IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
October 2019 Vol.:16, Issue:3

Current Perspective in Antimicrobial Resistance: New Antibiotics and Alternative Strategies

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Submission: 25 September 2019
Accepted: 30 September 2019
Published: 30 October 2019

Keywords: Antimicrobial resistance, Vaccine, Natural products, Alternative Strategies

ABSTRACT

Antimicrobial resistance (AMR) is the ability of a microorganism (like bacteria, viruses, and some parasites) to stop an antimicrobial such as antibiotics, antivirals and antimalarials from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases, resulting in prolonged illness, disability, and death. Without effective antimicrobials for prevention and treatment of infections, medical procedures such as organ transplantation, cancer chemotherapy, diabetes management and major surgery (for example, caesarean sections or hip replacements) become very high risk. The alarming increase of multidrug resistant pathogens makes the discovery process of new antibacterial compounds to continue the conventional strategies. The approaches based on conventional and new targets and the alternative routes based on targeting non-multiplying bacteria, non-cultural bacteria, antimicrobial peptides, lysins, probiotics, vaccines or bacteriophages discussed here brought together as much information as possible on the subject and will hopefully help specialists work hand-in-hand in the discovery of novel antibacterials.

INTRODUCTION

Antimicrobial resistance has been a major clinical and public health problem within the lifetime of most people living today. Antimicrobial resistance (AMR) is the ability of a microbe to resist the effects of medication previously used to treat them. Further antibiotics were discovered and went on to revolutionize healthcare, becoming the bedrock of many of the greatest medical advances of the 20thcentury. More recently, advances in antiviral developments over the past 20 years have transformed HIV from a probable death sentence into a largely manageable lifelong condition.

Antimicrobial resistance (AMR) can also be referred to as drug resistance. AMR develops when micro-organisms (bacteria, fungi, parasites, or viruses) no longer respond to a drug to which they were previously susceptible. Microorganisms that develop antimicrobial resistance are sometimes referred to as “superbugs”. Antimicrobial resistance is just one of the many adaptive traits that resilient bacterial subpopulations may possess or acquire; enabling them to out-compete and out-survive their microbial neighbor’s and overcome host strategies aimed against them.

Resistance to antimicrobials is a natural process that has been observed since the first antibiotics were discovered. Major life threatening infectious diseases such as TB (Tuberculosis), HIV (Human immunodeficiency virus) and malaria. Antimicrobial drugs are medicines that are active against a range of infections, such as those caused by bacteria (antibiotics), fungus (antifungal), viruses (antivirals), and parasites (including antimalarial). [1]

Resistance has increasingly become a problem in recent years because the pace at which we are discovering novel antibiotics has slowed drastically, while antibiotic use is rising. And it is not just a problem confined to bacteria, but all microbes that have the potential to mutate and render our drugs ineffective. The great strides forward made over the past few decades to manage malaria and HIV could be reversed, with these diseases once again spiraling out of control.

The variation in the AMR problems of individual countries is linked to huge differences in how heavily they use antimicrobial drugs. Overuse and misuse of antimicrobials is facilitated in many places by their availability over the counter and without prescription, but even where this is not the case prescribing practices vary hugely between (and often within) countries.

Such issues are only made worse by large quantities of counterfeit and sub-standard antimicrobials permeating the pharmaceuticals markets in some regions.

As with all infectious diseases, the speed and volume of intercontinental travel today creates new opportunities for antimicrobial-resistant pathogens to be spread globally. Such mixing of different microbes, particularly bacteria, provides them with opportunities to share their genetic material with each other, creating new resistant strains at an unprecedented pace.[2]

HISTORY OF ANTIBIOTIC RESISTANCE

Antibiotics revolutionized medicine in the 20th century, and have together with vaccination led to the near eradication of diseases such as tuberculosis in the developed world. Their effectiveness and easy access led to overuse, especially in livestock raising, prompting bacteria to develop resistance. [3]

Penicillin, the first natural antibiotic discovered by Alexander Fleming in 1928. Before the early 20thcentury, treatments for infections were based primarily on medicinal folklore. Mixtures with antimicrobial properties that were used in treatments of infections were described over 2000 years ago. Many ancient cultures used specially selected mold and plant materials and extracts to treat infections.

Alexander Fleming, the first sulfonamide and first commercially available antibacterial, Prontosil, was developed by a research team led by Gerhard Domagk in 1932 at the Bayer Laboratories of the interessenGemeinschaft Farben conglomerate in Germany. Domagk received the 1939 Nobel Prize for Medicine for his efforts. Prontosil had a relatively broad effect against Gram-positive cocci, but not against enterobacteria. Research was stimulated apace by its success. The discovery and development of this sulfonamide drug opened the era of antibacterials.[4]

In 1939, coinciding with the start of World War II, Rene Dubos reported the discovery of the first naturally derived antibiotic, tyrothricin, a compound of 20% gramicidin and 80% tyrocidine, from B. brevis. It was one of the first commercially manufactured antibiotics universally and was very effective in treating wounds and ulcers during World War II. Commonly used antibiotics and timeline of antibiotic resistance are depicted in table 1 and 2.

TABLE No. 1: COMMONLY USED ANTIBIOTICS

Antibiotic class	Example(s)	Target	Mode of resistance
β-lactams	Penicillins (ampicillin) Cephalosporins (cephamycin) Penems (meropenem) Monobactams (aztreonam)	Peptidoglycan biosynthesis	Hydrolysis Efflux Altered target
Aminoglycosides	Gentamicin Streptomycin Spectinomycin	Translation	Phosphorylation Acetylation Nucleotidylation Efflux Altered target
Glycopeptides	VancomycinTeicoplanin	Peptidoglycan biosynthesis	Reprogramming of peptidoglycan biosynthesis
Tetracyclines	Minocycline Tigecycline	Translation	Monooxygenation Efflux Altered target
Macrolides	Erythromycin Azithromycin	Translation	Hydrolysis Glycosylation Phosphorylation Efflux Altered target
Lincosamides	Clindamycin	Translation	Nucleotidylation Efflux Altered target
Streptogramins	Synercid	Translation	C-O lyase (type B streptogramins) Acetylation (type A streptogramins) Efflux Altered target
Oxazolidinones	Linezolid	Translation	Efflux Altered target
Phenicols	Chloramphenicol	Translation	Acetylation Efflux Altered target
Quinolones	Ciprofoxacin	DNA replication	Acetylation Efflux Altered target
Pyrimidines	Trimethoprim	C1 metabolism	Efflux Altered target
Sulfonamides	Sulfamethoxazole	C1 metabolism	Efflux Altered target
Rifamycins	Rifampin	Transcription	ADP-ribosylation Efflux Altered target
Lipopeptides	Daptomycin	Cell membrane	Altered target
Cationic peptides	Colistin	Cell membrane	Altered target Efflux

TABLE No. 2: TIMELINE OF ANTIBIOTIC RESISTANCE

Timeline of antibiotic resistance	
1940	Penicillin-R Staphylococcus antibiotic resistance identified
1943	Penicillin antibiotic introduced
1950	Tetracycline antibiotic introduced
1953	Erythromycin antibiotic introduced
1959	Tetracyclin-R Shigella antibiotic resistance identified.
1960	Methicillin antibiotic introduced
1962	Methicillin R Staphylococcus antibiotic resistance identified
1965	Penicillin-R pneumococcus antibiotic resistance identified
1967	Gentamycin antibiotic introduced
1968	Erythromycin R-Streptococcus antibiotic resistance identified
1972	Vancomycin antibiotic introduced
1979	Gentamycin-R enterococcus antibiotic resistance identified
1985	Imipenam and ceftazidim antibiotic introduced
1987	Ceftazidim -R enterobacteriaceae antibiotic resistance identified
1988	Vancomycin- R enterococcus antibiotic resistance identified
1996	Levofloxacin antibiotic introduced , Levofloxacin R pneumococcus antibiotic resistance identified
1998	Imipenam R enterobacteriaceae
2000	XDR tuberculosis antibiotic introduced, Linezolid antibiotic introduced
2001	Linezolid R-Streptococcus antibiotic resistance identified
2002	Vancomycin- R Staphylococcus antibiotic resistance identified
2003	Daptomycin antibiotic introduced
2009	Ceftriaxone R-neisseriagonorrhoeae antibiotic resistance identified
2010	Ceftaroline antibiotic introduced
2011	Ceftaroline R Staphylococcus antibiotic resistance identified

The era of antibacterial treatment began with the discovery of arsphenamine, first synthesized by Alfred Bertheim and Paul Ehrlich in 1907, and used to treat syphilis. The first systemically active antibacterial drug, prontosil was discovered in 1933 by Gerhard Domagk, for which he was awarded the 1939 Nobel Prize. All classes of antibiotics in use today were first discovered prior to the mid-1980s.[5]

ANTIMICROBIAL RESISTANCE IN TUBERCULOSIS, HIV, MALARIA

Antimicrobial resistance is the ability of microbes to resist the effects of drugs that is, the germs are not killed, and their growth is not stopped.

TUBERCULOSIS

Tuberculosis is a severe airborne disease caused by bacterial infection. Its situation is worsened due to the presence of multidrug resistant (MDR) strains of mycobacteria tuberculosis, the causative agent of disease. From the guidelines of the WHO, the first-line drugs for tuberculosis treatment include isoniazid, rifampicin, pyrazinamide and ethambutol. First-line drugs are mainly bactericidal, highly efficacious and relatively less toxic compared with other anti-tuberculosis drugs.

Mechanism of resistance:

Intrinsic drug resistance; in Mycobacterium tuberculosis has been attributed to its unique cell wall properties, including the tuberculosis presence mycolic acid, fatty acid and hydrophobic barrier and also beta- lactamase enzymes responsible for resistance to antibiotics.

Acquired drug resistance occurs when a microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible. Acquired drug resistance is caused mainly by spontaneous mutation in chromosomal gene, and the selective growth of such drug-resistant mutants may be promoted during sub optimal drug therapy.[6]

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Human immunodeficiency virus (HIV) infects cells of the immune system, destroying or impairing their function. HIV infection is relentless destruction of the immune system leading to onset of the acquired immunodeficiency syndrome (AIDS). Resistant strain of the HIV virus emerge rapidly if only one antiviral drug is used, newer drugs are needed because of the continuing emergence of the drug-resistant HIV strains.[7]

Mechanism of resistance:

The HIV virion has two properties that increase its ability to develop ARV resistance. The basic mechanisms include.

- A modulator effect at drug binding site.
- An enzymatic activity that can remove drug from its binding site
- A size change in the drug binding site causing inability to compete for the enzyme.

MALARIA

Malaria is a disease caused by a parasite and kills around one million people every year worldwide, the evolution of drug-resistant parasites has led to certain antimalarial drugs becoming ineffective. Chloroquine plus primaquine remains the first-line regimen for radical cure of vivax malaria in most regions.

Mechanism of resistance:

In general, resistance appears to occur through spontaneous mutation, in some drugs by point mutation. Chloroquine is remarkable antimalarial drug; it is rapidly active against the blood stages of all four species of human malaria. It related to non- enzymatic Inhibition of haem polymerization a necessary defense mechanism for the parasite.[8]

MULTI DRUG RESISTANCE

Multidrug resistance is antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drugs. The types most threatening to public health are MDR bacteria that resist multiple antibiotics; other types include MDR viruses, fungi, and parasites.[9]

Common multidrug-resistant organisms are usually bacteria:

- ❖ Vancomycin-Resistant Enterococci (VRE)
- ❖ Methicillin-Resistant Staphylococcus aureus (MRSA)
- ❖ Extended-spectrum β-lactamase (ESBLs) producing Gram-negative bacteria
- ❖ Klebsiella pneumonia carbapenemase (KPC) producing Gram-negatives
- ❖ Multidrug-Resistant gram negative rods (MDR GNR) MDRGN bacteria such as Enterobacter species, E.coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa

❖ Multi-drug-resistant tuberculosis[10]

EMERGING TRENDS IN ANTIMICROBIAL DRUG DEVELOPMENT

The need for new antimicrobial agents is greater than ever because of the emergence of multidrug resistance in common pathogens, the rapid emergence of new infections, and the potential for use of multidrug-resistant agents in bioweapons.

NEW ANTIBIOTICS FROM NATURAL PRODUCTS

Natural products have historically been of crucial importance in the identification and development of antibacterial agents. Interest in these systems has waned in recent years, but the rapid emergence of resistant bacterial strains has forced their re-evaluation as a route to identify novel chemical skeletons with antibacterial activity for elaboration in drug development.[11] Natural products have played a major role in antibiotic drug discovery since 1941 when penicillin was introduced to the market, and currently, natural products are again the most important source for promising drug candidates.

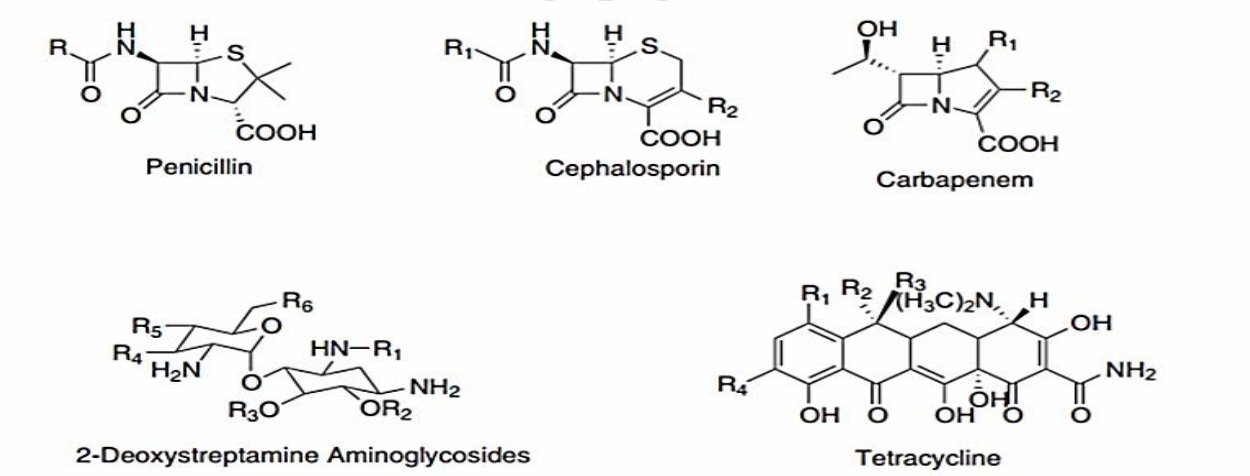

Figure No. 1: Antibiotics from natural sources

The antibiotics provide unique challenges to the drug discovery sector; the choice of chemical matter early in the discovery process is of paramount importance to success. Traditionally, natural products, i.e., genetically encoded small molecules, produced by bacteria and fungi have been the dominant source of clinically used antibiotics. In contrast, completely synthetic chemical scaffolds are in the minority. On the other hand, a survey of some of the most prescribed drugs targeting human biology reveals different situation-

synthetics medicine. The reasons for this discrepancy reflect the fact that bacterial cells have evolved complex and redundant obstacles to penetration and retention of compounds, as noted above.[12]

Natural products on the other hand are also products of evolution that have very often been selected precisely for their ability to enter bacterial cells. Natural products continue to provide new chemical structures with high levels of antibacterial activity. The structural modularity of many natural product-derived antibacterials allows a building-block approach which can be exploited for the rapid construction of new chemical systems. Reinvigoration of this approach is fully justified as a fast route to address the AMR problem.[13]Some natural and semisynthetic antibiotics used in clinical use are listed in table 3 and table 4.

TABLE No. 3: NATURAL ANTIBIOTIC IN CLINICAL USE

Chemical scaffold	Example of clinically used drugs
Beta-Lactam	Penicillins: amoxicillin, ampicillin, Cephalosporins: cephalexin
Glycopeptide	Vancomycin
Macrolide	Erythromycin, clarithromycin
Lincosamide	Clindamycin
Aminoglycoside	Gentamicin, tobramycin, amikacin
Streptogramin	Synercid
Tetracycline	Doxycycline, minocycline
Rifamycin	Rifamycin
Lipopeptide	Aptomycin
Cationic peptide	Colistin

TABLE No. 4: SYNTHETIC ANTIBIOTICS IN CLINICAL USE

Antibiotic class	Example
Sulfonamide	Sulfamethoxazole
Diaminopyrimidine	Trimethoprim
Fluoroquinolone	Ciprofloxacin, levofloxacin
Oxazolidinone	Linezolid
Nitroimidazole	Metronidazole

The history of natural-product based antibiotic discovery using cell-based growth inhibition screens has been more often than not one of rediscovery of known scaffolds rather than completely novel compounds. The discarding of these known scaffolds during the discovery process, a process called dereplication, is laborious, involving the purification and characterization of the active metabolites from complex mixtures. The challenge of dereplication also points to another difficulty with natural products, which is compound availability. In many cases, the natural product serves as a lead for further modification by medicinal chemists to impart better drug-like properties. These challenges to a revival in natural products as privileged leads in antibiotic discovery are significant; however, they can, and are, being overcome. Recent advances in microbial genomics and understanding of metabolite production are combining to solve the dereplication problem. [14]

INDOLE BASED ANTIBIOTICS

Antibiotic resistance represents a worldwide threat. According to a new report issued by the Centers for Disease Control and Prevention each year more than two million people get infections that are resistant to antibiotics and at least 23,000 people die as a result. In particular, methicillin resistant S.aureus (MRSA) is a major community-acquired as well as nosocomial pathogen causing skin and soft tissues infections, respiratory disease and more serious illness like pneumonia, endocarditis and sepsis.[15]The most important factor in antibiotic multi-drug resistance (MDR) is the irresponsible use of antibiotics. This led the medical community to ask for urgent development of novel drugs to fight resistant strains.

In particular, the S.aureusNorA efflux pump, which belongs to the major facilitator superfamily (MFS) of transport proteins, can contribute to quinolone-based drug resistance by removal of these drugs thus lowering their effective concentrations. The major obstacle in designing specific inhibitors of the NorA pump is that the three dimensional structure of the protein is not yet known. Some molecules are well-known inhibitors of NorA, such as reserpine, which is frequently used as reference compound in inhibition studies. Unfortunately, although reserpine is an approved drug for other targets, it cannot be used for NorA inhibition because of its neurotoxic effect at the required concentrations.[16]

The indole moiety has been shown to be promising with respect to the EPI activity of a compound. The compound 5-nitro-2-phenyl-(1H)-indole, known as INF55, is one of the first identified indole-based inhibitors of NorA and is capable of producing a 4-fold increase in the

susceptibility of S.aureus to ciprofloxacin at a concentration of 1.5µg/ml. [17]the structure of indole based antibiotic is shown in figure 2.

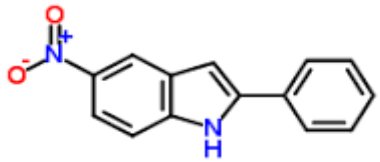

Figure No. 2: Indole based Antibiotics

GLYCOPEPTIDE MODIFICATION

The Glycopeptide antibiotics (GPAs) are essential for the control of infectious disease caused by Gram-positive pathogenic bacteria; Vancomycin, teicoplanin and telavancin are some examples of Glycopeptide.GPAs act by binding to the N-acyl-D-Ala-D-Ala termini of peptidoglycan and its precursor.

This interaction physically blocks cell wall biosynthetic enzymes, inhibiting cell growth and division. They remain essential drugs for the treatment of life-threatening infections caused by important human pathogens such as Staphylococcus aureus and Enterococcus species. The antibiotics consist of a heptapeptide scaffold that is matured to the active antibiotic via a series of tailoring enzymes that oxidatively catalyze 3−4 intramolecular cyclizations and a variety of modifications including glycosylation, halogenation, acylation, etc. The first reported GPA biosynthetic gene clusters revealed a predicted set of nonribosomal peptide synthetase units required for assembling the peptide scaffold along with genes encoding amino acid and sugar biosynthesis, self-resistance, export, and tailoring enzymes. Various tailoring enzymes including glycosyltransferases, methyltransferases, sulfotransferases, halogenases, and acyl-transferases decorate the heptapeptide scaffolds of GPAs. The modifications resulting from the action of these enzymes have been shown or speculated to impart stability, increase solubility, affect dimerization constants, limit conformational flexibility, avoid degradation, and evade resistance.[18]

Unfortunately, the dwindled pipeline of new antibiotics into the market and the emergence of glycopeptide-resistant enterococci and other resistant bacteria are increasingly making effective antibiotic treatment difficult. The structures of some glycopeptides antibiotics are depicted in figure 3.

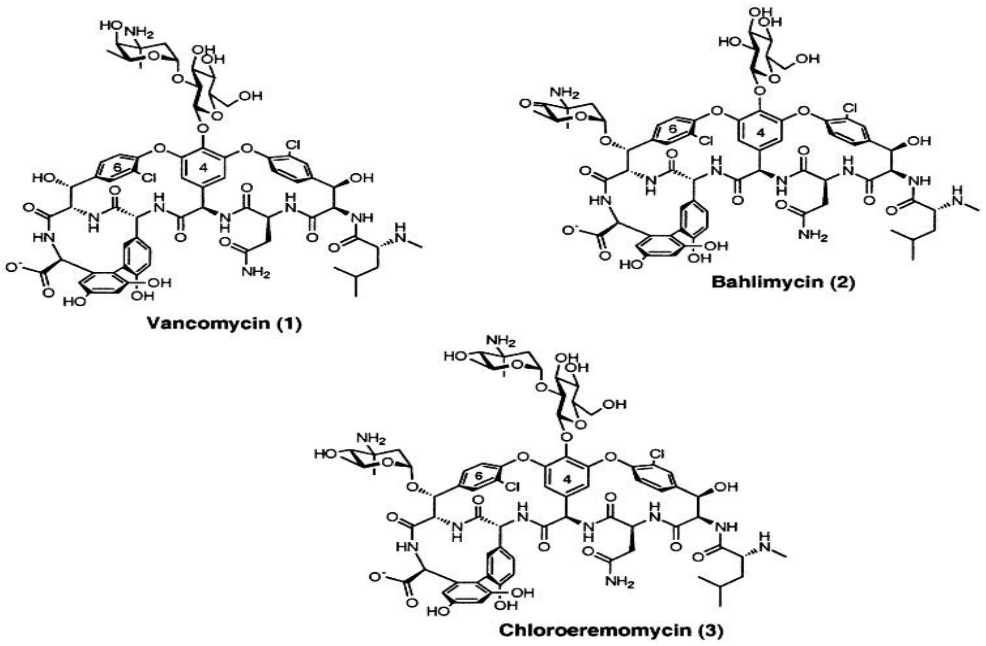

Fig. 3: Glycopeptide antibiotics

COMBINATORIAL BIOSYNTHESIS

Combinatorial biosynthesis can be defined as the modification of biosynthetic pathways by combining natural or engineered biosynthetic enzyme activities from disparate sources. This process exploits the substrate promiscuity of biosynthetic machinery to produce "unnatural" natural products with altered structures expanding the structural diversity of natural products and improving their pharmaceutical properties.[19] Natural products that are difficult to obtain from the natural producers can also be produced in greater quantities utilizing combinatorial biosynthesis. Structures of some antibiotics obtained from natural sources are depicted in figure 1. In general, combinatorial biosynthesis encompasses both precursor-directed biosynthesis incorporating a chemically synthesized precursor into a natural product by the endogenous natural biosynthetic catalysts and mutasynthesis, where the gene producing a natural building block is disrupted allowing for the incorporation of modified precursors more efficiently.[20]

Importance of derivatives produced from modified precursors;

- expand the diversity and
- Novelty of the natural products available for screening in drug discovery programs. [21]

In addition, natural products have evolved to meet the needs of their original host and have not necessarily been optimized for desirable drug properties in humans leading to potential side effects. Therefore, many natural products require structural modification to optimize their pharmaceutical properties. Although chemical modification has been successful in expanding and optimizing the structural diversity, it is a difficult and laborious process for complex natural products. Therefore, there is increasing interest in combinatorial biosynthesis as an efficient alternative tool for the structural diversification of natural products and lead optimization.[22, 23]

SUGAR CONTAINING ANTIBIOTICS

The high chemical diversity and complexity of natural products derived from actinomycetes is generated by assembling diverse chemical scaffolds such as polyketide (PK), peptide, carbohydrate, nucleoside or alkaloids. In particular, some typical natural products generated by polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) biosynthetic machinery represent a useful family of pharmacologically relevant NCEs. Structural variation within these core scaffolds (aglycones) is affected by the diversified incorporation of a number of related building blocks resulting in different structural features and pharmacological properties.[24]

Biosynthesis of sugar-containing antibiotics includes;

- the assembly of an aglycone core
- biosynthesis of an activated form of the sugar moiety (i.e., a nucleotide diphosphate [NDP]-activated sugar) and
- delivery of one or more NDP-sugars to the aglycone core by a glycosyltransferase (GT)

The sugar moiety usually alters the pharmacological properties of the parent scaffold and is also responsible for certain interactions with molecular targets. A significant number of these sugar-containing molecules are clinically useful as anti-infectives, anti-proliferatives, or as therapeutics for other forms of human diseases.

OTHER SUGAR CONTAINING ANTIBIOTICS

❖ Biosynthetic features of peptidyl nucleoside antibiotics and their combinatorial biosynthesis:

Peptidyl nucleoside antibiotics are a large family of microbial natural products derived from nucleosides and nucleotides. Due to the vital roles of nucleosides and nucleotides in most fundamental cellular metabolic processes, peptidyl nucleosides exhibit a broad spectrum of biological activities, such as antibacterial, antifungal, antiviral, insecticidal, immunosuppressive, and antitumor activities. Among them, antifungal antibiotics, including nikkomycin X (73) and polyoxin B (74), target cell wall biosynthesis Moreover; they are nontoxic to mammals, making them valuable antifungal agents. Structurally, this group is comprised of a di- or tri-peptide scaffold including the non-proteinogenic amino acid residue hydroxypyridyl-homothreonine (HPHT) and an amino hexuronic acid that is N-glycosidically linked to a uracil or a 4-formyl-4-imidazolin-2-one base.[25]

❖ Biosynthetic features of sugar-containing alkaloids and their combinatorial biosynthesis:

Examples of such indolocarbazole include rebeccamycin (85), a DNA-topoisomerase I inhibitor, and staurosporine (86), a polykinase inhibitor.

Therefore, great efforts have been made to generate indolocarbazole derivatives with improved properties for cancer treatment. Some semi-synthetic 86 derivatives, including N-benzoylstaurosporine have entered into clinical trials for the treatment of cancer, neurodegenerative disorders, and diabetes-associated pathologies. In general, the indolocarbazole core scaffold is derived from two tryptophan units with the incorporated carbon skeleton intact while the sugar moiety was derived from glucose and methionine. The sugar can be attached to this indolocarbazole moiety through one (as in 85) or two (as in 86) N-glycosidic bonds.

Next, diverse “sugar plasmids” capable of synthesizing several deoxysugars were expressed in the aglycone-producing recombinant for subsequent glycosylation, thus generating a total of eight new glycosylated indolocarbazole analogs. Among them, L-olivosylindolocarbazole shows anti-tumor activity.[26]

Although traditional methods of chemical synthesis still remain indispensable for developing new antibiotics, combinatorial biosynthesis can be a complementary approach to provide large numbers of natural product analogs (or libraries) that would be costly or difficult to access by chemical methods alone.

ALTERNATIVE STRATEGY FOR ANTIMICROBIAL RESISTANCE

There is a wide array of other possible alternatives currently being researched and developed. Some alternatives aim to prevent infection, as vaccines do, others to replace antibiotics as treatment, and still, others to make antibiotics more effective or reduce the likelihood of resistance arising by being taken alongside them. Following are alternatives, which have the potential to come to market within the next ten years: antibodies, probiotics, lysins, wild-type and engineered bacteriophages, immune stimulation, and peptides.

ANTIMICROBIAL PEPTIDES

Antimicrobial peptides (AMPs) are part of the innate immune response found among all classes of life; Antimicrobial peptides have been demonstrated to kill Gram negative and Gram positive bacteria, enveloped viruses, fungi and even transformed or cancerous cells.

Unlike the majority of conventional antibiotics, it appears as though antimicrobial peptides may also have the ability to enhance immunity by functioning as immunomodulators.

Antimicrobial peptides are a unique and diverse group of molecules, which are divided into subgroups on the basis of their amino acid composition and structure. Antimicrobial peptides are generally between 12 and 50 amino acids. These peptides include two or more positively charged residues provided by arginine, lysine or, in acidic environments, histidine, and a large proportion of hydrophobic residues. The peptides have a variety of antimicrobial activities ranging from membrane permeabilization to action on a range of cytoplasmic targets.

The modes of action by which antimicrobial peptides kill microbes are varied and may differ for different bacterial species. Some antimicrobial peptides kill both bacteria and fungi, e.g., psoriasin kills E. coli and several filamentous fungi. The cytoplasmic membrane is a frequent target, but peptides may also interfere with DNA and protein synthesis, protein folding, and cell wall synthesis. The initial contact between the peptide and the target organism is

electrostatic, as most bacterial surfaces are anionic, or hydrophobic, such as in the antimicrobial peptide Piscidin. Their amino acid composition, amphipathicity, cationic charge and size allow them to attach to and insert into membrane bilayers to form pores by 'barrel-stave', 'carpet' or 'toroidal-pore' mechanisms. Alternately, they may penetrate into the cell to bind intracellular molecules which are crucial to cell living. Intracellular binding models includes inhibition of cell wall synthesis, alteration of the cytoplasmic membrane, activation of autolysin, inhibition of DNA, RNA, and protein synthesis, and inhibition of certain enzymes.[27]

In general, the antimicrobial activity of these peptides is determined by measuring the minimal inhibitory concentration (MIC), which is the lowest concentration of drug that inhibits bacterial growth.

CHEMICALLY NOVEL AGENTS

The antibiotics are well-tested strategies of selection of compounds from natural and non-natural sources and modifying existing classes. Natural compounds, such as penicillin, have been discovered by scientific observation, or by searching for such compounds. These natural compounds have provided basic structures such as 6-aminopenicillanic acid, which chemists have used to produce analogues, such as amoxicillin. Novel compounds were also developed from the non-natural chemical route, for instance, prontosil (the precursor of sulpha drugs), metronidazole, isoniazid and oxazolidinones. Arguably, quinolones may have been created through the non-natural chemical route, although they are originally derived from quinine.[28]

If modern medicine is to continue in its present form, novel families of antibiotics must enter the marketplace at regular intervals. Although analogues of existing families, which kill resistant bacteria, prolong the life of each family for a number of decades, eventually this well of discovery dries out. Within the next 10 years, screening of whole bacteria against novel natural and chemical compound libraries may produce new antibiotics. Genomics, non-culturable bacteria, bacteriophages and non-multiplying bacteria may also be a source of novel compounds.[13]

PHAGE THERAPY

Phage therapy or viral phage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections. Phage therapy has many potential applications in human medicine as well as dentistry, veterinary science, and agriculture.

Bacteriophages are much more specific than antibiotics. They are typically harmless not only to the host organism but also to other beneficial bacteria, such as the gut flora, reducing the chances of opportunistic infections. They have a high therapeutic index, that is, phage therapy would be expected to give rise to few side effects. Because phages replicate in vivo, a smaller effective dose can be used. Phages tend to be more successful than antibiotics where there is a biofilm covered by a polysaccharide layer, which antibiotics typically cannot penetrate. Phages are currently being used therapeutically to treat bacterial infections that do not respond to conventional antibiotics.[29]

LYSINS

Lysins, also known as endolysins or murein hydrolases, are hydrolytic enzymes produced by bacteriophages in order to cleave the host's cell wall during the final stage of the lytic cycle. Lysins are highly evolved enzymes that are able to target one of the five bonds in peptidoglycan (murein), the main component of bacterial cell walls, which allows the release of progeny virions from the lysed cell. These enzymes are being used as antibacterial agents due to their high effectiveness and specificity in comparison with antibiotics, which are susceptible to bacterial resistance.

ENZYBIOTIC

Enzybiotics are an experimental antibiotic approach employing enzymes to combat pathogenic bacterial infections. Many of the enzymes used as enzybiotics are lysins, enzymes derived from bacterial viruses (or bacteriophages) used to release progeny bacteriophage from infected bacteria, though other natural or synthetic enzymes may be used. Enzybiotic approaches have attempted to incorporate bacteriophage-derived lysins to kill bacterial cells. One particular lysin, isolated from phage P68 of Staphylococcus aureus, has shown antimicrobial activity against its host species when used along with the antibiotic gentamicin.[30]

PROBIOTICS

Probiotics are microorganisms that are believed to provide health benefits when consumed. The term probiotic is currently used to name ingested microorganisms associated with benefits for humans and animals. The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes. A significant expansion of the potential market for probiotics has led to higher requirements for scientific substantiation of putative benefits conferred by the microorganisms.[31]

Alternative antimicrobial strategies in the treatment and prevention of gastrointestinal infections are the application of probiotics and their anti-microbial metabolites such as bacteriocins. Probiotics are largely administered through functional foods and as dietary supplements (pharmaceuticals) or biotherapeutics. Lactic acid bacteria are the most important probiotic micro-organisms because they are autochthonous in the human gastrointestinal tract of healthy people. A considerable number of health benefits have been postulated as a result of the probiotic intake, including modification of gut microflora, prevention of pathogen colonization, stimulation of gut immunity, reduction in inflammatory reactions, prevention of colon cancer, alleviation of lactose intolerance, lowering of serum cholesterol and reduction of food allergies.

Mode of action of probiotics includes:

- ✓ antagonistic effects against pathogenic microorganisms in intestinal tract
- ✓ alteration of microbial metabolism in the intestinal tract
- ✓ stimulation of immunity and increase of nutritional value

Probiotics are considered to be generally safe, but they may cause bacteria-host interactions and unwanted side effects in certain cases.

VACCINE

A vaccine is a biological preparation that provides active acquired immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins or

one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as a threat, destroy it, and keep a record of it so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters.[32]

Vaccines have historically been the most effective means to fight and eradicate infectious diseases. Limitations to their effectiveness, nevertheless, exist. Sometimes, protection fails because the host's immune system simply does not respond adequately or at all. Lack of response commonly results from clinical factors such as diabetes, steroid use, HIV infection or age. It also might fail for genetic reasons if the host's immune system includes no strains of B cells that can generate antibodies suited to reacting effectively and binding to the antigens associated with the pathogen.

VACCINES FOR TB, MALARIA AND HIV

The immense burden that TB, malaria and HIV have on the world, collectively causing more than five million deaths a year16, means the need for effective preventative vaccines is urgent. Efforts have been underway for more than a decade, but with limited success so far. There are currently quite a few vaccines in the pipeline but many are early-stage, meaning – statistically – a low chance of bringing to market a successful vaccine in the near future. Even if some are successful we may well be waiting many years, if not decades, for them to come to market. It is therefore crucial that efforts in this area are increased to reduce the burden that these diseases place on several million people.

A. Tuberculosis (TB)

The vaccines may be the single biggest contributor to overcoming the huge global problem of TB. There is only one vaccine against TB today, the Bacille-Calmette-Guérin (BCG). It was developed in the 1920s and is given to children to prevent more severe forms of TB, though it does not offer complete protection from the disease.

B. HIV

There are currently no HIV vaccines. Research and development has moved back into early-stage, preclinical and phase I trials there have been some recent vaccine candidate failures. This means that we are still quite far from a usable vaccine, though there are some encouraging signs.

C. Malaria

In a major advancement, the vaccine candidate known as Mosquirox was approved last year by the European Medicines and is the first vaccine ever licensed for use against a parasite. Though the efficacy of the vaccine is relatively low, it still represents a milestone achievement in the fight against malaria.[33]

FUTURE PERSPECTIVE

Growing concern about antibiotic resistance is propelling the urgent modification of existing antibiotics and parallel development of newer antibiotics. Phytochemicals isolated from natural sources and then chemically synthesized via modifications are likely to provide the most effective antimicrobial drugs in the near future.[34]New compounds that target bacterial virulence can be developed to control the enormous threat posed by multi-drug resistance. A better understanding of the structure, function and action mechanism of existing and newly identified AMPs will lead to their being fine-tuned by proper design to work against MDR pathogens. Phages may also play a major role in treating bacterial infections in humans. Combined treatment of phages with antibiotics is likely to be a future choice. To develop new classes of antibiotic or antimicrobial agents with different modes of action against MDR pathogens. Combinational drug use is extensively used to treat bacterial infection, but even this combinational dose pattern may lead to resistance among pathogens.[35]

CONCLUSION

The alarming increase of multidrug resistant pathogens makes the discovery process of new antibacterial compounds to continue the conventional strategies of targeting known metabolic pathways or bacterial complex processes (replication, transcription and translation of genetic code), which have delivered well validated targets. It seems that the route of analogues of the existing antibiotics will offer the majority of new antibiotics with increased potential to enter the marketplace. At the same time, there is a great need for developing new strategies for antibiotic drugs discovery, as resistance mechanisms of bacteria to some families of antibiotics become more sophisticated. These strategies are based either on new bimolecular targets coming from bacterial cellular processes with still unknown biochemical mechanism, multiplicity of targets, or innovative solutions. The approaches based on conventional and new targets and the alternative routes based on targeting on-multiplying bacteria, non-culturable bacteria, or bacteriophages discussed here brought together as much information as

possible on the subject and will hopefully help specialists work hand-in-hand in the discovery of novel antibacterials. Until novel resistance-breaking antibiotics are developed, educational programs based on enhanced hygiene, reduction of misuse and abuse of antibiotics, eradication of unjustified and inappropriate antibiotic prescriptions and of self-medication should also be considered as important factors to limit the problem of antimicrobial resistance.

REFERENCES

1. O'Neill J. Antimicrobial resistance. 1st ed. London: Review on Antimicrobial Resistance; 2014.
2. Wright G. Molecular mechanisms of antibiotic resistance. Chemical Communications. 2011;47(14):4055.
3. Aminov R. A Brief History of the Antibiotic Era: Lessons Learned and Challenges for the Future. Frontiers in Microbiology. 2010; 1.
4. Fleming A. On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to Their Use in the Isolation of B. influenza. Clinical Infectious Diseases. 1980; 2(1):129-139.
5. Wright P, Seiple I, Myers A. The Evolving Role of Chemical Synthesis in Antibacterial Drug Discovery. Angewandte Chemie International Edition. 2014; 53(34):8840-8869.
6. Zignol M, Hosseini M, Wright A, Weezenbeek C, Nunn P, Watt C et al. Global Incidence of Multidrug-Resistant Tuberculosis. The Journal of Infectious Diseases. 2006; 194(4):479-485.
7. ClavelFHance A. HIV Drug Resistance. New England Journal of Medicine. 2004; 350(10):1023-1035.
8. Ridley R. Haemozoin formation in malaria parasites: is there a haem polymerase? Trends in Microbiology. 1996; 4(7):253-254.
9. Boucher H, Talbot G, Bradley J, Edwards J, Gilbert D, Rice L et al. Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. Clinical Infectious Diseases. 2009; 48(1):1-12.
10. Li XNikaido H. Efflux-Mediated Drug Resistance in Bacteria. Drugs. 2009; 69(12):1555-1623.
11. Lipinski C, Lombardo F, Dominy B, Feeney P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews. 1997; 23(1-3):3-25.
12. Bérdy J. Bioactive Microbial Metabolites. The Journal of Antibiotics. 2005; 58(1):1-26.
13. Morrison KHergenrother P. Natural products as starting points for the synthesis of complex and diverse compounds. Nat Prod Rep. 2014; 31(1):6-14.
14. Walsh CFischbach M. Natural Products Version 2.0: Connecting Genes to Molecules. Journal of the American Chemical Society. 2010; 132(8):2469-2493.
15. Lowy F. Staphylococcus aureus Infections. New England Journal of Medicine. 1998;339(8):520-532.
16. Piddock L. Multidrug-resistance efflux pumps — not just for resistance. Nature Reviews Microbiology. 2006; 4(8):629-636.
17. Poole K. Efflux pumps as antimicrobial resistance mechanisms. Annals of Medicine. 2007;39(3):162-176.
18. Kennedy J. Mutasynthesis, chemo biosynthesis, and back to semi-synthesis: combining synthetic chemistry and biosynthetic engineering for diversifying natural products. Nat Prod Rep. 2008; 25(1):25-34.
19. Wong FKhosla C. Combinatorial biosynthesis of polyketides—a perspective. Current Opinion in Chemical Biology. 2012; 16(1-2):117-123.
20. Ang E, Sun H, Liu Z, Zhao H. Recent advances in combinatorial biosynthesis for drug discovery. Drug Design, Development and Therapy. 2015; 823.
21. Winter JTang Y. Synthetic biological approaches to natural product biosynthesis. Current Opinion in Biotechnology. 2012; 23(5):736-743.
22. Li JVederas J. Drug Discovery and Natural Products: End of an Era or an Endless Frontier? Science. 2009; 325(5937):161-165.
23. CraggGNewman D. Natural products: A continuing source of novel drug leads. BiochemicaletBiophysicalActa (BBA) - General Subjects. 2013;1830(6):3670-3695.24.

24. Zhan J. Biosynthesis of Bacterial Aromatic Polyketides. Current Topics in Medicinal Chemistry. 2009; 9(17):1598-1610.
25. Elshahawi S, Shaaban K, Kharel M, Thorson J. A comprehensive review of glycosylated bacterial natural products. ChemSoc Rev. 2015; 44(21):7591-7697.
26. Süssmuth R, Müller J, von Döhren H, and Molnár I. Fungal cyclooligomer depsipeptides: From classical biochemistry to combinatorial biosynthesis. Nat Prod Rep. 2011; 28(1):99-124.
27. Reddy K, Yedery R, Aranha C. Antimicrobial peptides: premises and promises. International Journal of Antimicrobial Agents. 2004; 24(6):536-547.
28. Fernandez P. Antibacterial discovery and development—the failure of success? Nature Biotechnology. 2006; 24(12):1497-1503.
29. Keen E. Phage Therapy: Concept to Cure. Frontiers in Microbiology. 2012; 3.
30. Veiga-Crespo P, Ageitos J, Poza M, Villa T. Enzybiotics: A Look to the Future, Recalling the Past. Journal of Pharmaceutical Sciences. 2007; 96(8):1917-1924.
31. Rijkers G, de Vos W, and Brummer R, Morelli L, Corthier G, Marteau P. Health benefits and health claims of probiotics: bridging science and marketing. British Journal of Nutrition. 2011; 106(09):1291-1296.
32. TRANSLATION OF AN Address ON THE GERM THEORY. The Lancet. 1881; 118(3024):271-272.
33. RappuoliRAderem A. A 2020 vision for vaccines against HIV, tuberculosis and malaria. Nature. 2011; 473(7348):463-469.
34. PokrovskayaVBaasov T. Dual-acting hybrid antibiotics: a promising strategy to combat bacterial resistance. Expert Opinion on Drug Discovery. 2010; 5(9):883-902.
35. West water C, Kasman L, Schofield D, Werner P, Dolan J, Schmidt M et al. Use of Genetically Engineered Phage to Deliver Antimicrobial Agents to Bacteria: an Alternative Therapy for Treatment of Bacterial Infections. Antimicrobial Agents and Chemotherapy. 2003; 47(4):1301-1307.