Mechanisms responsible for the emergence of carbapenem resistance in Pseudomonas aeruginosa

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Abstract

Pseudomonas aeruginosa (P. aeruginosa) is an opportunistic pathogen associated with a range of nosocomial infections. This microorganism is noted for its intrinsic resistance to antibiotics and for its ability to acquire genes encoding resistance determinants. Among the beta-lactam antibiotics, carbapenems with antipseudomonal activity are important agents for the therapy of infections due to P. aeruginosa. The development of carbapenem resistance among P. aeruginosa strains is multifactorial. Plasmid or integron-mediated carbapenemases, increased expression of efflux systems, reduced porin expression and increased chromosomal cephalosporinase activity have all been defined as contributory factors. Phenotypic tests and molecular techniques are used for the characterization of the resistance determinants. The isolation of carbapenem resistant strains is alarming and requires the implementation of strict infection control measures in order to prevent the spread of carbapenemase encoding genes to unrelated clones or to other bacterial species. Hippokratia 2012, 16, 4: 303-307

Key words Pseudomonas aeruginosa, carbapenem resistance, carbapenemases, efflux systems, OprD

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Introduction

Pseudomonas aeruginosa (P. aeruginosa) is an important cause of nosocomial infections that can be particularly severe in immunocompromised patients. These pathogens are common causative agents of pneumonia, bacteremia, urinary tract, skin and soft tissue infections. The increasing isolation in healthcare settings of P. aeruginosa strains non-susceptible to most anti-pseudomonal agents is due to a number of factors, including its innate resistance to a variety of antimicrobial agents, its ability to acquire resistance determinants and the increased use of antibiotics, which promotes the selection of resistant clones. Carbapenems have a broad spectrum of antibacterial activity and are used as last resort drugs for the treatment of infections caused by multiresistant P. aeruginosa isolates. This extraordinary microorganism however, often possesses the necessary mechanisms to overcome the activity of almost all the available antibiotics.

Carbapenem use for P. aeruginosa infections

Carbapenems are the most effective antimicrobial agents against gram-positive and gram-negative bacteria including P. aeruginosa. Carbapenems bear a penamic together with the beta-lactam ring and, like all other beta-lactams, they inhibit bacterial cell wall synthesis by binding to and inactivating Penicillin Binding Proteins (PBPs). This unique molecular structure offers them their exceptional stability to many beta-lactamases including AmpC and most of the extended spectrum beta-lactamases (ESBLs).

Seven antibiotics belong to the carbapenem family (Figure 1), each one presenting particular characteristics that influence their way of administration and their usefulness as anti-pseudomonal agents.

Imipenem, for example, is susceptible to renal dehydropeptidase I and is administered in combination with cilastatin which acts as a dehydropeptidase I inhibitor. Panipenem is a carbapenem antibiotic that was developed in Japan in 1993 and is available to date only in the Asian market. Similar to imipenem, panipenem is used in combination with betamipron in order to overcome the inactivating effect of dehydropeptidase I.

Meropenem is stable to dehydropeptidase I inactivation but is less efficient than imipenem against gram-positive microorganisms. On the other hand, meropenem is more active against gram-negative bacteria especially against P. aeruginosa because it passes more swiftly through the OprD porin.
The Asian biapenem has a similar molecular structure to imipenem bearing a methyl group in 1B position. It shows a broad spectrum of activity especially against an aerobes and is stable to dehydropeptidase I inactivation. Ertapenem has longer half-life and has been introduced in Europe and North America as the one-daily-dose carbapenem for the treatment of community acquired infections. Even though it is efficient against Enterobacteriaceae, it shows little or no activity against P. aeruginosa and Acinetobacter spp. The large ertapenem molecule has higher affinity for the P. aeruginosa efflux systems and cannot pass easily through the porins of non-fermenters.

Doripenem like the other carbapenems, has a broad spectrum of activity and has been proven to perform better against P. aeruginosa than imipenem and the same as meropenem. Studies showed that the in vitro effect of doripenem against P. aeruginosa is more efficient than that of the other carbapenems; however, carbapenem-resistant P. aeruginosa strains are non-susceptible to all antibiotics of this category.

Tebipenem, the newest carbapenem is under development in Japan. Tebipenem is the active form of tebipenem pivoxil and is formed by the addition of a new side chain in position 2C of the biapenem molecule. Tebipenem is the carbapenem with the highest biocompatibility and can be administered per os. Despite its advantages, tebipenem like ertapenem, is not active against P. aeruginosa.

From “just beta-lactam resistance” to carbapenem resistance

P. aeruginosa exhibits intrinsic resistance to several beta-lactam antibiotics and may acquire additional resistance mechanisms due to mutational events or the acquisition of transferable genetic elements. Intrinsically, P. aeruginosa expresses chromosomally-encoded inducible AmpC beta-lactamase and several important efflux pump systems that export antibiotics, biocides, dyes, detergents, metabolic inhibitors, organic solvents and molecules involved in bacterial cell to cell communication.

Carbapenem resistance mechanisms have immerged under the pressure of carbapenem use in clinical settings and may be classified as enzymatic, mediated by carbapenemases (beta-lactamases hydrolyzing carbapenems among other beta-lactams), and non enzymatic. Carbapenem resistance however, develops frequently due to the concomitant presence of more than one mechanisms.

Carbapenemases in P. aeruginosa isolates

Beta-lactamases are classified according to a structural and a functional classification. Ambler’s structural classification comprises four molecular classes: A) The Extended Spectrum Beta-Lactamases (ESBL) that are inhibited by clavulanic acid, B) the Metallo-Beta-Lactamases (MBL), C) the Cefalosporinases (AmpC) and D) the Oxacillinases (OXA). Classes A, C and D share a common characteristic: Their enzymes bear serine in their active center whereas the MBLs bear Zn\(^{2+}\). Serine enzymes cleave the amide bond of the beta-lactam ring thus inactivating the antibiotic, while MBLs catalyze the same chemical reaction, using one or two divalent Zn\(^{2+}\) cations. More precisely, the MBL active site orients and polarizes the beta-lactam bond to facilitate nucleophilic attack by zinc-bound water hydroxide.

All types of transferable carbapenemases, except SIM-1 (Seoul imipenemase), have been detected in P. aeruginosa isolates around the world. Among them, the MBLs are considered as the most clinically important for P. aeruginosa. Most genes encoding MBLs are found as gene cassettes in integrons and are transferable. Furthermore, more resistance genes for other antibiotic classes can be present in the same integrons contributing thus in the development of a multi-drug resistant phenotype.

IMP (active on imipenem) and VIM (Verona in-
treon-encoded metallo-β-lactamase) type MBLs are spread through all continents after their first identification in Japan 23 and Italy 24 respectively while other metallo-enzymes have been detected sporadically. SPM-1 (Sao Paolo metallo-β-lactamase) has caused serious outbreaks in Brazil 20 and has also been recently found in Basel 20 in an isolate recovered by a patient previously hospitalized in Brazil. GIM-1 (German imipenemase) and AIM-1 (Australian imipenemase) have been reported from Germany 21 and Australia 22 and did not spread elsewhere whereas the first and to date only report for NDM-1 (New Delhi Metallo-β-lactamase) in P. aeruginosa was made from Serbia 23.

Beta-lactamases of P. aeruginosa with carbapenemase activity are classified apart from class B- in the remaining three enzymatic classes as well. Ambler class A carbapenemase KPC (Klebsiella pneumoniae carbapenemase) was first reported in P. aeruginosa isolates in Colombia 24 and unlike to KPC-producing Klebsiella pneumoniae, KPC-producing P. aeruginosa did not reach other continents except Latin America. Due to their high rates of carbapenem hydrolysis, KPCs make powerful resistance determinants that do not need additional mechanisms such as efflux or impermeability. Enzymes GES/IBC (Guiana extended spectrum) / (Integron-borne cephalosporinan) of the same enzymatic class possess carbapenemase activity which may become clinically important when combined with diminished outer membrane permeability or efflux over-expression. For P. aeruginosa, GES-2 has been reported in South Africa 25 and IBC-2 in Greece 26.

Class C beta-lactamas are not carbapenemases. They possess however a low potential of carbapenem hydrolysis and their overproduction combined with efflux systems over-expression and/or diminished outer membrane permeability has been proven to lead to carbapenem resistance 27.

Finally, class D carbapenemases like OXA-198 28 are rare for P. aeruginosa and do not have the same clinical impact as for Acinetobacter baumannii 29.

Detection of carbapenemases may be performed by polymerase chain reaction (PCR) using specific primers for each carbapenemase-encoding gene. Phenotypic assays however are also used for class A and B enzymes. Ethylene-diamine-tetraacetic acid (EDTA) is a polyamine carboxylic acid that binds metal ions like Zn2+ and can inactivate the metallo-beta-lactamases. EDTA is used for the detection of class B enzymes by a carbapenem-EDTA disc synergy test or the comparison of the inhibition halo of a carbapenem and a carbapenem+EDTA disc. For the detection of class A carbapenemases a double disc synergy test may be performed using clavulanic acid with carbapenems, aztreonam and third generation cephalosporins. Especially for P. aeruginosa cloxacillin (200μg cloxacillin/1ml) is dissolved into Mueller-Hinton agar to eliminate any possible AmpC beta-lactamase interference 20.

**Efflux pumps over-expression**

Efflux pump systems are an extremely important cause of multi-drug resistance for P. aeruginosa. They are tripartite systems that are composed by 1) a protein transporter of the cytoplasmic membrane that uses energy in the form of proton motif force to transport drugs and other substances through the inner membrane, 2) a periplasmic connective protein and 3) an outer membrane protein component with a barrel configuration. This way, the most commonly observed pump system, MexAB-OprM (Multidrug efflux system AB- Outer membrane protein M), consists of the MexB pump, the MexA linker lipoprotein and the OprM exit portal 31.

MexAB-OprM together with MexXY-OprM act synergistically with low outer membrane permeability conferring intrinsic multi-drug resistance to P. aeruginosa. In addition to this intrinsic resistance, these and some other additional systems (MexCD-OprJ, MexEF-OprN, MexJK-OprM, MexVW-OprM) promote acquired multidrug resistance as a consequence of hyper expression of the efflux genes by mutational events 32. Substrates for these efflux pumps are commonly quinolones, aminoglycosides and meropenem while imipenem and cefazidime are not usually affected. However, when efflux pump over-expression coexists with low outer membrane permeability resistance to other carbapenems develops as well 33.

The CCCP-test is used for the phenotypic detection of efflux pumps over-expression 24. Carbonyl cyanide m-chlorophenyl hydrzone (CCCP) is a pump inhibitor that can be added in Mueller-Hinton agar during its preparation. The strain is inoculated in an agar plate with CCCP as well as in a plate without CCCP, and a meropenem disc is placed for both inoculations. The result is considered positive if the inhibition zone of meropenem is wider in the agar plate with CCCP than the one in the plate without pump inhibitor.

In PCR, primers for mexB and mexY can be used to detect MexAB-OprM and MexXY-OprM systems respectively although the efflux pumps over-expression is certified performing reverse transcriptase real time PCR that shows the presence and also the quantitative expression of mRNAs from these genes 35.

**Diminished outer membrane permeability**

Carbapenems enter into the periplasmic space of P. aeruginosa through the OprD outer membrane porin formerly known as D2 porin. The porin loss probably by a mutational event of the OprD gene leads to imipenem resistance 36. Furthermore, in strains with OprD downregulation 37, reduced susceptibility to meropenem is observed while other beta-lactams are not affected 38. Diminished expression or loss of the OprD porin is rather frequent during imipenem treatment 39.

A carbapenem resistant, beta-lactam susceptible phenotype is characteristic for diminished expression of OprD even though such phenotypes will not be observed among multi-resistant isolates. Traditionally, the
loss of OprD is determined by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electroforesis (SDS-PAGE) for the separation of the membrane’s proteins9 while later studies use reverse transcriptase real time PCR with specific primers for the OprD gene48.

Concluding remarks
Nosoconial infections due to multi-drug resistant *P. aeruginosa* are a significant cause of morbidity and mortality in hospital settings. *P. aeruginosa* strains harboring carbapenem resistance mechanisms compromise severely the selection of appropriate treatments because of the fact that carbapenem resistance is commonly associated with resistance to other antibiotic classes. Consequently, the detection of carbapenem-resistant strains and the implementation of strict infection control measures become critical for limiting the spread of the underlying resistance mechanisms.

Conflict of interest
The authors declare no conflicts of interest.

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