Current advances on bacterial pathogenesis inhibition and treatment strategies

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Bacterial pathogenesis is a multi-factorial process that is regulated by the production of virulence factors, enabling bacteria to cause various infectious diseases. For many years, antibiotics have been successfully applied for fast treatment of bacteria-mediated diseases, offering an effective infection transmission control. However, it has become clear in recent years that the overuse of antibiotics leads to an increased emergence and spread of multi-drug resistant microorganisms. As a consequence, bacteria cause life threatening infections and increased mortality, morbidity, length of hospitalization and health care costs. There is a need to decrease the antibiotic usage and to develop new effective antimicrobial strategies to prevent and treat certain infections. This review summarizes recent advances in attenuation of bacterial virulence and treatment of infections. Prevention strategies that provide minimal evolutionary stress and no resistance development such as interference with bacterial cell-to-cell signalling (quorum sensing) pathways and virulence mechanisms are reviewed. Moreover, new advances on antimicrobial agents such as antimicrobial peptides, bacteriophages, nanoantibiotics and natural polyphenols as well as a new strategy of resistance genes disruption using the bacterial adaptive immune system as a potential therapeutic tool, are also considered.

Keywords:bacteria virulence; multi-drug resistance; virulence factors; anti-virulence strategies; emerging therapeutics

1. Introduction

Among the 100 trillion cells that constitute the human body, only 1 in 10 is actually human. The remaining cells are microorganisms such as bacteria and viruses [1]. These microorganisms are harmless and live in perfect balance with human body, playing an important role in supporting and maintaining vital functions such as our immune and digestive systems [1]. However, when this balance is broken and the delicate ecosystems that bacteria carefully construct in different parts of human body are disrupted, bacteria become pathogenic, causing infection diseases. The introduction of antibiotics in the early 20th century initiated a new era in the treatment of microbial infections. They were the most successful drug ever introduced saving countless lives, extending life span and permitting previously deadly medical procedures. To kill bacteria, antibiotics target different cell components and use different mechanisms of action such as the inhibition of cell wall synthesis (lactams and glycopeptides), protein production (macrolides, aminoglycosides, tetracyclines) and nucleic acids synthesis (fluoroquinolones, rifampin) [2, 3]. Antimicrobial agents such as sulfonamides and folic analogues also disrupt essential metabolic pathway for folic acid synthesis followed by the inhibition of DNA synthesis [3].

Although antibiotic strategies are highly effective in the treatment of bacterial infections, they have been responsible for a substantial evolutionary stress caused on bacterial population and the emergence of drug and multi-drug resistance [2, 3]. Dangerous bacterial species such as the methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) have emerged due to antibiotic overuse. This resistance development placed the bacterial infections as a leading cause of death worldwide and is now one of the greatest challenges of the twenty-first century.

Nevertheless, the increasing understanding of bacterial pathogenesis and intercellular communication has been a valuable tool to develop new strategies in the treatment of bacteria-mediated diseases. Alternative approaches through inhibition of bacterial pathogenesis thus causing less evolutionary stress on bacteria population have been also studied. This review provides a brief overview of bacterial virulence as a new target for attenuation of bacterial pathogenesis and treatment of the acquired infections. Promising anti-virulence strategies based on the interference with the virulence factors disruption are outlined. As alternative therapeutic approaches special focus is given to the antimicrobial peptides, bacteriophages, natural polyphenols and nanoantibiotics as promising antimicrobial agents, in addition to a novel strategy that targets resistance gene disruption in bacteria.

2. Bacterial pathogenesis and the virulence mechanisms

During pathogenesis bacteria use an array of virulence factors to cause disease in the human host, acting individually or simultaneously at different stages of the infection [4]. This is a multi-factorial process that depends on the immune status of the host, the nature of the species or strains (virulence factors) and the number of organisms in the initial exposure [5]. Once bacteria find the suitable site for colonization they initiate the expression of target genes followed by the production of virulence factors that invade the host cells and trigger the infection process [2]. These virulence factors include: i) membrane proteins that play an important role in adhesion and binding to host cells, facilitating the

colonization, biofilm formation and intercellular communication [6]; ii) secretory proteins such as toxins, which kill or change signal transduction in mammalian cells and are responsible for some host cell-bacteria communication [7]; iii) specialized secretion systems that resemble a syringe and are used by bacteria to inject toxins (effectors) into the host cell [8]; iv) polysaccharide capsules that surround the bacterial cell and have anti-phagocytic properties [9]; v) cell wall and outer membrane components, like peptidoglycan layer and lipopolysaccharide, mainly in Gram-negative bacteria that protect against complement-mediated lysis and are potent inducer of inflammation [10]; and vi) a group of other virulence factors that include proteins involved in biofilm formation and siderophores [4]. Biofilm formation confers pathogenic bacteria increased resistance to convectional antibiotics and host defences mechanisms [11]. Pathogenic bacteria, such as *P. aeruginosa, E. coli*, *Staphylococci* and *Mycobacterium* are able to form biofilms on living (lungs, burn wounds and urinary tract) or nonliving surfaces, such as distinct medical devices (indwelling catheters, artificial hips and contact lenses) causing intractable infections [11-13]. Understanding how pathogenic bacteria use virulence factors to interact with their hosts and originate the disease is a prerequisite to define new targets for vaccines and drug development.

3. Inhibition of bacterial pathogenesis - anti-virulence strategies

A novel approach for the inhibition of bacterial pathogenesis and treatment of bacterial infections involves targeting the virulence factors. These anti-virulence strategies interfere with the process of infection before the host damage occurs. Bacterial virulence is blocked specifically, without killing or inhibiting bacterial growth, causing less than the traditional antibiotics evolutionary pressure for the development of resistant genes. Moreover, by preventing the expression or activity of virulence traits, the bacteria are less prone to colonize the host. Among the main anti-virulence strategies are these targeting the inhibition of: i) toxins; ii) bacterial adhesion to the host cell; iii) specialized bacterial secretory systems; iv) organism-specific virulence gene expression; and v) organism-specific cell-to-cell signalling (i.e. quorum-sensing) (Table 1). By targeting these virulence factors the infection progression is interrupted and the host immune system is able to eliminate the pathogen. These strategies are discussed below.

Table 1 Anti-virulence strategies

Targets	Anti-virulence agents	Mode of action	Reference
Toxins production	Antibodies	Neutralize anthrax toxin	[14]
	Toxin analogues	Block toxin activation	[15]
Bacterial adhesion	Pilicides	Interfere with pilus formation	[16]
	Mono- and oligosaccharides	Block carbohydrate - specific site of pili/fimbrae	[17, 18]
Specialized secretory systems	Inhibitory molecules	Inhibit toxin secretion, interaction with host cells or functional assembly	[19]
Quorum sensing	Gram – negative bacteria:		
	SAM analogues	Block AHLs production	[20]
	Enzymes	Inactivation of AHLs	[21-25]
	Antagonists	Target transcriptional receptor inhibition	[26-28]
	Gram – positive:		
	Apolipoprotein B	Inactivate AIPs	[29]
	AIP analogues	Block membrane associated receptors	[30, 31]

3.1. Inhibition of bacterial toxins

Toxins are usually identified as the first virulent factors to be secreted during bacterial pathogenesis acting directly or indirectly on the host cells [2, 4, 10]. The most studied toxin systems are those of *B. anthracis* (anthrax toxin) and *E. coli* (shiga toxin). They are known as AB toxins and consist of two subunits A and B with distinct functions in infection development. B-binding subunit is crucial for bacterial adhesion and binds to a specific host receptor, while A-active subunit exhibits selective enzymatic activity and injures the host cells [2, 10]. Understanding the mechanisms that toxins use to interact with the host has been a valuable tool to define several inhibition strategies and combat bacterial pathogenesis. For example, the neutralization of the toxins using monoclonal antibodies has been considered as an attractive treatment approach providing rapid and extensive protection [19, 32-34]. Monoclonal antibodies isolated from chimpanzees were shown to inhibit the binding subunit of anthrax toxin therefore preventing and treating the anthrax infection [14]. Moreover, analogues of the toxin binding subunits successfully blocked the activation of A subunit and

prevented the invasion of host cells [15, 35]. Current strategies to attenuate pathogenesis involve the blocking of the intracellular uptake of the active part of the toxin or the inhibition of its active site [36].

3.2. Inhibition of bacterial adhesion

Many pathogenic bacteria use specific structures such as pilus, fimbrae and flagella to attach and colonize the host cells [37-39]. For instance, uropathogenic *E.coli,* which cause life–threatening urinary tract infections, use a long multisubunit appendage (pilus) to attach more efficiently to the urogenital epithelia and to prevent its washing by the urine flow [40]. This mechanism of action indicates that by affecting the bacterial adhesion a decrease of the risk of infections development may be attained. Several compounds, called pilicides were shown to interfere with pilus formation resulting in a non-functional structure that fails to bind the host and consequently decrease the bacterial colonization and prevent the pathogenesis [16]. Mono or oligosaccharides that interact specifically with the carbohydrate–specific site on the pili/fimbrae structures were also able to block the bacterial adhesion [17, 18].

3.3. Inhibition of bacterial secretory systems

During pathogenesis bacteria use secretory systems to transport and inject the toxins (effectors) into target cells. These systems have been considered as potential targets for novel anti-virulence therapeutic agents [2, 17]. Inhibition of the secretory systems has been accomplished using small molecules that prevent their functional assembly, toxins secretion or interaction with the host cells [2, 41].Various studies revealed that some compounds were capable of inhibiting the secretion systems and attenuate the virulence of *S. typhimurium*, *Pseudomonas*, *Francisella* and reduce their pathogenesis [42]. For example, acylated hydrazones of salicylaldehydes were found to efficiently inhibit the secretory system of *Chlamydia* and prevent pathogenesis [43].

3.4. Inhibition of organism-specific virulence gene expression

Targeting the virulence gene expression has been mainly researched on *V. cholerae*. This bacterium is responsible for some of the largest episodes of diarrhoeal disease pandemics and different approaches to treat the infection that it causes have been extensively studied. During infection, *V. cholerae* secretes two virulence factors: the cholera toxin, a protein that causes watery diarrhoea and the toxin-coregulated pilus (TCP), a thin, flexible, filamentous appendage on the surface of bacterial cells that colonize the small intestine [44]. A small molecule 4-[N-(1,8-naphthalimide)]-n-butyric acid (virstatin) was shown to efficiently inhibit virulence regulation in *V. cholerae* [45]. This was the first study showing that a targeted anti-virulence approach could prevent cholera disease in animal models and the mechanism by which this inhibition occurs has been since then studied extensively [46].

3.5. Quorum sensing inhibition strategies

The expression of the virulence factors in important human and plant pathogens such as *P. aeruginosa*, *S.aureus*, *E.coli, S. typhimurium, Erwinia*, and *A. tumefaciens,* among others is regulated by a process called quorum sensing (QS) [47-49]. QS allows bacteria to regulate community-wide behaviours including biofilm formation, virulence, conjugation, sporulation, and swarming motility [50]. This is possible due to a mechanism of cell-to-cell communication that is based on the production, secretion, and detection of small signalling molecules, called autoinducers (AIs). The QS systems used by Gram-negative and Gram-positive bacteria differ in the type of QS signalling molecules they use and in the signal transduction systems (Fig. 1). The most intensively studied AIs used by Gram–negative bacteria are acyl homoserine lactones (AHLs) (Table 2). The AHLs signalling molecules are produced by AHLs synthases, which use adenosyl-methionine (SAM) as a source for lactone ring formation and acyl-carrier proteins (ACP) as a source for the side fatty acid chain of AHLs. On the other hand, Gram-positive bacteria use linear or cyclic oligopeptide signals, called autoinducing peptides (AIPs) (Table 2) [12]. These AIPs are produced as precursor propeptides in the intracellular compartment, further processed by a membrane-bound endopeptidase (Fig.1-green circle) and secreted to the extracellular environment as mature AIPs [51].

Targeting QS systems in Gram-negative and Gram-positive bacteria constitutes a novel pharmacological approach to control bacteria virulence and biofilm formation. In recent years, the development of new anti-quorum sensing drugs that have the advantage to affect bacterial behaviours, but do not kill or inhibit their growth has been gaining ground [50, 52-54]. This would allow the host defense system to eliminate attenuated bacteria or substantially increase the effect of co-administered antibiotics. Different strategies aiming the QS signalling interruption in Gram-negative and Gram-positive are discussed below.

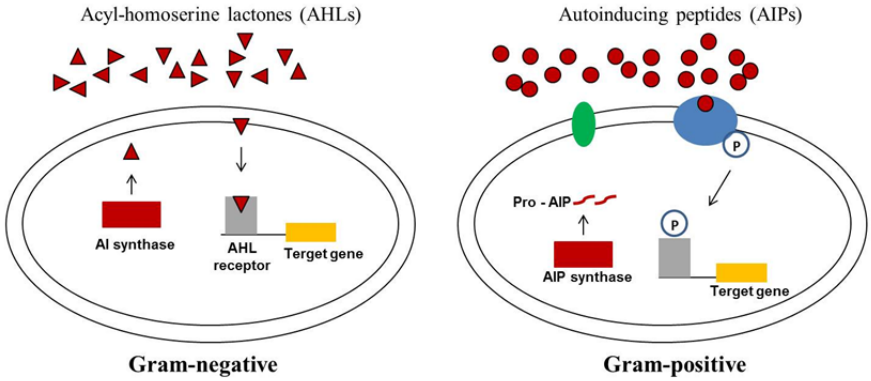


Fig. 1 Quorum sensing systems in bacteria. Gram-negative bacteria (left) secrete AHLs (red triangles) that in threshold concentrations penetrate into the cells and activate the cognate AHL receptor and induce the QS regulated genes expression. Gram-positive bacteria (right) produce mature AIPs (red circles) that further interact with a transmembrane histidine kinase receptor activating the target gene expression via autophosphorylation of the trascriptional regulator.

3.5.1. Gram-negative bacteria

Blockage of AIs synthesis

A potential target for inhibiting QS pathway is the blockage of AHL production in Gram-negative bacteria. This inhibition strategy is the least investigated and relies on the development of structural analogues of the substrates for AHL synthases - S-adenosyl methionine (SAM) and acyl-carrier protein (ACP) [55]. Structural analogues of SAM such as L/D-S-adenosylhomocysteine and sinefungin have been found to suppress the AHL synthesis and inhibit the first step in QS signalling [20]. The inhibitory activity of these analogues has been proved only *in vitro* and they have not been tested *in vivo* yet. Other enzymes in living systems, however, also use SAM and its analogues could potentially cause side effects [53, 55].

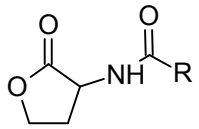
Inactivation of AIs in Gram-negative bacteria

The inactivation of AHLs by degrading them has been regarded as a promising strategy for pathogenesis inhibition. AHLs degradation does not allow signal accumulation in extracellular environment and consequently QS-regulated virulent genes are not expressed [47]. Altering AIs in extracellular surrounding provides minimal evolutionary stress on pathogens and would eliminate the risk of resistance development. Enzymes such as acylase, lactonase, and oxidoreductases are known to selectively inactivate AHLs involved in Gram-negative bacteria QS communication systems (Fig. 2).

First evidences that these enzymes were able to quench QS signals was attained with lactonase- and acylase-producing bacterial species such as *Bacillus sp.* 240B1, *Bacillus* strain COT1, *A. tumefaciens, B. thuringiensis, Arthrobacter* sp. IBN110, *B. thuringiensis*, *B. cereus*, *B. mycoides, V. paradox*, *Anabaena*, *Ralstonia*, *Rhodococcus.* The enzymes extracted from these bacteria were capable of degrading AHL molecules [21, 54, 56] and have been also found to interrupt the QS signalling of Gram-negative bacteria in a process called quorum quenching (QQ) [21, 57]. Dong *et al*. reported an AHL-lactonase produced by *Bacillus sp*. and encoded by the *aiiA* gene, that was able to degrade the ester bond in the AHL lactone ring [21]. In fact, the expression of *aiiA* gene encoded in plants has shown to attenuate the virulence of the plant pathogen *E. carotovora*. *In vitro* studies demonstrated that lactonase effectively degraded a range of AHL derivatives and could be used to quench QS and prevent pathogenesis [21, 58]. Moreover, there is evidence that lactonase and antibiotics applied together can be used to block not only the pathogenesis but also the multi-drug resistance in clinical isolates such as *P. aeruginosa* [59]. By cleaving the amide bond between the homoserine lactone and the fatty acid chain the acylase-producing bacteria *V. paradoxusm* was also able to degrade AHL signals [22, 56]. Lin *et al.* have demonstrated that the expression of acylase encoded by genes from *Ralstonia* in pathogenic *P. aeruginosa* PAO1 efficiently suppressed the production of QS regulated virulence factors (elastase and pyocianin), decreased its swarming motility and attenuated the pathogenesis to *C. elegans* [22]. Lately, Huang *et al.* demonstrated that the soil *P. aeruginosa* PAI-A are also capable to degrade long AHLs as a sole energy source for growth in selective conditions [60]. Acylase I isolated from kidney was also shown to be capable to deacylate *in vitro* model AHL molecules including *N*-butyryl- and *N*-octanoyl-l-homoserine lactones, and thereby inhibit the QS pathway in *P. aeruginosa* and reduce the formation of pathogenic biofilms on a polystyrene surface [23].

Table 2 Chemical structure of natural autoinducers.

Gram - negative
Acyl homoserine lactones

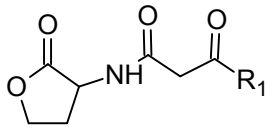


R=C_3H_7
P. aeruginosa; A. hydrophila; A. salmonicida [61, 62]

R=C_3H_6OH
V. harveyi [63]

R= C_5H_{11}
C.violaceum;P.aureofaciens; Y. pseudotuberculosis [64-66]

R=C_7H_{15}
V. fischeri; *B. cenocepacia* [67, 68]



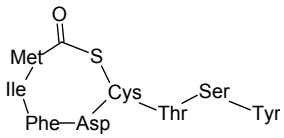
R_1=C_3H_7
V.fischeri;E.carotovora;E. chrysanthemi [67, 69,70]

R_1=C_5H_7
A.tumefaciens [71]

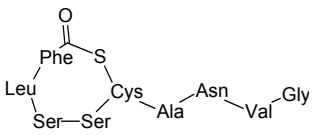
R_1=C_7H_{15}
P.putida; Yersinia pestis [72, 73]

R_1=C_9H_{19}
P. aeruginosa [61]

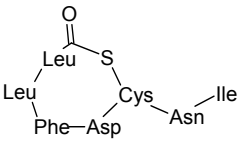
Gram - positive
Auto-inducing peptides



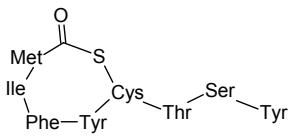
AIP-I



AIP-II

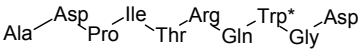


AIP-III

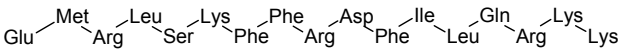


AIP-IV

(*S.aureus*)



ComX *(B. subtilis)* [74]



CSP (*S. mutans*) [75]

Another group of QQ enzymes that has been reported includes oxidoreductases produced by species as *R. erythropolis, Burkholderia sp. GG4* [56, 76]. Oxidoreductases "confuse" bacterial signalling pathways through the modification of the 3C keto group of the acyl side chain of AHLs into hydroxyl group [76]. Upon modification, AHL fails to bind to the cognate transcription regulator and further activation of QS regulated genes do not occur [56]. An oxidoreductase produced by *Burkholderia sp. GG4* has also been demonstrated to modulate the signalling molecule 3-oxo-C6-HSL and attenuate *E. carotovora* virulence [24].

Another family of human enzymes called paraoxonases (PONs) was recently reported to exhibit lactonase activity disrupting AHL-mediated QS systems [77]. Teiber *et al.* reported PONs activity on QS molecules produced by *P. aeruginosa* and other species including *Burkholderia*, *Yersinia*, *Serratia* and *Aeromonas* [25].

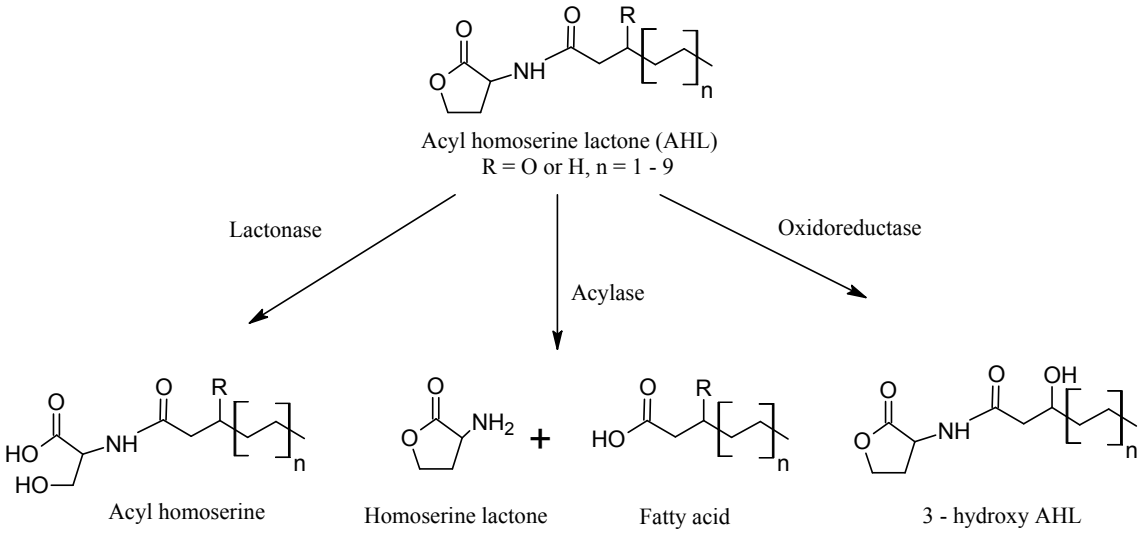


Fig. 2 Enzymatic degradation of AHL signals by AHL-lactonase and AHL-acylase. Oxidoreductases inactivate AHL by substituting the oxo group at the C3 with hydroxyl group [21].

Blocking of the receptors in QS pathways

Although the mechanism of AHLs binding to the receptors is not clear, numerous compounds have been reported to block the binding of these QS molecules to the cognate receptor in QS signalling [53]. Some examples of different strategies used to block the receptors include the development of antagonists that are based on: i) AHL analogues, ii) structurally unrelated antagonists, and iii) natural QS inhibitors.

Several reports have been published concerning the AHL analogues ability to block the receptors and therefore act as receptor antagonists. The development of these analogues is usually achieved through modification of the AHL acyl side chain, lactone ring or both the lactone ring and the side chain [55]. Strategies such as: the replacement of the C3 atom by a sulphur atom [78], the introduction of aromatic side chains such as phenylpropionyl and phenoxyacetyl groups or of a bulky group [79], the replacement of the AHL carboxamide bond with sulfonamide bond, or even the introduction of sulfone or sulfoxide group have been regarded as effective approaches to induce antagonistic activity [53, 80]. Development of AHL antagonists through lactone ring modifications have been less reported. By changing the lactone functional group with ketones, alcohols and amines has shown to provide antagonistic effect against the transcriptional regulators in *P. aerugionosa* [53]. Ishida *et al.* synthesized acyl cycloalkylamide analogues of AIs and observed that *N*-octanoyl cyclopentylamide and its structural analogue *N*-decanoyl cyclopentylamide interfered with AHL-mediated QS systems in *P. aeruginosa* [26].

Other compounds structurally unrelated to AHL were screened using bioassay reporter strains for their capability to inhibit bacterial quorum sensing. Several chemical compounds including 4-nitro-pyridine-*N*-oxide (4-NPO), P-benzoquinone, 2,4,5-tri-bromo-imidazole and 3-nitro-benzen-sulfonamide were found to successfully interfere with QS. Among these chemical antagonists, the most effective was 4-NPO [55]. The 4-NPO significantly reduced the expression of virulence genes regulated by QS systems in *P. aeruginosa* and also decreased its biofilm formation [27, 55].

Numerous quorum sensing inhibitors (QSI) have been extracted from natural sources such as plants, herbs and fungi [55]. Plants and fungi naturally produce QS inhibitors as their first line of defence against pathogenic bacteria, inhibiting their colonisation and attenuating their virulence [55]. The screening of QSI produced by *Penicillium* species revealed that penicillic acid and patulin produced by *Pe. radicicola* and *Pe. coprobium,* are the most effective antagonists of LasR and RhlR receptors of *P. aeruginosa* QS systems [55]. Moreover, *in vitro* study of *P. aeruginosa* biofilm formation in presence of patulin and tobramycin, showed increased sensitivity to antibiotics [78]. A number of QS inhibitors extracted from fruits have proved to interfere with bacterial QS and considered as alternative anti-infective agents. For instance, studies performed *in vitro* using *V. harveyi* screening bioassay have shown that the grapefruit juice inhibited AHLs signalling [81]. Further investigation showed that furocoumarins, presented in grapefruit could also prevented the biofilm formation of *E. coli* O157:H7, *S. typhimurium* and *P. aeruginosa* [81]. Rasmussen *et al.* reported a number of plants and herbs extracts including garlic, carrot, bean, water lily, chamomile, habanero and propolis capable to suppress the QS regulated virulence genes. The most effective was found to be the garlic extract containing at least three different inhibitors of the QS pathway. Moreover, a synergistic effect of the garlic extract and tobramycin was also reported to reduce biofilm formation [28]. Adonizio *et al.* demonstrated that extracts of several south Florida medical plant species including *B. buceras C. erectus*, *C. viminalis* attenuated *P. aerugionsa* PAO1 pathogenesis inhibiting the virulence factors production [82]. Moreover, plant-derived polyphenols have been also shown to affect the QS and biofilm formation. The inhibition mechanism of QS by these compounds is still not well understood. However, there are evidences that (-)-epigallocatechingalate (EGCG), ellagic acid, tannic acid, and

pyrogallol interfere with AHL-dependent QS systems [78]. EGCG showed significant inhibitory effect on pathogenic *E. coli* O157:H7 suppressing the QS-regulated genes to express the virulence factors [83]. Another extracts form *Camellia sinensis* (Green tea) demonstrated anti-QS activity modulating the expression of virulence factors in *P.aeruginosa* PAO1[84].

The halogenated furanone compounds (or fimbrolides) are a largely investigated group of QS inhibitors isolated from red macroalga *D. pulchra* [53, 78]. This alga produces more than 30 furanones as secondary metabolites that were shown to interfere with AHL-based QS signalling systems by inhibiting the swarming motility of *S. liquefaciens* and *P. mirabilis* [78]. Indeed, natural furanones have been shown to target QS systems also in *V. fischeri*, *V. harveyi*, *S. ficaria* and other bacteria [53]. The furanone - (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H) produced by *D. pulchra*, in particular, inhibited pathogenic phenotypes of *E. coli* such as swarming motility and biofilm formation [85]. Nevertheless, natural furanones with significant activity over a number of bacterial species failed against P. *aeruginosa*. In contrast, synthetic derivatives of naturally occurring furanones (i.e. halogenated furanones) increased the survival time of mice infected with lethal *P. aeruginosa* [86]. It has to be pointed out, however, that the halogenated furanones are too reactive and might be toxic to the human cells [87].

QSIs (antagonists) are not intrinsically antimicrobial agents, but rather cause the bacteria to be more susceptible to antimicrobials and raise the immune responses of the host. Moreover, their combination with classical antibiotics and/or novel antimicrobials is a possible strategy to generate new hybrid therapeutics with complimentary modes of action.

3.5.2. Gram-positive bacteria

The interference with AIP-mediated quorum sensing systems in Gram-positive bacteria relies on different targets. Gram-positive bacteria possess a two–component QS system consisting of a membrane-bounded histidine kinase receptor and a responsive regulator (Fig. 1). Other components of AIP-mediated signalling systems including AIP synthases, activators, efflux AIP transport systems, transcriptional regulators are also considered as targets [53]. Understanding the mechanisms of receptor activation by QS molecules have led to the development of new QSI that shut down the entire QS pathway and attenuate bacterial pathogenesis.

Peterson *et al.* reported apolipoprotein B as a sequester of AIPs from *S. aureus* preventing the activation of the receptor and therefore the expression of virulence genes [29]. Several AIP-mediated QS systems have been extensively studied including the agr system of pathogenic *S. aureus* and the agr-like system of *E. faecalis,* both possessing structurally similar AIP signals. The agr–quorum sensing system of *S. aureus* uses various thiolactone – containing peptides to control the pathogenesis [2, 53]. This system consists of an AgrC histidine sensor kinase receptor that selectively interact with AIP, which results in the activation of the AgrA transcriptional regulator via phosphorylation [53].

Based on the discovery that the AIP protein side chain (also called tail) is crucial for receptor binding and activation, it is reasonable to think that its synthesized analogues might inhibit bacterial signalling [53]. Otto *et al.* reported different AIPs derivatives of *S. epidermidis* that successfully suppressed the agr–controlled production of the virulence factor δ-toxin and α–toxin, and it did not affect the bacterial growth [30]. *S. aureus* uses second signalling system that regulates the activation of agr-system, known as RNA–III activating peptide (RAP) mediated system [53, 55]. The (RAP)–mediated pathway was successfully disrupted by the RNA III inhibiting peptide (RIP), a heptapeptide originally isolated from *S. xylosus* [31, 88]. *In vivo* studies have demonstrated the inhibitory activity of synthetic RIP (amide form of the originally isolated one) by reducing *S. aureus* infections such as cellulitis, septic arthritis, keratitis, osteomyelitis and mastitis [88, 89]. A synergistic effect of RIP and antibiotics has been also reported to act against biofilm formation of *S. aureus* [88]. There is evidence that a non peptide analogue of RIP, known as 2′,5-di-*O*-galloyl-D-hamamelose (hamamelitannin), a natural product of *Hamamelis virginiana* (witch hazel) also interferes with the QS pathway in *S. aureus* and *S. epidermidis*, and reduces the risk of infection [90].

The greatest advantage of the discussed anti-virulence strategies targeting virulence factors and signalling pathways in pathogenic bacteria is the reduced evolutionary pressure for emergence of resistance. Those strategies affect only the pathogens and reduce the risk for harmful to the mammalian cells side effects, unlike the conventional therapeutics. An interaction, however, with beneficial microbiota in human gastrointestinal tract should not be excluded. It is also likely that bacteria may develop resistance to anti-virulence agents using alternative routes. Therefore, the understanding of bacterial virulence is a key issue for the rationale design of such therapeutic agents. Creating anti-virulence drugs that are efficient against broad spectrum of bacteria without adverse side effects is highly challenging.

4. Emerging therapeutic strategies

In contrast to the anti-virulence strategies that interfere with the process of infection before the host damage occurs, therapeutic strategies are intended to act when the infection is already established. Alternatives to the antibiotic treatments are presented below.

4.1. Antimicrobial peptides

Antimicrobial peptides (AMPs) have long been considered as efficient alternatives to conventional antibiotics to fight multi-drug resistant pathogens and to prevent pathogenic biofilm formation [91]. These compounds were initially identified as host defence modulators in organisms such as frogs and insects and further isolated from tissues and cell types in many other living organisms [91-93]. AMPs share common properties including: a net positive charge under physiological conditions, amphiphilicity containing cationic and hydrophobic amino acids [91, 94] and efficiency in low concentrations [95]. Despite their mode of action is not fully understood, it is known that these compounds possess different mechanisms of action towards mammalian and microbial cells [93]. It is believed that upon contact with microbial cell membrane, AMPs form a secondary structure, allowing insertion of hydrophobic components into the membrane lipid domains, which results in disruption of the membrane structure. They are extremely rapid in killing bacteria and decrease the risk of resistance development compared to conventional antibiotics. Disruption of cell membrane can be achieved by different pores-forming mechanisms such as barrel-stave, toroidal pore, and carpet models [93, 96]. AMPs are also reported to interfere with other intracellular mechanisms including inhibition of cell wall synthesis, protein inhibition or action on DNA or RNA [96].

Protegrins, a natural AMP isolated from porcine leucocytes, was shown to possess broad-spectrum antibacterial activity against *E. coli, S. aureus, P.aeruginosa, C. trachomatis, N. gonorrhoeae*, yeasts (*C. albicans*) and viruses (HIV-1) by interacting with their cell membranes [94]. Magainins found in the skin of Xenopus laevis, also exhibited broad-spectrum selective antibacterial activity against Gram-negative and Gram-positive bacteria while analogues of magainins were active against malignant melanoma *in vivo* and effectively treated *E. coli* infections in mice [97]. Arenicins, AMPs produced by marine polychaeta *Arenicola marina* showed bactericidal properties against pathogenic Gram-positive bacteria such as S. *aureus, L. monocytogenes, B. megaterium*, and Gram-negative bacteria such *E. coli* and *A. tumefaciens* and against fungus *C. albicans* [98]. The combination of AMPs with conventional antibiotics have been shown to induce a synergistic effect preventing drug-resistance development and reduce the treatment antibiotic dosage [91]. Anantharaman *et al.* demonstrated the synergistic effect of designed antibacterial peptides with non-peptide antibiotics such as rifampin and kanamycin against pathogenic *E. coli* [99]. In fact, the number of studies that focus on antibiotic and AMPs combinations as an alternative approach to control multi-drug resistance has been increasing. Enhanced antibacterial effect against opportunistic human pathogen *P. aeruginosa* was observed *in vitro* when tachyplesin III and colistin were applied together, and *in vivo* when alpha-helical antimicrobial peptides and rifampicin were combined [100, 101].

AMPs have also been reported to act upon bacterial biofilm. Wang *et al.* showed that chrysophsin-1, a cationic antimicrobial peptide, could be used as an alternative drug in preventing and treating pathogenic *S. mutans* biofilms, the major causative agent for dental caries [102]. Natural AMP, called LL-37 and several of its synthetic fragments successfully inhibited biofilm formation of *P. aeruginosa* PAO1 strain [103]. Moreover, several bovine AMPs at sub-inhibitory concentration did not exhibit bactericidal effect against bacteria, but were able to inhibit biofilm formation. These peptides were shown to be efficient agents to eradicate pre-formed biofilm and for the treatment of cystic fibrosis diseases [104].

There are evidences that bacteria can also develop resistance to AMPs by proteolytic inactivation or changes in the cell membrane surfaces. It is believed, however, that the process is longer and less common when compared with conventional antibiotics [91]. Nevertheless, the mechanisms of AMPs interaction and the potential resistance development have to be well investigated [92].

4.2. Polyphenols protective agents and therapeutic potential

Polyphenols are bioactive compounds extracted from plants that have numerous pharmacological properties, such as antimicrobial, antioxidant, antiviral and anticancer activities [105]. Epidemiological studies suggests that increased dietary intake of polyphenol-rich fruits and vegetables promote human health [106].

The antibacterial activity of phenolic compounds is thought to be due to a direct interference with bacterial growth or through the inhibition of virulence factors production, resulting in attenuated pathogenesis [107]. For instance, Panduratin A is a natural chalcone compound derived from the rhizomes of fingerroot (Boesenbergia rotunda) that was shown to exhibit antimicrobial activity against clinical isolates of *Staphylococcus* and *Enterococcus* (*E. faecalis and E. faecium*), with a MIC about 2 µg/mL [108]. Epigallocatechin-3-gallate, a derivative of non-flavonoid gallic acid, was shown to possess a broad spectrum of antibacterial activity against both Gram–positive (meticillin-resistant *S.aureus* and *S. mutans*) and Gram–negative bacteria [109, 110]. Polyphenolic compounds have also been shown to act synergistically with antibiotics. Flavonoids combined with antibiotics have been shown to be effective against drug-resistant pathogens by decreasing the antibiotic concentration dosage and eliminating the target pathogen [111]. Moreover, evidences that polyhenolic extracts are also promising anti-biofilm agent due to their ability to inhibit bacterial growth and virulence factors production have been arising. Cranberry polyphenolic extracts were shown to prevent initial adhesion and further maturation of *E. coli*, *E. faecalis* and specific uropathogenic isolates of P-fimbriated *E.coli* [112, 113]. Non-flavonoid gallic acid, in particular, eradicated *E. coli, P.aeruginosa, S. aureus* and *L.*

monocytogenes biofilms in more than 70 % as well as inhibited the bacterial motility [114]. In vitro experiments using biofilm-producing enterococcal strains showed strong anti-biofilm activity of Panduratin A [108].

4.3. "Nanoantibiotics" a new trend for treating infections

Nanotechnology is another approach for the development of novel non-traditional antimicrobial agents. This new paradigm for the effective treatment of infectious diseases designs new antimicrobial drugs, called nanoantibiotics, that possesses many advantages over other antimicrobial agents including increasing effectiveness against drug resistant species, lack of adverse effects and overcoming resistance development interfering with multiple biological pathways [115]. These nanoantibiotics either show antimicrobial activity by themselves or elevate the effectiveness and safety of conventional antibiotics administration creating high local concentrations [116, 117]. Antimicrobial NPs could cause mechanical perturbation of the bacterial membrane and even access the cell "unrecognized" as a treat by the defense system of the bacteria.

Metal and metal oxides nanoparticles (NPs) such as zinc, silver, gold, aluminum, copper, zinc oxide, titanium oxide, nitric-oxide have been studied for their antibacterial ability [118-120]. The mechanisms of action by which these NPs kill bacteria involve: i) production of reactive oxygen species e.g. OH^-, H_2O_2, and O_2 (ROS), ii) disturbance of the bacterial cell wall membrane, iii) inhibition of intracellular enzymes activity and DNA synthesis, and iv) interruption of energy transduction [121, 122]. Silver has always been considered as a potent antimicrobial agent and in the form of nanoparticles its unique properties are magnified [123]. Silver particles were capable to inhibit the bacterial growth and biofilm formation of pathogenic *E. coli* and *P. aeruginosa* PAO1[124]. With a broad-spectrum of antibacterial activity ZnO NPs were highly effective against medically relevant bacteria species. The mechanism of antibacterial action of ZnO is thought to be due to ionic zinc or reactive oxygen species (ROS) generation upon contact with bacteria [125].

Apart from bearing antimicrobial activity on its own, NPs are also promising platform for efficient antibiotic delivery due to their unique physicochemical properties including small and controllable size, functionalizable structure and increased surface to volume ratio, which allow high drug loading and better interaction with bacterial and host cells [122]. Moreover, nanocarriers induce targeted delivery, better solubility of water insoluble drugs, prolonged drug circulation and longer therapeutic effect [126]. Different NP platforms such as liposomes, polymeric NPs, solid lipid NPs and dendrimers, among others, have been used to facilitate the delivery of antimicrobials to the infection site [127]. For instance, when antibiotics such as penicillin, gentamicin, streptomycin, or ciprofloxacin are encapsulated in liposomes, an improved antibacterial activity against multi-drug resistant bacteria is observed in comparison to their free forms. Gentamicin encapsulated in polyethyleneglycol (PEG) liposomes had an enhanced therapeutic effect against resistant *K. pneumonia in vitro*. Liposome-encapsulated tobramycin showed antibacterial activity at sub-MIC concentrations against several bacteria including *P. aeruginosa*, *E. coli* and *S.aureus* [128]. Although the nanoantibiotics constitute promising strategies to overcome antibiotic resistance and treat the infectious diseases the clinical use of NPs in recent years appears to be limited by their potential toxicity [129].

4.4. Disruption of resistant genes

Recently, systems called clustered regularly interspaced short palindromic repeats (CRISPR) have been identified in prokaryotes. CRISPR function as a bacteria prokaryotic immune system, playing an important role in their resistance to exogenous genetic elements such as phages and other invaders (plasmids) [130, 131]. The resistance development occurs when the short sequence of an invader genetic material is inserted in CRISPR sequence array. The RNA transcribed from the CRISPR array is then processed by Cas proteins into RNA-based spacers in a mechanism known as interference stage of action of CRISPR/Cas sytem [132, 133]. The spacers are often acquired from plasmid and phage DNA sequences. This acquisition of spacers into the bacterial CRISPR array guides the system to constantly cleave nucleic acid molecules harboring these sequences. Thus, the system is competent in adaptively and specifically targeting invaders. CRISPR/Cas adaptive immune system can also target antibiotic resistant genes. In recent years this system has been studied in order to decrease the acquirement of antibiotic resistant genes after its transfer in certain species [134]. There are some evidences that rational designing of the system based on the insertion of short DNA sequences may be used to specifically target desired DNA molecules, such as those encoding resistance determinants and therefore eliminate the spread of antibiotic resistance [132, 135]. Marraffini *et al.*have shown that interfering with CRISPR prevents the routes of plasmids carrying the resistant genes and eliminates the spread of resistance in pathogenic *staphylococci* [136].

4.5. Bacteriophage therapy to treat infections

The discovery of bacterial viruses, called bacteriophages or phages, by Frederick and Félix d'Hérelle in 1915 and 1917, marked the beginning of a treatment strategy of bacterial infections. The early clinical studies using phage therapy have only been carried out in Eastern Europe and the results published mainly in Russian, Georgian, and Polish journals [137]. In these countries, phage therapy has been successfully applied to cure bacteria-mediated infections since the

beginning of their discovery [138]. To treat various infections, formulations including tablet for administration, liquids for spraying or injection have been developed [139].

The appearance of antibiotic resistant bacteria and subsequent severe consequences on humans health renewed the interest in bacteriophages as promising alternative antibacterial agents [140, 141]. Bacteriophages possess a polyhedral protein coat, called capsid, that encapsulates nucleic acids either DNA or RNA, and a tail that is required for the attachment and invasion of the host cells [141, 142]. The phages recognise specific receptors on bacterial surface inject their genetic material into the cells, start to multiply and at the end of its growth cycle kill bacteria via lysis [13, 142]. Following cell lysis, virions (e.g. virus particles) are released that can infect bacteria located on other sites in the body [141]. This means that the phages can increase their number in the presence of the target, an interesting phenomenon not observed among antimicrobial agents [141]. Other advantage of phage therapy is the specific phage-bacteria interaction targeting only harmful bacteria without affecting beneficial and/or human cells. However, major drawback is that phage therapy needs correct identification of target bacterial strain [139, 140]. Nevertheless, with the development of better diagnostic approaches, the identification of the pathogens nowadays could be achieved faster and more accurately making the selection of phage for specific treatment easier [140].

Recent studied have shown that phage therapy was successfully used to treat infections caused by resistant bacteria such as *K.pneumonia* and *P. aeruginosa* [138, 143] and in combination with appropriate antibiotic treatment was shown to act synergistically on biofilms-associated infections of *S. aureus* and *P. aeruginosa* species [144]. The combination of phage therapy and antibiotics is regarded as a possible strategy to decrease resistance development [145].The possibility that bacteria develop resistance to phages through mutation of the specific receptor on the bacterial cell surface or inactivation of phage nucleic acid using CRISPR adaptive immune system should be also considered [13, 131]. Moreover, phages can induce immune response in the human body and release upon bacterial lysis toxins causing a toxic shock [142]. Recently, the immunogenicity of phages was shown to be mitigated when phages with reduced immunogenicity and lacking infectivity are used for targeted drug delivery. Drug-carrying phages were shown to be not toxic in mice, and the unique drug loading via an aminoglycoside linker greatly reduced the immunogenicity of the phages [146].

5. Conclusions

For more than 50 years antibiotics have been saving lives from many infectious diseases, being one of the most successful drugs ever introduced. However, their overuse resulted in increased emergence of multi-drug resistant bacteria and is now a major threat for human beings. New alternative approaches to prevent and cure bacteria-mediated infections using novel anti-virulence and therapeutic agents are under investigation. Understanding the mechanisms of bacterial pathogenesis and drug resistance development provided new insights in the field of drug discovery. Novel strategies that control the initial stages of bacterial pathogenesis using molecules capable to attenuate virulence mechanisms have the potential to prevent infections and overcome the antibiotics resistance. On the evidence that the anti-virulence agents do not kill bacteria but control bacterial virulence, it is believed that the host immune system will be capable of overcoming any infection without the need of antibiotic treatment. In the case when the human organism cannot overcome the infection, non-conventional treatments including AMPs, polyphenols, bacteriophages, and "nanoantibiotics" may be applied. Combination of these therapeutics using different types/ratios could also result in a synergistic effect in the treatment of multi-drug resistant bacteria. Non-conventional therapeutics kill bacteria via different mechanism and do not induce evolutionary stress which decreases the opportunity of resistance development. Despite of some drawbacks of these approaches such as the high cost, low bioavailability, insufficient pharmacokinetic data, possibility to affect the beneficial microbiota and opportunity to induce host immune response it is believed that continued investigations will lead to a deeper understanding of bacterial virulence, antibacterial strategies and therapeutics that will ultimately result in efficient pathogenesis inhibition and treatment.

6. References

[1] Relman, D. A., Microbiology: Learning about who we are. *Nature* 2012, *486*, 194-195.

[2] Rasko, D. A., Sperandio, V., Anti-virulence strategies to combat bacteria-mediated disease. *Nature Reviews Drug Discovery* 2010, *9*, 117-128.

[3] Tenover, F. C., Mechanisms of Antimicrobial Resistance in Bacteria. *The American journal of medicine* 2006, *119*, S3-S10.

[4] Wu, H.-J., Wang, A. H. J., Jennings, M. P., Discovery of virulence factors of pathogenic bacteria. *Current Opinion in Chemical Biology* 2008, *12*, 93-101.

[5] Chiang, S. L., Mekalanos, J. J., Holden, D. W., In vivo genetic analysis of bacterial virulence. *Annual review of microbiology* 1999, *53*, 129-154.

[6] Proft, T., Baker, E. N., Pili in Gram-negative and Gram-positive bacteria - structure, assembly and their role in disease. *Cellular and molecular life sciences : CMLS* 2009, *66*, 613-635.

[7] Keim, P., Mock, M., Young, J., Koehler, T. M., The International Bacillus anthracis, B. cereus, and B. thuringiensis Conference, "Bacillus-ACT05". *Journal of bacteriology* 2006, *188*, 3433-3441.

[8] Galán, J. E., Cossart, P., Host-pathogen interactions: a diversity of themes, a variety of molecular machines. *Current Opinion in Microbiology* 2005, *8*, 1-3.
[9] Vivanco, J. M., Baluška, F., *Secretions and Exudates in Biological Systems*, Springer 2012.
[10] Finlay, B. B., Falkow, S., Common themes in microbial pathogenicity revisited. *Microbiology and Molecular Biology Reviews* 1997, *61*, 136-169.
[11] Mah, T.-F. C., O'Toole, G. A., Mechanisms of biofilm resistance to antimicrobial agents. *Trends in microbiology* 2001, *9*, 34-39.
[12] Kalia, V. C., Purohit, H. J., Quenching the quorum sensing system: Potential antibacterial drug targets. *Critical Reviews in Microbiology* 2011, *37*, 121-140.
[13] Richards, J. J., Melander, C., Controlling bacterial biofilms. *Chembiochem : a European journal of chemical biology* 2009, *10*, 2287-2294.
[14] Chen, Z., Moayeri, M., Zhou, Y.-H., Leppla, S.*, et al.*, Efficient neutralization of anthrax toxin by chimpanzee monoclonal antibodies against protective antigen. *The Journal of infectious diseases* 2006, *193*, 625-633.
[15] Sharma, S., Thomas, D., Marlett, J., Manchester, M., Young, J. A. T., Efficient neutralization of antibody-resistant forms of anthrax toxin by a soluble receptor decoy inhibitor. *Antimicrobial agents and chemotherapy* 2009, *53*, 1210-1212.
[16] Berg, V., Almqvist, F., Pilicides-small molecules targeting bacterial virulence. *Organic & Biomolecular Chemistry* 2007, *5*, 1827.
[17] Barczak, A. K., Hung, D. T., Productive steps toward an antimicrobial targeting virulence. *Current Opinion in Microbiology* 2009, *12*, 490-496.
[18] Hartmann, M., Lindhorst, T. K., The bacterial lectin FimH, a target for drug discovery - Carbohydrate inhibitors of type 1 fimbriae-mediated bacterial adhesion. *European Journal of Organic Chemistry* 2011, 3583-3609.
[19] Chen, Z., Moayeri, M., Purcell, R., Monoclonal antibody therapies against anthrax. *Toxins* 2011, *3*, 1004-1019.
[20] Parsek, M. R., Val, D. L., Hanzelka, B. L., Cronan, J. E., Greenberg, E. P., Acyl homoserine-lactone quorum-sensing signal generation. *Proceedings of the National Academy of Sciences of the United States of America* 1999, *96*, 4360-4365.
[21] Dong, Y. H., Wang, L. H., Xu, J. L., Zhang, H. B.*, et al.*, Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. *Nature* 2001, *411*, 813-817.
[22] Lin, Y. H., Xu, J. L., Hu, J., Wang, L. H.*, et al.*, Acyl-homoserine lactone acylase from Ralstonia strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Molecular Microbiology* 2003, *47*, 849-860.
[23] Xu, F., Byun, T., Dussen, H.-J., Duke, K. R., Degradation of N-acylhomoserine lactones, the bacterial quorum-sensing molecules, by acylase. *Journal of Biotechnology* 2003, *101*, 89-96.
[24] Chan, K.-G., Atkinson, S., Mathee, K., Sam, C.-K.*, et al.*, Characterization of N-acylhomoserine lactone-degrading bacteria associated with the Zingiber officinale (ginger) rhizosphere: Co-existence of quorum quenching and quorum sensing in Acinetobacter and Burkholderia. *BMC Microbiology* 2011, *11*, 51.
[25] Teiber, J. F., Horke, S., Haines, D. C., Chowdhary, P. K.*, et al.*, Dominant role of paraoxonases in inactivation of the Pseudomonas aeruginosa quorum-sensing signal N-(3-oxododecanoyl)-L-homoserine lactone. *Infection and Immunity* 2008, *76*, 2512-2519.
[26] Ishida, T., Ikeda, T., Takiguchi, N., Kuroda, A.*, et al.*, Inhibition of quorum sensing in Pseudomonas aeruginosa by N-acyl cyclopentylamides. *Applied and Environmental Microbiology* 2007, *73*, 3183-3188.
[27] Vanoyan, N., Walker, S. L., Gillor, O., Herzberg, M., Reduced Bacterial Deposition and Attachment by Quorum-Sensing Inhibitor 4-Nitro-pyridine-N-oxide: The Role of Physicochemical Effects. *Langmuir* 2010, *26*, 12089-12094.
[28] Rasmussen, T. B., Bjarnsholt, T., Skindersoe, M. E., Hentzer, M.*, et al.*, Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. *Journal of bacteriology* 2005, *187*, 1799-1814.
[29] Peterson, M. M., Mack, J. L., Hall, P. R., Alsup, A. A.*, et al.*, Apolipoprotein B Is an innate barrier against invasive Staphylococcus aureus infection. *Cell host & microbe* 2008, *4*, 555-566.
[30] Otto, M., Süßmuth, R., Vuong, C., Jung, G., Götz, F., Inhibition of virulence factor expression in Staphylococcus aureus by the Staphylococcus epidermidis agr pheromone and derivatives. *FEBS Letters* 1999, *450*, 257-262.
[31] Balaban, N., Giacometti, A., Cirioni, O., Gov, Y.*, et al.*, Use of the quorum-sensing inhibitor RNAIII-inhibiting peptide to prevent biofilm formation in vivo by drug-resistant Staphylococcus epidermidis. *Journal of Infectious Diseases* 2003, *187*, 625-630.
[32] Fei Wang, P. R., Ivy Jiang, Ritsuko Sawada-Hirai, Shu Man Sun, Rebecca Nedellec, Phillip R. Morrow, Angray S. Kang, Human monoclonal antibodies that neutralize anthrax toxin by inhibiting heptamer assembly - WestminsterResearch. 2004.
[33] Tzipori, S., Sheoran, A., Akiyoshi, D., Donohue-Rolfe, A., Trachtman, H., Antibody therapy in the management of shiga toxin-induced hemolytic uremic syndrome. *Clinical microbiology reviews* 2004, *17*, 926-941.
[34] Radjainia, M., Hyun, J.-K., Leysath, C. E., Leppla, S. H., Mitra, A. K., Anthrax toxin-neutralizing antibody reconfigures the protective antigen heptamer into a supercomplex. *Proceedings of the National Academy of Sciences of the United States of America* 2010, *107*, 14070-14074.
[35] Wang, F., Ruther, P., Jiang, I., Sawada-Hirai, R.*, et al.*, Human monoclonal antibodies that neutralize anthrax toxin by inhibiting heptamer assembly. *Human Antibodies* 2004, *13*, 105-110.
[36] Goldman, M. E., Cregar, L., Nguyen, D., Simo, O.*, et al.*, Cationic polyamines inhibit anthrax lethal factor protease. *BMC Pharmacology* 2006, *6*, 8.
[37] Allen, W. J., Phan, G., Waksman, G., Pilus biogenesis at the outer membrane of Gram-negative bacterial pathogens. *Current Opinion in Structural Biology* 2012, *22*, 500-506.
[38] Kirov, S. M., Bacteria that express lateral flagella enable dissection of the multifunctional roles of flagella in pathogenesis. *FEMS Microbiology Letters* 2003, *224*, 151-159.
[39] Hamada, S., Amano, A., Kimura, S., Nakagawa, I.*, et al.*, The importance of fimbriae in the virulence and ecology of some oral bacteria. *Oral Microbiology and Immunology* 1998, *13*, 129-138.
[40] Bien, J., Sokolova, O., Bozko, P., Role of Uropathogenic Escherichia coli Virulence Factors in Development of Urinary Tract Infection and Kidney Damage. *International journal of nephrology* 2012, *2012*, 681473.

[41] Zigangirova, N. A., Zayakin, E. S., Kapotina, L. N., Kost, E. A., *et al.*, Development of Chlamydial Type III Secretion System Inhibitors for Suppression of Acute and Chronic Forms of Chlamydial Infection. *Acta naturae* 2012, *4*, 87-97.
[42] Felise, H. B., Nguyen, H. V., Pfuetzner, R. A., Barry, K. C., *et al.*, An Inhibitor of Gram-Negative Bacterial Virulence Protein Secretion. *Cell Host & Microbe* 2008, *4*, 325-336.
[43] Bailey, L., Gylfe, Å., Sundin, C., Muschiol, S., *et al.*, Small molecule inhibitors of type III secretion in Yersinia block the Chlamydia pneumoniae infection cycle. *FEBS Letters* 2007, *581*, 587-595.
[44] Matson, J. S., Withey, J. H., DiRita, V. J., Regulatory networks controlling Vibrio cholerae virulence gene expression. *Infection and Immunity* 2007, *75*, 5542-5549.
[45] Hung, D. T., Shakhnovich, E. A., Pierson, E., Mekalanos, J. J., Small-molecule inhibitor of Vibrio cholerae virulence and intestinal colonization. *Science (New York, N.Y.)* 2005, *310*, 670-674.
[46] Shakhnovich, E. A., Hung, D. T., Pierson, E., Lee, K., Mekalanos, J. J., Virstatin inhibits dimerization of the transcriptional activator ToxT. *Proceedings of the National Academy of Sciences of the United States of America* 2007, *104*, 2372-2377.
[47] L. Caetano M. Antunes, R. B. R. F., Michelle M. C. Buckner and B. Brett Finlay, Quorum sensing in bacterial virulence. *Microbiology* 2010, *156*, 2271-2282.
[48] Barnard, A. M. L., Bowden, S. D., Burr, T., Coulthurst, S. J., *et al.*, Quorum sensing, virulence and secondary metabolite production in plant soft-rotting bacteria. *Philosophical Transactions of the Royal Society B: Biological Sciences* 2007, *362*, 1165-1183.
[49] Von Bodman, S. B., Bauer, W. D., Coplin, D. L., Quorum Sensing in Plant-Pathogenic Bacteria. *Annu Rev Phytopathol* 2003, *41*, 455-482.
[50] Rutherford, S. T., Bassler, B. L., Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harbor perspectives in medicine* 2012, *2*.
[51] Parker, C. T., Sperandio, V., Cell-to-cell signalling during pathogenesis. *Cellular Microbiology* 2009, *11*, 363-369.
[52] Hentzer, M., Givskov, M., Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. *The Journal of clinical investigation* 2003, *112*, 1300-1307.
[53] Ni, N., Li, M., Wang, J., Wang, B., Inhibitors and antagonists of bacterial quorum sensing. *Medicinal Research Reviews* 2009, *29*, 65-124.
[54] Roche, D. M., Byers, J. T., Smith, D. S., Glansdorp, F. G., *et al.*, Communications blackout? Do N-acylhomoserine-lactone-degrading enzymes have any role in quorum sensing? *Microbiology* 2004, *150*, 2023-2028.
[55] Rasmussen, T. B., Givskov, M., Quorum sensing inhibitors: A bargain of effects. *Microbiology* 2006, *152*, 895-904.
[56] Hong, K.-W., Koh, C.-L., Sam, C.-K., Yin, W.-F., Chan, K.-G., Quorum Quenching Revisited—From Signal Decays to Signalling Confusion. *Sensors* 2012, *12*, 4661-4696.
[57] Dong, Y.-H., Xu, J.-L., Li, X.-Z., Zhang, L.-H., 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* 2000, *97*, 3526-3531.
[58] Wang, L. H., Weng, L. X., Dong, Y. H., Zhang, L. H., Specificity and Enzyme Kinetics of the Quorum-quenching N-Acyl Homoserine Lactone Lactonase (AHL-lactonase). *Journal of Biological Chemistry* 2004, *279*, 13645-13651.
[59] Kiran, S., Sharma, P., Harjai, K., Capalash, N., Enzymatic quorum quenching increases antibiotic susceptibility of multidrug resistant Pseudomonas aeruginosa. *Iranian Journal of Microbiology* 2011, *3*, 1-12.
[60] Huang, J. J., Han, J. I., Zhang, L. H., Leadbetter, J. R., Utilization of Acyl-Homoserine Lactone Quorum Signals for Growth by a Soil Pseudomonad and Pseudomonas aeruginosa PAO1. *Applied and Environmental Microbiology* 2003, *69*, 5941-5949.
[61] Smith, R. S., Iglewski, B. H., P. aeruginosa quorum-sensing systems and virulence. *Current Opinion in Microbiology* 2003, *6*, 56-60.
[62] Swift, S., Karlyshev, A. V., Fish , L., Durant, E. L., Winson, M. K., Chhabra, S. R., Williams, P., Macintyre, S., Stewart, G. S. Quorum sensing in Aeromonas hydrophila and Aeromonas salmonicida: Identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. *J Bacteriol* 1997;179(17):5271–5281. .
[63] Cao, J. G., Meighen, E. A., Purification and structural identification of an autoinducer for the luminescence system of Vibrio harveyi. *The Journal of biological chemistry* 1989, *264*, 21670-21676.
[64] Zhang, Z., Pierson, L. S., A second quorum-sensing system regulates cell surface properties but not phenazine antibiotic production in Pseudomonas aureofaciens. *Applied and environmental microbiology* 2001, *67*, 4305-4315.
[65] McClean, K. H., Winson, M. K., Fish, L., Taylor, A., *et al.*, Quorum sensing and Chromobacterium violaceum: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiology (Reading, England)* 1997, *143 (Pt 12)*, 3703-3711.
[66] Atkinson, S., Throup, J. P., Stewart, G. S., Williams, P., A hierarchical quorum-sensing system in Yersinia pseudotuberculosis is involved in the regulation of motility and clumping. *Molecular microbiology* 1999, *33*, 1267-1277.
[67] Lupp, C., Ruby, E. G., Vibrio fischeri uses two quorum-sensing systems for the regulation of early and late colonization factors. *Journal of bacteriology* 2005, *187*, 3620-3629.
[68] Sokol, P. A., Sajjan, U., Visser, M. B., Gingues, S., *et al.*, The CepIR quorum-sensing system contributes to the virulence of Burkholderia cenocepacia respiratory infections. *Microbiology (Reading, England)* 2003, *149*, 3649-3658.
[69] Welch, M., Dutton, J. M., Glansdorp, F. G., Thomas, G. L., *et al.*, Structure activity relationships of Erwinia carotovora quorum sensing signaling molecules. *Bioorganic & Medicinal Chemistry Letters* 2005, *15*, 4235-4238.
[70] Nasser, W., Bouillant, M. L., Salmond, G., Reverchon, S., Characterization of the Erwinia chrysanthemi expI-expR locus directing the synthesis of two N-acyl-homoserine lactone signal molecules. *Molecular microbiology* 1998, *29*, 1391-1405.
[71] Haudecoeur, E., Faure, D., A fine control of quorum-sensing communication in Agrobacterium tumefaciens. *Communicative & integrative biology* 2010, *3*, 84-88.
[72] Fekete, A., Kuttler, C., Rothballer, M., Hense, B. A., *et al.*, Dynamic regulation of N-acyl-homoserine lactone production and degradation in Pseudomonas putida IsoF. *FEMS Microbiology Ecology* 2010, *72*, 22-34.

[73] Kirwan, J. P., Gould, T. A., Schweizer, H. P., Bearden, S. W., *et al.*, Quorum-sensing signal synthesis by the Yersinia pestis acyl-homoserine lactone synthase YspI. *Journal of bacteriology* 2006, *188*, 784-788.
[74] Thoendel, M., Horswill, A. R., Chapter 4 - Biosynthesis of Peptide Signals in Gram-Positive Bacteria, in: Allen, I. L., Sima, S., Geoffrey, M. G. (Eds.), *Advances in Applied Microbiology*, Academic Press 2010, pp. 91-112.
[75] Jarosz, L. M., Deng, D. M., van der Mei, H. C., Crielaard, W., Krom, B. P., Streptococcus mutans competence-stimulating peptide inhibits Candida albicans hypha formation. *Eukaryotic cell* 2009, *8*, 1658-1664.
[76] Uroz, S., Chhabra, S. R., Cámara, M., Williams, P., *et al.*, N-acylhomoserine lactone quorum-sensing molecules are modified and degraded by Rhodococcus erythropolis W2 by both amidolytic and novel oxidoreductase activities. *Microbiology* 2005, *151*, 3313-3322.
[77] Camps, J., Pujol, I., Ballester, F., Joven, J., Simó, J. M., Paraoxonases as potential antibiofilm agents: Their relationship with quorum-sensing signals in gram-negative bacteria. *Antimicrobial Agents and Chemotherapy* 2011, *55*, 1325-1331.
[78] Kociolek, M. G., Quorum-Sensing Inhibitors and Biofilms. *Anti-Infective Agents in Medicinal Chemistry (Formerly `Current Medicinal Chemistry - Anti-Infective Agents)* 2009, *8*, 315-326.
[79] Geske, G. D., Wezeman, R. J., Siegel, A. P., Blackwell, H. E., Small molecule inhibitors of bacterial quorum sensing and biofilm formation. *Journal of the American Chemical Society* 2005, *127*, 12762-12763.
[80] Castang, S., Chantegrel, B., Deshayes, C., Dolmazon, R., *et al.*, N-Sulfonyl homoserine lactones as antagonists of bacterial quorum sensing. *Bioorganic & Medicinal Chemistry Letters* 2004, *14*, 5145-5149.
[81] Girennavar, B., Cepeda, M. L., Soni, K. A., Vikram, A., *et al.*, Grapefruit juice and its furocoumarins inhibits autoinducer signaling and biofilm formation in bacteria. *International Journal of Food Microbiology* 2008, *125*, 204-208.
[82] Adonizio, A., Kong, K.-F., Mathee, K., Inhibition of quorum sensing-controlled virulence factor production in Pseudomonas aeruginosa by South Florida plant extracts. *Antimicrobial Agents and Chemotherapy* 2008, *52*, 198-203.
[83] Kang-Mu, L. E. E., Wan-Seok, K. I. M., Jeesun, L. I. M., Sunyoung, N. A. M., *et al.*, Antipathogenic properties of green tea polyphenol epigallocatechin gallate at concentrations below the MIC against enterohemorrhagic escherichia coli O157:H7. *Journal of Food Protection* 2009, *72*, 325-331.
[84] K. Mihalik a, D. W. C. b., S. H. Crixell a, R. J. C. McLean c, D. A. Vattem a*, Quorum sensing modulators of Pseudomonas aeruginosa characterized in Camellia sinensis. *Asian Journal of Traditional Medicines* 2008, *3*.
[85] Ren, D., Sims, J. J., Wood, T. K., Inhibition of biofilm formation and swarming of Escherichia coli by (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone. *Environmental Microbiology* 2001, *3*, 731-736.
[86] Wu, H., Song, Z., Hentzer, M., Andersen, J. B., *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*, 1054-1061.
[87] Chenia, H. Y., Anti-Quorum Sensing Potential of Crude Kigelia africana Fruit Extracts. *Sensors (Basel, Switzerland)* 2013, *13*, 2802-2817.
[88] Liaqat, I., Bachmann, R. T., Nasim Sabri, A., Edyvean, R. G. J., Biggs, C. A., Investigating the effect of patulin, penicillic acid and EDTA on biofilm formation of isolates from dental unit water lines. *Applied microbiology and biotechnology* 2008, *81*, 349-358.
[89] Giacometti, A., Cirioni, O., Gov, Y., Ghiselli, R., *et al.*, RNA III inhibiting peptide inhibits in vivo biofilm formation by drug-resistant Staphylococcus aureus. *Antimicrobial agents and chemotherapy* 2003, *47*, 1979-1983.
[90] Kiran, M. D., Adikesavan, N. V., Cirioni, O., Giacometti, A., *et al.*, Discovery of a quorum-sensing inhibitor of drug-resistant staphylococcal infections by structure-based virtual screening. *Molecular pharmacology* 2008, *73*, 1578-1586.
[91] Park, S.-C., Park, Y., Hahm, K.-S., The role of antimicrobial peptides in preventing multidrug-resistant bacterial infections and biofilm formation. *International journal of molecular sciences* 2011, *12*, 5971-5992.
[92] Yeaman, M. R., Yount, N. Y., Mechanisms of antimicrobial peptide action and resistance. *Pharmacological reviews* 2003, *55*, 27-55.
[93] Brogden, K. A., Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nature reviews. Microbiology* 2005, *3*, 238-250.
[94] Rodziewicz-MotowidBo, S., Mickiewicz, B., Greber, K., Sikorska, E., *et al.*, Antimicrobial and conformational studies of the active and inactive analogues of the protegrin-1 peptide. *The FEBS journal* 2010, *277*, 1010-1022.
[95] Hassan, M., Kjos, M., Nes, I. F., Diep, D. B., Lotfipour, F., Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance. *Journal of applied microbiology* 2012, *113*, 723-736.
[96] Chan, D. I., Prenner, E. J., Vogel, H. J., Tryptophan- and arginine-rich antimicrobial peptides: Structures and mechanisms of action. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 2006, *1758*, 1184-1202.
[97] Jacob, L., Zasloff, M., Potential therapeutic applications of magainins and other antimicrobial agents of animal origin. *Ciba Foundation symposium* 1994, *186*, 197-216.
[98] Ovchinnikova, T. V., Shenkarev, Z. O., Nadezhdin, K. D., Balandin, S. V., *et al.*, Recombinant expression, synthesis, purification, and solution structure of arenicin. *Biochemical and Biophysical Research Communications* 2007, *360*, 156-162.
[99] Anantharaman, A., Rizvi, M. S., Sahal, D., Synergy with rifampin and kanamycin enhances potency, kill kinetics, and selectivity of de novo-designed antimicrobial peptides. *Antimicrobial agents and chemotherapy* 2010, *54*, 1693-1699.
[100] Cirioni, O., Ghiselli, R., Silvestri, C., Kamysz, W., *et al.*, Efficacy of tachyplesin III, colistin, and imipenem against a multiresistant Pseudomonas aeruginosa strain. *Antimicrobial agents and chemotherapy* 2007, *51*, 2005-2010.
[101] Cirioni, O., Silvestri, C., Ghiselli, R., Orlando, F., *et al.*, Protective effects of the combination of alpha-helical antimicrobial peptides and rifampicin in three rat models of Pseudomonas aeruginosa infection. *The Journal of antimicrobial chemotherapy* 2008, *62*, 1332-1338.
[102] Wang, W., Tao, R., Tong, Z., Ding, Y., *et al.*, Effect of a novel antimicrobial peptide chrysophsin-1 on oral pathogens and Streptococcus mutans biofilms. *Peptides* 2012, *33*, 212-219.
[103] Nagant, C., Pitts, B., Nazmi, K., Vandenbranden, M., *et al.*, Identification of peptides derived from the human antimicrobial peptide LL-37 active against biofilms formed by Pseudomonas aeruginosa using a library of truncated fragments. *Antimicrobial agents and chemotherapy* 2012, *56*, 5698-5708.

[104] Pompilio, A., Crocetta, V., Scocchi, M., Pomponio, S., *et al.*, Potential novel therapeutic strategies in cystic fibrosis: antimicrobial and anti-biofilm activity of natural and designed ±-helical peptides against Staphylococcus aureus, Pseudomonas aeruginosa, and Stenotrophomonas maltophilia. *BMC Microbiology* 2012, *12*, 145.
[105] Francesko, A., Fernandes, M. M., Tzanov, T. Gallic acid implications in health: multi-therapeutic and protective agent, edited by Thompson, M. A. and Collins, P. B., in *Handobook on Gallic Acids: Natural Occurrences, Antioxidant Properties and Health Implications*. Nova Science Pub Inc, 2013, ISBN-13: 9781626189218.
[106] David Vauzour, A. R.-M., Giulia Corona, Maria Jose Oruna-Concha and Jeremy P. E. Spencer, Polyphenols and Human Health: Prevention of Disease and Mechanisms of Action. *Nutrients* 2010, *2 (11)*, 1106-1131.
[107] Daglia, M., Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology* 2012, *23*, 174-181.
[108] Rukayadi, Y., Han, S., Yong, D., Hwang, J.-K., In vitro antibacterial activity of panduratin A against enterococci clinical isolates. *Biological & Pharmaceutical Bulletin* 2010, *33*, 1489-1493.
[109] Gordon, N. C., Wareham, D. W., Antimicrobial activity of the green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) against clinical isolates of Stenotrophomonas maltophilia. *International Journal of Antimicrobial Agents* 2010, *36*, 129-131.
[110] Du, X., Huang, X., Huang, C., Wang, Y., Zhang, Y., Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. *Journal of Dentistry* 2012, *40*, 485-492.
[111] W. Bylka, I. M., N.A. Pilewski, , Natural Flavonoids as Antimicrobial Agents. *JANA* 2004, *7*, 24-31.
[112] Eydelnant, I. A., Tufenkji, N., Cranberry Derived Proanthocyanidins Reduce Bacterial Adhesion to Selected Biomaterials. *Langmuir* 2008, *24*, 10273-10281.
[113] Foo, L. Y., Lu, Y., Howell, A. B., Vorsa, N., The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated Escherichia coli in vitro. *Phytochemistry* 2000, *54*, 173-181.
[114] Borges, A., Saavedra, M. J., Simões, M., The activity of ferulic and gallic acids in biofilm prevention and control of pathogenic bacteria. *Biofouling* 2012, *28*, 755-767.
[115] Huh, A. J., Kwon, Y. J., Nanoantibiotics : A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. 2011, *156*, 128-145.
[116] Hajipour, M. J., Fromm, K. M., Akbar Ashkarran, A., Jimenez de Aberasturi, D., *et al.*, Antibacterial properties of nanoparticles. *Trends in Biotechnology* 2012, *30*, 499-511.
[117] Allahverdiyev, A. M., Kon, K. V., Abamor, E. S., Bagirova, M., Rafailovich, M., Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents. *Expert review of anti-infective therapy* 2011, *9*, 1035-1052.
[118] Guzman, M., Dille, J., Godet, S., Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. *Nanomedicine : nanotechnology, biology, and medicine* 2012, *8*, 37-45.
[119] Mubarak Ali, D., Thajuddin, N., Jeganathan, K., Gunasekaran, M., Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. *Colloids and surfaces. B, Biointerfaces* 2011, *85*, 360-365.
[120] Maness, P.-C., Smolinski, S., Blake, D. M., Huang, Z., *et al.*, Bactericidal Activity of Photocatalytic TiO2 Reaction: toward an Understanding of Its Killing Mechanism. *Applied and environmental microbiology*.
[121] Li, W.-R., Xie, X.-B., Shi, Q.-S., Zeng, H.-Y., *et al.*, Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. *Applied microbiology and biotechnology* 2010, *85*, 1115-1122.
[122] Xie, Y., He, Y., Irwin, P. L., Jin, T., Shi, X., Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni. *Applied and environmental microbiology* 2011, *77*, 2325-2331.
[123] Prabhu, S., Poulose, E., Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *International Nano Letters* 2012, *2*, 32.
[124] Radzig, M. A., Nadtochenko, V. A., Koksharova, O. A., Kiwi, J., *et al.*, Antibacterial effects of silver nanoparticles on gram-negative bacteria: Influence on the growth and biofilms formation, mechanisms of action. *Colloids and Surfaces B: Biointerfaces* 2013, *102*, 300-306.
[125] Ma, H., Williams, P. L., Diamond, S. A., Ecotoxicity of manufactured ZnO nanoparticles A review. 2013, *172*, 76-85.
[126] Zhang, L., Gu, F. X., Chan, J. M., Wang, A. Z., *et al.*, Nanoparticles in medicine: therapeutic applications and developments. *Clinical pharmacology and therapeutics* 2008, *83*, 761-769.
[127] Zhang, L., Pornpattananangku, D., Hu, C. M. J., Huang, C. M., Development of nanoparticles for antimicrobial drug delivery. *Current medicinal chemistry* 2010, *17*, 585-594.
[128] Drulis-Kawa, Z., Dorotkiewicz-Jach, A., Liposomes as delivery systems for antibiotics. *International journal of pharmaceutics* 2010, *387*, 187-198.
[129] Wim H. J. De , B. P. J., Drug delivery and nanoparticles: Applications and hazards. *Int J Nanomedicine* 2008, *3*, 133-149.
[130] Hale, C. R., Zhao, P., Olson, S., Duff, M. O., *et al.*, RNA-Guided RNA Cleavage by a CRISPR RNA-Cas Protein Complex. *Cell* 2009, *139*, 945-956.
[131] Szczepankowska, A., Chapter 8 - Role of CRISPR/cas System in the Development of Bacteriophage Resistance, in: Małgorzata, Ł., Wacław, T. S. (Eds.), *Advances in Virus Research*, Academic Press 2012, pp. 289-338.
[132] Garneau, J. E., Dupuis, M.-È., Villion, M., Romero, D. A., *et al.*, The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* 2010, *468*, 67-71.
[133] Richter, C., Chang, J. T., Fineran, P. C., Function and Regulation of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) / CRISPR Associated (Cas) Systems. *Viruses* 2012, *4*, 2291-2311.
[134] Sapranauskas, R., Gasiunas, G., Fremaux, C., Barrangou, R., *et al.*, The Streptococcus thermophilus CRISPR/Cas system provides immunity in Escherichia coli. *Nucleic acids research* 2011, *39*, 9275-9282.
[135] Hale, C. R., Majumdar, S., Elmore, J., Pfister, N., *et al.*, Essential Features and Rational Design of CRISPR RNAs that Function with the Cas RAMP Module Complex to Cleave RNAs. *Molecular Cell* 2012, *45*, 292-302.
[136] Fellman, M., Blocking The Spread Of Antibiotic Resistance In Bacteria. December 18, 2008.
[137] Sulakvelidze A., A. Z., Morris Glenn J. , Bacteriophage Therapy. *Antimicrobial agents and chemotherapy* 2001, *45*, p. 649–659.

[138] Morello, E., Saussereau, E., Maura, D., Huerre, M.*, et al.*, Pulmonary bacteriophage therapy on Pseudomonas aeruginosa cystic fibrosis strains: first steps towards treatment and prevention. *PloS one* 2011, *6*, e16963.
[139] Potera, C., Phage renaissance: new hope against antibiotic resistance. *Environmental health perspectives* 2013, *121*, 48-53.
[140] Sulakvelidze, A., The challenges of bacteriophage therapy. *Industrial Pharmacy* 2011, 14-18.
[141] Abedon, S. T., Kuhl, S. J., Blasdel, B. G., Kutter, E. M., Phage treatment of human infections. *Bacteriophage* 2011, *1(2)*, 66-85.
[142] Inal, J. M., Phage therapy: A reappraisal of bacteriophages as antibiotics. *Archivum Immunologiae et Therapiae Experimentalis* 2003, *51*, 237-244.
[143] Chhibber, S., Kaur, S., Kumari, S., Therapeutic potential of bacteriophage in treating Klebsiella pneumoniae B5055-mediated lobar pneumonia in mice. *Journal of Medical Microbiology* 2008, *57*, 1508-1513.
[144] Yilmaz, C., Colak, M., Yilmaz, B. C., Ersoz, G.*, et al.*, Bacteriophage therapy in implant-related infections: an experimental study. *The Journal of bone and joint surgery. American volume* 2013, *95*, 117-125.
[145] Lu, T. K., Collins, J. J., Dispersing biofilms with engineered enzymatic bacteriophage. *Proceedings of the National Academy of Sciences of the United States of America* 2007, *104*, 11197-11202.
[146] Vaks, L., Benhar, I., In vivo characteristics of targeted drug-carrying filamentous bacteriophage nanomedicines. *Journal of Nanobiotechnology* 2011, *9*, 58.

