomonas aeruginosa*	Intranasal administration of phages	Successful treatment	[154]
Ulcers and wounds	Human	*Proteus vulgaris*	Topical application of phages	Successful treatment	[173]
Meningitis	Murine	*Escherichia coli*	Intraperitoneal or subcutaneous administration of phages.	Successful treatment	[174]
Sepsis	Murine	*Acinetobacter baumannii*	Intraperitoneal administration of phages	Successful treatment	[175]
Bacteremia	Murine	*Enterococcus faecium*	Intraperitoneal administration of phages	Successful treatment	[176]
Ileocecitis	Hamster	*Clostridium difficile*	Oral administration of phages	Successful treatment	[177]
Dysentery	Human	*Shigella dysenteriae*	Oral administration of phages	Successful treatment	[178]
Cholera	Human	*Vibrio cholerae*	Oral administration of phages	Successful treatment	[178]
Pneumonia	Murine	*Streptococcus pneumoniae*	Intraperitoneal administration of Cpl-1 lysin enzyme	Successful treatment	[179]
Bacteremia	Murine	*Streptococcus pyogenes*	Intraperitoneal administration of PlySs2 lysin enzyme	Successful treatment	[179]
In vitro	*In vitro*	*Bacillus anthracis*	Application of PlyG lysin enzyme	Significant reduction in bacterial density	[180]
Endophthalmitis	Murine	*Staphylococcus aureus*	Application of Ply187 lysin as eye drops	Successful treatment	[181]
Bacteremia	Murine	*Acinetobacter baumannii*	Administration of PlyF307 lysin enzyme	Successful treatment	[182]
In vitro	*In vitro*	*Pseudomonas aeruginosa* and *Salmonella typhimurium*	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.

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