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A century of antibiotic resistance: targets, mechanisms and susceptibility testing

Stephen Hawking famously said, «Intelligence is the ability to adapt to change». Indeed, bacteria proved to be smart, having been in constant evolution, skillfully responding to the (selective) pressure imposed by antibiotics with useful modifications that earned some of them the precious title of multi-drug resistant (MDR).

Almost a century after the discovery of penicillin, as foreseen by its far-sighted discoverer, antibiotic resistance constitutes a growing global threat with potentially major consequences, both on the ability to treat patients and on our capacity of infection prevention and control. The increased consumption of antibiotics both in humans and in animals (food industry), together with inappropriate prescribing, contributed to the growing of this problem. Consequently, antibiotics passed from representing the «wonder-drug» with healing powers to the most feared of evils. This was worsened by the small pipeline of new molecules developed in the last 30 years, which contributed to the need of implementation of antibiotic stewardship programs to control the use of antibiotics. With infectious diseases specialists on one side, busy limiting prescriptions and teaching how to better adapt the antibiotic spectrum, microbiologists have been deploying increasingly advanced technologies in order to rapidly detect resistance and allow clinicians a faster fine-tuning of antibiotic treatment.

Antibiotic molecules

The past two years aside, where COVID19 claimed the headlines as «new kid on the block», the enter on the market of new antimicrobial molecules has often been the hit of the day on newspapers, as they have been coming with promising novel actions against MDR «super bugs».

Antibiotics can be divided into four main classes, according to their site of action: i) the cell wall, ii) the cell membrane, iii) the protein synthesis or i) the nucleic acid synthesis (Table 1).

Each class share the same target, but they can differ by i) their ability to reach their target inside the bacteria, ii) their capacity to resist hydrolysis by bacterial enzymes, iii) their action spectrum (narrow versus large) and iv) their pharmacological properties.

Some of them deploy a bactericidal effect, being capable of killing bacteria because acting on a defense or replication mechanism essential for germ survival. Most of the molecules inhibiting the synthesis of peptidoglycan, an essential component of the bacterial wall, are bactericidal. Among these, we find beta-lactamines (such as penicillin, cephalosporins or carbapenems), glycopeptides (such as vancomycine) and fosfomycin. Another bactericidal effect is to block some specific enzymes (such as topoisomerase, RNA polymerase) deputed to the deoxyribonucleic acid (DNA) synthesis: examples of molecules in this class are quinolones (such as ciprofloxacine) and rifampicine. A third bactericidal action is the alteration of the permeability of the cytoplasmic membrane, effect deployed by antibiotics such as polymixines, an «old» class of molecules that has been revisited in the past years as rescue molecule when dealing with MDRs. Finally, some other antibiotics can induce bacterial death by inhibiting the protein synthesis, like aminoglycosides.

Not all molecules have the capacity of directly killing the bacteria: several classes are defined bacteriostatic for their ability to inhibit bacterial replication. This can function both as support for the immune system, which can effectively complete the job by killing the pre-hit bacteria, or as a booster in combination with other bactericidal molecules. Here we find molecules like tetracyclines (such as doxycycline) and macrolides (azythromicine, clarythromicine), which (like aminoglycosides) are capable of entering the cell wall of the bacteria using energy-dependent transport mechanisms in ribosomal sites, which subsequently leads to the inhibition of the protein synthesis. Another intra-cellular target is the synthesis of folates (vitamins essential for the synthesis of DNA), mechanisms used by molecules such as sulphonamides and trrimethoprimes, which work as metabolite analogues.

Researchers keep looking for new mechanisms in order to develop molecules that might hit these or other targets and spare the antibiotics we (and bacteria) already know. An example is a novel mechanism of action, recently discovered in Switzerland (University of Zurich), targeting an outer membrane protein, essential for Gram-negative bacteria, thus destroying the integrity of the bacterial membranes: the target is promising, but no molecules have been released to the market yet.

Mechanisms of resistance

We can distinguish four main mechanisms of bacterial resistance (Table 2), which can be intrinsic (always expressed) or induced (expressed under stress such as antibiotic exposure) and can occur mainly because of natural resistance, gene transfer or mutations.

The first mechanism is the reduction of permeability of the cell membrane. This is the most common intrinsic resistance mechanisms, but can also be induced by antibiotic use, with the effect of preventing the entry of the antibiotic into the bacterial cell. This mechanism can take place through different mechanisms: by modifying the number of porins (water-filled channels) in bacterial membrane, by inducing structural, chemical or polar modification of the wall, as well as collaborating with the bacterial «community» and creating a biofilm, which entangle the contact with antibiotics.

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Mechanism of action	Target	Antibiotic class	Effect on bacteria	Example of molecules
Cell wall synthesis inhibition	Wall penicillin-binding protein (PBP)	ß-lactams: penicillins, cephalosporines, carbapenems	Bactericidal	Penicillin G, amoxicillin, cephalosporin, meropenem
	Wall peptidoglycan	Glycopetides	Bactericidal	Vancomycin
	30s ribosomial subunit (intra-cellular)	Aminogycosides	Bactericidal	Streptomycin, amikacin, gentamicin
		Tetracyclines	Bacteriostatic	Tetracycline, doxycycline
Disruption of cell membrane	Lipopolysaccharides (external membrane of Gram-negative bacteria)	Polymixins	Bactericidal	Colistine, polymyxin B
Protein synthesis inhibition	50s ribosomial subunit (intra-cellular)	Macrolides	Bacteriostatic	Erythromycin, azithromycin
		Oxazolidinones	Bacteriostatic	Linezolid
		Rifamycin	Bactericidal	Rifampin
Nucleic acid synthesis inibition/ disruption	Nucleic acid	Fluoroquinolones	Bactericidal	Levofloxacin, ciprofloxacin
		Nitroimidazoles	Bactericidal	Metronidazole
	Enzymes for acid folic synthesis	Sulfonamides and trimethoprim	Bacteriostatic	Sulfamethoxazole, trimethoprim

Table 1. Classes of antibiotics and action mechanisms.

The second mechanism is the push of antibiotics outside the cell by means of efflux pumps, which are transporters whose genes are usually chromosomally encoded and then either naturally expressed or expressed under stressful circumstances.

While these first two mechanisms are often part of natural resistance, other mechanisms can be acquired by different means: most commonly by horizontal genes transfer, as well as by random mutations on their DNA. Horizontal gene transmission is often plasmid-mediated via conjugation but can also take place by transfer of free DNA (transformation) or, less frequently, by means of bacteriophages (microscopic viruses capable of infecting bacteria) via transduction. As consequence of these gene acquisition, we can find the production of enzymes capable of inactivating the antibiotic molecule: a typical example are the enzymes called beta-lactamases, inactivating penicillin and its derivates. Finally, bacteria can resist antibiotics by modifying the antibiotic target (penicillin binding proteins, cell wall, ribosomes or enzymes for acid nucleic synthesis), making impossible for the molecules to bind at the surface or in the inside of the bacterial cell.

No matter what the resistance method used, some clones of resistant bacteria selected by the pressure of antibiotics spread in the environment and to other people, and this is how clusters (or epidemics) of MDR infections are generated.

General resistance mechanisms	Principal classes involved	Example of mechanism detail
Cell permeability reduction	ß-lactams	Reduction in wall porins
	Glycopetides	Thickening of cell wall
	Aminoglycosides	Changes in wall polarity
	Aminoglycosides	Changes in wall polarity
Efflux pumps	ß-lactams	Pumping antibiotics out of bacterial cells.
	Aminoglycosides	
	Tetracyclines	
	Macrolides	
	Oxazolidinones	
	Fluoroquinolones	
	Sulfonamides and trimethoprim	
Molecule inactivation	ß-lactams	Production of ß-lactamases enzymes
	Aminoglycosides	Production of aminoglycosides modifying enzymes
	Tetracyclines	Antibiotic structure modification
	Fluoroquinolones	Antibiotic chemical modifications
Antibiotic target modification	ß-lactams	Modification in penicillin binding proteins (PBP)
	Glycopetides	Modification in wall peptidoglycan
	Aminoglycosides	Mutations in ribosomes
	Tetracyclines	Protection of ribosomes
	Macrolides	Mutations in ribosomes
	Oxazolidinones	Ribosomial chemical modifications
	Fluoroquinolones	Modification in enzymes for acid nucleic synthesis
	Sulfonamides and trimethoprim	Overproduction/reduction in enzymes for acid folic synthesis

Table 2. Summary of principal bacteria resistance mechanisms
Readapted from Reygaert WC. AIMS Microbiol. 2018.

Susceptibility testing and resistance detection

In lack of a wide pipeline of new molecules, innovations and advances in antimicrobial susceptibility testing (AST) and antimicrobial resistance (AMR) detection play an essential role in helping clinicians to preserve the currently available antibiotics. The main AST and AMR detection methods are summarized in Table 3.

AST employs a phenotypic approach, which mostly relies on pure bacterial cultures in contact with different con-

centrations of antibiotics: when the bacteria are resistance to a certain concentration of antibiotic, the growth will stop and the arrest will be visible. Molecules can be tested through different methods: agar (solid media) or broth (liquid media) dilution, disk diffusion (measuring the diameter of inhibition zone around the disk with a pre-determined concentration of antibiotic) or gradient tests (decreasing concentrations of antibiotics contained in a single strip with different marked levels). Based on bacterial growth, the major limitation for most of phenotypic tests is the speed. For this reason, rapid AST methods have been investigated, and several promising technologies are under study. Rapid phenotypic tests are already on the market. They are capable to detect the presence or absence of a specific enzyme link to resistance through chromogenic media, changing colors according to the susceptibility. Nanomotion technologies instead, are based on fluctuations caused by the nanomotion due to metabolically active bacteria: through cantilevers (micro-electromechanical sensors), they are capable of detecting the changes in nanomotion caused by the exposure to a drug, without the need to wait for bacterial growth on cultures. Other examples of new phenotypic methods, which rely on the combination of imaging and artificial intelligence, are the use of nuclear magnetic resonance or mass spectroscopy to detect susceptibility based on a specific image pattern. Flow cytometry is also a technique that has been readapted for the study of antibiotic susceptibility and resistance detection.

Aside from phenotypic AST, there is also the possibility of directly detecting some specific genes associated with resistance, as well as mutations or variations in genes' expression. These are known as molecular methods for AMR detection and are based on nucleid-acid amplification (genetics), sequence databases and bioinformatics (genomics). They represent the future of AMR detection, but nowadays they are still limited by prohibitive costs and long turnaround-times, making them a luxury that only research centers can afford.

Testing	Type of method	Method details
Antibiotic susceptibility testing	Classical phenotypic	Solid media dilution
		Liquid media dilution
		Disk diffusion
		Gradient strip tests
		Chromogenic media
	Innovative phenotypic	Nanomotion technologies
		Nuclear magnetic resonance
		Mass spectrometry
		Flow cytometry
Antimicrobial resistance detection	Genetic	Nucleic-acid amplification
	Genomic	Next-generation sequencing

Table 3. Principal available methods for antibiotic susceptibility and antimicrobial resistance testing.

Conclusion

The several promising laboratory technologies being developed and researched are a precious help to compensate the lack of novel molecules in the antimicrobial pipeline. Nevertheless, the reduction of turnaround time for detection of AMR can have a very different impact according to the epidemiology of resistance present in the country and it cannot supply one hundred percent the need on new «weapons». Indeed, we are still left with the question: where did these resistant bacteria come from? Why they are endemic is some countries and others are able, instead, to keep their rate under control? The possibility of non-humans (animals, environment) reservoirs has been widely debated, but large studies have disproved this hypothesis and evidence seems in favor of human-human transmission and infection control habits as main responsible for the increase of antimicrobial resistance.

A lot is still to investigate, but now it is our turn to show intelligence, to adapt to change, to fight back antibiotic resistance, to develop revolutionary methods for AST, to update the pipeline of new antimicrobials and to learn how to use antibiotics appropriately. Humans and bacteria will continue to evolve adapting to each other, that's the beauty of nature, but the fight against «super-bugs» is not over yet: who do you think is going to win?

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