An emerging crisis of antibiotic resistance: In search of alternative antimicrobial sources

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Antimicrobials produced by some of the microorganisms, plants, and animals impede bacterial growth or kill infectious microorganisms. Members of the *Actinobacteria* alone produce ~80% of the total antimicrobial compounds. Recently the overuse and misuse of natural, synthetic, and chemically modified antibiotics have posed a serious threat to human health as well as the environment. Therefore, screening of newer and natural antimicrobial agents from microbial, plant, and animal sources has been continually broadening. Terrestrial plants have vast ability to synthesize aromatic substances such as alkaloids, phenolic compounds, tannings, triterpenoids and terpinenes to defend against predation by microorganisms, insects, and animals. In this chapter, we will review recent works on naturally isolated antimicrobial compounds, such as antimicrobial peptides, enzymes, phytochemicals, plant and herbal extracts as potential antimicrobial agents for different infectious diseases. We will also review the mode of action of these antimicrobial agents and their target organisms in relation to bacterial, viral, fungal, and protozoan mediated human diseases.

Keywords antimicrobials; natural products; human pathogens

1. Concerns over the usage of antimicrobials

Since the discovery of penicillin, both natural and synthetic antimicrobials have made significant contributions to human health. Synthetic antimicrobials are widely used in veterinary medicine and in broad, non-therapeutic uses such as disinfectants, pesticides, and preservatives. However, these applications are unsafe due to a continued rise in antibiotic-resistant infections. As a result, there has been renewed interest in the search for novel alternative approaches as well as resources to screen, identify, and isolate natural antimicrobials.

1.1. Rising threats of emerging antimicrobial-resistant pathogens

The prolonged use of antibiotics and other antimicrobials has led to the rise in resistant microorganisms, such that standard treatments are not only ineffective, but resistant infections may spread worldwide. This resistance is unfortunately a consequence of a variety of reasons, including inappropriate and excessive use of antibiotics, gradual decline in the research and development of new antibiotics, and the absence of strict guidelines for their application. The rise in antimicrobial-resistance, the pressure to reduce the use of antimicrobials, and the increasing difficulty and cost of developing effective antibiotics have also discouraged investment by pharmaceutical companies.

1.2. Impacts on the environment

Another important area of concern is the effect of antibiotic residues in the environment. Although environmental antibiotic contamination can be largely attributed to human antibiotic usage, applications of antibiotics in crops, livestock, poultry, and aquaculture also contribute significantly. Antibiotics and their metabolites enter the environment through antibiotic-containing manure, excretion by animals, and/or direct application. Entire bodies of water such as streams, lakes, and rivers will be contaminated through surface run-off and leaching. The concentrations of these antibiotics accumulate over time and have detrimental effects on biodiversity and ecosystems.

1.3. Human health issues

The prevalence of antimicrobial and antibiotic use has contributed to an environment where resistant organisms are selected for and allowed to flourish. A high percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA). With the increase of resistant pathogens, human health also suffers as the infections they cause often fail to respond to conventional treatments. In light of the significance of antimicrobial resistant pathogens, this chapter aims to review the current literature on natural antimicrobials, their modes of action, and future emerging aspects of research.

2. Natural antimicrobial products

The problem of antimicrobial-resistance has prompted research for the discovery of new drugs to control bacterial infections, ranging from novel antibiotic sources to elaborate combinations between commercially available

antimicrobials. Amphipathic peptides with antimicrobial activity are widely distributed throughout nature and are produced by both prokaryotes and eukaryotes. A list of natural antimicrobial products, their sources, and the microorganisms susceptible to these antimicrobials are listed in Table 1.

2.1. Antimicrobials produced by microorganisms

Microorganisms are an important source of antimicrobial secondary metabolites, which include sulfonamides, penicillins, streptomycins, tetracyclines, macrolides, glycopeptides, and cephalosporins. The majority of commercially used natural antibiotics are produced by fungi and spore-forming bacteria. A large number of antimicrobial products such as tetracyclines, aminoglycosides, macrolides, chloramphenicol, ivermectin, rifamycins, and other non-β-lactam antibiotics have been isolated and studied from *Streptomyces*, a genus within Actinobacteria. In fact, Actinobacteria produce ~10,000 known antibiotics, and ~50% of these are produced by *Streptomyces* alone [1]. Many other microbes have also been shown to produce substances that inhibit the growth of other organisms. For example, *Penicillium* and *Cephalosporium* produce not only β-lactam antibiotics such as penicillin and cephalosporin, but also the base molecules for synthetic antibiotics such as amoxicillin and ampicillin.

Lantibiotics are antimicrobial peptides produced by Gram-positive bacteria. These peptides contain post-translationally modified N-terminal leader sequences [2]. They kill common food spoilage organisms such as *Listeria monocytogenes* and *Clostridium botulinum*, and show promising activity against resistant *S. aureus* and enterococcal infections [3]

2.2. Antimicrobials produced by plants

Plant-derived antimicrobials include secondary metabolites and aromatic substances, which subsequently serve as defense mechanisms against predation by microorganisms, insects and herbivores. Furthermore, they are successful in the treatment of infectious diseases, and do not exhibit side effects commonly associated with synthetic antimicrobials.

The major groups of antimicrobial phytochemicals can be divided into several categories that include phenolic compounds, alkaloids, lectins, polypeptides, terpenoids, polyacetylenes, and essential oils as listed in Table 1.

2.2.1. Phenolic compounds

Phenolic compounds not only denature enzymes but also bind to minerals, vitamins and carbohydrates required for the growth of microorganisms. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds that are effective against bacteria. The leaves of *Piper regnellii* produce phenolic compounds that exhibit strong antibacterial and antifungal properties [4]. Kanzonol C and its analogues have been isolated from the twigs of *Dorstenia barteri*, and show inhibitory activities against both bacteria and fungi [5].

Quinones are most commonly derived from aromatic compounds in plants, but are also present in certain types of bacteria and fungi. Previous studies have shown that quinones exhibit antifungal activity against *Colletotrichum*, and they are useful in agricultural fungal pathogen control. The root bark of the African Border Tree, *Newbouldia laevis,* contains newbouldia quinone, which has exhibited *in vitro* antimicrobial activity against different bacterial species and *Candida*. Quinones, however, are most notable for their industrial application in the production of hydrogen peroxide.

Flavonoids are commonly found in citrus fruits, tea, wine, and dark chocolate, and are identified as potent antimicrobial agents. For example, flavonoids extracted from *Coccinia cordifolia* exhibit strong antimicrobial activity against Gram-positive bacteria (*Sarcina lutea*, *Bacillus subtilis* and *S. aureus*), Gram-negative bacteria (*Salmonella typhi*, *Shigella dysenteriae* and *E. coli*), and certain fungi (*Candida albicans*, *Aspergillus niger* and *Penicillium notatum*) [6]. Another flavonoid, apigenin, isolated from *Ranunculus scleratus*, is active against *S. typhi* and *Agrobacterium tumefaciens*, [7, 8] and also exhibits potent toxic activity against several strains of MRSA. Amentoflavones from *Selaginella tamariscina* show good inhibitory activity against the fungal pathogens, *Candida albicans*, *Saccharomyces cerevisiae* and *Trychophyton beigelii,* indicating its broad-spectrum antibiotic potential [9].

Tannins are found in almost every plant part: bark, wood, leaves, fruits, and roots. Tannin-rich pomegranate peels have been used as a remedy for diarrhea and dysentery in traditional medicine. Recent studies have also shown that tannic acid can be utilized in the leather industry as a substitute antimicrobial agent in the pickling process [10].

Coumarins are secondary metabolites that are widely distributed in members of the Rutaceae (citrus) family. Although they are quite toxic to the liver and kidneys, coumarins have been used to prevent recurrences of cold sores caused by herpes simplex virus (HSV-1) in humans. Another study has shown that hydroxylcinnamic acids, related to coumarins, are inhibitory to Gram-positive bacteria [11]. A 6,7-dimethoxycoumarin isolated from *Ranunculus laetus* has shown inhibitory activity against *Pseudomonas aeruginosa*, *S. aureus, E. coli* and *B. subtilis* [7].

2.2.2. Terpenoids

Terpenoid is a class of secondary metabolites with carbon backbones composed of isoprene units that contains ~36,000 compounds which exhibit significant antiviral, antibacterial, antimalarial, anti-inflammatory, and anticancer activity. Cinnamodial, a diterpenoid isolated from the leaves and bark of *Pleodendron costaricense*, exhibits strong activity

against *Alternaria alternata*, *C. albicans* D10, and *Wangiella dermatitidis* [12]. The diterpenoid Hardwickiic acid, obtained from the stem bark of *Irvingiaga bonensis*, exhibits potent activity against both Gram-negative and Gram-positive bacteria [13]. Oleanolic acid, along with another pentacyclic triterpenoid, canthic acid, has been isolated from the root bark of *Newbouldia laevis* and show broad-spectrum antimicrobial activity against many Gram-positive and Gram-negative bacterial species, as well as *Candida* strains [13].

2.2.3. Alkaloids

Alkaloids are a group of naturally occurring compounds produced by plants that are used for the plant's defense mechanism. They often contain one or more carbon rings along with a nitrogen atom. The position of the nitrogen atom differs among alkaloids, and determines their properties. Morphine, which is isolated from the opium poppy *Papaver somniferum*, was the first clinically relevant example of an alkaloid, and its derivatives include codeine and heroin. Furthermore, many plant families are known to produce a wide variety of antimicrobial alkaloids such as the glycoalkaloid, solamargine, which are potentially useful against HIV infection, as well as AIDS associated intestinal infections.

The plant genus *Berberis* produces a commonly used alkaloid, berberine, which is considered a strong antibiotic that suppresses the growth of a wide range of tumor cells. Novel berberine triazoles have also been shown to exhibit strong antibacterial and antifungal activities which are comparable to the reference drugs chloromycin, norfloxacin and fluconazole [14].

2.2.4. Lectins and polypeptides

Lectins are carbohydrate-binding proteins which are believed to play a role in cell-to-cell binding. Although lectins inhibit the growth of bacteria and fungi, recent interest in lectins has been intrigued by their anti-HIV properties [15]. A study has shown that a jacalin-related lectin, BanLec, isolated from banana, binds to high mannose carbohydrate such as those found on virus envelope proteins, and hence could be used to prevent their transmission [16]. Some larger lectins including mannose-specific lectins from several plants, MAP30 from bitter melon, and GAP31 from *Gelonium multiflorum* have been shown to inhibit proliferation of viral diseases such as HIV and cytomegalovirus.

2.2.5. Oxylipins

Oxylipins are products of fatty acid pathways which have varied effects in different organisms. For example, they exhibit different physiologic effects such as modulating vascular resistance or inflammation. In certain organisms, such as plants, oxylipins (polyacetylenes) are often utilized in defense mechanisms against microbes and pests. As an example, a polyacetylene compound isolated from *Bupleurum salicifolium* inhibits the growth of *B. subtilis* and *S. aureus*, but not other organisms, including Gram-negative bacteria. Similarly, polyacetylene compounds extracted from *Panax ginseng* exhibit significant antibacterial activity [17]. In clinical settings, oxylipins and polyacetylene compounds can be used for the treatment of liver diseases and malaria.

2.2.6. Essential oils

Essential oils are aromatic compounds obtained from plant materials such as flowers, fruits, herbs and leaves, and have been used clinically to treat respiratory tract infections and colds. A majority of essential oils exhibit some level of antibacterial activity against one or more microorganisms and have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. For example, peppermint and orange oils exhibit anticancer activity [18], and lavender oil exhibits strong antibacterial and antifungal activity [19].

2.3. Antimicrobials from animal tissues and animal products

Natural compounds, isolated from certain insect and animal species have been shown to exhibit antimicrobial activity [20]. Since these antimicrobials act through a variety of mechanisms including disruption of cell membranes and interference with metabolism, microbial resistance against these compounds is much more unlikely. In addition, many antimicrobials are found in common animal products, such as lysozymes in egg whites, lactoperoxidase in milk, and more prevalent antimicrobial peptides.

2.3.1. Antimicrobials produced by animals

Over 1,500 small antimicrobial peptides have been identified in animals [20], and these peptides perform a wide variety of functions in the cell, including antimicrobial defense against bacteria, viruses, and fungi. Since these antimicrobial peptides are conserved in their structure, function and modes of action, synthetic antimicrobial peptides may be used clinically to prevent or treat infections. Furthermore, these peptides have been shown to have bactericidal effects, against not only strains resistant to conventional antibiotics, but also against mycobacteria (including *M. tuberculosis*), enveloped viruses (such as HIV), fungi, and cancerous cells.

2.3.2. Antimicrobials found within animal products

Milk: Along with its inherent nutritional value, there has been great interest in milk's ability to ward off microbial infections. This is due to a wide array of antimicrobial factors, most notably lactoferrin, lactoperoxidase, lysozymes, and immunoglobulins [21]. The latter of these will not be discussed extensively; however, immunoglobulins are glycoproteins produced by plasma cells used by the immune system as antibodies against foreign particles, such as bacteria and viruses.

Lactoferrin: Lactoferrin is an iron-binding glycoprotein, found in large quantities in exocrine mammalian secretions such as milk, tears, saliva, and seminal fluid. Lactoferrin is able to inhibit growth for a wide range of microorganisms, including a number of Gram-negative and Gram-positive bacteria, such as *S. aureus*, *Bacillus* spp. and *L. monocytogenes*. The antimicrobial mechanism of lactoferrin has been attributed to its ability to deprive bacteria of iron, which is essential for growth, as these organisms have a high iron requirement. Studies have also shown that lactoferrin exerts a direct, bactericidal effect on certain streptococcal mutants and *Vibrio cholerae*, independent of iron-deprivation [22].

Lactoperoxidase: Lactoperoxidase is a glycoprotein that contains a heme group and is an effective antimicrobial agent. This is due to its ability to inhibit vital metabolic enzymes.

Lysozymes: Lysozymes are found in egg whites, human tears, saliva, milk, and other body fluids, and are important for their ability to hydrolyze β-1,4-glycosidic linkages between the two components of peptidoglycan in bacterial cell walls. There are three different types of lysozymes, including C-lysozyme, G-lysozyme, and I-lysozyme [23], which usually function in association with lactoferrin or immunoglobulin A.

Albumin: Albumin is found in egg whites and contains a number of proteins with antimicrobial properties including bacteriolytic lysozymes. These antimicrobial effects may be attributed to several mechanisms, including bacterial cell lysis, metal binding, and vitamin binding.

Honey: Honey contains alkaloids, flavonoids, phenolics, and peptides/proteins, and exhibits strong antimicrobial activity [24]. In addition, glucose oxidase present in honey provides a continual yet slow release of hydrogen peroxide, which plays an important role in decreasing inflammation and wound healing.

3. Classification of antimicrobials on the basis of mode of action

Antimicrobial classification is based on chemical structures and functions. Based on chemical structure, antibiotics are classified into different groups, which include carbohydrate-containing antibiotics (streptomycin), macrocyclic lactones (erythromycin), quinones and related antibiotics (tetracycline), amino acid and peptide antibiotics (penicillin), heterocyclic antibiotics containing oxygen (monensin), heterocyclic antibiotics containing nitrogen (polyoxins), aromatic antibiotics (chloramphenicol), alicyclic derivatives (cycloheximide), and aliphatic antibiotics (fosfomycins). Similarly, antimicrobial compounds from plants are also classified into phenolics and polyphenols (apigenin), terpenoids (fridelin), alkaloids (berberine), lectins, and polypeptides (fabatin), polyacetylenes (17-hydroxypanaxacol), and essential oils (eugenol).

Antibiotics are classified based on the cellular components or biosystems they affect. Generally, they either induce cell death (bactericidal) or inhibit cell growth (bacteriostatic). Most current bactericidal antimicrobials inhibit cell wall, DNA, RNA, or protein synthesis. Antibiotic-induced cell death has been associated with double-stranded DNA breaks following treatment with inhibitors of topoisomerase II [25], arrest of DNA-dependent RNA synthesis following treatment with rifamycins [26], cell envelope damage and loss of structural integrity following treatment with inhibitors of cell wall synthesis, and with cellular energetics, ribosome binding, and protein mistranslation following treatment with inhibitors of protein synthesis. Since the discovery of penicillin, the increasing prevalence of drug-resistant bacteria [27] has made it crucial to better understand the multilayered mechanisms by which currently available antibiotics kill bacteria or impede bacterial growth, as well as to explore alternative antibacterial therapies.

3.1. Inhibition of peptidoglycan synthesis

The bacterial cell consists of layers of peptidoglycan, a covalently cross-linked polymer matrix. The degree of peptidoglycan cross-linking is correlated with the structural integrity of the cell. The integrity of the peptidoglycan layer is maintained by the activity of transglycosylases and penicillin-binding proteins (transpeptidases), which add disaccharide pentapeptides to extend the glycan strands of existing peptidoglycan molecules and cross-link adjacent peptide strands of immature peptidoglycan units, respectively. β-lactams and glycopeptides are the classes of antibiotics that interfere with specific steps in homeostatic cell wall biosynthesis. Treatment with cell wall synthesis inhibitors can result in changes to cell configuration, induction of cell-stress responses, and ultimately, cell lysis. For example, β-lactams (including penicillins, carbapenems, and cephalosporins) block the cross linking of peptidoglycan units by inhibiting the peptide bond formation reaction that is catalyzed by penicillin-binding proteins (PBPs).

β-lactam is an analogue of the terminal D-alanyl-D-alanine dipeptide of peptidoglycan and acts as a substrate for PBP during the acylation phase of cross-link formation. Penicilloylation of the PBP active site blocks the hydrolysis of the drug, thereby disabling the enzyme. In contrast, most actinobacterium-derived glycopeptide antibiotics inhibit

peptidoglycan synthesis by binding peptidoglycan and blocking transglycosylase and PBP activity [28]. In this regard, glycopeptides act as steric inhibitors of peptidoglycan maturation and reduce the mechanical strength of the cell. However, some chemically modified glycopeptides have been shown to directly interact with transglycosylase enzymes. β-lactams can be used to treat Gram-positive and Gram-negative bacteria, whereas glycopeptides are effective against only Gram-positive bacteria due to their low permeability. The mechanism of killing by peptidoglycan synthesis inhibitors has been mainly due to the lysis event.

β-lactams and glycopeptides are commonly used to treat bacterial infections in humans. Penicillin, a member of β-lactams, can be used to treat against bacterial infections, including *Chlamydia trachomatis* (the etiological agent of the sexually transmitted disease, Chlamydia), *Helicobacter pylori* (etiological agent of the infection of gastric ulcers and gastritis), *Borrelia burgdorferi* (etiological agent of Lyme disease) and many others. Cephalosporin, another representative of β-lactam antibiotics, can also be used to treat against a variety of infections, including *Streptococcus pneumoniae* (the most common etiological agent for bacterial meningitis), *Staphylococcus aureus* (etiological agent for Staph infections), and many others. Carbapenem, another type of β-lactam antibiotic, has a much wider spectrum of targets than penicillin or cephalosporin and can be used for most of the same targets even when the target bacteria can produce β-lactamase. Vancomycin, a type of glycopeptide, can be used to treat most Gram-positive bacterial infections, even with multiple resistance to antibiotics; however, it is used as a "last resort" treatment due its platelet aggregating effects.

3.2. Disruption of cell membrane function

The cell membrane of gram-negative bacteria is a trilamellar structure that binds the bacterial protoplasm, and is composed of a phospholipid bilayer. The integrity of the cytoplasmic and outer membranes is vital to bacteria, and compounds that disorganize these membranes rapidly kill the cells. Some antibiotics change the permeability of the plasma membrane, resulting in loss of metabolites. These antibiotics disorganize the structure or inhibit the function of bacterial membranes by interacting with phospholipids.

The fungal cell wall differs drastically from the mammalian cell wall, so that it may be used as a convenient target, especially for mammalian fungal infections. The polyene antibiotics are the only group of antifungal antibiotics that directly target the plasma membrane via a specific interaction with the main fungal sterol, ergosterol. The sterols which are present within the fungal cell membrane can bind with the polyene-based drugs, leading to disruption of the cell membrane. A disruption of the equilibrium of free sterols to sphingolipids by natamycin may also be responsible for the inhibition of ergosterol-dependent protein functions [29]. Unlike other polyene antibiotics, the mode of action of natamycin is not based on the ergosterol-dependent permeabilization of the plasma membrane, and instead alters the membrane properties [30].

3.3. Transcription: Inhibition of RNA polymerase

The inhibition of RNA synthesis by the rifamycin class of semi-synthetic bactericidal antibiotics has a devastating effect on prokaryotic nucleic acid metabolism and is a potent means of bacterial death [26]. Rifamycins and other compounds of the ansamycin group inhibit DNA-dependent transcription by stably binding with high affinity to the β-subunit (encoded by *rpoB*) of a DNA-bound and actively transcribing RNA polymerase [31]. The β-subunit is located in the channel that is formed by the RNA polymerase–DNA complex, from which the newly synthesized RNA strand emerges. Rifamycins uniquely require RNA synthesis to not have progressed beyond the addition of two ribonucleotides; this is attributed to the ability of the drug molecule to sterically inhibit nascent RNA strand initiation [32]. Although rifamycins are not thought to block the elongation step RNA, a class of RNA polymerase inhibitors has been discovered that could inhibit it. Mutagenesis of *Streptomyces meditterranei* has led to the isolation and characterization of more potent rifamycin forms, including the clinically relevant rifamycin SV and rifampicin, which are able to penetrate the matrix formed in biofilms [33]. Rifamycins are considered bactericidal against Gram-positive bacteria and bacteriostatic against Gram-negative bacteria, a difference that has been attributed to drug uptake and not to affinity of the drug with the RNA polymerase β-subunit. Furthermore, they are among the first-line therapies used against mycobacterial diseases such as mycobacterium avium complex, leprosy and tuberculosis because they efficiently induce mycobacterial cell death.

3.4. Translation: Inhibition of protein synthesis

Drugs that inhibit protein synthesis are among the broadest classes of antibiotics and can be divided into two subclasses: the 30S inhibitors and 50S inhibitors. 30S ribosome inhibitors include tetracyclines and aminocyclitols. Tetracyclines work by blocking the access of aminoacyl tRNAs to the ribosome [34] and are commonly used to treat various bacterial infections including, pneumonia, chlamydia, gonorrhea, urinary tract infections and acne. The aminocyclitol class comprises aminoglycosides (streptomycin, kanamycin and gentamicin) and spectinomycin which bind the 16S rRNA component of the 30S ribosome subunit. Binding of aminoglycosides to the ribosome does not stop translation but instead promotes protein mistranslation through the addition of inappropriate amino acids into elongating peptide

strands. Aminocyclitols, despite being some of the first clinical antibiotics, retained their importance in fighting bacterial infections and are mainly used against Gram-negative bacteria. 50S ribosome inhibitors include macrolides (erythromycin), lincosamides (clindamycin), streptogramins (quinupristin), amphenicols (chloramphenicol) and oxazolidinones (linezolid) [35], and these inhibitors work by physically blocking either the initiation of protein translation or translocation of peptidyl tRNAs, The mechanism by which these drugs act involves blocking the access of peptidyl tRNAs to the ribosome and subsequent blockage of the peptidyltransferase elongation reaction by steric inhibition. This model also accounts for the phenomenon that these classes of drugs lose their antibacterial activity when elongation has progressed beyond a crucial length [36].

Among ribosome inhibitors, naturally derived aminoglycoside is the only class that is broadly bactericidal. Macrolides, streptogramins, spectinomycin, tetracyclines, chloramphenicol and macrolides are typically bacteriostatic but can also be bactericidal in certain species. For example, chloramphenicol has been shown to effectively kill *S. pneumonia* and *Neisseria meningitides*, and the macrolide, azithromycin, has exhibited bactericidal activity against *Haemophilus influenza*. This variability among certain species is most likely due to the sequence differences among bacterial species in the variable regions of the highly conserved ribosomal proteins and RNAs.

3.5. Inhibition of DNA replication

DNA synthesis requires the modulation of chromosomal supercoiling via strand breakage and rejoining reactions. Compounds that directly interfere with DNA-associated enzymatic processes exhibit sufficient selectivity for use as antimicrobials. The synthetic quinolones, including the clinically relevant fluoroquinolones target DNA topoisomerase complexes. Quinolones are derivatives of nalidixic acid, introduced in the 1960s to treat urinary tract infections. The quinolone class of antimicrobials interferes with the maintenance of chromosomal topology by targeting topoisomerase II and topoisomerase IV, stopping these enzymes at the DNA cleavage stage and preventing strand rejoining. Despite the general functional similarities between topoisomerase II and topoisomerase IV, their susceptibility to quinolones varies across bacterial species [37]. For example, several studies have shown that topoisomerase IV is the primary target of quinolones in Gram-positive bacteria, whereas in Gram-negative bacteria, their primary target is topoisomerase II.

Fluoroquinolones are a class of broad-spectrum antibiotics that are mostly used in bacterial infections that show resistance to other older antibiotics. To prevent the development of multidrug resistant bacterial infections, current fluoroquinolones are recommended for limited treatment of hospital acquired *S. pneumonia*, and not as a first line treatment.

Several studies have shown that preventing the induction of the DNA stress response (SOS response) enhances killing by quinolones [38]. Preventing the activation of the SOS response has also been shown to reduce the formation of drug-resistant mutants by blocking the induction of error-prone DNA polymerases, homologous recombination and horizontal transfer of drug-resistance genes [39].

3.6. Blocking the synthesis of essential metabolites

Both sulfonamides and trimethoprim block the key steps in folate synthesis (folic acid pathway), which is a cofactor in the biosynthesis of nucleotides. The sulfonamides are the first synthetic antimicrobial agents with a wide spectrum for the prevention and cure of bacterial infections. Sulfonamide antagonizes *p*-aminobenzoic acid (PABA), an essential metabolite, by competitively inhibiting the condensation of PABA with dihydropteridine to form dihydropteroic acid. This is the first step in the biosynthesis of tetrahydrofolic acid, and it prevents the subsequent synthesis of folic acid. Clinically, sulfonamides are broad-spectrum antibiotics, used to treat a variety of Gram-positive and Gram-negative bacterial infections which cause diseases such as bacterial meningitis, long-lasting bronchitis, and traveler's diarrhea.

Another such example is trimethoprim. Trimethoprim is a competitive inhibitor of dihydrofolic acid reductase, and it blocks a step in the biosynthesis of tetrahydrofolic acid reductase, an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid. It can be used to treat infections by *E. coli*, *H. influenza* and *Klebsiella pneumonia*, which cause a variety of diseases such as pneumonia, traveler's diarrhea, and urinary tract infections.

3.7. Identification of antimicrobial targets: new genomic and antisense approaches

The majority of our current antibiotics originated from screening and identifying antimicrobial agents from natural sources. This approach was effective for several decades, but since the mid-1970s, no novel class of broad-spectrum antibiotics has been discovered. The emergence of antibiotic resistant bacteria has created an urgent need for new antimicrobial classes that are unaffected by resistance mechanisms. Therefore, new methods must be employed to discover novel antibiotics.

Access to whole or near-complete gene sets from multiple organisms, in combination with state-of-the-art bioinformatics techniques, greatly facilitates the selection of novel antibacterial targets. Genomics is used to select potential antibacterial targets [40] and provides insights into pathogenesis [41] and antibiotic resistance. The genomic-based antibacterial target may include ligand receptors, enzymes or other metabolites. Certain characteristics are required in target identification. The target should be unique to the pathogen of interest, and should be essential for its

survival. In the genomic approach targets are identified via bioinformatics and expression profiling of their predicted open-reading frames using DNA microarrays. Essentiality of the potential targets will be confirmed using knockout mutants [42]. Antisense approaches are also important in the control of microbial targets. The targets may be silenced [43] or be down-regulated by antisense RNA.

4. Surveillance of antimicrobial use and new antimicrobial therapy

The surveillance of antimicrobial use, especially antibiotics, has become a very notable issue in recent years. It has especially been pushed to the forefront of clinical medicine due to concerns over broad and rapid antimicrobial resistance among pathogenic organisms – partially due to overprescribing or overuse of antimicrobials. For instance, organisms such as bacteria isolated in clinical settings are tested for antibiotic susceptibility, so that a targeted treatment can be obtained, and resistance patterns can be avoided. In terms of surveillance of antimicrobials, it is clear that there has been a drastic increase in use – especially of antibiotics – over the past few decades. This has contributed to an environment where hardy and resistant organisms are selected for and allowed to flourish. This may lead to a future in which our antimicrobials will be unable to adequately treat infections. Therefore, the modern approach to these concerns has been two-fold: limit antibiotic use and develop new antimicrobials to combat resistant organisms.

The world has been heading towards a path of antibiotic resistance since the discovery of pencillin by Alexander Fleming in the early 1900s. It is apt to note that most organisms are no longer susceptible to penicillin and have not been for many years; in fact, the first resistance patterns to penicillin were likely noticed in the 1940's soon after its introduction. The mechanisms of resistances varied but most involve the degradation or inhibition of the β-lactam ring such as via a β-lactamase enzyme. Modified antibiotics from penicillin were eventually developed to create larger β-lactam rings that would avoid degradation. However, resistance was even developed to these. The mechanisms of antibiotic resistance have been found to be more varied than just enzymatic degradation. For instance, efflux pumps are proteins usually located within bacterial cell membranes that dispel unwanted materials or toxins from the interior of a cell. Most, if not all, bacteria contain efflux pumps, suggestive of a common ancestral link [44]. These efflux pumps can dispel antibiotics from a cell interior or can undergo changes to do so. Another diverse mechanism of antibiotic resistance has to do with the formation and proliferation of biofilms. This method differs from conventional intracellular or unicellular innate resistance methods involving plasmids and mutations and rather involves multicellular strategies such as increased antimicrobial tolerance and resistance to phagocytosis [45].

As an example of an antimicrobial resistance pattern, the antibiotic vancomycin was often used as a drug of last resort until recently. Due to the continued overuse and incorporation of vancomycin, significant problems have begun to emerge recently with this antibiotic, including poor bioavailability, increased bacterial resistance, and dosing issues. For instance, a recent study retrospectively examined the outcomes of 320 patients with MRSA bacteremia who were treated with vancomycin [46]. Among these patients, 52.5% experienced vancomycin failure based on the study set points, meaning that vancomycin was ineffective in eradicating the underlying bacteremia.

There are new antibiotics that have been developed or are in the pipeline that are already being used to combat resistant strains. Quinupristin-dalfopristin is an antibiotic that is gaining more recognition and study in recent years. Its potency is increased due to its synergistic combination of two different drugs. More specifically, quinupristin binds to a nearby site on the 50S ribosomal subunit and prevents elongation of the polypeptide while dalfopristin binds to the 23S portion of the 50S ribosomal subunit and changes it conformation, enhancing the efficacy and binding of quinupristin by 100 times. This antibiotic has great efficacy in treating a variety of Gram-positive conditions, primarily skin based. Future studies will likely push this and other antibiotics to the forefront of treatment of many resistant bacteria.

Daptomycin is another antibiotic that has gained traction in recent years. Its exact mechanism of action is unknown but it appears to bind to bacterial membranes and cause rapid depolarization. This rapid depolarization leads to the inhibition of DNA, RNA, and protein synthesis and thereby leads to bacterial cell death [47]. As such, it differs markedly from most other antibiotics in the market. It is derived from a naturally occurring saprotroph, *Streptomyces roseosporus* and its activity is still comparably higher than vancomycin when compared in the serum. Daptomycin is currently approved by the FDA for the treatment of complicated skin infections caused by β-hemolytic streptococci, *Enterococcus faecalis,* and *S. aureus* and studies on daptomycin have shown remarkable efficacy in the treatment of soft tissue infections in MRSA.

Outside of quinupristin-dalfopristin and daptomycin, there are other relatively new antibiotics that have been released including, linezolid, tigecycline, and ceftobiprole. However, since previous antibiotic resistance can aid in faster development of resistance to new antibiotics, development and research efforts may need to be increased [48]. For instance, one study suggests that increased vancomycin use may affect daptomycin efficacy. More specifically, one mechanism of resistance to vancomycin was found to be through a thickened cell wall that formed in an attempt to resist large vancomycin molecules. A follow-up study showed that although the mechanisms of action of daptomycin and vancomycin differ considerably, both antibiotic molecules possess large molecular weights and must pass cell wall barriers to act. These thickened cell walls could therefore cause decreased diffusion and a clogging effect through which resistance to these two and possibly other antibiotics could be conferred [49].

The development of these resistance patterns involves factors such as horizontal gene transfers, mutations, plasmids, and transposons. Although resistance is often thought of mainly in terms of antibiotics, since they are so widely utilized, they are by no means the only antimicrobials that are experiencing microbial resistance. For instance, antivirals undergo resistance via similar mechanisms such as enzymatic mutations.

Environments that promote the selection of mutating and adapting bacteria enhance the tendency towards resistance. However, this is a problem that does not necessarily have a clear solution. Changing prescribing practices does not seem to be necessarily appropriate as individuals should not suffer if there is a clear bacterial infection or the clinical suspicion is high. However, it is also clear that the status quo will not necessarily work particularly if the pipeline flow of new antibiotics slows down from its historic pace. And even though it could be argued that there is or may be an overuse of antimicrobials, it is apparent that antibiotic resistance is a factor that is present in natural situations and the environment itself. As such, we are left with a very difficult and rapidly approaching situation with few realistic answers.

Table 1 List of natural antimicrobials by source, class, and susceptibility.

Organism	Antimicrobial Class	Susceptible Microorganisms
Microorganism		
Bacillus polymyxa	Polypeptides	Gram-negative bacteria
Bacillus subtilis	Phospholipids	Gram-positive bacteria
Fischerella	Ambiguine I Isonitriles	Bacteria
Micromonospora	Macrolides and Peptides	Bacteria
Nostoc commune	Noscomins	Bacteria
Nostoc flagilliforme	Nostoflans	Virus
Nostoc muscorum	Phenolic compounds	Bacteria
Oscillatoria nigrovirdis	Viridamide A	Protozoa
Penicillium	β-Lactams	Gram-positive bacteria
Salinispora arenicola	Microlides	Bacteria
Streptomyces erythreus	Macrolides	Bacteria
Streptomyces griseus	Aminoglycosides	Bacteria
Streptomyces lincolnensis	Lincomycins	Bacteria
Streptomyces mediterranei	Rifamycins	Bacteria
Streptomyces nodosus	Polyenes	Fungi
Streptomyces noursei	Macrolides	Fungi
Streptomyces	Tetracyclines	Bacteria
Streptomyces venezuelae	Chloramphenicols	Bacteria
Streptomyces	Pyrrolosesquiterpenes	Bacteria
Streptomyces psommoticus	Polyketides	Bacteria, fungi
Streptomyces	Quinones and Microlides	Bacteria, fungi
Plant		
Abrus schimperi	Quinones	*Leishmania donovani*
Aeglemarmelos	Terpenoids	Fungi
Allium cepa	Sulfoxides	Bacteria, *Candida*
Allium sativum	Sulfoxides and Terpenoids	Bacteria, fungi, protozoa
Aloe barbadensis, Aloe vera	Complex mixture	Corynebacterium, *Salmonella*
Aloysiatri phylla	Terpenoids	*M. tuberculosis*, *S. aureus*
Anacardium pulsatilla	Polyphenols	*P. acnes*, bacteria, fungi
Archidendron Jiringa	Lectins	Bacteria, fungi
Arctium lappa	Terpenoids,and Polyacetylenes	Bacteria, fungi, viruses
Artemisia dracunculus	Terpenoids and Polyphenols	Viruses
Berberis vulgaris	Alkaloids	Bacteria and protozoa
Camellia sinensis	Flavonoids and Terpenoids	Bacteria, fungi, protozoa, virus
Cannabis sativa	Alkaloids	Bacteria and viruses
Capsicum annuum	Terpenoids	Bacteria
Carum carvi	Coumarins	Bacteria, fungi, viruses
Cassia angustifolia	Anthraquinones	*S. aureus*
Centella asiatica	Terpenoids	*M. leprae*
Citrus sinensis	Terpenoids	Fungi
Citrus paradisa	Terpenoids	Fungi
Croton pullei	Alkaloids	Bacteria, fungi

Crotolaria pallida	Peptides	Bacteria
Coccinia cordifolia	Flavonoids	Bacteria
Coriandrum sativum	Essential oils	Bacteria, fungi
Curcuma longa	Terpenoids	Bacteria, protozoa
Dorstenia barteri	Phenolics	Bacteria, fungi
Galium odoratum	Coumarins	Bacteria, fungi, protozoa, virus
Helichrysum gymnocomum	Phenolics	Bacteria
Hydrastis canadensis	Alkaloids	Bacteria, Protista, Plasmodium
Lawsonia	Quinones	*M. tuberculosis*, Bacteria
Mahonia aquifolia	Alkaloids	Plasmodium, Trypansomes
Malus sylvestris	Flavonoid derivatives	Bacteria, fungi, protozoa
Medicago sativa	Flavonoids	Gram-positive bacteria
Melissa officinalis	Polyphenols	Viruses
Mentha piperita	Terpenoids	Bacteria, fungi, protozoa
Mentha pulegium	Essential oils	Gram-positive bacteria
Momordica charantia	Essential oils	Bacteria, fungi, protozoa
Ocimum basilicum	Terpenoids	*Salmonella*, bacteria
Oleaeuropaea	Aldehydes	Bacteria, fungi, protozoa
Papaver somniferum	Alkaloids and others	Bacteria, fungi, protozoa
Panax ginseng	Polyacetelenes	Bacteria, Fungi
Peganum harmala	Alkaloids	Bacteria, fungi
Phyllanthus mullerianus	Essential oils	Bacteria, fungi
Piper bête, Piper nigrum	Essential oils and Alkaloids	Bacteria, fungi, protozoa
Quercus rubra	Polyphenols and Flavonoids	Bacteria, fungi
Ranunculus laetus	Coumarins	Bacteria
Ranunculus scleratus	Flavonoids	*Salmonella*, *Agrobacterium*
Rauvolfia serpentina	Alkaloids	Bacteria, fungi, protozoa
Rhamnus purshiana	Polyphenols and Anthaquinones	Virus, bacteria, fungi
Ricinus communis	Essential oils	Bacteria, fungi, protozoa
Rosmarinus officinalis	Terpenoids	Bacteria, fungi, protozoa
Santolinachamae cyparissus	Essential oils	*Candida*, *Schitosoma*
Satureja montana	Essential oils and Terpenoids	Bacteria, fungi, protozoa, virus
Schinus terebinthifolius	Terpenoids	Bacteria, fungi and protozoa
Thymus vulgaris	Terpenoids and Polyphenols	Viruses, bacteria, fungi
Tussilago farfara	Essential oils and Flavonoids	Bacteria, fungi, protozoa
Vicia faba	Thionins	Bacteria
Withania somnifera	Lactones	Bacteria, fungi
	Animal/Animal Product	
Amphibians	Magainins, dermaseptins, Buforins and Brevinins	Bacteria, fungi, protozoa
Cattle	Indolicidins	Bacteria
Hemipteran	Thanatins	Bacteria, fungi
Human	Histatins	Bacteria, fungi
Pig and Insects	Cecropins	Bacteria, fungi, virus
Mammals and Insect	Defensins	Bacteria, fungi, protozoa
Pig	Protegrin I	Bacteria, fungi, virus
Egg	Cystatins, Avidins, G2 Globulins	Bacteria, fungi
Honey	Complex mixture	Fungi, bacteria
Milk	Lactoferrins	Bacteria, fungi

References

[1] Lazzarini A, Cavaletli L, Toopo G, Mirinelli F. Rare genera of Actinomycetes as potential producers of new antibiotics. *Antonie van Leeuwenhock*. 2000;78:399-405.

[2] Bierbaum G, Sahl HG. Lantibiotics: mode of action, biosynthesis and bioengineering. *Current Pharmaceutical Biotechnology*. 2009;10:2–18.

[3] Cotter PD, Hill C, Ross RP. Bacterial lantibiotics: strategies to improve therapeutic potential. *Current Protein & Peptide Science*. 2005;6:61-75.

[4] Koroishi AM, Foss SR, Cortez DA, Ueda-Nakamura T, Nakamura CV, Dias Filho BP. In vitro antifungal activity of extracts and neolignans from *Piper regnellii* against dermatophytes. *Journal of Ethnopharmacology*. 2008;117:270–277.

[5] Mbaveng AT, Ngameni B, Kuete V, Simo IK, Ambassa P, Roy R, Bezabih M, Etoa FX, Ngadjui BT, Abegaz BM, Meyer JL, Lall N, Beng VP. Antimicrobial activity of the crude extracts and five flavanoids from the twigs of *Dorstenia barteri* (Moracae*). Journal of Ethnopharmacology*. 2008;116:483-489.

[6] Khatun S, Pervin F, Karim MR, Ashraduzzaman M, Rosma A. Phytochemical screening and antimicrobial activity of *Coccinia cordifolia* L. plant. *Pakistan Journal of Pharmaceutical Sciences*. 2012;25(4):757-61.

[7] Hussain J, Hussain H, Shinwari ZK, Ahmad I, Hussain ST, Ahmad VU. Antibacterial activity of the Chemical constituents from *Ranunculus laetus*. *Chemistry of Natural Compounds*. 2009;45(5):720-721.

[8] Bissa S, Bohra A. Evaluation of Antibacterial Potential of *Ranunculus sceleratus*. *Botany Research International*. 2012;5:10-13.

[9] Jung HJ, Sung WS, Yeo SH, Kim HS, Lee IS, Woo ER, Lee DJ. Antifungal effect of amentoflavone derived from *Selaginella tamariscina*. *Archives of Pharmacal Research*. 2006;29(9):746–751.

[10] Reddy M, Gupta S, Jacob M, Khan S, Ferreira D. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L. *Planta Medica*. 2007;73:461-467.

[11] Fernandez MA, Garcia MD, Saenz MT. Antibacterial activity of the phenolic acids fraction of *Scrophularia frutescens* and *Scrophularia sambucifolia*. *Journal of Ethnopharmacology*. 1996;53:11–14.

[12] Amiguet VT , Petit P, Ta CA, Nunez R, Sanchez-Vindas PS, Alvarez PL, Smith ML, Arnason JT, Durst T. Phytochemistry and antifungal properties of the newly discovered tree *Pleodendron costaricense*. *Journal of Natural Product*. 2006;69:1005-1009.

[13] Kuete V, Eyong KO, Folefoc GN, Beng VP, Hussain H, Krohn K, Nkengfack AE. Antimicrobial activity of the methanolic extract and of the chemical constituents isolated from *Newbouldia laevis*. *Die Pharmazie - An International Journal of Pharmaceutical Sciences*. 2007;62:552-556.

[14] Zhang SL, Chang JJ, Damu GL, Fang B, Zhou XD, Geng RX, Zhou CH. Novel berberine triazoles: synthesis, antimicrobial evaluation and competitive interactions with metal ions to human serum albumin. *Bioorganic & Medical Chemistry Letters*. 2013;23:1008-1012.

[15] De Bolle MF, Osborn RW, Goderis IJ, Noe L, Acland D, Hart CA,Torrekens S, Van Leuven F, Broekart NF. Antimicrobial properties from *Mirablis jalapa* and *Amaranthus caudalus*: expression, processing, localization and biological activity in transgenic tobacco. *Plant Molecular Biology*. 1996;31:993-1008.

[16] Swanson MD, Winter HC, Goldstein IJ, Markovitz DM. A lectin isolated from bananas is a potent inhibitor of HIV replication. *Journal of Biological Chemistry.* 2010;285(12):8646-8655.

[17] Fukuyama N, Shibuya M, Orihara Y. Antimicrobial polyacetylenes from *Panax ginseng* hairy root culture. *Chemical and Pharmaceutical Bulletin.* 2012;60:377-380.

[18] Arias BA, Ramon-Laca L. Pharmacological properties of citrus and their ancient and medieval uses in the Mediterranean region. *Journal of Ethnopharmacology*. 2005;97:89-95.

[19] Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine*. 2006;6:39.

[20] Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature.* 2002;415:389-395.

[21] Leon-Sicairos N, Lopez-Soto F, Reyes-Lopez M, Godinez-Vargas D, Ordaz-Pichardo C, de la Garza M. Amoebicidal Activity of Milk, Apo-lactoferrin, sIgA and Lysozyme. *Clinical Medicine and Research*. 2006;4:106-113.

[22] Valenti P, Antonini G. Lactoferrin: an important host defense against microbial and viral attack. *Cellular and Molecular Life Sciences*. 2005;62:2576-2587.

[23] Parisien A, Allain B, Zhang J, Mandeville R, Lan CQ. Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. 2007;104:1-13.

[24] Halawani E, Shohayeb M. Survey of the antibacterial activity of Saudi and some international honeys. *Journal of Microbiology and Antimicrobial*. 2011;3: 94-101.

[25] Drlica K, Malik M, Kerns RJ, Zhao X. Quinolone-mediated bacterial death. *Antimicrobial Agents and Chemotherapy.* 2008;52:385–392.

[26] Floss HG, Yu TW. Rifamycin-mode of action, resistance, and biosynthesis. *Chemical Reviews.* 2005;105:621–632.

[27] Taubes G. The bacteria fight back. *Science.* 2008;321:356-361.

[28] Kahne D, Leimkuhler C, Lu W, Walsh C. Glycopeptide and lipoglycopeptide antibiotics. *Chemical Reviews.* 2005;105:425–448.

[29] Guan XL, Souza CM, Pichler H, Dewhurst G, Schaad O, Kajiwara K, Wakabayashi H, Ivanova T, Castillon GA, Piccolis M, Abe F, Loewith R, Funato K, Wenk MR, Riezman H. Functional interactions between sphingolipids and sterols in biological membranes regulating cell physiology. *Molecular Biology of the Cell*. 2009;20:2083–2095.

[30] te Welscher YM, van Leeuwen MR, de Kruijff B, Dijksterhuis J, and Breukink E. Polyene antibiotic that inhibits membrane transport proteins. *Proceedings of the National Academy of Sciences.* 2012;109(28):11156-11159.

[31] Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. *Cell.* 2001;104:901-912.

[32] McClure WR, Cech CL. On the mechanism of rifampicin inhibition of RNA synthesis. *Journal of Biological Chemistry.* 1978;253:8949-8956.

[33] Guillot PV, Gualtieri M, Bastide L, Leonetti JP. *In vitro* activities of different inhibitors of bacterial transcription against *Staphylococcus epidermidis* biofilm. *Antimicrobial Agents and Chemotherapy*. 2007;51:3117-3121
[34] Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*. 2001;65:232–260.
[35] Katz L, Ashley GW. Translation and protein synthesis: macrolides. *Chemical Reviews*. 2005;105:499-528.
[36] Tenson T, Lovmar M, Ehrenberg M. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. *Journal of Molecular Biology*. 2003;330:1005-1014.
[37]Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiology and Molecular Biology Reviews.* 1997;61:377-392 .
[38]Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell.* 2007;130:797-810.
[39]Guerin E, Cambray G, Sanchez-Alberola N, Campoy S, Erill I, Da Re S, Gonzalez-Zorn B, Barbe J, Ploy MC, Mazel D. The SOS response controls integron recombination. *Science.* 2009;324:5930-1034.
[40]Monaghan RL, Barrett JF. Antibacterial drug discovery-then, now and the genomics future. *Biochemical Pharmacology*. 2006;71:901-909.
[41]Polissi A, Soria MR. Functional genomics of bacterial pathogens: from post-genomics to therapeutic targets. *Molecular Microbiology*. 2005;57:307-312.
[42]Pucci MJ. Use of genomics to select antibacterial targets. *Biochemical Pharmacology*. 2006;71:1066-1072.
[43]Singh SB, Phillips JW, Wang J. Highly sensitive target-based whole-cell antibacterial discovery strategy by antisense RNA silencing. *Current Opinion in Drug Discovery & Development.* 2007;10:160–166.
[44]Webber MA, Piddock LJ. The importance of efflux pumps in bacterial antibiotic resistance. *Journal of Antimicrobial Chemotherapy*. 2003;51(1): 9-11.
[45]Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet*. 2001;358(9276):135-138.
[46]Kullar R, Davis R, Levine DP, Rybak MJ. Impact of Vancomycin Exposure on Outcomes in Patients With Methicillin-Resistant Staphylococcus aureus Bacteremia: Support for Consensus Guidelines Suggested Targets. *Clinical Infectious Diseases*. 2011;52(8): 975-981.
[47]Schriever CA, Fernandez C, Rodvold KA, Danziger LH. Daptomycin: a novel cyclic lipopeptide antimicrobial. *American Journal of Health-System Pharmacy*. 2005; 62(11):1145-1158.
[48]Ambrose PG, Hammel JP, Bhavnani SM, Rubino CM, Ellis-Grosse EJ, Drusano GL. Frequentist and Bayesian pharmacometric-based approaches to facilitate critically needed new antibiotic development: overcoming lies, damn lies, and statistics. *Antimicrobial Agents and Chemotherapy*. 2012;56(3):1466-1470.
[49]Cui L, Tominaga E, Neoh H-m, Hiramatsu K. Correlation between Reduced Daptomycin Susceptibility and Vancomycin Resistance in Vancomycin-Intermediate *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*. 2006;50(3):1079-1082.

