Brief Insight about Carbapenem Resistance in Gram-Negative Bacteria

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Article History: Received 10th June, Accepted 5th July, published online 10th July 2023

Abstract

Background: The global effort to develop new antibiotics or modify existing ones to fight resistant pathogens globally is now huge. Antibiotic resistance evolves when the bacteria can escape the effect of antibiotics by different mechanisms, like neutralizing the antibiotics, pumping them outside of the cell, or modifying their outer structure resulting in inhibition of the drugs. The mechanisms of antibiotic resistance are categorized into four groups: intrinsic resistance in which bacteria can change their structures or components, another way is acquired resistance, where bacteria can acquire new resistance genes and DNA from other resistant bacteria. Furthermore, genetic changes in the DNA which can alter the production of protein leading to different components and receptors that cannot be recognized by the antibiotic, and finally DNA transfer through a horizontal gene transfer between bacteria via transformation, transduction or conjugation. Evidence suggests that patients who are infected by carbapenem-resistant pathogens have an increased likelihood of morbidity and mortality compared with those infected by susceptible pathogens, which is likely due to administration of antibiotics with suboptimal or no activity against these organisms. Thus, recognizing the risk of carbapenem resistance, particularly in the most vulnerable patient populations, and/or early detection of specific carbapenem resistance mechanisms are critical to reduce the risk of mortality, length of hospitalization, and associated costs. The alarming level of carbapenem resistance has presented particular challenges for the management of a variety of infections caused by nonfermenters because of the low permeability of the outer bacterial membrane to several antibiotics, including, but not limited to, the carbapenems

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Keywords: Carbapenem Resistance, Gram-Negative Bacteria

Introduction

Since the discovery of penicillin by Fleming in 1929, a large number of antibacterial agents have been developed and have had a huge impact on human health and the mortality rates of humans around the world. Widespread excessive dispensing and irresponsible use of antibiotics has resulted in the development of resistant strains. Unfortunately, most antibiotics are available over the counter in the developing

countries and can be dispensed without prescription; therefore, patients and general public education are crucially needed **(1)**.

The global effort to develop new antibiotics or modify existing ones to fight resistant pathogens globally is now huge. Antibiotic resistance evolves when the bacteria can escape the effect of antibiotics by different mechanisms, like neutralizing the antibiotics, pumping them outside of the cell, or modifying their outer structure resulting in inhibition of the drugs. The mechanisms of antibiotic resistance are categorized into four groups: intrinsic resistance in which bacteria can change their structures or components, another way is acquired resistance, where bacteria can acquire new resistance genes and DNA from other resistant bacteria. Furthermore, genetic changes in the DNA which can alter the production of protein leading to different components and receptors that cannot be recognized by the antibiotic, and finally DNA transfer through a horizontal gene transfer between bacteria via transformation, transduction or conjugation **(2)**.

Gram-negative bacteria can cause serious diseases in humans, especially in immuno-compromised individuals. Nosocomial infections caused by Gram-negative bacilli (GNB) are the most challenging issue for health care professionals due to resistance to antibiotics. Resistant GNB is responsible for most of the cases of ventilator-associated pneumonia, catheter-related bloodstream infections and other ICU-acquired sepsis such as urinary tract infections. The major Gram-negative bacteria that cause complications are *Enterobacteriaceae* and non-fermenting GNB (*Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*) **(2)**.

The mechanism of antimicrobial resistance in GNB arises from the expression of antibiotic inactivating enzymes and non-enzymatic paths which may result from increasing the intrinsic resistance due to mutations in chromosomal genes (such as increasing the expression of antibiotic-inactivating enzymes, efflux pumps or target modifications) or acquired by transfer of mobile genetic elements carrying resistance genes such as plasmid encoding β-lactamases, aminoglycosides modifying enzymes, or non-enzymatic mechanisms like Qnr (plasmid-borne quinolone resistance gene) for fluoroquinolone (FQ) resistance in *Enterobacteriaceae* **(3)**.

Enterobacteriaceae resistance to third generation cephalosporins is now above 10%, and 2-7% for carbapenem. This is because of the rapid spread of extended-spectrum β-lactamase (ESBL) producing strains. Carbapenem resistance rates for *klebsiella pneumonia* are above 25% while 20 to 40% is for *P. aeruginosa* and 40 to 70% ICU acquired infections being carbapenem-resistant for *A. baumannii* **(3)**.

Thienamycin, the first reported carbapenem, was isolated from *Streptomyces cattleya* in 1976. Despite showing significant potency, clinical use was limited by its instability in water. Since then, medicinal chemistry optimization has yielded a number of carbapenems, namely imipenem, meropenem, doripenem, ertapenem, biapenem and tebipenem, which have been approved for clinical use. However, biapenem and tebipenem are approved for use only in Japan. Tebipenem is currently under development in the USA and has completed Phase III clinical trials **(4)**.

Carbapenem resistance in gram-negative bacteria

Carbapenem resistance in gram-negative bacteria has become a worldwide problem. The 2017 World Health Organization (WHO) global priority list of pathogens ranks carbapenem-resistant Enterobacteriaceae (CRE), carbapenem-resistant *Pseudomonas aeruginosa*, and carbapenem-resistant *Acinetobacter baumannii* in the highest priority category (ie, critical). To address this global epidemic, identification and ongoing surveillance of carbapenem-resistant gram-negative bacteria are needed **(5)**.

Evidence suggests that patients who are infected by carbapenem-resistant pathogens have an increased likelihood of morbidity and mortality compared with those infected by susceptible pathogens, which is likely due to administration of antibiotics with suboptimal or no activity against these organisms.

Thus, recognizing the risk of carbapenem resistance, particularly in the most vulnerable patient populations, and/or early detection of specific carbapenem resistance mechanisms are critical to reduce the risk of mortality, length of hospitalization, and associated costs. The alarming level of carbapenem resistance has presented particular challenges for the management of a variety of infections caused by nonfermenters because of the low permeability of the outer bacterial membrane to several antibiotics, including, but not limited to, the carbapenems **(6)**.

The concerns surrounding CRE-related infections have recently been mitigated to some degree by the approval of new β-lactam–β-lactamase inhibitor combination therapies, which demonstrate activity against strains with specific underlying resistance mechanisms; however, on-therapy resistance has already been reported. The use of older agents, such as tigecycline or colistin, is frequently associated with unclear efficacy and/or toxicity issues. It is clear that understanding specific mechanisms underlying carbapenem resistance and monitoring local epidemiology would lead to more effective treatment of infections caused by carbapenem-resistant gram-negative bacteria **(7)**.

- **Mechanisms of carbapenem resistance**

1. Enzymatic Hydrolysis

One key mechanism of carbapenem resistance is hydrolysis of carbapenems by carbapenemase enzymes, which are encoded mainly on plasmids and are highly transmissible. The Ambler classification system categorizes β-lactamase enzymes into 4 groups (ie, A, B, C, D) based on their central catalytic domain and substrate preference. Of these, classes A, B, and D include carbapenemases, whereas class C enzymes hydrolyze primarily cephalosporins. Enzymes in classes A, C, and D have serine in the active catalytic site, whereas class B enzymes are metallo-β-lactamases (MBLs) with zinc in the active site **(8)**.

Among the newer agents, avibactam inhibits class A (eg, *Klebsiella pneumoniae* carbapenemase [KPC]), class C (eg, ampicillin chromosomal cephalosporinase [AmpC]), and only some class D (eg, oxacillin carbapenemase/oxacillinase [OXA]–48) serine-β-lactamases, but does not significantly inhibit the activity of class B MBLs (eg, imipenemase metallo-β-lactamase [IMP], Verona integron-encoded metallo-β-lactamase [VIM], New Delhi metallo-β-lactamase [NDM]). Similarly, vaborbactam inhibits class A and C enzymes but not those belonging to class B and D **(9)**.

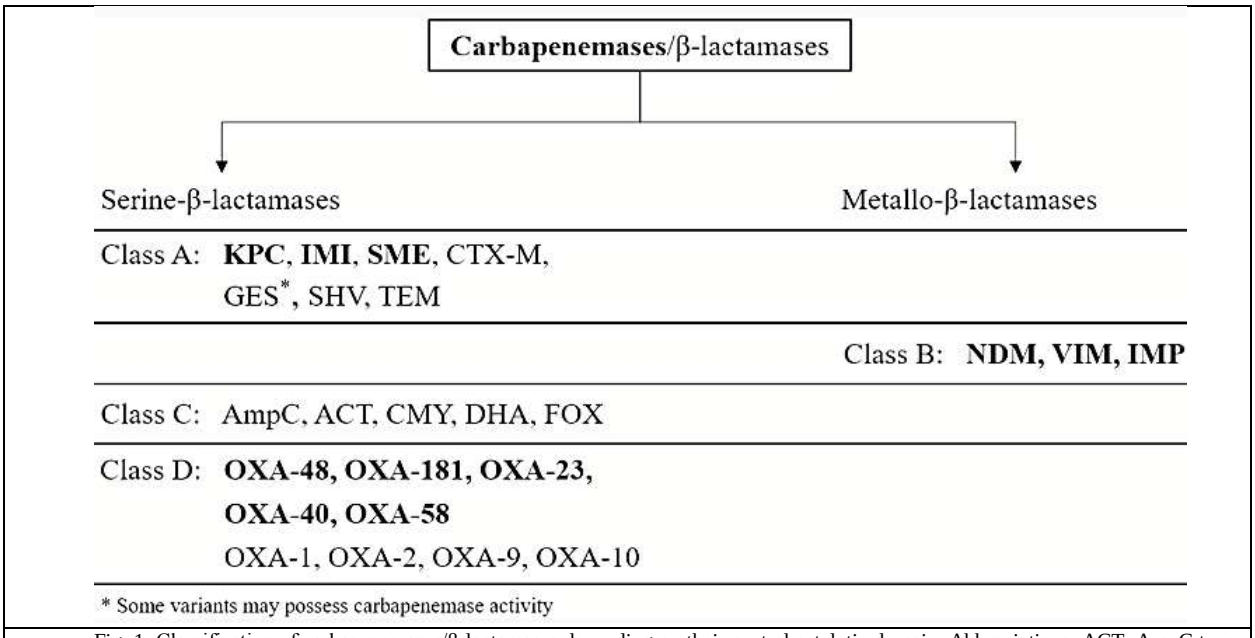

Fig. 1. Classification of carbapenemases/β-lactamases depending on their central catalytic domain. Abbreviations: ACT, AmpC type β-lactamase; AmpC, ampicillin chromosomal cephalosporinase; CMY, cephamycin-hydrolyzing β-lactamase; CTX-M, cefotaxime-hydrolyzing β-lactamase–Munich; FOX, plasmid-mediated class C β-lactamase; GES, Guiana extended-spectrum β-lactamase; IMI,

imipenem-hydrolyzing β-lactamase; IMP, imipenemase metallo-β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA, oxacillin carbapenemase/oxacillinase; SHV, sulfhydryl variant of the TEM enzyme; SME, *Serratia marcescens* enzyme; TEM, Temoneira class A extended-spectrum β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase. **(5)**.

Although most class A enzymes do not exhibit intrinsic carbapenemase activity, this group of enzymes includes the prevalent KPC. All (class B) MBLs possess carbapenemase activity, and this group includes the acquired VIM, IMP, and NDM enzymes that may be found in many gram-negative species **(8)**. Class C includes AmpC β-lactamase enzymes that are not carbapenemases per se, as their hydrolytic activity against carbapenems is very weak or nonexistent, but that can play a role in resistance to carbapenems in the context of permeability defects **(10)**.

This is true, in particular, for many enterobacterial species that naturally produce a class C cephalosporinase (such as *Enterobacter* species, *Serratia marcescens*, *Proteus* species, *Providencia* species, *Morganella morganii*, and *Hafnia alvei*) and *P. aeruginosa*. Class D (also termed oxacillin carbapenemase [OXA enzymes]) enzymes constitute a heterogeneous group of β-lactamases with significant carbapenemase activity, especially OXA-48–type enzymes in Enterobacteriaceae and OXA-23, frequently found in *A. baumannii*. *Stenotrophomonas maltophilia* has intrinsic carbapenem resistance due to the presence of a chromosomally encoded MBL, namely L1 **(11)**.

2. Other Carbapenem Resistance Mechanisms

Nonenzymatic carbapenem resistance mechanisms include:

- loss of expression of porin-encoding genes, mutations in chromosomally encoded porin genes (such as OprD).
- overexpression of genes encoding efflux pumps (such as MexAB-OprM, MexXY-OprM, or MexCD-OprJ), particularly in *P. aeruginosa*. Porins are nonspecific channels in the outer membrane of gram-negative bacteria that permit the passive transport of hydrophilic small molecules and nutrients (and also some antibiotics) across the otherwise impermeable membrane. Porin loss and efflux pump overexpression associated with carbapenem resistance may also contribute to cross-resistance to other β-lactams and other antibiotic classes **(11)**.
- Target modifications: mutations or other modifications that alter the production level or the binding affinity of penicillin-binding proteins, mechanisms that have been observed rarely in *Escherichia coli*, *P. aeruginosa*, and *A. baumannii* **(12)**.

- **Diagnostics**

Both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) annually define the susceptibility breakpoints to commercially available carbapenems, including doripenem, ertapenem, imipenem, and meropenem for gram-negative species, although EUCAST no longer provides doripenem breakpoints. When a strain is found to be nonsusceptible to carbapenems (ie, intermediate or resistant), the mechanism of resistance is still unknown. Thus, to confirm the production of carbapenemases and/or presence of other mechanisms, further biochemical assays and/or gene-based tests must be performed **(13)**.

Determining the mechanism of carbapenem resistance can help in the selection of the most appropriate antibiotic therapy early in the treatment of gram-negative infections. For therapeutic decision making, the rapid turnaround time (defined as 1 day or as short as <2 hours) would be particularly beneficial in reducing length of hospitalization and/or time spent in the intensive care unit. Both

biochemical and molecular technologies are widely available, with endorsement from CLSI, EUCAST, and/or the US Food and Drug Administration **(13)**.

The biochemical assays include the Carba NP, its derivative Blue Carba, and β Carba tests, which are inexpensive and confirm phenotypically carbapenemase-producing organisms (but not other resistance mechanisms). These methods are based on the expression of any carbapenemase enzyme during bacterial growth in culture (ie, up to 24–48 hours), and use imipenem or meropenem as a substrate, which is then hydrolyzed by the carbapenemase. The colorimetric positive signal may be obtained in <1 hour (eg, Carba NP) and can be used directly from clinical samples (blood cultures, infected urine). Furthermore, specific inhibitors of carbapenemase activity can be included, such as avibactam, vaborbactam, or ethylenediaminetetraacetic acid **(14)**.

Further biochemical assays include the carbapenemase inactivation method, which is also inexpensive, and the matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF) technology, which may be cost-effective in large centers and hospitals. However, all of these methods described above, besides having some specificity or sensitivity issues, are also unable to identify the exact carbapenemase enzyme and require growth of bacteria **(15)**.

The specific assays used to detect the presence of known carbapenemase genes located on plasmids, or porin channel or efflux pump mutations, are normally gene based and amplify the potential genes present by the use of oligomer primers and probes. Commercially available polymerase chain reaction (PCR) tests include Check-Direct carbapenemase-producing Enterobacteriaceae (CPE) assays (Check-Points, Wageningen, the Netherlands), Xpert Carba-R (Cepheid, Sunnyvale, California), EazyPlex SuperBug ID complete A/B (Amplex, Giessen, Germany), and the very recent point-of-care GenePOC technology (GenePOC, Quebec City, Canada) **(16)**.

All 4 methods can detect KPC, NDM, and VIM encoding genes with 100% sensitivity, and OXA-48–type carbapenemases (including OXA-181) with 83%–100% sensitivity; however, only Xpert Carba-R detects IMP-1. Turnaround time is usually the same day. The commercial microarrays allow for the detection of a much higher number of target genes than PCR with 100% sensitivity and typically include bacterial identification targets as well as resistance markers (eg, KPC, NDM, OXA, VIM, IMP, Guiana extended-spectrum β-lactamase [GES], German imipenemase [GIM], and São Paulo metallo-β-lactamase [SPM] carbapenemases). Currently available systems include Verigene (Luminex, Austin, Texas), BioFire FilmArray (Salt Lake City, Utah), and the Check-Points systems. Whole genome sequencing allows detection of either carbapenemase genes or other resistance-associated mutations and may also play a role as the technology becomes less expensive and more widespread. However, such an approach requires a significant expertise and adequate equipment, which is not systematically available, and a precise knowledge of combined resistance mechanisms (eg, mutations, level of expression) **(17)**.

Some of these rapid gene-based assays, such as the Xpert Carba-R platform or BioFire FilmArray, have the potential for direct specimen sampling (eg, nasal swab, rectal swab, sputum, wound specimen, blood, urine) without the need for culturing, allowing appropriate treatment to be initiated as soon as the carbapenemase resistance mechanism has been identified and minimizing the risk of treatment failure associated with empiric antimicrobial therapy **(18)**.

Despite the technological advances in molecular and biochemical rapid diagnostics, there are 2 fundamental considerations: (1) a negative test does not imply that the organism is carbapenem susceptible, as it may still be resistant due to nonenzymatic mechanisms; (2) conversely, the presence of a gene does not systematically imply the organism is carbapenem resistant, owing to the level of expression of the resistance gene; and (3) a positive biochemical test will not identify the specific carbapenemase enzyme.

Consequently, only phenotypic tests relying on actual growth inhibition provide a full susceptibility picture **(5)**.

- **Global epidemiology of carbapenem-resistant pathogens**

Although data are limited for some regions, the overall burden of disease caused by carbapenem-resistant pathogens is similar in most regions (ie, Asia-Pacific, the Indian continent, Europe, North America, and Latin America), with nonfermenters being the most problematic pathogens followed by a relatively lower proportion of CREs. Data of both large surveillance studies and smaller hospital investigations demonstrate similarity in carbapenem resistance rates irrespective of the methodology used to detect the mechanism of resistance or the antibiotic used. The reported rates of carbapenem resistance seem to be considerably higher for nonfermenters (frequently >60%) than for fermenters (frequently <10%) across regions **(19)**.

- **Novel treatment strategies for carbapenemase-producing organisms (CPO):**

Treatment of CPO, especially carbapenemase-producing carbapenem resistant enterobacterales (CP-CRE) , remains difficult. Patients with CP-CRE infection suffer unacceptably high mortality, emphasizing the need for novel diagnostics and therapies. Studies performed to date demonstrate a bias to report trials of successful combination chemotherapy, informed largely by results from in vitro studies. In most trials targeting CP-CRE, combination therapies have included the use of **(*i*)** colistin (polymyxin E) and a carbapenem; **(*ii*)** colistin and tigecycline, or colistin and fosfomycin; or **(*iii*)** double carbapenem therapy. Interestingly, it was also shown in vitro that dual carbapenem combinations might work against carbapenemase-producing strains, with significant synergies observed when using imipenem and another carbapenem **(7)**.

What about new drugs in development ?

Avibactam is a synthetic non-β-lactam, bicyclic diazabicyclooctane (DBO) β-lactamase inhibitor that inhibits the activities of Ambler class A and class C β-lactamases and some Ambler class D enzymes. Avibactam closely resembles portions of the cephem bicyclic ring system and has been shown to bond covalently to β-lactamases. Against carbapenemase-producing *K. pneumoniae*, the addition of avibactam significantly improves the activity of ceftazidime in vitro (~4-fold MIC reduction). In surveillance studies, the combination of ceftazidime with avibactam restores in vitro susceptibility against all extended-spectrum β-lactamases and most KPCs tested. Studies comparing outcomes of infections with KPC-producing gram-negative bacteria treated with ceftazidime-avibactam as monotherapy or in combination with colistin are ongoing. **(15)**.

Patients treated with ceftazidime-avibactam vs colistin (monotherapy or combination) had a higher probability of a better outcome as compared to patients treated with colistin. **(15)**.

Relebactam, combined with imipenem/cilistatin, will soon be evaluated in clinical studies. In vitro studies indicate that imipenem/cilistatin-relebactam is comparable to ceftazidime-avibactam. The role of the combination of imipenem vs ceftazidime remains to be defined. The US Food and Drug Administration (FDA) recently approved ceftazidime-avibactam based on data obtained in Phase 2/3 trials of complicated urinary tract infections and intra-abdominal infections (ceftazidime-avibactam combined with metronidazole). Despite encouraging results, the FDA cautioned that ceftazidime-avibactam should be reserved for situations when there are limited or no alternative drugs for treating an infection. The concern

was that resistance to ceftazidime-avibactam would emerge in KPC-producing strains. Regrettably, resistance is already being reported due to mutations occurring in the KPC enzyme and porin changes **(20)**.

In summary, combination chemotherapies seem to be effective against KPC-producing bacteria, but we still need to design the right trial to answer the fundamental question as to why. We also need to carefully examine new drugs in the pipeline, and use clinical trials to define their best use. Other drugs in development are summarized in Table 1. It is noted that there are some drugs specifically targeted for MBL producers (aztreonam-avibactam and cefidericol); these developments are awaited in earnest. Novel combinations (ceftazidime-avibactam paired with aztreonam) are also being explored. In addition, the optimization of pharmacokinetic and pharmacodynamic parameters is essential for ensuring efficacy in difficult-to-treat infections **(21)**.

Table 1. Novel Agents in Development for Treating Carbapenem-Resistant Organisms, Including Carbapenemase-Producing Organisms and Those Resistant to Carbapenems by Other Mechanisms

Antibiotic	Drug Class	Intended Indication/Activity/Comments
Aztreonam-avibactam	Monocyclic-β-lactam and BLI	Gram-negative bacteria expressing ESBLs, serine-based carbapenemases, and MBLs
Cefiderocol	Siderophore-β-lactam (cephalosporin)	•cUTI, carbapenem-resistant gram-negative bacterial infections •Active against MBL-producing strains
Ceftaroline fosamil-avibactam	Cephalosporin and BLI	Currently undefined, likely CAP
Eravacycline	Tetracycline	•cIAI and cUTI •Multidrug-resistant gram-negative rods
Imipenem/cilistatin-relebactam	Carbapenem and BLI	•cUTI •cIAI •HAP •Active against ESBLs and KPCs
LYS228	Monobactam	MBL-producing Enterobacteriaceae including CRE
Meropenem-vaborbactam	Carbapenem and cyclic boronic acid BLI	•cUTI •CRBSI •HAP •VAP •cIAI due to CRE
Plazomycin	Aminoglycoside	•cUTI •CRBSI •HAP •VAP •cIAI due to CPOs and CRE

cUTI :complicated urinary tract infection
cIAI : complicated intra-abdominal infections
CRBSI : catheter related bloodstream infections
HAP: hospital acquired pneumonia
CAP : community acquired pneumonia
CPO: carbapenemase-producing organisms
VAP: ventillator associated pneumonia
CRE: carbapenem-resistent enterobacterales

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