Chapter

1

FIGHTING ANTIMICROBIAL RESISTANCE IN ESKAPE PATHOGENS

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1.1. INTRODUCTION

Antimicrobial resistance (AMR) is the ability of a microorganism to withstand antimicrobial compounds. The evolution of AMR is a natural phenomenon that results from bacterial gene mutations or the acquisition of exogenous resistance genes present in mobile genetic elements that are able to propagate horizontally between bacteria. The use of antimicrobial agents and the transmission of antimicrobial-resistant microorganisms are the most important factors that lead to the emergence and expansion of AMR [1].

Bacteria can acquire several mechanisms that make them resistant to various families of antibiotics. This can have severe consequences when the suitable antibiotic treatment to fight the infection is lacking [1].

AMR problems require collective efforts at the country level, as well as close international teamwork. In Europe, the European Antimicrobial Resistance Surveillance Network is the prime system for monitoring AMR in bacteria that are etiological agents of serious infections [1]. The European Centre for Disease Prevention and Control (ECDC) reported that resistance to multiple antibiotics is an increasing worry in the EU and stated that 'With increasing resistance even to last-line antibiotics we face a frightening future where routine surgery, childbirth, pneumonia and even skin infections could once again become life-threatening' [2].

The Centers for Disease Control and Prevention (CDC) reported that at least 2 million people are infected with a resistant microorganism every year in the United States which results in at least 23,000 individuals dying every year [3].

Multidrug resistance is one of the three principal concerns to global public health [4]. Several factors are responsible for this situation, such as an increase in the global use of antibiotics [5], the absence of widely-used best practices in the management and training of antibiotic administration [5,6], the inappropriate use of antibiotics (*i.e.* insufficient dosage and prescriptions to treat mild bacterial or viral infections) [7] and the extensive and lawless use of antibiotics in animals to enhance meat production [8]. Another significant factor in the rise of antibiotic resistance is the propagation of resistant strains among the population or from other environmental sources [3]. Finally, a lack of sufficient knowledge about the mechanisms involved in bacterial tolerance and persistence is associated with AMR [9-11].

In 2016, the World Health Organization (WHO) generated a priority list of pathogens with the principal aims of allocating funding to facilitate the global coordination of research and promoting strategies to identify new active antiinfective agents against multidrug-resistant (MDR) pathogens. Several factors were considered in the creation of this list, including mortality, healthcare load, community charge, the prevalence of resistance, the 10-year

tendency of resistance, transmission, prevention in the community, prevention in healthcare institutions, the ability to treat, and the pipeline. This priority list included carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, as well as third-generation cephalosporin- and carbapenem-resistant Enterobacteriaceae [12]. In relation to Gram-positive bacteria, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* were also present [12]. Pathogens that cause community-acquired infections, such as clarithromycin-resistant *Helicobacter pylori* and fluoroquinolone-resistant *Campylobacter* spp., *Neisseria gonorrhoeae* and *Salmonella typhi*, were also included [12].

1.2. PREVENTION

In terms of prevention, the CDC affirms that avoiding the development of infections reduces the levels of antibiotics used and reduces the propagation of resistant cells. The CDC is trying to prevent infections caused by antibiotic-resistant bacteria in healthcare settings, the community and food. The CDC works to avoid antibiotic resistance in healthcare settings by supplying a method to identify resistance, prescribing models at diverse scales and giving recommendations to healthcare facilities and laboratories through infection-control guidelines. To prevent antibiotic resistance in the community, the CDC is trying to implement systems to follow infections and their changes in resistance, organise teams in regional areas and at a national level and manage the transmission of infections. To prevent antibiotic-resistant foodborne infections, the CDC collaborates with health departments, with the Food and Drug Administration (FDA) (which regulates antibiotics, foods, animal feed and other products) and also with the U.S. Department of Agriculture (which is responsible for the regulation of meat, poultry and egg products). However, prevention is only the first step, and the development of new diagnostic tools to detect specific mechanisms of bacterial AMR is also an important issue in the fight against AMR.

1.3. ESKAPE PATHOGENS

The ESKAPE pathogens (*E. faecium*, *S. aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* spp.) are the main causes of nosocomial infections around the world. The majority of these pathogens are MDR isolates, which is one of the biggest challenges in clinical practice [4]. The main reason is that they are responsible for a dramatic increase in morbidity and mortality in infected patients, hence their prompt detection is vital [13].

Understanding the resistance mechanisms in these bacteria is not only critical for the development of new antimicrobial agents or other anti-infective treatments [4] but also for the development of new diagnostic techniques.

On one hand, the ability to detect antibiotic-resistance genes and their low turnaround times has made molecular methods a reference for the diagnosis of multidrug resistance. These methods have a high clinical and epidemiological impact [13]. Multiplex real-time polymerase chain reaction (PCR) merits special attention because it is able to quickly identify multiple pathogens and various resistant genes in a sample [14-19].

Moreover, the study of the bacterial mechanisms of tolerance and persistence to stress conditions (including antimicrobial agents), which could occur before the development of resistance, might provide the key to the fight against AMR. Although antibiotic-treatment failure is typically attributed to resistance, it has long been realised that other mechanisms (*i.e.* tolerance and persistence) help bacteria to survive antibiotic exposure. Several authors examined the progression from tolerant to resistant populations, observing that, as always, tolerance goes before resistance. This infers that avoiding the evolution of tolerance may be a new strategy for decelerating the occurrence of resistance [9]. The molecular mechanisms involved in the development of tolerant or persistent bacterial cells are numerous and include RNA polymerase, sigma S (RpoS) and the general stress response, oxidant tolerance (*i.e*. reactive oxygen species (ROS), energy metabolism or efflux pumps, the bacterial DNA damage (SOS) response, the quorum sensing (QS) system or bacterial communication, the 5',3'-bis-guanosine penta/tetraphosphate [(p)ppGpp] network and toxin–antitoxin modules [10,20].

The minimum duration for killing (MDK), which is a quantitative measure of tolerance that can be extracted from time-kill curves, was proposed by Brauner *et al.* as a means to distinguish between the various strategies of bacterial survival under antibiotic stress. In clinical practice, the MDK concept may be helpful for different objectives, such as adjusting effective treatments depending on the specific survival strategies employed by the etiological agent and their duration [21].

1.4. NEW ANTIINFECTIVE TREATMENTS

The increased prevalence of antibiotic-resistant bacteria is one of the principal global health problems, therefore the discovery and development of new molecules and antimicrobial treatments is a main objective for the World Health Organization (WHO) [22]. In 2016, to raise awareness of the need for new antibiotics, WHO member states requested a priority list of antibiotic-resistant bacteria to perform research and develop new and beneficial

antiinfective treatments. The alternative antiinfective treatments against MDR pathogens are classified into the following seven groups: a) new drugs, b) phage therapy (including derivates), c) antivirulence therapy, d) lysins, e) antibodies, f) probiotics and g) immune stimulation (Figure 1).

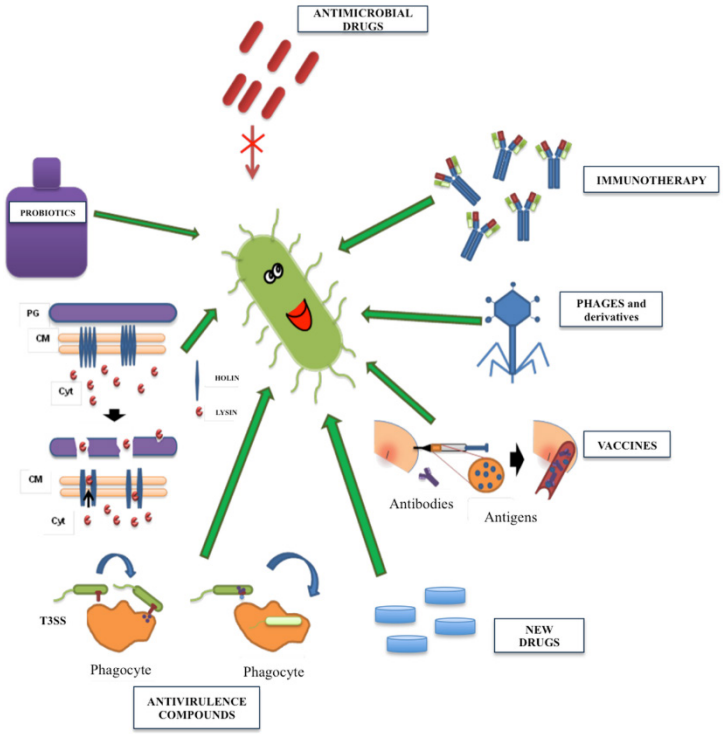

Figure 1. New antiinfective treatments. Due to the lack of effective antimicrobial agents to combat MDR bacteria, other anti-infective treatments should be considered. Immunotherapy, phage therapy and its derivatives, vaccines, new drugs, antivirulence compounds and probiotics, as shown in this figure, are alternative treatments to fight against infections caused by MDR bacteria.

a) New drugs

Since 2000, three new classes of human-use antibiotics have been launched on the market, one of which is restricted to topical use. 'Gap innovation' has been used to explain the absence of new structural types in the antibacterial arsenal since 1962 [20].

Two recent reports, one by the Infectious Diseases Society of America (IDSA) [24] and the other by the ECDC [25], demonstrated that there are a few candidate drugs in the pipeline that offer benefits over existing drugs and that a few of these drugs will treat infections caused by the ESKAPE pathogens. The

goal of the IDSA is to lay the foundations of a sustainable and global antibacterial drug R&D enterprise with the short-term capacity to develop 10 new, safe and effective antibiotics by 2020. To achieve this objective, the IDSA has released a new teamwork called the '10 x 20' initiative. Specifically, the IDSA will sustain the development of 10 new systemic antibacterial drugs by means of discovering new drug classes as well as exploring potential new compounds from existing antibiotic families [26].

b) Phage therapy (including derivatives)

Bacteriophages (viruses that specifically infect bacteria) were discovered and used as antimicrobial agents during the 1920s; however, they stopped being used following the appearance of antibiotics. Nevertheless, they have continued to be used in the Soviet Union for decades. This 'forgotten cure' employs natural viruses that infect bacteria, and are present in all ecosystems, but are unable to infect eukaryotic cells [27].

The literature has described the use of living phages as a treatment for lethal infectious diseases caused by Gram-positive and Gram-negative bacteria. Another finding in the field of bacteriophage therapy is the possibility of treating with genetically-modified and nonreplicating phages. Moreover, bacteriophages are potential adjuvants of antibiotic therapy. Phages encoded with lysosomal enzymes are also efficient at treating infectious diseases [28].

Several animal studies have demonstrated the efficacy and safety of phage therapy in the treatment of different infections [29-31]. In humans, potential applications of phages include the phage-mediated prevention and phage treatment expanding from conventional phage therapy, treatment with phage enzymes (*e.g.* endolysins) and the use of phages as adjuvants of antibiotics. Lysins represent a new class of anti-infective agents that are obtained from bacteriophages. They are bacterial hydrolytic enzymes of the cell wall that are capable of selectively and rapidly [≥3 log colony-forming units (CFU) in 30 min] killing specific Gram-positive bacteria. They also provide a targeted therapeutic proposal that produces a limited effect in other bacteria. The potential for lysin resistance in bacteria should be low due to the direction of the highly-conserved peptidoglycan components [32]. Endolysins against Gram-negative pathogens were recently characterised and developed [33-35]. In *S. aureus,* the application of lytic proteins to treat severe infections such as bacteraemia or endocarditis has already been studied. Furthermore, the structure and mechanism of action of these proteins have also been examined to better understand their ability to inhibit the infection and to modify them to improve their activity [36].

Interestingly, the use of *P. aeruginosa* and *A. baumannii* phages together could inhibit QS systems [37,38]. These data highlight a new field in phage therapy.

In conclusion, phage therapy is a safe alternative for the treatment of infections caused by MDR pathogens. It can also be used in combination with existing antibiotics to enhance their effect; however, there are currently no approved phage applications for humans, and further clinical trials are needed in this area in the near future [27,39].

c) Antivirulence therapy

The objectives of the antivirulence procedures are to reduce the use of antibiotics, reduce the appearance of antibiotic resistance and protect the beneficial flora. Antivirulence agents do not exert strong selective pressures on bacteria that benefit the evolution of resistance and persistence mechanisms and, because they do not have an impact on viability, they should not alter the beneficial microbiota [40]. Several techniques can be used to identify possible antivirulence compounds, including the scraping of natural products, the structural modification of native ligands and the *in silico* coupling and high-throughput screening (HTS) of chemical libraries. Research in this field has increased dramatically in recent years; however, the first antivirulence compound has yet to arrive.

Antivirulence strategies for ESKAPE pathogens tend to target: (a) specific virulence factors (*e.g.* type three secretion system (T3SS) and enterotoxins), (b) master virulence regulators and signals (*e.g.* two-component systems and QS, such as acetylases and lactonases) [41] or (c) resistance to host defences and antibiotics (*e.g.* capsules, staphyloxanthin and biofilms).

Vila-Farrés *et al.* proposed an innovative approach to tackling MDR bacteria. The outer membrane protein A (OmpA) is a beta-barrel porin that is highly conserved among bacterial species, especially Gram-negative bacteria. These authors studied the efficacy of OmpA inhibitors in the prevention of infection both *in vitro* and *in vivo* [42].

Table 1. Examples of ESKAPE antivirulence targets and their inhibitors. Adapted from Maura *et al.* [43] and Beceiro *et al.* [40].

Inhibitor	Virulence mechanism	Target	Pathogen	Refs
Morin hydrate	Toxins	Hla	*S. aureus*	[44]
Phosphonoacetamide derivative		Staphyloxanthin		[45]
Menthol		Enterotoxins		[46]

Table 1. (continued)

Inhibitor	Virulence mechanism	Target	Pathogen	Refs
Per-6-(3-aminopropylthio)-*β*-cyclodextrin		Protective antigen (PA) heptamer pore	*Bacillus anthracis*	[47]
2®-2[(4-Fluoro-3-methyl-phenyl) sulfonylamino]-*N*-hydroxy-2-(tetrahydro-2H-pyran-4-yl) acetamide		Lethal factor (LF) subunit	*B. anthracis*	[48]
Cisplatin		LF subunit	*B. anthracis*	[49]
Synsorb-Pk		Gb3	Enterohemorrhagic *E. coli* (EHEC)	[50,51]
Chlorogenic acid	Adhesion and colonisation	Sortase A	*S. aureus*	[52]
Bicyclic-2-pyridones (pilicides)		PapD		[53]
Virstatin		ToxT		[54]
Black pepper oil	Biofilms		*S. aureus*	[55]
TAGE-triazole conjugates			*A. baumannii, S. aureus* and *P. aeruginosa*	[56]
GarO (garlic ointment)			*K. pneumoniae S. aureus, Staphylococcus epidermidis, P. aeruginosa, A. baumannii*	[57]
Mix of sugars		Adhesion	*P. aeruginosa*	[58]
Ebselen	C-di-GMP	Diguanylate cyclase	*P. aeruginosa*	[59]
Acylated hydrazones of salicylaldehydes	Bacterial secretory system	Yop/T3SS	*Chlamydia* and *Shigella sp.*	[60,61]
2-imino-5-arylidene thiazolidinone		Sip/T3SS	*Salmonella Pseudomonas* and *Yersinia sp.*	[62]
Diarylacrylonitrile		Sortase A	*S. aureus*	[63]

Table 1. (continued)

Inhibitor	Virulence mechanism	Target	Pathogen	Refs
Anti-PcrV antibody		PcrV protein/T3SS	*P. aeruginosa*	[64]
Fascioquinol E	Capsules	CpsB phosphatase	*S. aureus*	[65]
Triazines		Transport/ export machinery	*K. pneumoniae*	[66]
C14-TOA (3-acyltetronic acid)	QS system	Agr system	*S. aureus*	[67]
Benzimidazol derivate M64		MvfR (PqsR)	*P. aeruginosa*	[68]
Furanones (C-30), patulin, salicylic acid, *etc.*		AHLs	*Pseudomonas sp.*	[69-71]
Thiophenones		IcaC, LrgB	*S. epidermidis*	
Substrate analogues 3/oxo C12 D10		LasR	*Pseudomonas sp.*	[72]
Substrate analogues/C4		LuxR	*Vibrio fischeri*	[72,73]
Halogenated compounds			*Pseudomonas sp.* *Escherichia coli* *S. aureus*	[74-76]
Catechins (galloyl group)		LuxS system	*Vibrio harveyi* and *Eikenella corrodens*	[77,78]
Tomatidine		Agr system	*S. aureus*	[79]
26 % hydroxypropyltrimethyl ammonium chloride chitosan (HACC)-loaded, gentamicin-loaded polymethylmethacrylate (PMMA) (chitosan derivative)		*icaAD-icaR*	*S. aureus*	[80]
Salicylate		*marA/fimB*	*E. coli*	[81]
LED 209		QscE		[81]

Table 1. (continued)

Inhibitor	Virulence mechanism	Target	Pathogen	Refs
(Z)-4-bromo-5-(bromomethylene)-3-methylfuran-2-(5H)-one		AI-2	*E. coli*	[82]
RNA III-inhibiting peptide and its analogues		Target of RNAIII-activating protein (TRAP)	*S. aureus*	[83-85]
Synthetic autoinducer of autocamptide-2 related inhibitor peptide (AIP) and analogues (I–IV)		RNA III	*S. aureus*	[83]
AIP-nonfunctional peptide analogues		Agr receptors	*S. aureus*	[86]
Lactonases and acylases		AHL-based autoinducers	*P. aeruginosa*	[76]
Cell extracts and secretion products		*las*-and *rhl*-QS systems	*P. aeruginosa*	[76]
Compounds obtained from food and plant sources			*P. aeruginosa*	[76]
Acyl-adenylate derivates	Iron metabolism	Aryl acyl adenylating enzymes	*Mycobacterium tuberculosis* and *Yersinia pestis*	[87,88]
Piridine derivative/ HTS 85K		BasE	*A. baumannii*	[89]
I-A09	Activity against the host immune response	mPTPB	*M. tuberculosis*	[90]
Sulfamoyl D-Ala		DItA	*B. subtilis*	[91]
1-(1-Naphthylmethyl)-piperazine, phenyl-arginine-*β*-naphthylamide	Resistance-nodulation-division (RND) efflux pumps	RND efflux pumps	*Vibrio cholera*	[92]
Trifluoromethyl lactones (12 compounds)		RND efflux pumps	*Chromobacterium violaceum* and *E. coli*	[93]

d) Antibodies

In 1890, Von Behring and Kitasato were the first to use the blood of rabbits to neutralise toxins [94]. Since then, serum therapy has been widely used to treat infectious diseases such as pneumococcal pneumonia, meningococcal meningitis, dysentery and erysipelas. However, serum therapy has since been relegated to being used to treat scarce pathologies (*i.e.* hepatitis, measles or toxin-induced diseases) due to the high number of adverse effects observed. Following serum therapy, and as a result of advances in the field of immunology, the use of antibodies against a specific pathogen or virulence factor was implemented. Antibodies constitute a traditional boarding in infectious diseases that without question is not directly connected with resistance; however, the identification of determinants (which are involved in virulence) is relatively conserved among strains and could potentially be attractive to study in relation to resistance to multiple antibiotics [27].

e) Probiotics

Probiotics are live microorganisms that provide benefits for the health of the host when they are administered in suitable amounts [95]. The most commonly used probiotics are usually bacterial strains (*i.e. Lactobacillus* or *Bifidobacterium*) or fungal isolates of the normal microbiota (*i.e. Saccharomyces boulardii*). Probiotics interact through many paths, such as antimicrobial activity with growth inhibition or the expression of bacterial virulence agents. Probiotics generate acids that lower the pH of the local environment [96] and toxins that suppress the growth of other bacteria [27].

f) Immune stimulation

Attempting research and development to find the next generation of antibacterial drugs is essential; however, vaccines, in combination with the proper use of current antibiotics, are starting to be recognised as pivotal and potent tools to attenuate AMR [97]. Bacterial infections can be avoided with the preventive use of bacterial vaccines. As a consequence, antibiotic prescriptions will be reduced and the selective pressure of the drug that gives rise to resistant strains will be minimised. In addition, the beneficial effects of vaccines on AMR have also been perceived with viral vaccines, such as those that prevent influenza [98]. Such vaccinations can reduce inappropriate antibiotic prescriptions for a viral disease and avoid the bacterial superinfections that would require antibacterial therapy.

Newly developed vaccines (*i.e.* vaccines against infections caused by *Clostridium difficile* or *S. aureus*), pneumococcal-conjugate vaccines with wide serotype coverage and vaccines to avoid infections due to Gram-negative

bacteria promise to manage these severe diseases, encourage the reduced use of antibiotics and avoid AMR [99].

A new vaccine candidate (D-alanine auxotroph) against staphylococcal disease was recently developed [100]. Moreover, these authors also developed *A. baumannii*, *P. aeruginosa* and *S. aureus* mutants that were D-glutamate auxotrophic strains and proved their efficacy as whole-cell vaccines *in vivo* [101].

1.5. CONCLUSION

Many factors may be necessary to overcome MDR bacteria ('superbugs') including focusing on their prevention, the detection and development of new treatments where clinical involvement is essential, innovation and research. Collective global action is needed to manage the crisis of antibiotic resistance through the balancing of innovation access and stewardship.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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