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Carbapenemase-Producing Organisms: A Global Scourge!

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Abstract

The dramatic increase in the prevalence and clinical impact of infections caused by bacteria producing carbapenemases is a global health concern. These carbapenemase-producing organisms (CPO) are especially problematic when encountered in members of the family *Enterobacteriaceae*. Due to their ability to readily spread and colonize patients in health care environments, preventing

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the transmission of these organisms is a major public health initiative and coordinated international efforts are needed to contain the risk of infection. Central to the treatment and control of CPO are phenotypic- (growth-/biochemical-dependent) and nucleic acid-based carbapenemase detection tests that identify carbapenemase activity directly or their associated molecular determinants. Importantly, bacterial isolates harboring carbapenemases are often resistant to multiple antibiotic classes resulting in limited therapy options. Emerging agents, novel antibiotic combinations and treatment regimens offer promise for management of these infections. This review highlights our current understanding of CPO with emphasis on their epidemiology, detection, treatment, and control.

Keywords

Carbapenem-producing organisms; Carbapenem-resistant *Enterobacteriaceae*; Carbapenemase; Metallo-beta-lactamase; Carbapenemase detection tests; Whole-genome sequencing; Antimicrobial therapy

Introduction

One of the most concerning forms of antimicrobial resistance (AMR) is resistance to the carbapenems, especially when observed in members of the family *Enterobacteriaceae*. A primary mechanism of carbapenem resistance in Gram-negative bacteria is acquired carbapenemases, enzymes that hydrolyze these antibiotics. In this review, the epidemiology, laboratory detection, approaches to combat widespread dissemination, and treatment strategies for carbapenemase-producing organisms (CPO), especially carbapenemase-producing carbapenem-resistant *Enterobacteriaceae* (CP-CRE), will be discussed.

The Biology and Epidemiology of CPO

Phenotypic resistance to carbapenems in Gram-negative bacteria commonly results from acquisition of carbapenemases, or production of cephalosporinases combined with mutations that decrease permeability of the bacterial cell wall to entry of carbapenems. CPO may exhibit significant variation in carbapenem minimum inhibitory concentration (MIC) values depending on their permeability status, the rate of carbapenem hydrolysis by the associated enzyme, and the level of gene expression [1]. Carbapenemases belong to Ambler classes A, B, or D, with class A and D enzymes possessing a serine-based hydrolytic mechanism, and class B enzymes requiring one or two zinc ions for their catalytic activity [1]. There is a rare instance of class C beta-lactamase that can hydrolyze imipenem (CMY-10) [2]. Globally distributed in many genera of bacteria, certain carbapenemases are typically associated with specific regions or countries (Figure 1). However, in an era of widespread international travel and exposure to medical care, the association between a specific resistance mechanism and a given region or country may change, creating an urgent need for routine local and national surveillance.

The class A *Klebsiella pneumoniae* carbapenemase (KPC) has been extensively reported in *K. pneumoniae* and other *Enterobacteriaceae*, but has also been identified in other Gram-negative pathogens including *Pseudomonas aeruginosa* [3]. KPC-producing *K. pneumoniae*

are widespread in the United States, but are also endemic in some European countries such as Greece and Italy (Figure 1) [4].

Class B beta-lactamases, or metallo-beta-lactamases (MBL), are commonly identified in *Enterobacteriaceae* and in *P. aeruginosa* [5]. Among the MBLs, New Delhi Metallo-beta-lactamase (NDM)-, Verona Integron-encoded Metallo-beta-lactamase (VIM)-, and Imipenemase Metallo-beta-lactamase (IMP) enzymes, are the most frequently identified worldwide (Figure 1) [5]. IMP-producers are mainly detected in China, Japan, and Australia, mostly in *Acinetobacter baumannii*. VIM-producers are most often found in Italy and Greece (*Enterobacteriaceae*), and in Russia (*P. aeruginosa*) [6,7].

Acquired class D carbapenem-hydrolyzing beta-lactamases are commonly reported in *A. baumannii* (mainly OXA-23-, OXA-40-, and OXA-58-like enzymes), but not in *P. aeruginosa*. OXA-48 and derivatives (*e.g.*, OXA-181 and OXA-232), have been detected in *Enterobacteriaceae*, hydrolyze narrow-spectrum beta-lactams and weakly hydrolyze carbapenems, but spare broad-spectrum cephalosporins [8]. OXA-48-producing *Enterobacteriaceae* have been endemic in Turkey since 2004, and are now also frequently discovered in several European countries (*e.g.*, France and Belgium), and across North Africa (Figure 1) [9]. Ten variants of OXA-48 beta-lactamases are acknowledged and are increasingly reported worldwide [9], notably among nosocomial *K. pneumoniae* and community *Escherichia coli* isolates [10].

Carbapenemase genes are often located on mobile genetic elements further enhancing their spread. For example, the widespread dissemination of the $bla_{\text{OXA-48}}$ gene was shown to be related to a successful and epidemic plasmid that conjugates at high rates within *Enterobacteriaceae* [11].

Other less common carbapenemases belonging to a variety of molecular classes (*e.g.*, class A FRI-1 and IMI-like beta-lactamases, class B SPM-1 and GIM-1, and class D OXA-198) are reported sporadically and are found in specific species, likely because the corresponding genes are located on narrow host-range plasmids or chromosomes, which makes wide diffusion unlikely [10,12].

Laboratory Detection of CPO

Detection of carbapenemase-mediated carbapenem resistance is essential for patient management, infection control, and public health surveillance. The diversity of these enzymes and the range of associated susceptibility phenotypes makes detection challenging. Selection of a carbapenemase detection test (CDT) is contingent on several factors: epidemiology, diagnostic performance, labor intensity, complexity, and cost. The relative importance of turnaround time depends on whether the assay will be employed for therapeutic decision making and/or infection control or surveillance studies.

CDTs are broadly differentiated into two groups: phenotypic- (growth-/biochemical-dependent) and nucleic acid-based. Phenotypic assays monitor carbapenemase activity through a variety of methods: growth of a susceptible reporter strain following drug inactivation by a carbapenemase-producing test strain, observation of a pH change after

beta-lactam ring hydrolysis, detection of carbapenem hydrolysis products, or via inhibition with small molecules. In contrast, nucleic acid assays detect genetic determinants associated with carbapenemases.

The modified Hodge test (MHT) is probably the most extensively described CDT used in *Enterobacteriaceae*. This assay demonstrates acceptable sensitivity for most carbapenemases, especially KPC enzymes, but low sensitivity for NDM-producing strains [13,14]. Additionally, it has poor specificity; isolates encoding cephalosporinases in conjunction with porin mutations often produce false-positive results [13,15]. While the MHT is inexpensive and uncomplicated to perform, it is often difficult to interpret and requires an additional 24-hour growth step after AST results are obtained.

Conceptually akin to the MHT, the carbapenem inactivation method (CIM) assesses growth of a susceptible reporter strain around a carbapenem disk previously incubated with a suspension of a suspected carbapenemase-producing test strain. If the test strain produces a carbapenemase, drug in the disk will be inactivated thus allowing growth of the reporter strain up to the edge of the disk, whereas a zone of growth inhibition indicates the antibiotic in the disk remains active, thus the test strain lacks carbapenemase activity. CIM sensitivity is reported to be between 98 and 100% [16,17], but again this technique requires an additional 24-hour culture step. A modified version of the CIM (mCIM) was evaluated in a multi-center study, demonstrating 97% sensitivity and 99% specificity for detection of carbapenemase production in *Enterobacteriaceae* [18]. Based on those data, the mCIM was added to the CLSI M100 document as a reliable method for detection of carbapenemase production in *Enterobacteriaceae* [19].

The Carba NP test (RAPIDEC® CARBA NP, bioMérieux, Durham, NC), its derivatives, and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), monitor the hydrolysis of carbapenems using bacterial extracts [20,21] and produce same-day results. In the Carba NP test, carbapenemase-dependent hydrolysis of imipenem causes a decrease in pH, registered by a pH indicator as a color change. The test exhibits excellent sensitivity [20], although the recognition of OXA-48-producing isolates may be challenging [17,22]. To aid in early identification, the Carba NP test has been successfully extended to detect the presence of CPO in positive blood cultures even before isolation of organism on solid media, providing value for antibiotic stewardship [23].

MALDI-TOF MS can identify carbapenem degradation products following incubation of a bacterial protein extract with a carbapenem substrate. Overall, the sensitivity of MALDI-TOF MS for this purpose is high, and sensitivity for OXA-48-producing isolates is enhanced by inclusion of bicarbonate in the reaction buffer [22]. Notwithstanding the promise of mass spectrometry-based assays, because they are complex to perform and interpret, widespread implementation in clinical microbiology laboratories may be unfeasible.

Conventional AST methods such as broth microdilution, disk diffusion, and gradient diffusion can be modified to detect different classes of carbapenemases by performing them in the absence and presence of small molecule inhibitors including phenylboronic acid, which inhibits serine active site enzymes, and ethylenediaminetetraacetic acid, an inhibitor

of MBL activity. These assays have reportedly high sensitivities and specificities [24,25,26,27,28], are inexpensive, and generally easy to implement and interpret but require overnight incubation.

Nucleic acid-based CDT include commercially available and laboratory-developed PCR and microarray platforms to detect carbapenemase genes in bacterial isolates or directly from clinical specimens. They exhibit clinically relevant sensitivities and specificities and have same-day turnaround times [29,30,31,32,33], but are typically associated with high costs. In the setting of changing epidemiology or emergence of novel enzymes, the specificity of targeted PCR- or microarray-based platforms could be a shortcoming.

Whole-genome sequencing (WGS) platforms potentially represent the ultimate molecular CDT by interrogating the entire genomic content, chromosomal and extrachromosomal, of a bacterium to identify carbapenem resistance determinants [34,35,36]. Furthermore, WGS data provide an opportunity to query for extra information, including strain relatedness, plasmid types encoding the carbapenemase, other factors influencing carbapenem resistance (*e.g.*, porin mutations), presence of additional resistance factors, and data can be analyzed in near real-time or archived for future inquiry. Notwithstanding the power and promise of WGS, these assays are still the purview of advanced clinical microbiology and public health laboratories, and require considerable expertise to perform and interpret. As algorithms improve, costs decrease, and commercialized options are brought to market, the clinical workforce is likely to become increasingly proficient at performing and interpreting these data allowing WGS to gain wider acceptance.

WGS for Investigation of the Epidemiology and Diversity of CPO

Recent studies indicate that WGS, combined with hospital epidemiology, may facilitate the tracking of transmissions within healthcare facilities with the level of precision necessary to guide the modification of infection control procedures and limit the spread of healthcare-associated infections [36,37,38,39]. One example is the National Institutes of Health Clinical Center outbreak in which a single patient colonized on admission with KPC-producing *K. pneumoniae* was eventually linked to CPO colonization in 18 additional patients. The epidemiologic data could not discriminate between undetected transmission from the index patient or introduction of a second strain. The extensive genetic similarity among KPC-producing *K. pneumoniae* in the United States prevented a definitive match to the index patient using standard outbreak investigation tools such as pulsed-field gel electrophoresis or repetitive element PCR. WGS revealed direct linkage of the index patient, with transmission originating from three different anatomic sites [34], indicating silent colonization, even in immunocompromised patients. In another healthcare-related outbreak, WGS was instrumental in identifying limited healthcare-associated transmission of CRE against a background of sporadic introduction of multiple other strains [36]. In other studies, WGS was key in determining the phylogeny of carbapenem resistant *Enterobacter* species and how gene regulation by insertion sequence elements impacted carbapenem and multidrug resistance in *A. baumannii* [40,41]. WGS has also been used to create a reference set capturing the diversity of plasmids and mobile elements that carry the KPC gene [36,42].

Novel Treatment Strategies for CPO

Treatment of CPO, especially CP-CRE, remains problematic. 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 [43].

In an early study performed at a tertiary care center, Qureshi and colleagues reported that 28-day mortality was 13.3% in the combination therapy group (colistin and another agent) compared with 57.8% in the monotherapy group (P= 0.01), and that combination regimens were independently associated with better survival (P=0.02) [44]. Additionally, a multi-center retrospective cohort study conducted in three large Italian teaching hospitals examined death within 30 days of the first positive blood culture among 125 patients with blood stream infections caused by KPC-producing *K. pneumoniae* [45]. That investigation found 54.3% mortality in the monotherapy arm versus 34.1% mortality in the combination therapy group (P = 0.02); triple combination therapy (tigecycline, colistin, and meropenem) was associated with lowest mortality (P = 0.01). This study also revealed that patients infected by CP-CRE with imipenem MIC values of ≥4 μg/mL had worse outcomes than patients whose isolates had an MIC value of ≤2 μg/mL. The “dividing line” appears to be an MIC value between 2 and 4 μg/mL and predicted differences in mortality were notable (16.1% versus 76.9%; P <0.01); each imipenem MIC doubling dilution increased the probability of death two-fold.

In a subsequent review of 20 clinical studies involving 414 patients, Tzouvelekis and colleagues reported that a single active agent resulted in mortality rates not significantly different from those observed in patients administered no active therapy [46]. Consistent with the notions reported above, combination therapy with two or more agents active *in vitro* was superior to monotherapy, providing a clear survival benefit (mortality rate, 27.4% versus 38.7%; P <0.001). The lowest mortality rate (18.8%) was observed in patients treated with carbapenem-containing combinations.

In contrast, Falagas and partners in 2014 reported the largest meta-analysis performed to date [47], examining 20 studies involving 692 patients. Surprisingly, the authors reported 50% mortality in patients treated with tigecycline and gentamicin, 64% mortality for tigecycline and colistin, and 67% mortality for carbapenems and colistin. This comprehensive analysis called into question the conclusions drawn from the earlier retrospective, nonrandomized studies, and emphasized that unexplained molecular heterogeneity, and non-uniform microbiology testing might be confounding results. These differences suggest that studies concluding the superiority of combination therapy over monotherapy may not be sufficiently rigorous for us to accept their conclusions.

What about new drugs in development? Avibactam is a synthetic non-beta-lactam, bicyclic diazabicyclooctane beta-lactamase inhibitor (DBO), that inhibits the activities of Ambler class A and C beta-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 beta-lactamases. Against carbapenemase-producing *K. pneumoniae*, the addition of avibactam significantly improves the activity of ceftazidime *in vitro* (~four-fold MIC reduction). In surveillance studies, the combination of ceftazidime with avibactam restores *in vitro* susceptibility against all ESBLs 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. An important study comparing the outcomes of patients infected with CRE treated with colistin vs. ceftazidime/avibactam was recently performed [48]. Patients initially treated with either ceftazidime-avibactam or colistin for CRE infections were selected from the Consortium on Resistance Against Carbapenems in *Klebsiella* and other Enterobacteriaceae (CRACKLE), a prospective, multicenter, observational study. Thirty-eight patients were treated first with ceftazidime-avibactam and 99 with colistin either as monotherapy or combination therapy. 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. This study strengthens the notion that treatment with a highly active agent as monotherapy in the appropriate clinical setting may be better than therapy with a less desirable agent singly or in combination.

Relebactam, also a DBO, combined with imipenem/cilistatin, will soon be evaluated in clinical studies [49]. *In vitro* studies indicate that imipenem/cilistatin-relebactam is comparable to ceftazidime-avibactam. The role of the combination of imipenem versus ceftazidime with different DBOs remains to be defined.

The United States Food and Drug Administration (FDA) recently approved ceftazidime-avibactam based on data obtained in Phase II/III 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 [50,51]

In summary, combination chemotherapies seem to be effective against KPC-producing bacteria (Table 1) [49], 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 2. The reader will note 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 [52]. In addition, optimizing pharmacokinetic and pharmacodynamic parameters are essential for ensuring efficacy in difficulty to treat infections. Activities such as testing in hollow fiber

models, prolonged or continuous infusion are being aggressively evaluated to optimize drug dosing [53,54,55].

Monitoring and Control of Carbapenemase-Producing Organisms

Approaches to addressing the rapid intercontinental spread of CPO and other multi-resistant organisms include surveillance and judicious use of infection prevention and control (IPC) practices. There is evidence that IPC efforts at the local and country-wide level are effective in reducing transmission of CPO [56], and the role of IPC in the overall control of CPO cannot be overemphasized. Regarding surveillance at a global level, the Global Antimicrobial Resistance Surveillance System (GLASS) program was launched in 2015 as part of the WHO Global Action Plan on AMR to support a standardized approach to collection, analysis, and sharing of AMR data to inform local and national decision-making, and provide the evidence base for action and advocacy. Another approach that has been suggested is the application of the International Health Regulations (IHR), which represents a legal framework for international efforts to reduce the risk from public health threats that may spread between countries [57]. IHR requires countries to report certain disease outbreaks, including smallpox, wild-type poliomyelitis, severe acute respiratory syndrome, new types of influenza, or any public health event of international concern (PHEIC) which may include “new or emerging antibiotic resistance” [57]. The rationale for declaring AMR, specifically CPO, as a PHEIC has been reported previously [58], and includes multi-drug resistance, propensity for rapid spread, absence of geographic/political boundaries, presence in *E. coli* (the most common cause of urinary tract infection globally), presence in microbes of high public health importance, namely *Salmonella*, *Shigella*, and *Vibrio* species, and carriage of resistance traits on very mobile broad-host range plasmids [59]. The emergence of plasmid-mediated colistin resistance in *Enterobacteriaceae* has created a potential scenario of pan-resistant CRE [60].

Although application of IHR to CPO may have potential benefits including increasing surveillance and response capacities to address the spread of AMR on a global basis [58], a counter reaction argues that it is difficult to appreciate how the global spread of AMR constitutes an “extraordinary event” and that it is neither pragmatic nor within the framework of the IHR to consider it a PHEIC [61]. The only PHEICs declared to date include H1N1 2009 global influenza pandemic, Ebola virus disease in 2014, and the recent clusters of microcephaly and neurological abnormalities associated with Zika virus. In addition to global efforts underway, country-specific guidelines, including The *Combating Antibiotic Resistant Bacteria* report and the President's Council of Advisors on Science and Technology strategic plans, provide practical recommendations to the United States government to facilitate addressing the problem of antimicrobial resistance. Canada and the European Union have made similar commitments.

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Summary

Infections caused by carbapenemase-producing bacteria have experienced unprecedented intercontinental spread and proliferation and continue to be a therapeutic challenge. The genetic features that facilitate widespread dissemination are becoming increasingly understood. Control requires efficient laboratory detection and treatment, and a coordinated international response.

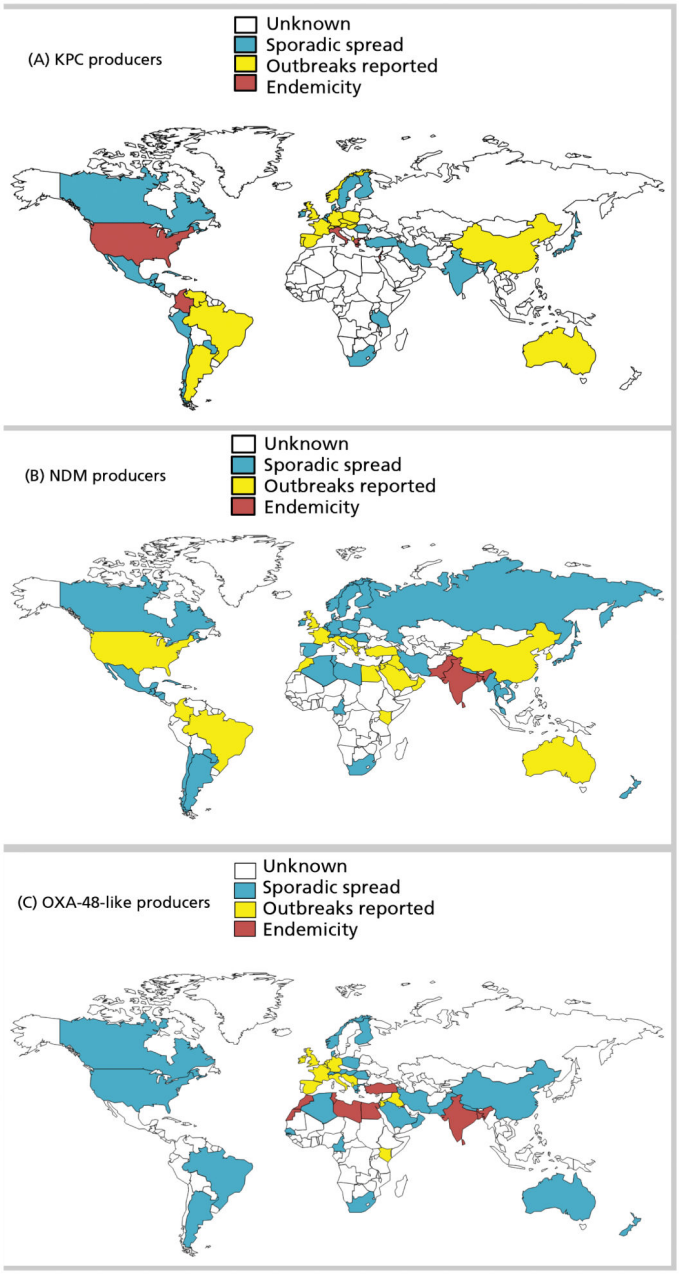

Figure 1.

Worldwide distribution of carbapenemases. A) KPC producers in *Enterobacteriaceae* and *P. aeruginosa*. B) NDM producers in *Enterobacteriaceae* and *P. aeruginosa*. C) OXA-48 producers in *Enterobacteriaceae*.

Table 1

Clinical regimens used in observational studies for treating carbapenem-resistant *Klebsiella pneumoniae* where carbapenemase is identified [45].

Beta-lactamases present	Regimen	Improved survival versus monotherapy
KPC- and MBL- producing *K. pneumoniae*	Carbapenem and tigecycline, plus aminoglycoside or colistin; Carbapenem and tigecycline; Carbapenem and aminoglycoside; Carbapenem and colistin	Yes
KPC-producing *K. pneumoniae*	Colistin and aminoglycoside; Colistin and tigecycline; Colistin and quinolone; Colistin and carbapenem; Carbapenem and carbapenem	Yes

Table 2

Novel agents in development for treating carbapenem-resistant and CPO

Antibiotic	Drug class	Intended Indication/Activity/Comments
Cefiderocol	Siderophore-β-lactam (cephalosporin)	Complicated urinary tract infections (cUTIs), carbapenem-resistant Gram-negative bacterial infections Active against metallo-beta-lactamase producing strains
ceftaroline fosamil/avibactam	Cephalosporin and DBO BLI.	Currently undefined.
Eravacycline	Tetracycline	cIAI and cUTI Multi-drug resistant organisms (MDRO)
Imipenem/cilistatin/relebactam	Carbapenem and DBO beta-lactamase inhibitor (BLI).	cUTIs, intra-abdominal infections (cIAI), hospital acquired pneumonia (HAP) Active against ESBLs and KPCs
Meropenem-vaborbactam	Carbapenem and cyclic boronic acid beta-lactamase inhibitor	cUTI, catheter-related bloodstream infections, HAP/ventilator-associated bacterial pneumonia (VAP), cIAI due to CRE
Plazomycin	Aminoglycoside	cUTI, catheter-related bloodstream infections, HAP/ventilator-associated pneumonia, cIAI due to CPO and CRE