Mechanisms of Carbapenem Resistance

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Carbapenem antibiotics are the most effective antimicrobials for the treatment of infections caused by the most resistant bacteria. They belong to the category of β-lactams that include the penicillins, cephalosporins, monobactams and carbapenems. This class of antimicrobials has a broader spectrum of activity than most other beta-lactams antibiotics and are the most effective against Gram-positive and Gram-negative bacteria. All β-lactams antibiotics have a similar molecular structure: the carbapenems together with the β-lactams.

Keywords: carbapenem antimicrobials

1. Introduction

Carbapenem have a penicillin-like five-membered ring, but the sulfur at C-1 in the five-membered ring is replaced by a carbon atom and a double bond between C-2 and C-3 has been introduced [1]. For carbapenems, the characteristic setting of the side chain in the trans position instead of the cis position, commonly found in other β-lactams, made them insensitive to the effects of β-lactamases. The PBPs 1a, 1b 2 and 3 are the principal targets of inhibition and PBPs 2 and 3 are specific for Gram-negative bacteria. Beta-lactam antibiotics can kill or even inhibit susceptible bacteria. The concentration of an antibiotic at the site of infection must be sufficient to inhibit the growth of the offending microorganism. If host defenses are impaired, antibiotic-mediated killing may be required to eradicate the infection. The concentration of the drug at the site of infection must not only inhibit the organism, but must also remain below the level that is toxic to human cells. If this can be achieved, the microorganism is considered to be susceptible to the antibiotic. The cell walls of bacteria are essential for their normal growth and development. Peptidoglycan is a heteropolymeric component of the cell wall that provides rigid mechanical stability. Gram-positive bacteria have as many as 40 layers of peptidoglycan, in Gram-negative bacteria, there appears to be only one or two layers. The surface structure in Gram-negative bacteria is more complex and the inner membrane, which is analogous to the cytoplasmic membrane of Gram-positive bacteria, is covered by the outer membrane, lipopolysaccharide and capsule. The outer membrane functions are an impenetrable barrier for some antibiotics. Other pharmacological differences that characterize the two types of bacteria are membrane permeability, efflux pumps, a large amount of different β-lactamase and the transpeptidation reaction, that occurs outside the cell membrane [1]. It is the last step in peptidoglycan synthesis that is inhibited by the β-lactam antibiotics [2].

2. Mechanism of Resistance

2.1. Decreased Permeability

Resistance to β-lactam drugs may be related to the inability of antibiotics to reach their sites of action by reducing the entry into their outer cell walls through the porin channels. Some small hydrophilic antibiotics such as β-lactams, as well as tetracycline, chloramphenicol and fluorochinolones diffuse through aqueous channels in the outer membrane that are formed by proteins (Omp) called porins [3]. The number, the form and quality of porins in the outer membrane are mutable among different Gram-negative bacteria. A significant example is P. aeruginosa which is intrinsically resistant to a wide variety of antibiotics due its reduced classic high-permeability porins' expression. Broader spectrum of β-lactam antibiotics such as ampicillin and amoxicillin and most of cephalosporins can diffuse through the pores in the E. coli outer membrane more rapidly than penicillin G. These are important instances of bacterial resistance to β-lactam antibiotics [4][5].

2.2. Overexpression of Efflux Pump

Active efflux pumps serve as another mechanism of resistance, removing the antibiotic from its site of action before it can act. This is an important mechanism of β-lactam resistance in *P. aeruginosa*, E. coli and Neisseria gonorrhoeae. Multidrug efflux pumps traverse both the inner and outer membranes of Gram-negative bacteria. The pumps are composed of a minimum of three proteins and are energized by the proton motive force and is produced by the respiratory enzymes and

oxidative phosphorylation. Increased expression of these pumps is an important cause of antibiotic resistance. The onset of the mechanism of antibiotic resistance may represent a genetic occurrence [6][7][8]. The mutation responsible for drug resistance is usually a modification at a specific site on bacterial chromosomes. The capture, accumulation and dissemination of resistance genes are largely due to the actions of mobile genetic elements (MGE), a term used to refer to elements that promote intracellular DNA mobility (e.g., from the chromosome to a plasmid or between plasmids) as well as those that enable intercellular DNA mobility [9]. Thus, the resistance can be transferred from a resistant microorganism to a new location in the same or different DNA to a sensitive microorganism [10]. Multidrug efflux pumps are incorporated into bacteria and their purpose is to transport antibiotics outside the outer membrane of bacteria. Moreover, drug-specific efflux mechanism is promoted by plasmids and others mobile transporters [11]. All these components participate in specific activity promoting horizontal genetic exchange and contribute to achieve and disseminate the resistance genes. Bacteria efflux proteins are proteins identified primarily in Gram-negative bacteria but also exist in Gram-positive bacteria. They are divided into seven families. The seven families included in the resistance-nodulation-division (RND) superfamily are: the heavy metal efflux (HME), the nodulation factor export family (NFE), the major facilitator (MF) superfamily, the SecDF protein-secretion accessory protein family, the hydrophobe/amphiphile efflux-2 family, the eukaryotic sterol homeostasis family and the hydrophobe/amphiphile efflux-3 family. The efflux pumps of the RND superfamily, such as AcrB of E. coli and MexB of *P. aeruginosa*, play a fundamental role in promoting multidrug resistance. These pumps are associated with outer membrane channel such as TolC of E. coli and OprM of P. aeruginosa belonging to the OMF family proteins, the AcrA of E. coli and MexA of P. aeruginosa that are included into the MFP family. These three groups of proteins are essential for drug efflux and the mere lack of one these give the entire system a non-functional status. All these elements are involved in the intercellular mechanism of antibiotic resistance. Efflux mechanism or to be more precise, genes encoding efflux pumps are not commonly transmitted by mobile genetic elements. Only a few plasmid-mediated efflux pumps have been described in recent years. The transcription of genes involved in the efflux pumps is checked by local regulators. The overexpression of efflux genes, that are at the basis of Gram-negative bacteria resistance, is regulated by the mutation mechanism.

2.3. Mutation and Transformation in Antibiotic Target Structures

Another mechanism which can develop antibiotic resistance is represented by the mutation of the antibiotic target. The resistance to streptomycin, to quinolones, to rifampin and others antibiotic groups is caused by a series of mutations that can occur in the gene encoding the target protein, the protein concerned with drug transport and/or with drug activation. [12] The archetypal example of this is penicillin resistance in S pneumoniae and which is conferred by mosaic penicillin-binding protein (pbp) genes encoding penicillin-insensitive enzymes. Mosaicism in the penA gene, which encodes a PBP, in N.gonorrhoeae has also been linked with high-level resistance to extended-spectrum cephalosporins. Every antibiotic has its specific target site, by binding to which exerts its inhibitory effect. Mutation of the target site can occur during an infection and a mutation into a single microorganism can induce resistance [13]. Genes encoding DNA gyrase or topoisomerase IV, within the bacterial chromosomal are responsible for quinolone resistance, this resistance is expanded above all after the therapeutic use of these antibiotics in Pseudomonas and Staphylococci [14]. The mechanism of mutation may occur in a functional target with a decreasing affinity for the antibiotics, which do not work completely and behave with a reduced efficiency (**Table 1**). Another mechanism of bacterial resistance to antimicrobial agents is the transformation. This is the capacity of transferring genetic information, involving uptake and incorporation of DNA, how it happens in altered penicillin-binding proteins (PBPs), that are mosaics of overseas DNA imported and incorporated from a streptococcus to a resident PBP gene [15].

Table 1. Molecular mechanisms of antibiotic resistance.

↓ Permeability	Outer membrane forms a permeability barrier (Gram positive >Gram negative). Down-regulation of porins or by the replacement of porins with more selective channels.
↑ Efflux	Bacterial efflux pumps actively transport many antibiotics out of the cell (multidrug resistance [MDR] efflux pumps).

Mutation and Transformation in Antibiotic Target Structures	Changes to the target structure that prevent efficient antibiotic binding: Transformation can confer antibiotic resistance by target protein modification through the formation of ‘mosaic’ genes. Acquisition of a gene homologous to the original target. Protection by modification of the target: Erythromycin ribosome methylase (erm). Chloramphenicol–florfenicol resistance (cfr) methyltransferase. Quinolone resistance (qnr) gene.

2.4. Class A Carbapenemases

Class A carbapenemases are chromosomally encoded (SME, NmcA, SFC-1, BIC-1, PenA, FPH-1, SHV-38), plasmid-encoded (KPC, GES, FRI-1), or both (IMI) [16]. Among these, the best known KPC (Klebsiella pneumoniae carbapenemase) was spread all around the world and has been isolated in most of the clinical enterobacterial species such as P. aeruginosa and A. baumannii. Generally, class A carbapenemases reduce susceptibility to imipenem for the bacteria sensitive to it and allow the hydrolysis of a broad variety of β-lactams, including carbapenems [17].

2.5. Class B Carbapenemases

The class B of the Ambler group belonging to the (MBLs) are clinically the most relevant carbapenemases. These are divided into three subclasses: B1, B2, and B3, but the largest number of clinically relevant MBLs belong to the B1 subclass, including the most frequently Verona integron-encoded MBL (VIM), imipenemase (IMP) and New Delhi MBL. (NDM) Those MBLs are generally located within different integron structures, which are connected with mobile plasmids or transposons facilitating the transfer of resistance genes between bacteria [18].

2.6. Class D Carbapenemases

Class D carbapenemases (Oxa-type β-lactamases, CHDLs) are a group of enzymes that have been discovered in A. baumannii and *K. pneumoniae* human strains [19]. These carbapenemases and specifically the OXA-48 and related variant are of clinical relevance because they make it difficult to treat infection [6]. In clinical therapy, the β-lactamase inhibitors (i.e., amoxicillin-clavulanic acid etc.) are effective for class A β-lactamases, but do not inhibit class D carbapenemases. Therefore, it is important in the next future to develop inhibitors of A. baumannii class D carbapenemases to make the carbapenem antimicrobials effective [18][20].

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