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New Generation Antibiotics/Antibacterials: Deadly Arsenal for Disposal of Antibiotic Resistant Bacteria

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Abstract

Inappropriate prescribing, lack of compliance in taking medicines and wise spread uncontrolled use of drugs led to emergence of multidrug resistance in clinically important infectious agents. Over 480000 new cases of multidrug-resistant tuberculosis (MDR-TB) were reported by WHO in the year 2013 in hundred countries world wide. Therefore, there is an urgent need for new generation antibacterial which can effectively and precisely act on drug resistant bacteria. Different strategies of development of resistance in bacteria involve the changes at molecular level like mutations, over expression of enzymes and efflux. So, the strategies of antibiotics development can include methods which can counteract at molecular level like antisense antibacterial and inhibition of quorum sensing. Bacterial gene rpoD found in Staphylococcus species is highly conserved and became basis in creation of antisense antibacterial against it. Lipid II class of antibiotics including teixobactin and antimicrobial peptides (AMPs), i.e. produced synthetically, have also shown promising results against the resistant bacterial strains. The present review summarizes the current scenario on research and development of new age antibiotics and techniques to tackle drug resistant bacterial infection.

Keywords: Teixobactin; Anti-microbial peptides; Ctriporin; Beta-Lactum

Introduction

The last century saw the emergence of antibiotics as wonder drugs which not only saved million lives but also revolutionsed the arena of advanced medical sciences. These drugs have not only reduced the mortality during routine surgery and childbirth to negligible, and but also reduced the stigma associated with HIV which was once considered as deadly disease. In recent times due to exposure to various xenobiotics/drugs, bacteria are evolving so rapidly that they have developed resistant against antibiotics/antibacterial and hence pose serious threat to health [1]. Resistance development limits the use of antibacterials and this increases the demand of introduction of new compounds [2,3]. When early resistance to penicillin was observed, second generation antibiotics, methicillin, cephalothin and imipenem were already developed [4]. In 1961, a methicillin resistant strain of *S. aureus* (MRSA) was observed [5] and at present worldwide, an estimated 2 billion peoples carry some form of *S. aureus* and of these up to 53 million (2.7% of carriers) are thought to carry MRSA [6]. Over the years it has become clear that bacteria can develop resistance to almost any antibiotic. Except a few antibiotics, for instance erythromycin and vancomycin, resistance was developed against majority of antibacterials only a few years after their introduction into clinical use [7,8].

The mechanism of development of resistance in bacteria is mainly consisting of three strategies- (i) overexpression of enzymes that can modify the antibiotic drug rendering the antibiotic inactive; (ii) mutation of the bacterial target site that allows the target site to maintain its functional role yet abrogates binding of drug to the target or transverse of the antibiotic across the bacterial cell wall; (iii) export of antibiotic drugs to the extracellular media via multidrug-resistant (MDR) efflux pumps or loss of porin channels resulting in lower permeability of antibiotics (Figure 1) [9]. For example-drugs such as β-lactams, are inactivated via the over expression of β-lactamases, which hydrolyze the antibiotics [10], antibiotics such as linezolidare and the streptogramin class rendered ineffective via the modification of 23S ribosomal RNA [11] and efflux pump proteins such as AcrB in *E. coli* [12] export antibiotics such as ciprofloxacin and tetracycline out of bacterial cells.

The aim of this review is to explore the current status of research and development of antibacterial compounds and techniques to tackle the bacterial infection.

Teixobactin

Teixobactin is the first of its kind of antibiotic that provides a

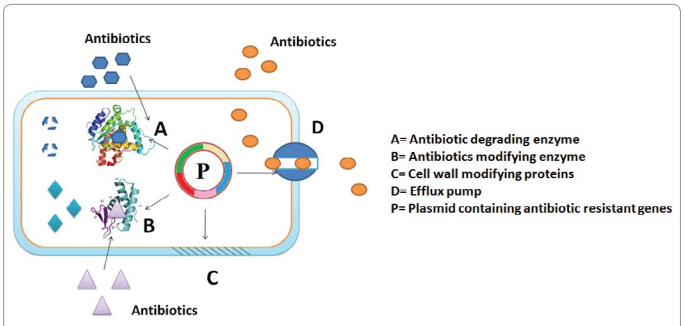

Figure 1: General mechanism of developing antibiotic resistance in bacteria.

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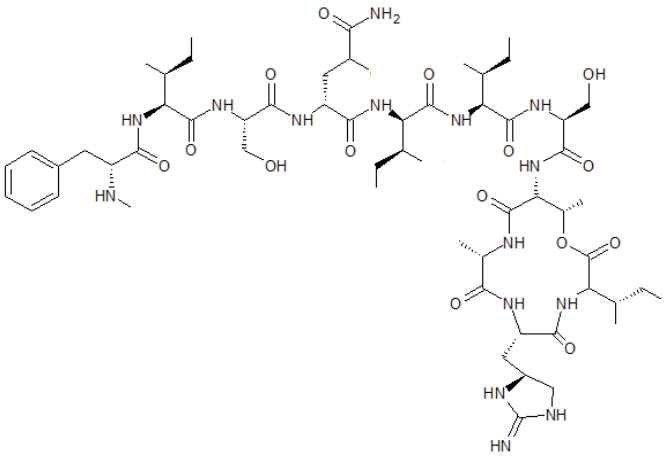

Figure 2: Structure of Teixobactin.

maiden entry to a more promising lipid II class of antibiotics (Figure 2). It is different from other known antibiotics like glycopeptides, lantibiotics and defensins in both its mode of action and structure [13-15]. Teixobactin is light of new hope in this era of incessantly growing antibiotics resistance; it is found out to be effective against a number of drug- resistant pathogenic microbes in some animal models of infection. Attack of teixobactin involves its binding to the wall of teichoic acid precursor that leads to the efficient lysis of cells and also the killing of cells by action of liberated autolysins [16]. Action of teixobactin is similar to the one other naturally occurring compound with a competent killing ability, 'acyldepsipeptide'. It converts the ClpP protease into a non-specific hydrolase which finally digests the cell [17]. Multiple targets are involved in the action of teixobactin out of which none of them is a protein.

Gram positive bacteria possess easily accessible lipid II which are poly prenyl precursors coupled to cell envelop and they represent a deadly weakness for antibiotic attack [18]. Among eubacteria pyrophosphate-sugar moiety of teixobactin target molecules is highly conserved. Gram-negative bacterium is one of those producers and its external membrane protects the bacterium from the re-entry of the compound. Henceforth, it is suggested by the study that the producer does not hires an alternative pathway for the synthesis of cell wall that would protect it from teixobactin. Therefore other bacteria couldn't borrow it. Horizontal transmission of a confrontation mechanism could sooner or later arise from some soil bacterium. The highly conserved teixobactin binding motif can take the form of an antibiotic modifying enzyme. Beta-lactams or aminoglycosides are those common antibiotics that codes for enzymes which attacks recurrently and they are unidentified for the vancomycin.

Newly discovered teixobactin is even less common than vancomycin. After its introduction into the clinic, it took years for vancomycin resistance to appear [19]. The lipid II modification pathway leading to vancomycin resistance possibly originated in the producer of vancomycin, *Amycolatopsis orientalis* [20]. Perhaps this could take even longer for resistance to better-protected teixobactin to emerge. The properties of teixobactin suggest that it evolved to minimize resistance development by target microorganisms. It is expected that additional natural compounds with similarly low susceptibility to resistance are present in nature and are waiting to be discovered [21]. However, teixobactin is not active against bacteria with an outer membrane like gram negative pathogens, particularly carbapenem resistant enterobacteriaceae, or those with New Delhi metallo-beta-lactamase 1 [22].

Antisense Antibacterial

Antisense antibacterials are short (about 10 to 20 bases), synthetic DNA analogs that constrains the essential genes expression at mRNA level in a sequence-specific manner [23]. Thereafter, antisense inhibition leads to bacteriocidal or bacteriostatic effect or restoration of bacterial susceptibility, which relies on the role of targeted gene. Synthetic antisense oligomers, particularly phosphorodiamidate morpholino (PMO) [24] and peptide nucleic acid (PNA) [25], possess favorable properties in light of antisense antibacterial application. It also includes enhanced biological stability, targeting specificity, binding affinity and access to an array of chemical modifications. Meanwhile, instead of simple mixture, cell penetrating peptides (CPP) can be covalently attached or conjugated at the end of PNA or PMO chain to upgrade cellular uptake of antisense oligodeoxynucelotides (ASODNs) without affecting Watson-Crick base paring between antisense oligomers and targeted RNAs [26]. Synthetic peptide- PNA or peptide-PMO conjugates targeting growth-essential genes shown to inhibit bacterial progression in pure culture and in infected tissue culture too. Therefore, a range of functional genes have been identified as potential targets [27]. However, only a few initial reports provided preliminary proof-of-principle support on antisense targeting of *S. aureus* genes for growth inhibitory effect (i.e., peptide-PNA targeting fabI [28] and phoB, fmhB, gyrA, plus hmrB.

Bacterial DNA-dependent RNA polymerase (RNAP) plays a vital role in transcription regulation and gene expression. Function of which requires coordination of a core enzyme (comprising five subunits α_2, β, β' and Ω) and an independent σ subunit that is reversibly employed by core enzyme [29]. The RNAP core enzyme is accountable for transcription elongation whereas different σs bind to different promoters to initiate transcription of genes of varied function. This irreversible inhibition of RNAP thereby causes cell death. It has engrossed much exploration for developing specific RNAP inhibitors (e.g., the rifamycins with fundamental clinical significance). The most developed σ70 family of σs, especially the primary σ70, is essential for initiating transcription of multiple genes in exponential growth cells [30], which to our knowledge has not previously been demonstrated for antisense target validation in *S. aureus*. The primary σ70s are found to be unique in structure, function as well as homology. The core regions of bacterial and eukaryotic RNAPs share structural and functional similarities, but the sequences of encoding genes are only partially homologous. Specifically, bacterial gene rpoD (encoding the primary σ70 of RNAP) shares the least homology in sequence with eukaryotic rpoD. Hence, in contrast to more conserved molecules, sequence-based drugs targeting rpoD products, including mRNAs, are less likely to cross react with host molecules. Most importantly, bacterial gene rpoD is highly conserved in identity and homologous in sequence among different pathogenic *Staphylococcus* species [31]. Such features are of distinctive advantages for developing narrow-spectra of anti-MRSA antisense agents.

Antimicrobial Peptides (AMPs)

In year 1939 Dubas discovered antimicrobial peptides [32]. Both Dubos and Hotchkiss identified an AMP in the following year which was named as gramicidin [33]. It was found to be very effective for topical treatment of wounds and ulcers [34]. AMPs, the major components of

innate immune system play a vital role in the host defense mechanism against environmental microorganisms. They are well versed in nature, existing in organisms from insects to plants and from microorganisms to mammals. AMPs have broad spectra of activity against infectious agents that includes Gram-negative and Gram-positive bacteria, fungi, viruses, and parasites too and rapid action. Cationic peptides are not affected by many antibiotic resistance mechanisms that now bound the use of other antibiotics [35,36]. Furthermore, in some of the cases, certain AMPs have been reported to kill antibiotic resistant bacteria e.g., both nisin (an AMP) and vancomycin (an antibiotic) kill bacteria by blocking their cell wall synthesis. However, MRSA (methicillin resistant *Staphylococcus aureus*) strain was reported to be resistant to vancomycin, while it was still sensitive to nisin [37].

In total, more than 5,000 AMPs have been discovered or synthesized up to date [38]. Natural AMPs can be found in both prokaryotes (e.g., bacteria) and eukaryotes (e.g., protozoan, fungi, plants, insects, and animals) [39-42] whereas in animals, AMPs are mostly found in the tissues and organs that are exposed to airborne pathogens. They are believed to be the first line of the innate immune defense [43,44] against viruses, bacteria, as well as fungi [40].

Most AMPs reported till date can be characterized on the basis of their secondary structures and classified into four types: β-sheet, α-helix, extended, and loop. Among these structural elements, α-helix and β-sheet structures are more common [45] and α-helical peptides are the most extensively studied AMPs (Table 1). In α-helix conformation the distance between two adjacent amino acids is around 0.15 nm and the angle between them with regard to the center is around 100 degree from the top view. The best known examples of α-helical AMPs are magainin, protegrin, cyclic indolicin and coiled indolicin [46]. β-sheet peptides are composed of at least two β-strands with disulfide bonds between these strands [47]

Researchers have identified, cloned and characterized a novel antimicrobial peptide from the venom of the scorpion *Chaerilus tricostatus* and named it as ctriporin [58]. The mature peptide of ctriporin was composed of 19 amino acid residues and possessed amidated C terminus. At low concentrations Ctriporin showed potent growth-inhibitory activity against standard *Candida albicans* and Gram-positive bacteria. Moreover, the *in vitro* treatment of clinically isolated pathogenic strains exhibited that ctriporin can also restrain antibiotic-resistant pathogens, including MRSA, methicillin-resistant coagulase-negative Staphylococcus (MRCNS), and penicillin- resistant *Staphylococcus epidermidis* (PRSE) strains. Ctriporin antimicrobial activity was explored in *in vivo* ointment application in topical treatment of mouse skin infection model. Finally, a standard Gram-positive bacterium, *S. aureus*, as a model bacterial strain is chosen to further explore the antimicrobial mechanism of ctriporin. All research about Ctriporin indicate it as an effective promising antimicrobial agent which act via the bactericidal mechanism of the rapid cell lysis [58].

S.No	Classes of AMPs	Examples
1.	Antiviral Peptides [48,49]	Heparan Sulfate (gllycosaminoglycan) Lactoferrin (cationic peptides)
2.	Antibacterial Peptides [37,50-52]	Buforin Drosocin, Pyrrhocoricin & Apidaecin Nisin
3.	Antifungal Peptides [53-55]	α helical(D-V 13K and P18) β sheet (Defensins) Indolicin
4.	Antiparasitic Peptides [56,57]	Magainin Cahelicidin

Table 1: Classifications of AMPs.

Inhibition of Quorum Sensing

Quorum sensing is a communication services or a system of communication between bacterial cells, whereby bacterial cells secrete and receipt signaling molecules from the local environment. A sufficient amount of inducer molecules is required to trigger the expression or suppression of specific genes responsible for bacterial activities like virulence gene expression, biofilm formation and resistance against antibiotic treatment [9]. Almost all quorum-sensing processes use micro molecules, known as autoinducers (AIs). Most frequently studied autoinducers belong to one of the following classes: acylated homoserine lactones (AHLs) used by Gram-negative bacteria (also sometimes called autoinducer-1 [AI-1]); peptide signals used by Gram-positive bacteria; and autoinducer-2 (AI-2) used by both Gram-negative and Gram-positive bacteria [59].

Inhibition of Quorum Sensing is one of the latest therapeutics development technologies that aim at targeting functions that are important for the infection. This technique has various potential benefits that include increase in range of bacterial targets, exertion of less selective stress and preservation of the host endogenous microbiome, which could ultimately result into the decreases resistance [60]. Several measures that were taken to inhibit regulation of virulence factors have engrossed on their interference with QS. Various efforts have been taken to discover the compounds along with inhibitory QS systems due to the vital role of QS in modulation and regulation of hundreds of virulence factors in bacteria. These compounds shall inhibit the synchronized expressions of virulence determinants without prying with bacterial growth because they could stay along with the base of an anti-pathogenic strategy and this would ultimately generate less resistance [61].Some bacteria species can produce enzymes called lactonases that can target and inactivate AHLs. Researchers have developed novel molecules which block the signaling receptors of bacteria, mBTL is a compound that has been shown to inhibit quorum sensing [62]. Furthermore, several research groups are analyzing and developing some compounds of natural origin (such as caffeine) as potential quorum sensing inhibitors [63].

Broadening the Spectrum of Beta-Lactam Antibiotics

The resistance of MRSA strain to all Beta-lactam class antibiotics limits treatment recourse for serious ailments caused by this organism. Researchers discover new agents that restore the activity of beta-lactams against MRSA, an approach that has led to the discovery of two classes of natural product antibiotics, a cyclic depsipeptide (krisynomycin) and a lipoglycopeptide (actinocarbasin) [64], which potentiate the activity of imipenem against MRSA strain COL. Researchers reported that these imipenem synergists are inhibitors of the bacterial type I signal peptidase SpsB, a serine protease that is required for the secretion of proteins that are exported through the Sec and Tat systems [65]. A synthetic derivative of actinocarbasin, M131, synergized with imipenem was prepared and exhibited both *in vitro* and *in vivo* potent efficacy. The *in vitro* activity of M131 extends to clinical isolates of MRSA but not to a methicillin-sensitive strain (Figure 3).

Synergy is restricted to beta-lactam antibiotics and is not observed with other antibiotic classes. The current propose is that the SpsB inhibitors synergize with beta-lactams by preventing the signal peptidase-mediated secretion of proteins required for beta-lactam resistance. Combinations of SpsB inhibitors and beta-lactams may expand the utility of these widely prescribed antibiotics to treat MRSA

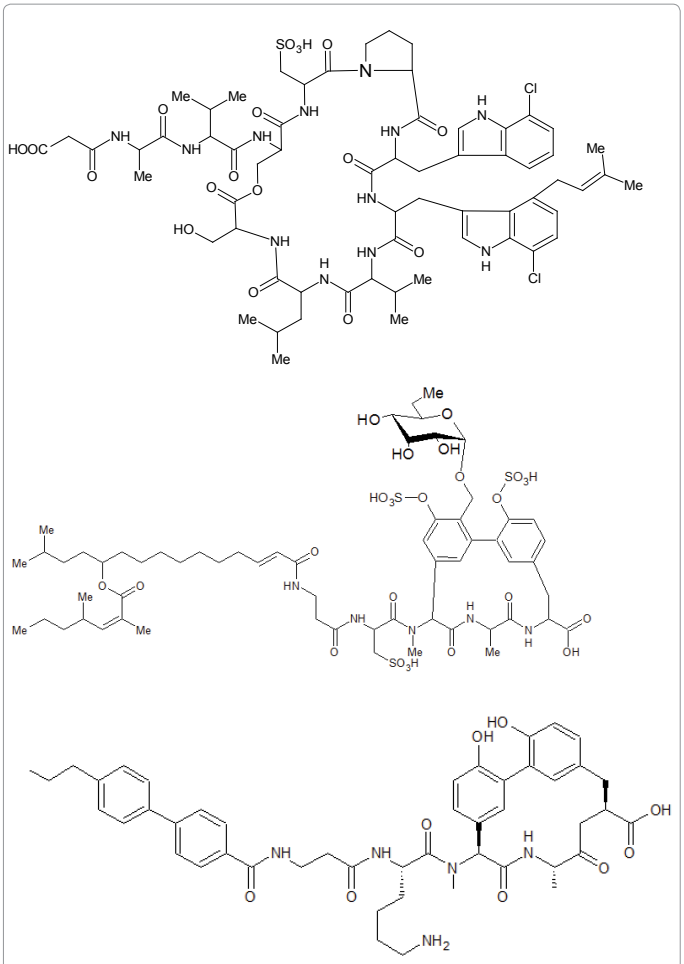

Figure 3: β- Lactam synergists. [A. Krisynomycin, B. Actinocarbasin and C. M131 synthetic derivative of actinocarbasin].

infections, analogous to beta-lactamase inhibitors which restored the utility of this antibiotic class for the treatment of resistant Gram-negative infections [64].

Nano Metals as Antibacterial Agents

Metals posess good thermal, electrical conductivity and chemical reactivity oweing to their large crystallographic suface area to volume ratio, hence can be potentially very toxic to the microbes. Due to their antimicrobial activity humans are using some metals since ages as antimicrobial agents in agriculture and medical uses e.g., silver has potential antimicrobial activity at unusual low concentrations [66]. Recently developed approaches in the domains of nanotechnology, especially the capability to produce metal oxide nanomaterials of definite size and shape, are liable to lead the development of new antibacterial agents [67]. Nanoparticles have received great attention due to their unique physical, chemical, and effective biological properties in various fields, including medicine. Considering these unique properties, nano-sized organic and inorganic particles are being generated for ultimate use in medical practices, such as metal oxides of zinc, copper, and iron in biomedical research [68,69].

Silver nanoparticles manifested biocide effect by anchoring and penetrating the bacterial cell wall and interact with sulfur- and phosphorus-containing biomolecules like DNA and silver ions strongly interacts with thiol groups of vital enzymes and inactivates them [70,71]. Silver exhibited more pronounced action against gram negative organisms than gram-positive bacteria and can inhibit growth of approximately 650 diesease causing agents. Silver nanoparticle based biocide showed that its antimicrobial effect is independent of acquisition of resistance against antibiotics [72]. Necessary metals such as copper also show similar properties but above some threshold levels [73,74]. The mode of action of the biocide activity is based on specific properties of metals and it can be activated by the metal reduction potential and the metal donor atom selectivity and/or speciation [66]. In addition, nanoparticles with smaller particle size have been reported to show good antimicrobial activity [75]. Antimicrobial activity of nanoparticles has largely been studied with human pathogenic bacteria such as *Escherichia coli* [76] and *Staphylococcus aureus* [77]. Moreover, these microbes seem to be highly sensitive to ZnO and CuO nanoparticles [75,78]. The cell can directly incorporate nanoparticles via endocytotic mechanisms and afterwards the cellular uptake of ions increases as ionic species are subsequently released within the cells by nanoparticle dissolution, a process often referred as "the Trojan horse mechanism". This high intracellular concentration gained after nanoparticle dissolution within the cell likely results in massive oxidative stress [79-81]. Recently bioactive glass BAG-S53P4 was reported to have anti-biofilm forming activity against MDR bacterial strains [82,83].

Conclusion

According to an estimate the number of deaths caused by bacterial infection is highest in the world. There is an urgent need of novel antimicrobial drugs to fight against infection because bacteria are evolving and developing with a greater pace. We are surrounded by endless possibilities to fight against bacterial infection but only some of them are explored yet. Out of all existent antimicrobial drugs only a few have been taken to the clinical trials only a very few reached to the market for public use.

Teixobactin is a very effective drug against many pathogenic micro-organisms which are resistant to the drugs currently in market. But it has some limitations for gram –ve bacteria. Anti-microbial peptides (AMPs) being a module of immune system though, can be synthesized for use as an antibiotics, which are very effective especially in topical treatment of ulcer etc. Cloned AMP ctriporin is a good example which a very effective role in inhibiting MRSA, MRCNS and PRSE. Molecular methods have also been employed for bacterial infection treatment involving antisense antibacterial and quorum sensing inhibitions which can easily overcome the resistance developed by micro-organisms and are very specific to its target. This can be a very sincere step in developing new antimicrobials.

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