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The Antibacterial Drug Discovery

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1. Introduction

An antibacterial is a compound or substance that kills or slows down the growth of bacteria. We usually associate the beginning of the modern antibacterial era with the names of Paul Ehrlich and Alexander Fleming. Infectious diseases are the leading causes of human morbidity and mortality for most of human existence. Antibacterials are probably one of the most successful forms of chemotherapy in the history of medicine. They save countless lives and make enormous contribution to the control of infectious diseases since the beginning of antibacterial era. Perhaps most of us born since the Second World War don't know how much enthusiasm, dedication, and hardship have been put in antibacterial drug discovery, and take the success of antibacterial agents too much for granted. Therefore, let's first look back what the human did to combat the infections before antibacterial era and how the outstanding scientists discovered so many efficient antibacterial agents used clinically today and led us enter the antibacterial era.

2. The history of antibacterial discovery

2.1. Pre-antibiotic era

Before the early 20th century, 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 [1]. Even the prehistoric peoples used a number of plants in wound treatment and it seems possible that many plants have the properties of antimicrobial effects [2; 3]. Tetracyclines can be incorporated into the hydroxyapatite mineral portion of bones as well as tooth enamel; once people take it, permanent markers of metabolically active areas will be left. Thus it is much conveniently to trace the exposure of these

antibacterials in ancient populations. It was found that the bone sample from Sudanese Nubian (A.D. 350 to 550) was labeled by the antibiotic tetracycline and their dietary regime contained tetracycline-containing materials by X-group cemetery and other advanced technologies [4; 5]. Moreover, another study showed that, bones from the Dakhleh Oasis, Egypt, in a late Roman period, exhibit discrete fluorochromelabelling, exactly like the teeth from patients treated with tetracycline [6]. A large number of customs and anecdotes can also reveal the occurrences of other antibacterials. One popular anecdote is about the antibiotic-like properties of red soil from the Hashemite Kingdom of Jordan. Interestingly, red soil was used for treating skin infections and diaper rash in the past and is still used in some communities today as an inexpensive alternative to antibiotics [7]. In fact, recently, many pharmaceutical antibiotics, such as streptomycin, actinomycin, erythromycin, vancomycin, nystatin and amphotericin, were produced from the soil actinomycetes [8].

The traditional Chinese medicine is the summary of experience about Chinese medical treatment over millennia and may contain a lot of unknown antibiotics [9]. Many traditional Chinese medicines were tested and found effective against four common oral bacteria [10]. Discovery of active components in the ancient herbs could enrich the arsenal of antimicrobials used by the mainstream medicine.

2.2. Foundation of the antibiotic era

Bacteria were first identified in the 1670s by van Leeuwenhoek, following his invention of the microscope. The relationship between bacteria and diseases gradually set up in the nineteenth century. Since then, researchers started to try and find effective antibacterial agents.

Paul Ehrlich is the father of chemotherapy and was honored with the Nobel prize due to the molecular side-chain theory of immunity. His concept of “magic bullet” is that the chemicals selectively target only disease-causing microbes but not the host cells. In 1906, Ehrlich, together with Berthel, developed hundreds of derivatives of Atoxyl, and finally discovered compound 606, a gold powder [9; 11]. In 1909, he found that Compound 606 could cure syphilis-infected rabbits in experiments; it could also improve terminal patients with dementia and cured early stage patients with infected sores [11]. It was publicly released as salvarsan in 1910. Despite the adverse side effects, salvarsan and its derivative neosalvarsan kept the status of the most frequently prescribed drug until the introduction of penicillin in the 1940s [12]. Amazingly, the chemical structure of salvarsan hadn't been known until 2005 [13].

The systematic screening approach introduced by Paul Ehrlich became the cornerstone of drug search strategies in the pharmaceutical industry. Sulfonamidochrysoidine (also named prontosil), the first commercially available antibiotic, was first synthesized by Bayer chemists Josef Klarer and Fritz Mietzsch in 1930s by this approach. Then Gerhard Domagk found its effect against *Streptococcus pyogenes* in mice [14]. Four years later he received the Noble Prize. Eventually prontosil was recognized as a precursor for a new class of antibacterial agents— sulfonamides.

The effect of mould on bacterial colonies hadn't been investigated until 19th century, although the antibacterial properties of mold had been known since ancient times. In 1921, Alexander Fleming observed some substances called lyzosomes which could dissolve bacteria. In 1928, he discovered that a specific mould species inhibited the development of Staphylococcus bacteria. The species was known as Pencilliumnotatum and the filtrate was called penicillin [15]. In 1940, Howard Florey and Ernst Chain worked out how to purify penicillin for clinical testing [16]. All the three researchers were awarded the Nobel Prize in 1945, and since then the era of antibiotics had been initiated. Penicillin became the top therapeutic molecule because of its widespread use and the magnitude of the therapeutic outcomes, and also because of the technologies developed for production of penicillin which became the basis for production of all subsequent antibiotics and other bioproducts in use today [17].

3. Classification

Antibacterials are commonly classified based on their mechanism of action or spectrum of activity. The main classes of antibacterial drugs target only four classical bacterial functions: bacterial-cell-wall biosynthesis (e.g., penicillin and vancomycin); bacterial protein biosynthesis (e.g., aminoglycoside and macrolide); DNA and RNA replication (e.g., ciprofloxacin and rifampin); and folate coenzyme biosynthesis (e.g., sulfamethoxazole) [18]. Antibacterials that target the cell wall or cell membrane or essential bacterial enzymes are more likely to be bactericidal; but generally the bacteriostatic is the antibacterial drugs that inhibits protein synthesis [19]. Another way to distinguish the antibiotics is based on their target specificity. The broad-spectrum antibiotic affects a wide range of disease-causing bacteria, including both Gram-positive and Gram-negative bacteria, in contrast to a narrow-spectrum antibiotic, which acts against specific families of bacteria. For example, ampicillin is a widely used broad-spectrum antibiotic.

4. Antibacterial resistance and its mechanisms

Bacterial resistance to antibacterial drugs increasingly becomes a major health and economic problem, eroding the discovery of antibiotics and their application to clinical medicine. As early as 1946, Alexander Fleming predicted that "There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria cannot react by in some way acquiring 'fastness' (resistance)." Today it is really the truth. Resistance to the antibiotics will emerge only a few years after it is introduced to clinic use [20]. Bacterial resistance is positively correlated with the use of antibacterial agents in clinical practice [21; 22]. Because any use of antibiotics can increase selective pressure in a population of bacteria, allowing survival of the resistant bacteria and death of the susceptible ones. We can find that pathogenic bacteria are resistant to practically all available antibacterial drugs. And many strains, which are informally called superbugs, are even resistant to several different antibiotics. Multidrug resistance has been found in *Pseudomonas aeruginosa* (*P. aeruginosa*),

Acinetobacter baumannii (*A. baumannii*), *E. coli*, and *Klebsiella pneumoniae* (*K. pneumoniae*), producing extended-spectrum β -lactamases (ESBL), vancomycin-resistant enterococci *Enterococcus faecium* (*E. faecium*) (VRE), MRSA, vancomycin-resistant *S. aureus* VRSA, extensively drug-resistant (XDR) *Mycobacterium tuberculosis* (*M. tuberculosis*), *Salmonella enterica* (*S. enterica*) serovar Typhimurium, *Shigella dysenteriae* (*S. dysenteriae*), *Haemophilus influenzae* (*H. influenzae*), *Stenotrophomonas*, and *Burkholderia* [23; 24].

Great amount of antibiotic is used in nonhuman niches, leading to the spread of resistant bacteria too. Antibiotics have been used for improving the production of livestock and poultry for more than 50 years [25]. The Institute of Food Technologists (IFT), once convened a panel of internationally renowned experts to address the concern that, the emergence of antimicrobial resistance may result from abuse in food production, manufacturing, and elsewhere [26].

Over the past several years, people struggled to search for the mechanisms of resistance. Therefore today there is a large pool of information about how drug resistances come out. Biochemical and genetic aspects of antibiotic resistance mechanisms are shown in Fig. 1.

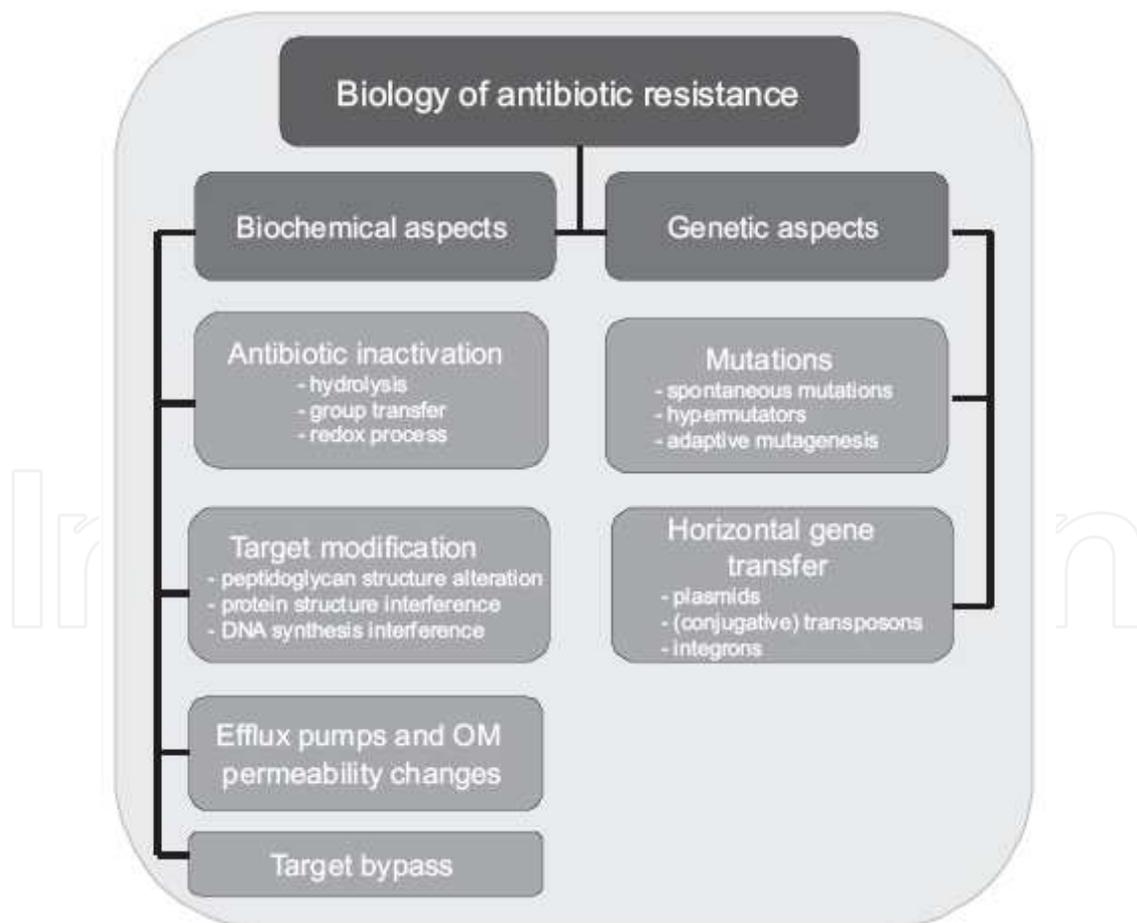


Figure 1. Kinds of antibiotic resistance mechanisms [85].

4.1. Genetics of antibiotic resistance

Resistance can be an intrinsic property of the bacteria themselves or it can be acquired. There are two main ways of acquiring antibiotic resistance: i) chromosomal mutations and ii) horizontal gene transfer. But the question is where the horizontal gene comes from? Some of these genes have an environmental origin and began their evolution before the antibiotic era; most likely, the primary genes originated and diversified within the environmental bacterial communities, then mobilized and penetrated into pathogens. [27; 28]

4.1.1. Mutations

4.1.1.1. Spontaneous mutations

These mutations occur randomly as replication errors or an incorrect repair of a damaged DNA in actively dividing cells, presenting an important mode of generating antibiotic resistance. They are also called the growth dependent mutations. Quinolone resistance in *Escherichia coli* resulted from the mutations in at least seven positions in the *gyrA* gene or three positions in the *parC* gene [29]. There are a large number of biochemical mechanisms of antibiotic resistance related to Spontaneous Mutations. For instance, Mutations in *mexR* can cause derepression of the *mexAB-oprM* multidrug efflux operon, causing a multidrug resistance phenotype in *Pseudomonas aeruginosa* [30].

4.1.1.2. Hypermutators

During a prolonged non-lethal antibiotic selective pressure a small bacterial population enters a transient state of a high mutation rate which is called hypermutable state. Hypermutators are found in many bacteria species such as *E. coli*, *S. enterica*, *Neisseria meningitidis* (*N. meningitidis*), *H. influenzae*, *S. aureus*, *Helicobacter pylori* (*H. pylori*), *Streptococcus pneumoniae* (*S. pneumoniae*), and *P. aeruginosa* [85]. Various studies suggested that hypermutations play an important role in acquisition of antibiotic resistance in pathogens [31; 32; 33].

4.1.1.3. Adaptive mutagenesis

Adaptive mutations arise in non-dividing or slowly dividing cells during the presence of a non-lethal selective pressure that favours them. A great number of antibiotic resistant mutants may come from this mutation process under bacterial natural conditions [85].

4.1.1.4. Horizontal gene transfer

Horizontal transfer of genetic material between bacteria is the most commonly used way to spread antibiotic resistance. In general, this exchange is accomplished mainly through the processes of transduction (via bacteriophages), conjugation (via plasmids and conjugative transposons), and transformation (via incorporation into the chromosome of chromosomal DNA, plasmids, and other DNAs) [34]. This type of genetic transfer not only occurred between closely related bacteria but can also occur between phylogenetically distant bacterial

genera, in particular between gram-positive and gram-negative bacteria [35]. Plasmid-encoded antibiotic resistance encompasses most classes of antibiotics in practice, such as aminoglycosides, cephalosporins and fluoroquinolones [36]. Transposons spread quicker than genes in chromosomes and are transferred by conjugation, transformation, or transduction [23; 24]. Integrons acquire and exchange exogenous DNA, known as gene cassettes, by a site-specific recombination mechanism. They can integrate stably into other DNAs where they deliver multiple antibacterial resistant genes in a single exchange. Resistance gene cassettes encoding the metallo- β -lactamases IMP and VIM confer resistance to the potent carbapenem β -lactams imipenem and meropenem [36].

4.1.2. Biochemistry of antibiotic resistance

As so many scientists have been struggling to study the biochemical mechanisms of antibiotic resistance, nowadays there is a large pool of related valuable information left. Biochemical mechanisms may be varied among different bacterial species, but can be mainly classified into four categories (Fig. 2). In fact, each of these four categories also contains an amazing diversity of resistance mechanisms. Sometimes a single bacterial strain may possess several types of resistance mechanisms. Each of the four main categories will be discussed respectively below.

4.1.2.1. Antibiotic inactivation

Biochemical strategies include enzymatic modification and redox mechanisms (which is less important and will not be elaborated in this paper). Enzymes can be divided into two general classes: those such as β -lactamases that degrade antibiotics and others that perform chemical transformations. The antibiotic β -lactam has a four-atom ring known as a beta-lactamin. The β -lactamase enzyme breaks that ring open, destroying the antibacterial properties of the drugs. β -lactamase consists of enzymes with a serine residue at the active site, and metalloenzymes with zinc ion as a cofactor and with a separate heritage [37]. β -lactamase enzymes are the most common and important weapons for Gram-negative bacteria to resist the antibiotics β -lactam [38]. The group transfer approaches are the most diverse and include the modification by acyltransfer, phosphorylation, glycosylation, nucleotidylation, ribosylation, and thiol transfer. They can inactivate antibiotics (aminoglycosides, chloramphenicol, streptogramin, macrolides or rifampicin) by chemical substitution. These modifications reduce the affinity of antibiotics to a target [85]. For example, enzymatic modification is the most prevalent mechanism to destroy aminoglycosides in clinic. Aminoglycoside modifying enzymes can be divided into three classes: acetyltransferases, nucleotidyltransferases, and phosphotransferases; they mainly catalyze the modification at –OH or –NH₂ groups of the 2-deoxystreptamine nucleus or the sugar moieties [39]. There are a large number of genes in the chromosomes and other mobile genetic elements coding for these enzymes which let the bacteria resist to more new antibiotics as well as horizontally spread their resistance among bacteria more easily. As a consequence, almost all pathogens are resistant to aminoglycosides through modifying enzymes [39].

4.1.2.2. Target modification

Another important resistance mechanism is the modification of antibiotic targets which makes the antibiotic unable to bind the targets properly. β -lactams target the bacterial enzymes of cell wall biosynthesis (the so-called penicillin-binding proteins, PBPs). Alterations in PBPs can reduce affinity for β -lactams, possibly causing β -lactam resistance in many bacteria strains, such as *H. influenzae*, *N. gonorrhoeae*, *N. meningitidis*, anaerobes, *S. dysenteriae* [40]. For instance, the *mecA* resistance gene which encodes PBP2a, a new penicillin binding protein with decreased affinity for oxacillin and most other β -lactam drugs, induces resistance to methicillin and oxacillin in *S. aureus* [41]. The resistance to antibiotics that interfere with protein synthesis or transcription is achieved by modification of the specific target. rRNAmethylases encoded by a number of genes modify the 16S rRNA molecule at specific positions critical for aminoglycosides binding [42]. Modification in the 23S rRNA component of the 50S ribosomal subunit also leads to resistance to the macrolide, lincosamide and streptogramin B group of antibiotics in many pathogen strains [43; 44]. Mutations of topoisomerase IV and gyrase genes can sufficiently alter affinity of fluoroquinolones to these enzymes [45].

4.1.2.3. Efflux pumps and outer membrane (OM) permeability

Efflux pumps Membrane proteins that export antibiotics from the cell and maintain their low intracellular concentrations are called efflux pumps. Drug efflux pumps play a key role in drug resistance not just because they can produce multidrug resistance but also because they can elevate level of other resistance mechanisms [46; 47]. Bacterial drug efflux transporters are currently classified into five families: the ATP-binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, and the resistance-nodulation-division (RND) superfamily [47]. Efflux transporters can be further classified into single or multicomponent pumps. Tetracycline and macrolide transporters are single component efflux systems that have narrow substrate profiles, while the RND family members have broader substrates and can pump out multiple structurally unrelated compounds [24; 46]. Efflux pumps exist in both Gram-positive and Gram-negative bacteria [48; 49]. MexAB-OprM efflux pumps in *Pseudomonas aeruginosa*, which belong to RND family, result in higher inhibitory concentration of a large number of antibiotics, such as penicillins, broad-spectrum cephalosporins, chloramphenicol, fluoroquinolones, macrolides, novobiocin, sulfonamides, tetracycline and trimethoprim, dyes and detergents [50; 51].

OM permeability The OM is an asymmetric bilayer: the phospholipid form the inner leaflet and the lipopolysaccharides (LPS) form the outer leaflet. OM of Gram-negative bacteria provides a formidable barrier that must be overcome by drugs. Drug molecules pass the OM by diffusion through porins or the bilayer, or by self-promoted uptake [85]. Small hydrophilic drugs (e.g., β -lactams), enter to the intracellular through the pore-forming porins, while macrolides and other hydrophobic drugs diffuse during their entry [52]. Some resistant clinical strains of *Neisseria meningitidis*, *K. pneumoniae* and *Enterobacter aerogenes* exhibit a noticeable porin variability resulting in decrease of antibacterial uptake [53]. Reduction of

LPS in the outer membrane of Polymyxin-resistant *P. aeruginosa* strains associates with resistance development [54].

4.1.2.4. *Target bypass*

This kind of resistance mechanisms is somewhat specific. Bacteria produce two kinds of targets: one is sensitive to antibiotics and the alternative one (usually an enzyme) that is resistant to inhibition of antibiotic. In ampicillin-resistant mutant *Enterococcus faecium* selected in vitro, bypass of the DD-transpeptidases by a novel class of peptidoglycan polymerases, the LD-transpeptidases, conveyed resistance to all β -lactams, except the carbapenems [55; 56].

5. What should we do?

5.1. Extending the lifespan of existing antibacterials

Although the emergency of antibiotic resistance seems inevitable, measures must be taken to prevent or at least delay this process. As mentioned above, many factors contribute to resistance, so we should adopt a complex approach. The most important way is to strictly control antibiotic misuse and overuse. Interestingly, the EU has implemented a comprehensive ban on the use of all antibiotics for growth promotion since 2006 [25]. And other developed countries also implement similar measures, but in many developing nations antibiotic use is relatively uncontrolled. As hospital-acquired infection is a major cause for antibiotic-resistance, strict antibiotic stewardship and policies should be adopted in the hospitals. For example, we can make some antibiotic policies to optimize the selection, dosing, route of administration, duration of the drug prescribed by the doctor, and limit the unintended consequences of antibiotic utilization [57].

5.2. New antibacterial drug discovery

As serious infectious diseases and multidrug resistance are emerging repeatedly, new antibiotics are needed badly to combat these bacterial pathogens, but the progress of discovery seems relatively slow. Most chemical scaffolds of antibiotics used now were just introduced between the mid-1930s and the early 1960s (fig 2). There are many reasons for this. The first is scientific. We have discovered the easy-to-find antibiotics. Now we have to work harder and think more cleverly to find new drugs. Another reason is commercial. Antibiotics are used much less than other drugs and the new antibiotic are just used to treat serious bacterial infections at most of the time. So antibiotics have a poor return on investment. In 2008 only five major pharmaceutical companies still kept their Enthusiasm in antibacterial discovery. It is most important to delink research and development costs from drug pricing and the return from investment on antibacterial discovery [58]. If the government could establish some subsidies and financial assistance schemes to compensate the cost, more drug companies will be attracted to this area.

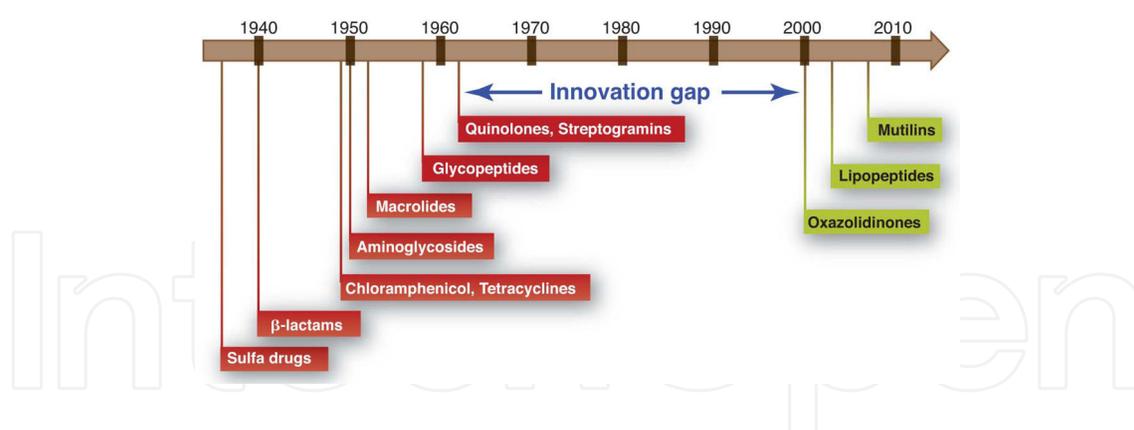


Figure 2. Innovation gap between 1962 and 2000 [59].

Despite the current grim situation in management of resistant bacteria, some new drugs have recently been approved by the FDA or are in late stages of the pipeline (Table 1, 2) [60]. The new drugs belong to the following classes of compounds: oxazolidinones, glycopeptides, ketolides, lycylcyclines, carbapenems and fluoroquinolones.

| CLASS OF COMPOUND | PHASE OF DEVELOPMENT | ANALOGS | MECHANISM OF ACTION | RESISTANCE MECHANISM | DRUG COMPANY |
|-------------------|----------------------|---|---|---|--|
| Oxazolidinones | FDA Approved 2000 | Linezolid, Radezolid, Torezolid, RWJ-416457 | Inhibits protein translation (initiation/elongation) | rRNA mutations | Pfizer, Rib-X, Trius Therapeutics, Johnson & Johnson |
| Glycopeptides | Phase III | Oritavancin, Dalbavancin, Telavancin | Inhibit peptidoglycan biosynthesis/transglycosylation | unidentified | Targanta/The Medicines Co., Pfizer, Theravance |
| Ketolides | Phase III | Cethromycin | Inhibits protein synthesis | rRNA dimethylation, ribosomal protein mutations | Advanced Life Sciences |
| Glycylcyclines | FDA Approved 2005 | Tigecycline, PTK0796 | Inhibits protein synthesis | Efflux pumps | Wyeth, Paratek Pharmaceuticals |
| Carbapenems | FDA Approved 2007 | Doripenem, Razupenem | Inhibits peptidoglycan biosynthesis | Carbapenemases, Efflux pumps, Porin mutations | Johnson & Johnson, Protez Pharmaceuticals |
| Streptogramins | Phase II | NXL103/XRP2868 | Inhibits protein translation | unidentified | Novexel |
| Fluoroquinolones | Preclinical | JNJ-Q2, finafloxacin | Inhibit type II topoisomerase | gyrA, parC mutations | Johnson & Johnson, MerLion Pharmaceuticals |

Table 1. New antibiotics of existing scaffolds

| DRUG NAME | TARGET/ MECHANISM OF ACTION | SPECTRUM OF ACTIVITY | PHASE OF DEVELOPMENT | DRUG COMPANY OR INNOVATOR |
|----------------------|--|---------------------------------------|-------------------------|--|
| Ceftobiprole | Tight binding to PBP2a | Gram-positive, Gram-negative | Phase III | Johnson & Johnson |
| Ceftaroline | Tight binding to PBP2a | Gram-positive, Gram-negative | Phase III | Forrest Laboratories |
| Iclaprim | Increased affinity to bacterial DHFR | Gram-positive, Gram-negative | Phase III | Arpida |
| Sulopenem | Binding to PBPs | Gram-negative | preclinical | Pfizer |
| BAL30376 | Monobactam/ β -lactamase inhibitor combination | Multi-drug resistant Gram-negative | preclinical | Basilea |
| Rx100472 | MethionyltRNA synthetase inhibitor | Gram-positive | preclinical | Trius Therapeutics |
| PC190723 | Cell division protein FtsZ | S. aureus | preclinical | Prolysis |
| MUT7307 | Enoyl-ACP FabI reductase (fatty acid biosynthesis) | Gram-positive, Gram-negative | preclinical | Mutabilis |
| Nitazoxanide | Inhibits vitamin cofactor of pyruvate:ferredoxin oxidoreductase (PFOR) | C. difficile | Phase II | Romark Laboratories |
| Fidaxomicin (OPT-80) | Inhibits RNA synthesis | C. difficile | Phase II | Optimer Pharmaceuticals |
| LED209 | Quorum sensing | S. typhimurium F. tularensis | preclinical | University of Texas South Western Medical Center, Dallas |
| BPH652 | Virulence factor (antioxidant) | MRSA | preclinical | University of Illinois, Chicago |
| Omiganan | Antimicrobial peptide; Depolarizes cytoplasmic membrane of bacteria | Gram-positive, fungi | Phase III | MIGENIX, Cadence pharmaceuticals |
| TMC207 | ATP synthase inhibition | M. tuberculosis | Phase II | Johnson & Johnson, Tibotec |
| CBR2092 | Dual pharmacophore | Gram-positive | Phase I | Cumbre |
| Amikacin | Novel drug delivery: inhaled nanoliposomes | P. aeruginosa biofilm | Phase II | Transave Inc. |

Table 2. New antibiotics in development

5.2.1. Tailoring existing scaffolds

It seems that there are many ways to search for new antibacterials, but the key question is: how to search for new antibacterial drugs and where to look for them? The most convenient method is to modify the existing scaffolds to generate their derivatives. All antibiotics approved between the early 1960s and 2000 were synthetic derivatives of the old scaffolds except carbapenems. Chemical modifications of old scaffolds may lead to improved bactericidal activities, better resistance profiles, safety, tolerability or superior pharmacoki-

netic/pharmacodynamic properties. There are four generations of β -Lactam antibiotics, all of which contains a β -lactam nucleus in their molecular structures. The second generation (e.g., cephalexin and cefaclor) and third generation (e.g., cefotaxime, ceftazidime) are not sensitive to plasmid-mediated broad-spectrum β -lactamases and have less allergic reactions, compared with the first generation (penicillins) [61]. The fourth-generation cephalosporins penetrate through the outer membrane of Gram-negative bacteria more easily and have low affinity for clinically important β -lactamases, so they have the advantage of killing many Gram-negative pathogens resistant to most third-generation [86]. Tigecycline is one of glycylcycline antibiotics derived from tetracycline and received approval from the US Food and Drug Administration for the treatment of skin, soft-tissue, and intra abdominal infections in 2005. Tigecycline can overcome the active efflux of drug from inside the bacterial cell and protection of ribosomes, which are two determinants of tetracycline resistance [62; 63]. But this approach is only a good short-term strategy to find new drugs, and but the benefit of these modified drugs will be offset quickly by the resistance to acquired through the horizontal acquisition or molecular evolution [9], which indicates that it is much more attractive to find novel chemical scaffolds.

5.2.2. Novel scaffolds

5.2.2.1. Explore new places

More than two-thirds of clinically used antibiotics come from natural products or their semi synthetic derivatives and most of them came out from soil actinomycetes. But recently researchers have shifted to underexplored ecological niches and bacterial species and found some new scaffolds. Compared to the terrestrial environment, the ocean remains an underexplored habitat with unparalleled biodiversity, leaving it the most promising place to yield new antibacterial metabolites. New antibacterial agents with novelty and/or complexity in chemical structure derived from marine bacteria have been elaborated clearly [64; 65]. Myxobacteria, a untapped bacterial strain, can produce many useful natural products which have great potential to develop into antibacterial drug [66].

5.2.2.2. The genomics

By the mid-1990s, pharmaceutical companies have little enthusiasm for making improvement to the existing antibacterials. Hundreds of bacterial genomes have been completely deciphered since 1995, among which are many important human pathogens, attracting large pharmaceutical companies back into antibacterial discovery [67]. Genomics influence various aspects of the antibiotic development, including new drug target identification, understanding the mechanism of antibiotic action, drug safety and efficacy assessment, bacterial resistance development, and so on [68]. Ecopia Biosciences was very skilled in using genome-scanning approach and discovered the new antibiotic scaffold ECO-0501 which is highly effective against a series of Gram-positive pathogens [59; 69]. GlaxoSmithKline also used a genomics-derived, target-based approach to screen for new drugs. They examined

more than 300 genes and employed 70 high-throughput screening campaigns over a period of 7 years, but unfortunately did not create a clinically used antibacterial [70].

5.2.2.3. *New targets*

It must be admitted that target-based genomic approach has not yielded satisfactory results, nevertheless, retooled target-based strategies can still play an important role in discovery process. Most antibiotic targets are limited to peptidoglycan synthesis, ribosomal protein synthesis, folate synthesis, and nucleic acid synthesis and topoisomerization. In the future we could continue to discover new antibiotics for these old targets through improvement of the existing scaffolds or even finding new scaffolds. For instance, Lipid II is a membrane-anchored cell-wall precursor that is essential for bacterial cell-wall biosynthesis; it is not only classical target for several old antibacterial classes, but is also targeted by the new antibiotics, such as lantibiotics, mannopeptimycins and ramoplanin [71]. Grouping targets by a common inhibitor scaffold rather than by function may lead to new targets; and as mentioned above, insights from outside the antibiotic arena are also important [59].

5.2.2.4. *Forward is back*

Compared with the fruitless target-based genomic approach, traditional whole-cell assays are more effective in antibiotic discovery. Just because it is not necessary to worry about cell permeability of a novel scaffold in the development process if whole-cell assays are used. As most of the existing libraries have already been used to screen for antibacterial drugs, libraries with new chemical diversity are extremely important in this approach. Sometimes, look for libraries that don't belong to antibacterial development areas may be useful. In fact, most pharmaceutical companies of other therapeutic areas have invested considerable resources in synthesizing small molecule libraries [59]. Candidates with a strong hit in a whole-cell antibacterial assay should be tested in the right animal model early in development, because *In vitro* experiment results are not always reliable. For example, Antimicrobial drug target type II fatty acid synthesis (FASII) is reported to be essential for their efficacy against infections caused by multiresistant Gram-positive bacteria. But another study showed that *Streptococcus agalactiae* and *S. aureus* could take up sufficient unsaturated fatty acids from human serum to obviate the essentiality of FAS II enzymes *in vivo* [72].

5.2.2.5. *Focus on spectrum*

Antibacterial spectrum is a major consideration when selecting a target for lead optimization. Permeability and target distribution determine the spectrum [73]. That is to say, the drug candidates should possess two properties at the same time: one is penetrating the cell and evading efflux pump systems, another is retaining potent activity at the molecular targets. However, since almost all targets of the antibacterials in clinical use are present in all bacteria, the antibacterial drug spectra are determined largely by the ability of permeability. Therefore, some compounds are just Gram-positive organism-selective and have no effect against Gram-negative pathogens which have a second membrane acting as a permeability barrier [74; 75]. Efflux pump inhibitors (EPIs) have been explored for broadening the anti-

bacterial spectrum and overcoming bacterial resistance. Although no clinically useful drugs have come out, extensive efforts have been made to test the effectiveness of EPIs across a range of in vitro and in vivo assays, especially the compound MC-207,110 [76].

'Broader is better' is the rule of antibacterial activity spectrum. But developing the agents with a narrower spectrum may be helpful in treating some special antibiotic resistant pathogens or the non-multiplying bacteria. One human squalene synthase inhibitor blocked staphyloxanthin biosynthesis in vitro, resulting in colorless bacteria which became more sensitive to killing by human blood and innate immune clearance [77]. Rifampicin is a standard antibiotic used for clearance of non-multiplying tuberculosis. Monoclonal antibodies (Mabs) have also become potential agents for narrow-spectrum antibacterial therapy. In clinical experiment *C. difficile* Mab combination MDX-066 and MDX-1388, which targets and neutralizes two main *C. difficile* toxins, can reduce the recurrence of *C. difficile* infection [78; 79]. A microbiologic diagnosis should be made before using these kinds of antibiotics for therapy. Such genus-selective agents may have the benefit of leaving more of the endogenous microflora unattacked compared with conventional antibiotics.

5.2.2.6. Other new methods

Bacteriophages

Bacteriophages and their fragments could kill the bacteria. They have been developed as antibacterials in humans, poultry and cattle industries, aquaculture and sewage treatment. This approach has novel mechanism of action that is completely different from current antimicrobials, but the problems are that quality control and standardization are difficult. Phage lysins, which are produced late in the viral infection cycle, can bind to cell wall peptidoglycan and rapidly induce Gram-positive bacteria lysis [80]. The sequencing of phages genomes may identify more proteins suitable for novel antibacterials [81; 82].

Other methods to find new drugs could be modulating immunity, developing monoclonal antibody for specific bacteria, designing antibacterial peptides (including antimicrobial peptides and compounds from animals and plants, the natural lipopeptides of bacteria and Fungi [83; 84]), and so on.

6. Conclusion and future issues

While the antibacterial resistance, especially multi-drug resistance continues to rise, what we should do is to investigate the potential mechanisms of drug resistance in bacteria and discover more effective antibacterials to deal with the terrible problems. Luckily there are several promising antibacterial drugs with novel mechanisms of action are in development and new types of targets have emerged. Also we need to be more precise in targeting the pathogens and limit the misuse of antimicrobials and other practices that accelerate the emergence of novel resistance mechanisms. The government must offer robust financial incentives for antibacterial R&D, and build a sustainable model for developing and using antibacterials.

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