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Carbapenemase-Producing Organisms: A Global Scourge

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The dramatic increase in the prevalence and clinical impact of infections caused by bacteria producing carbapenemases is a global health concern. Carbapenemase production is especially problematic when encountered in members of the family Enterobacteriaceae. Due to their ability to readily spread and colonize patients in healthcare environments, preventing the transmission of these organisms is a major public health initiative and coordinated international effort are needed. Central to the treatment and control of carbapenemase-producing organisms (CPOs) 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 CPOs with emphasis on their epidemiology, detection, treatment, and control.

Keywords. carbapenemase; carbapenemase detection tests; carbapenem-producing organisms; carbapenem-resistant Enterobacteriaceae; metallo-β-lactamase.

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 (CPOs), especially carbapenemase-producing carbapenem-resistant Enterobacteriaceae (CP-CRE), will be discussed.

THE BIOLOGY AND EPIDEMIOLOGY OF CPOs

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 [1]. CPOs 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].

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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 1 or 2 zinc ions for their catalytic activity [1]. There is a rare instance of class C β-lactamase that is reported to 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* is widespread in the United States, but is also endemic in some European countries such as Greece and Italy (Figure 1A) [4].

Class B β-lactamases, or metallo-β-lactamases (MBLs), are commonly identified in Enterobacteriaceae and *Pseudomonas aeruginosa* [5]. Among the MBLs, New Delhi metallo-β-lactamase (NDM) (Figure 1B), Verona integron-encoded metallo-β-lactamase (VIM), and imipenemase metallo-β-lactamase (IMP) enzymes are the most frequently identified worldwide [5]. IMP producing gram-negative bacteria 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].

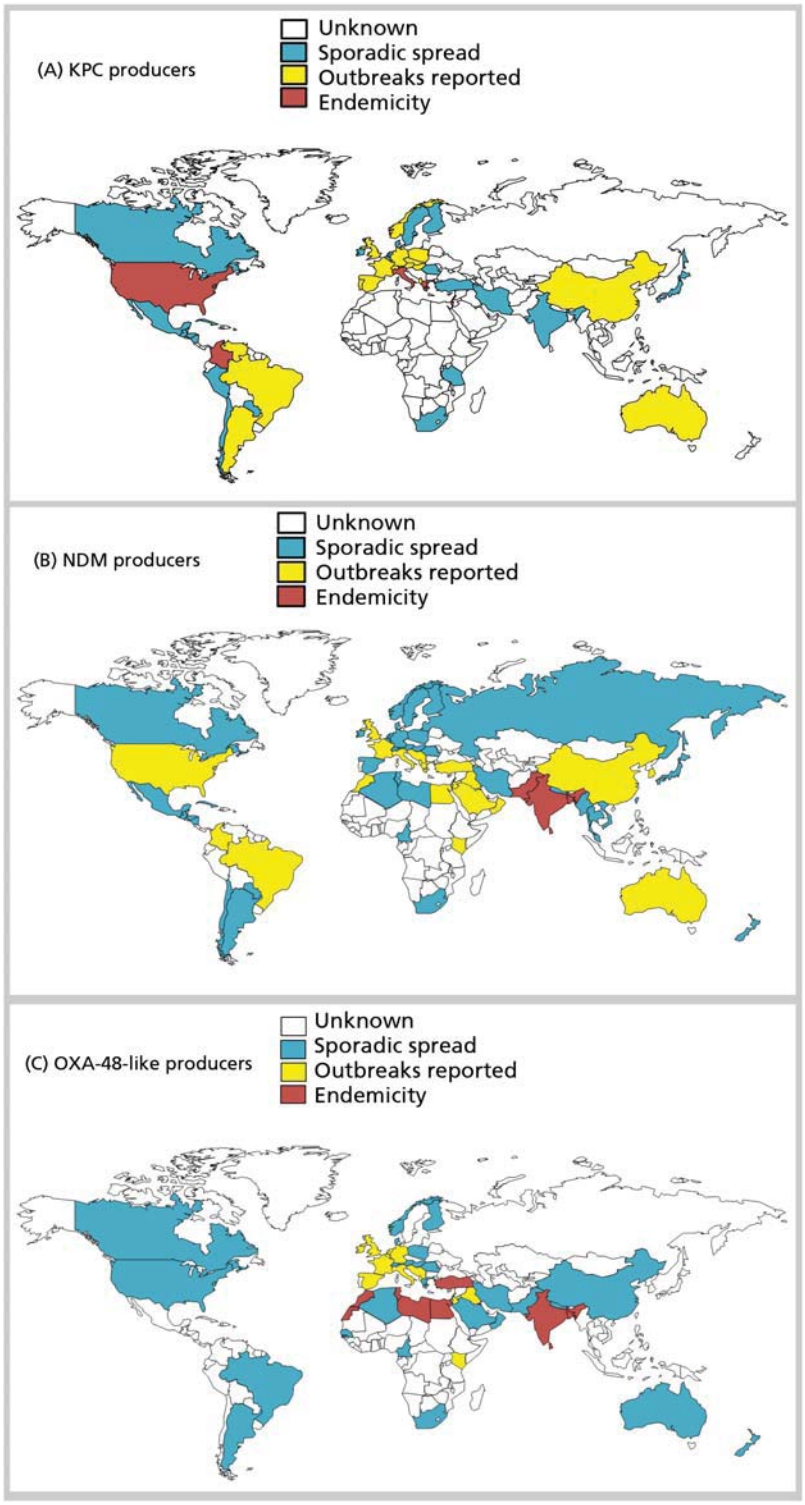

Figure 1. Worldwide distribution of carbapenemases. *A, Klebsiella pneumoniae* carbapenemase producers in Enterobacteriaceae and *Pseudomonas aeruginosa. B*, New Delhi metallo-β-lactamase producers in Enterobacteriaceae and *P. aeruginosa. C*, OXA-48–like producers in Enterobacteriaceae. Abbreviations: KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase-48.

Acquired class D carbapenem-hydrolyzing β-lactamases are commonly reported in *A. baumannii* (mainly OXA [oxacillinase]-23, OXA-24/40-, and OXA-58–like enzymes), but not in *P. aeruginosa*. OXA-48 and derivatives (eg, OXA-181 and OXA-232) have been detected in Enterobacteriaceae, hydrolyze narrow-spectrum β-lactams and weakly hydrolyze carbapenems, but spare broad-spectrum cephalosporins (ceftazidime, cefepime) [8]. OXA-48–producing Enterobacteriaceae are

endemic in Turkey (since 2004) and are frequently encountered in several European countries (eg, France and Belgium), and across North Africa (Figure 1C) [9]. Ten variants of OXA-48 β-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 (eg, class A FRI-1 and IMI-like β-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 CPOs

Detection of carbapenemase-mediated carbapenem resistance is essential for patient management, infection control, and public health efforts. The diversity of these enzymes and the range of associated susceptibility phenotypes make detection challenging. Selection of a carbapenemase detection test (CDT) is contingent on several factors: epidemiology, diagnostic performance, labor intensity, complexity, and cost. The 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 2 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 β-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 as isolates producing cephalosporinases in conjunction with porin mutations are often false-positive [13, 15]. Although the MHT is inexpensive and uncomplicated to perform, it is often difficult to interpret and requires an additional 24-hour growth step after antimicrobial susceptibility test (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 [16]. 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. In contrast, a zone of growth inhibition indicates the antibiotic in the disk remains active and the test strain lacks carbapenemase activity. CIM sensitivity is reported to be between 98% and 100% [16, 17], but again this technique typically requires an overnight culture step. A modified version of the CIM (mCIM) was evaluated in a multicenter 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 Clinical and Laboratory Standards Institute M100 document as a reliable method for detection of carbapenemase production in Enterobacteriaceae [19].

The Carba NP test (RAPIDEC CARBA NP, bioMérieux, Durham, North Carolina), its derivatives, and matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) monitor the hydrolysis of carbapenems using bacterial extracts and produce same-day results [20, 21]. 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 CP-CRE in positive blood cultures even before isolation of organism on solid media [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]. Despite the potential 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 [24–28] and are inexpensive and generally easy to implement and interpret, but require overnight incubation.

Nucleic acid–based CDTs include commercially available and laboratory-developed polymerase chain reaction (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 [29–33] but are typically associated with

high costs. PCR- and microarray-based platforms only detect certain carbapenemase genes and thus would not detect the emergence of new or previously uncommon carbapenemases.

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 [34–36]. Furthermore, WGS data provide an opportunity to query for extra information, including strain relatedness, plasmid types encoding the carbapenemase, other factors influencing carbapenem resistance (eg, porin mutations), and 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 CPOs

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 [34–39]. 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 CP-CRE 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 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 CP-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 CPOs

Treatment of CPO, especially 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 [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 ($P = .01$) and that combination regimens were independently associated with better survival ($P = .02$) [44]. Additionally, a multicenter retrospective cohort study conducted in 3 large Italian teaching hospitals examined death within 30 days of the first positive blood culture among 125 patients with bloodstream infections caused by KPC-producing *K. pneumoniae* [45]. That investigation found 54.3% mortality in the monotherapy arm vs 34.1% mortality in the combination therapy group ($P = .02$); triple combination therapy (tigecycline, colistin, and meropenem) was associated with lowest mortality ($P = .01$). 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% vs 76.9%; $P < .01$); each imipenem MIC doubling dilution increased the probability of death 2-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 2 or more agents active in vitro was superior to monotherapy, providing a clear survival benefit (mortality rate, 27.4% vs 38.7%; $P < .001$). 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 nonuniform 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-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. An important study comparing the outcomes of patients infected with CP-CRE treated with colistin vs ceftazidime-avibactam was recently performed [48]. Patients initially treated with either ceftazidime-avibactam or colistin for CP-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 vs ceftazidime with different DBOs 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 [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, the optimization of pharmacokinetic and pharmacodynamic parameters is essential for ensuring efficacy in difficult-to-treat infections. Activities such as testing in hollow fiber models, prolonged infusion, or continuous infusion are being aggressively evaluated to optimize drug dosing [53–55].

MONITORING AND CONTROL OF CPOs

Approaches to addressing the rapid intercontinental spread of CPOs and other multidrug-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 levels are effective in reducing transmission of CPOs [56], and the role of IPC in the overall control of CPOs 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

Table 1. Clinical Regimens Used in Observational Studies for Treating Carbapenem-Resistant *Klebsiella pneumoniae* Where Carbapenemase Is Identified

β-Lactamases Present	Regimen	Improved Survival vs Monotherapy
KPC- and MBL-producing *Klebsiella 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

Abbreviations: KPC, *Klebsiella pneumoniae* carbapenemase; MBL, metallo-β-lactamase.

Table 2. 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 DBO 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 DBO BLI	Currently undefined, likely CAP
Eravacycline	Tetracycline	•cIAI and cUTI •Multidrug-resistant gram-negative rods
Imipenem/cilistatin-relebactam	Carbapenem and DBO 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

Abbreviations: BLI, β-lactamase inhibitor; CAP, community acquired pneumonia; cIAI, complicated intra-abdominal infection; CPO, carbapenemase-producing organism; CRBSI, catheter-related bloodstream infection; CRE, carbapenem-resistant Enterobacteriaceae; cUTI, complicated urinary tract infection; DBO, diazabicyclooctane; ESBL, extended-spectrum β-lactamase; HAP, hospital-acquired pneumonia; KPC, *Klebsiella pneumoniae* carbapenemase; MBL, metallo-β-lactamase; VAP, ventilator-associated bacterial pneumonia.

efforts to reduce the risk from public health threats that may spread between countries [57]. The 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 CPOs, as a PHEIC has been reported previously [58] and includes multidrug 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 CP-CRE has created a potential scenario of pan-resistant Enterobacteriaceae [60].

Although application of IHR to CPOs may have potential benefits including increased 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 under way, 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 AMR. Canada and the European Union have made similar commitments.

Notes

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