# Carbapenem resistance: overview of the problem and future perspectives

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Ther Adv Infect Dis
(2016) 3(1) 15–21
DOI: 10.1177/2049936115621709



***Abstract***: Carbapenem resistance, mainly among Gram-negative pathogens, is an ongoing public-health problem of global dimensions. This type of antimicrobial resistance, especially when mediated by transferable carbapenemase-encoding genes, is spreading rapidly causing serious outbreaks and dramatically limiting treatment options. In this article, important key points related to carbapenem resistance are reviewed and future perspectives are discussed.

***Keywords***: antibiotic resistance, carbapenemases, carbapenems

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## Introduction

Beta-lactams are by far the most used antibiotics worldwide and include the penicillins, cephalosporins, monobactams and carbapenems. They all share a common beta-lactam ring and act similarly by binding to and inactivating the penicillin-binding proteins (PBPs), which are responsible for the formation of the bacterial cell wall.

Carbapenems, among the beta-lactams, are the most effective against Gram-positive and Gram-negative bacteria presenting a broad spectrum of antibacterial activity. Their unique molecular structure is due to the presence of a carbapenem together with the beta-lactam ring. This combination confers exceptional stability against most beta-lactamases (enzymes that inactivate beta-lactams) including ampicillin and carbenicillin (AmpC) and the extended spectrum beta-lactamases (ESBLs).

As they are highly effective against many bacterial species and less vulnerable to most beta-lactam resistance determinants, carbapenems are considered to be the most reliable last-resort treatment for bacterial infections. Furthermore, presenting fewer adverse effects, they are safer to use than other last-line drugs such as the polymyxins. For these reasons, the emergence and rapid spread through all continents of carbapenem resistance, mainly among Gram-negative bacteria, constitutes a global public-healthcare problem of major importance.

## Carbapenem-resistance determinants

It is noteworthy that resistance to carbapenems in some species is intrinsic. This is the case for example of *Stenotrophomonas maltophilia* that possesses the endogenous metallo-beta-lactamase (MBL) L1 [Sánchez, 2015], therefore the use of carbapenem antibiotics as a treatment for such infections is not considered. Intrinsic resistance to carbapenems, however, is not common among clinically important bacteria and for most of them carbapenem resistance is acquired by mutational events or gene acquisition *via* horizontal gene transfer.

Gram-positive bacteria become resistant to carbapenems and other beta-lactams through mutation-derived changes of their PBPs, while Gram-negatives commonly recruit other mechanisms to overcome the effect of carbapenem antibiotics. Certain species are able to prevent carbapenems reaching their PBPs by diminishing the permeability of their outer membrane. OprD for example, is an outer membrane porin of *Pseudomonas aeruginosa* through which carbapenems enter its periplasmic space where PBPs are located [Bonomo and Szabo, 2006]. Consequently, the diminished expression or loss of this porin leads to carbapenem resistance without concurrent resistance to other beta-lactams.

A different mechanism that may contribute to carbapenem resistance is the active expulsion of carbapenems out of the periplasmic space after their entrance. This is mediated by tripartite efflux pump systems composed of a protein

transporter of the cytoplasmic membrane, a periplasmic connective protein and an outer membrane porin [Schweizer, 2003]. Efflux pumps use energy in the form of proton motive force to transport various drugs and other substances out of the bacterial cell. The overexpression of efflux pumps that expel carbapenems, mostly meropenem, may lead to carbapenem resistance associated with multidrug resistance (MDR), since quinolones, penicillins, cephalosporins and aminoglycosides are common efflux pump substrates [Meletis *et al.* 2012].

Enzyme-mediated resistance to carbapenems is due to the production of beta-lactamases that are able to inactivate carbapenems together with other beta-lactam antibiotics and therefore called carbapenemases [Walsh, 2010; Poirel *et al.* 2007]. This type of resistance is the most important clinically because these enzymes hydrolyze all or almost all beta-lactams, confer high levels of carbapenem minimum inhibitory concentrations (MICs), are encoded by genes that are horizontally transferable by plasmids or transposons and are commonly associated with genes encoding for other resistance determinants.

All beta-lactamases are categorized into four molecular classes according to the Ambler classification [Ambler, 1980]. Among them, the class A enzymes KPC [Rapp and Urban, 2012], GES/IBC [Poirel *et al.* 2000; Giakkoupi *et al.* 2000], IMI/NMC-A [Walther-Rasmussen and Høiby, 2007], SFC-1 [Henriques *et al.* 2004], the class B MBLs IMP [Zhao and Hu, 2011b], VIM [Zhao and Hu, 2011a], NDM [Nordmann *et al.* 2011], SPM [Rossi, 2011], GIM [Castanheira *et al.* 2004], SIM [Lee *et al.* 2005], AIM [Yong *et al.* 2012], DIM [Poirel *et al.* 2010], FIM [Pollini *et al.* 2013], POM [Borgianni *et al.* 2015], and several class D (OXA-type) enzymes [Walther-Rasmussen and Høiby, 2006], possess the ability to hydrolyze at least partially a carbapenem antibiotic. Class C enzymes are not considered carbapenemases. It has been shown however that they possess a low potential of carbapenem hydrolysis and their overproduction may contribute to carbapenem resistance combined with diminished outer-membrane permeability and/or efflux pump overexpression [Quale *et al.* 2006].

The most effective carbapenemases, in terms of carbapenem hydrolysis and geographical spread, are KPC, VIM, IMP, NDM and OXA-48 types [Poirel *et al.* 2012]. KPCs inactivate all beta-lactam antibiotics and are only partially inhibited by beta-lactamase inhibitors like clavulanic acid, tazobactam and boronic acid. MBLs are able to hydrolyze all beta-lactams except aztreonam and are not inhibited by the aforementioned inhibitors. They bear zinc in their active centre, therefore their inhibition is achieved *in vitro* using metal chelators, such as ethylenediaminetetraacetic acid.

## Treatment options against carbapenem-resistant bacteria

While for Gram-positives there are still reliable alternatives to carbapenems (e.g. glycopeptides, daptomycin), carbapenem resistance in Gram-negative pathogens is dramatically limiting treatment options. Carbapenemase-producing Gram-negatives in particular are resistant to all or almost all beta-lactams, while commonly bearing at the same time genes encoding for resistance mechanisms against fluoroquinolones and/or aminoglycosides. Therefore, older agents, such as polymyxins and fosfomycin, which were rarely implemented in the past because of efficacy and/or toxicity concerns, together with the newer tigecycline, have become last-resort choices.

Based on the laboratory's antimicrobial susceptibility report, clinicians have to choose between the following schemes: (a) monotherapy using one of the possibly still active *in vitro* agents (these may be colistin, gentamycin, tigecycline and fosfomycin); (b) combination therapy without a carbapenem; (c) combination therapy with two or more drugs including at least one carbapenem, preferably when the carbapenem's MIC is $\leq 4$ mg/L [Miyakis *et al.* 2011] (this last option is considered as the most effective in terms of mortality according to the currently available studies [Tzouvelekis *et al.* 2014]).

Obviously, the involvement of previously almost abandoned antibiotics is not ideal. Even though the neurotoxicity of colistin has been questioned lately [Morrill *et al.* 2015], nephrotoxicity is a certain serious complication as it occurs in more than 40% of patients treated with polymyxins [Akajagbor *et al.* 2013].

Fosfomycin reaches high urinary concentrations for prolonged time periods and may be used against urinary tract infections [Kitchel *et al.* 2009; Peirano *et al.* 2011]. Its intravenous administration as monotherapy however in cases

of systemic infections may be problematic because of the potential for resistance development during treatment [Falagas *et al.* 2010].

Likewise, and despite the fact that many carbapenemase producers are susceptible *in vitro* to tigecycline, resistance to this drug is increasing rapidly [Sader *et al.* 2014]. Moreover, there are not enough data to support its effectiveness for infections caused by carbapenem-resistant bacteria when used as monotherapy [Morrill *et al.* 2015].

## Global spread of carbapenemases

Even though the existence of carbapenemases has been known for more than 20 years, their origin or probable physiological role are not yet known. IMP-1 was the first carbapenemase and the first MBL to be detected in 1991 in Japan [Watanabe *et al.* 1991], whereas VIM-1 was discovered later in 1997 in Verona, Italy [Lauretti *et al.* 1999]. Both IMP- and VIM-type enzymes have successfully spread to all continents since then [Walsh *et al.* 2005]. SPM was found in 2002 in Sao Paulo, Brazil causing serious outbreaks [Queenam and Bush, 2007], but remains mostly confined in South America. GIM, SIM, AIM, DIM, FIM and POM were reported only sporadically to date in contrast with NDM, which presented a spectacular global spread since its detection in 2008 in India [Yong *et al.* 2009]. NDM-producing microorganisms have been isolated with high frequency in healthcare facilities and environmental niches in the Indian subcontinent and disseminated through patient transfer or colonized travellers in Europe, North America, the Far East and Australia [Dortet *et al.* 2014]. Worryingly, a second probable epicentre seems to be located in the central Balkans without any obvious connection to that of India [Meletis *et al.* 2014a].

Among the class A carbapenem-hydrolyzing enzymes, KPCs presented the most rapid and geographically extended dissemination [Nordmann *et al.* 2009]. First found in the late 1990s in the New York City area in the USA [Yigit *et al.* 2001], they soon spread to bordering states [Endimiani *et al.* 2009], Latin America [Villegas *et al.* 2006], and Israel [Leavitt *et al.* 2007]. From there, they most probably entered neighbouring countries and through Greece to the rest of Europe, where its prevalence is high in southern countries but fortunately still remains low in northern ones. Another significant epicentre of KPC-encoding genes may be China, even though not enough data are known to assess its actual dissemination in that country [Tzouvelekis *et al.* 2012].

The main foci for OXA-48 producing bacteria are considered to be the Middle East [Carrër *et al.* 2010] and North Africa [Cuzon *et al.* 2010], however, isolations in Europe [Potron *et al.* 2011], Turkey [Carrër *et al.* 2008], India [Castanheira *et al.* 2011], Senegal [Moquet *et al.* 2011], and Argentina [Poirel *et al.* 2011], indicate an important global spread for this carbapenemase type as well.

## Reasons for the emergence and spread of carbapenem resistance

Antibiotic resistance is a phenomenon that follows antibiotic discovery and its main cause relies on the spectacular adaptability of bacteria to selective pressure. Indeed, bacteria are able to mutate promptly, to acquire genes horizontally and to 'collect' the necessary mechanisms for their survival if subjected to harmful factors.

Practically, resistance has emerged to all antibiotics used until now after a variable time period from their implementation and carbapenems are not the exception. It is reasonable however to consider that starting from the emergence of carbapenem resistance until its widespread dissemination, some human-related factors are playing a crucial role. These are mainly: (a) immoderate antibiotic prescription combined with the uncontrolled public access to antibiotics in many countries with poor sales regulations; (b) the lack of infection control measures in healthcare facilities once carbapenem resistance has emerged; (c) the use of subtherapeutic doses of antibiotics for the promotion of animal growth in the agricultural sector.

## Future perspectives

Carbapenem resistance in Gram-negative bacteria, especially when carbapenemases are involved, is the main contributing factor for MDR and usually the definitive step before pandrug resistance (PDR). Indeed, resistance to other last-resort drugs among carbapenemase producers may rapidly emerge when these agents are necessarily used in healthcare settings [Meletis *et al.* 2015b].

Moreover, it has been shown that carbapenem-resistant Gram-negative nosocomial pathogens will continue to evolve accumulating more carbapenem-resistance mechanisms [Meletis *et al.* 2014b], or more than one carbapenemase-encoding gene [Meletis *et al.* 2015a]. This will lead in many cases

to increased carbapenem MICs ruling out the best-to-date therapeutic choice against carbapenemase producers, which is the combined treatment including at least one carbapenem.

At this pace, untreatable infections could emerge on a large scale and the world may experience in some cases dramatic situations of the pre-antibiotic era. Already, clinicians in endemic areas routinely encounter patients with infections that do not respond to available treatments and laboratories often report MDR or even PDR bacteria. In the USA alone, 2 million people acquire serious infections due to antibiotic-resistant bacteria each year and according to the Centers for Disease Control and Prevention (CDC), 23,000 of them die as a result [CDC, 2013].

It is obvious that novel antibiotics are urgently needed. However, this is not easy. Pharmaceutical companies have to invest a great deal of money and time to scientific research and, even when they do, only one out of five drugs that reach the initial phase of testing in humans will receive approval from the US Food and Drug Administration [Hay *et al.* 2014]. Moreover, the development of antibiotics against MDR bacteria is not as profitable as developing drugs for other medical areas such as cancer or diabetes because only a relatively small number of patients contract such infections and these of course are not treated for long time periods [Meletis, 2014].

Despite the difficulties though, a new compound, ceftazidime-avibactam has been made available recently [Zasowski *et al.* 2015]. This drug is a combination of ceftazidime with a novel beta-lactamase inhibitor able to inhibit ESBLs, AmpC and class A carbapenemases including KPCs, but not MBLs. Although ceftazidime-avibactam is an important and promising addition to the antimicrobial armamentarium, resistance has already been observed in KPC-3-producing *Klebsiella pneumoniae* and *Enterobacter cloacae in vitro* [Livermore *et al.* 2015], and reported in a KPC-3-expressing *K. pneumoniae* clinical isolate [Humphries *et al.* 2015].

## Conclusion

Despite some serious efforts, mainly from industrialized countries, a definite solution to the problem still seems to be far off. Several parts of the world are already endemic for carbapenemase-encoding genes while the situation in others including the central Balkans and many African and Asian countries is not well documented.

Until a reliable alternative to carbapenems is found or the presence of carbapenemases effectively overcome, the rationalization of antibiotic use in both humans and animals, the application of strict infection control measures whenever carbapenem resistance is detected and the active surveillance for the presence of carbapenemase-encoding genes are of the outmost importance.

## Funding

The author received no financial support for the research, authorship, and/or publication of this article.

## Conflict of interest statement

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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