



REVIEW

Continuous Evolution: Perspective on the Epidemiology of Carbapenemase Resistance Among Enterobacterales and Other Gram-Negative Bacteria

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ABSTRACT

The global emergence of carbapenemase-producing bacteria capable of hydrolyzing the once effective carbapenem antibiotics is considered a contemporary public health concern. Carbapenemase enzymes, once constrained to isolates of *Klebsiella pneumoniae*, are now routinely reported in different bacteria within the Enterobacterales order of bacteria, creating the acronym CRE which now defines Carbapenem-Resistant Enterobacterales. CRE harboring different types of enzymes, including the most prevalent types KPC, VIM, IMP, NDM, and OXA-48, are now routinely reported and more importantly, are now frequently present in many infections world-wide. Defining and updating the contemporary epidemiology of both the US and global burden of carbapenem-

resistant infections is now more important than ever. This review describes the global distribution and continued evolution of carbapenemases which continue to spread at alarming rates. Informed understanding of the current epidemiology of CRE, coupled with advances in antibiotic options, and the use rapid diagnostics offers the potential for rapid identification and management of carbapenem-resistant infections.

PLAIN LANGUAGE SUMMARY

Carbapenems are a subclass of antibiotic used to treat infections caused by Gram-negative bacteria, particularly in resistant and multidrug-resistant (MDR) infections where penicillin and cephalosporins are no longer effective. However, carbapenem-resistant Enterobacterales (CRE) have emerged due to acquisition of carbapenemase enzymes, most prevalent types are KPC, VIM, IMP, NDM, and OXA-48; infections caused by these bacteria have disseminated globally in both the healthcare and community setting. Resulting in a significant public health issue and clinical burden, these CRE infections are associated with increased morbidity and mortality, in part because carbapenems are the last therapeutic line of defense against resistant and MDR bacterial infections. The author

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wanted to investigate current US and global epidemiology of carbapenem-resistant infections, identify factors driving changes, as well as diagnostic technologies, and reporting or surveillance methods in place to track trends and inform therapeutic protocols and development. Overall, carbapenemase enzymes originally only reported in one country or region in 2006–2007, by 2013 and onwards have spread not only to surrounding countries but to other continents, which has impacted antibiotic resistance patterns and susceptibility. Increasing human travel and environmental factors, such as livestock care, food distribution, sewage, and recreational water, have contributed to global dissemination of CRE. Active surveillance programs are key to tracking resistance in real time, in order to update susceptibility breakpoints and epidemiology, which can inform antibiotic treatment choices, management guidelines, and the development of new therapeutics. Together, these factors will help to identify, control, and treat the spread of carbapenem resistance.

Keywords: Carbapenem-resistant enterobacterales (CRE); Enterobacterales; Epidemiology; Global

Key Summary Points

Carbapenemase enzymes which confer resistance to carbapenem antibiotics are no longer only found in *Klebsiella pneumoniae* isolates, but are now routinely documented among different Enterobacterales bacteria, now labeled Carbapenem-Resistant Enterobacterales (CRE).

Carbapenem antibiotics are often used as empiric therapy in patients with serious infections caused by gram-negative bacteria, including resistant isolates, but this treatment is threatened by the global dissemination of CRE, which are now present in many infections worldwide.

The contemporary epidemiology of both the US and global burden of carbapenem-resistant infections needs defining and updating in order to identify, control, and treat the spread of carbapenem resistance.

Susceptibility breakpoints need to reflect the changes in bacterial susceptibility in order to inform and guide prescribing clinical practices, and for implementation in laboratory diagnostic technologies.

Global and national surveillance programs are needed to continuously track global resistance rates in real time, to facilitate effective antibiotic stewardship efforts and direct development of new therapeutic agents.

DIGITAL FEATURES

This article is published with digital features, including a summary slide and plain language summary, to facilitate understanding of the article. To view digital features for this article go to <https://doi.org/10.6084/m9.figshare.13469820>.

INTRODUCTION

Beta-lactam drugs are a cornerstone of antibiotic therapy, and widely used for Gram-negative infections. However, inactivation of the core structural beta-lactam ring, by beta-lactamase enzymes, has gradually eroded the efficacy of once effective and life-saving therapies. Carbapenems, a subclass of beta-lactams, have often maintained efficacy in the setting of cephalosporin and penicillin resistance because they contain a unique beta-lactam core that renders them resistant, and slow, to hydrolysis by many beta-lactamase enzymes. Thus, for decades, carbapenems have remained active against Gram-negative infections, and are frequently used as empiric therapy in patients with serious infections caused by Gram-negative bacteria.

Unfortunately, the security of carbapenem therapy is now challenged because of the dissemination of carbapenemase enzymes, which have been acquired by members of Enterobacterales family. Globally, these enzymes are becoming more frequent in healthcare and community settings. The dissemination of carbapenem-resistant Enterobacterales (CRE) now frequently challenge the efficacy of carbapenem therapies as the last line of defense against multi-drug resistant (MDR) organisms. As the molecular mechanisms of resistance continue to evolve, so too must our understanding of the epidemiology of carbapenem-resistant organisms and the therapeutic options needed to combat the threat of antimicrobial resistance. This review will focus on the evolving epidemiology CRE and overview of enzymatic carbapenemase-mediated CRE.

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

CLASSIFICATION AND EVOLUTION OF CARBAPENEM RESISTANCE

In 1913, 25 years before the release of penicillin, Ehrlich correctly assessed the capacity of bacteria to develop resistance to antibiotics [1]. Since Ehrlich's initial observations in 1913, the challenge of predicting and minimizing the scope of bacterial resistance has been a goal of modern medicine. Hearing Ehrlich's advice, Alexander Fleming himself predicted not only how useful antibacterial drugs would be but also how dangerous a world without them could be. In an interview shortly after winning the Nobel Prize in 1945 for discovering penicillin, Fleming said: "The thoughtless person playing with penicillin treatment is morally responsible for the death of the man who succumbs to infection with the penicillin-resistant organism." In 1940, Abraham and Chain described the first beta-lactamase enzyme (penicillinase) isolated from *Bacillus (Escherichia) coli*, well before the clinical use of penicillin [2]. Ironically, the enzyme was initially thought to present few clinical concerns because penicillin, largely

developed to treat staphylococcal disease, was not found to be associated with beta-lactamase enzymes [3]. The global dissemination of methicillin-resistant *Staphylococcus aureus*, at its height from 1995–2005, provides perspective for the relevance of contemporary concerns over CRE. Today, over 2000 unique protein sequences coding for beta-lactamases have been in both Gram-negative and Gram-positive organisms [4].

Nomenclature for beta-lactamases are initially based on amino acid sequences, defined as the Ambler classification, which divides beta-lactamases into 4 major classes A–D [5].

A second classification system, first proposed by Richmond and Sykes [6], classified beta-lactamases based on functional characteristics. Further functional classification of key beta-lactamases was proposed by Bush, Jacoby, and Medeiros, and relies on substrate and inhibitor activity profiles to organize enzymes in a 1–4 system that can be readily identified with their phenotype in clinical isolates [5]. Table 1 outlines the classification of carbapenemase enzymes.

Class A Beta-Lactamases

Until recent concerns about carbapenemase enzymes, Enterobacterales were generalizable for their susceptibility to common beta-lactamase inhibitors, clavulanic acid, tazobactam, and sulbactam. In 1965, a class A beta-lactamase reported from *E. coli* described the first plasmid-mediated beta-lactamase from a patient from Greece, which now bears the name "TEM" from the index patient in which it was reported [7]. Additional class A beta-lactamases in the form of SHV (sulfhydryl reagent variable) variants are now commonly found in beta-lactamase-resistant isolates of *E. coli* and *Klebsiella pneumoniae* associated with bloodstream infections, as well as hospital-acquired respiratory and urinary tract infections [5]. Within 2 years of the introduction of cefotaxime and ceftazidime, novel extended spectrum β -lactamases (ESBLs) were reported in clinical isolates [8]. Mutations in pre-existing *bla*_{TEM-1} and *bla*_{SHV-1} genes gave rise to the emergence of ESBLs capable of

Table 1 Classification of carbapenemase enzymes

Ambler Structural Class	Bush-Jacoby group	Enzyme name	Resistance profile	Examples	Examples of active antimicrobial agents	Common bacterial species involved
A	2f	Serine carbapenemase	Carbapenems, cephalosporins, aztreonam	KPC, GES, SME-1	Ceftazadime-avibactam mipenem-relebactam eropenem-vaborbactam cefidercol	KPC: <i>K. pneumoniae</i> , <i>E. coli</i> , <i>Enterobacter</i> spp.
B	3a	Metallo-beta lactamase	All beta-lactams except aztreonam	IMP, NDM, VIM, IND	Cefidercol [△]	NDM: <i>K. pneumoniae</i> , <i>E. coli</i> VIM: <i>K. pneumoniae</i>
D	2df	Carbapenemase	Penicillins (variable hydrolysis)	OXA	Cefidercol [△]	IMP: <i>K. pneumoniae</i> OXA48: <i>K. pneumoniae</i> , <i>E. coli</i> , <i>Enterobacter</i> spp.

Kazmierczak et al. [58] reported activity of cefidercol against KPC-positive ($n = 75$); MIC_{50/90} 1/2 µg/ml. NDM positive ($n = 12$); 4/8 µg/ml. OXA-48 ($n = 32$); 0.5/2/µg/ml. VIM positive ($n = 27$); MIC_{50/90} 1/4 µg/ml
KPC Klebsiella pneumoniae carbapenemase, MBL metallo-β-lactamase, NDM New Delhi metallo-β-lactamase, VIM Verona integrin-encoded metallo-β-lactamase, IMP, imipenemase, OXA oxacillinase, GES Guiana-extended-spectrum β-lactamase, SEM *Serratia marcescens* enzyme

hydrolyzing extended-spectrum cephalosporins with an oxyimino side chain. These cephalosporins include cefotaxime, ceftriaxone, and ceftazidime, as well as the oxyimino-monobactam aztreonam. Cefotaximases, characterized by CTX-M genes arose by plasmid transfer from pre-existing chromosomal ESBL genes from the bacteria *Kluyvera* spp., [9] CTX-M ESBLs now represent a large proportion of global ESBL strains and are the dominant ESBLs in the US, particularly among urinary tract infections [10, 10].

Class A Serine Carbapenemases

The non-metallo beta-lactamases can hydrolyze carbapenems as well as cephalosporins, penicillin, and aztreonam [9], marking the evolution of beta-lactamase activity from primarily targeting penicillins and cephalosporins to enzymes that hydrolyze carbapenems. Representative of class A serine carbapenemases include the enzymes NMC-A, IMI, SME, and KPC. Table 1 outlines the classification of beta-lactamases capable of hydrolyzing carbapenem antibiotics.

KPC enzymes have emerged as the most well-known, and well-described of the class A serine carbapenemases, which, while initially described in *K. pneumoniae*, are now readily detected in many members of the Enterobacteriaceae family. They confer resistance to all beta-lactams (excluding, carbapenems, and monobactams). Gram-negative bacteria harboring *bla*KPC genes can be carried on mobile genetic elements such as transposons (e.g., Tn4401b) and multiple plasmid (IncFII, IncL/M, and IncN) types [9]. Organisms expressing KPC genes are often concurrently resistant to other classes of antibiotics, such as quinolones and aminoglycosides, thus creating highly MDR organisms [12].

Class B Beta-Lactamases

Metallo-beta-lactamases (MBLs) have been isolated from *Acinetobacter* spp. and *Pseudomonas* spp., and are increasingly associated with members of the Enterobacteriales. such as *K. pneumoniae*, *K. oxytoca*, *E. coli*, and *Enterobacter* spp. MBLs are commonly associated with the Verona integron–encoded metallo-β-lactamase (VIM), *Pseudomonas* (IMP)-type and the New Delhi MBL (NDM) [13, 13] (52). MBLs are commonly expressed from mobile genetic elements such as integrons, plasmids and transposons, which have contributed to the spread of MBLs. MBLs effectively hydrolyze beta-lactamases and are not inhibited by beta-lactamase inhibitors (clavulanic acid, tazobactam, vaborbactam). They also differ from KPCs (serine carbapenemases) because they contain a bivalent metal ion, commonly zinc (Zn^{2+}) ions which coordinate histidine/cytosine/asparagine residues at the active site [15]. Genetic insertion of entire sequences upstream of the *bla*NDM-1 gene among bacteria in the Enterobacteriales seems to suggest that NDM variants likely evolved from *Acinetobacter baumannii* [16]. Currently, > 20 NDM types have been identified [17], yet expansion of NDMs do not appear to be due to proliferation of a single dominant clone. NDMs have been found in several epidemic clones, including *K. pneumoniae* ST11 and ST147 and *E. coli* ST131 and ST101, which are known to

harbor other β-lactamase genes and antibiotic resistance determinants [12]. IMP-type metallo-β-lactamases are among the most common families of acquired carbapenemases detected from Enterobacteriaceae and have been reported mainly in East Asia, including Japan [18]. Although IMP-type metallo-β-lactamases are class B beta-lactamases, their hydrolytic properties for carbapenems are weak, often resulting in elevated resistance to meropenem while retaining imipenem susceptibility [19, 19]. This feature has been implicated in the spread of *bla*IMP harboring plasmids in Japan, as determined after screening the antibiotic susceptibility of infectious bacteria to imipenem as a representative carbapenem [21].

Class C, Serine-Based Cephalosporinases

AmpC β-lactamase expressors are common to members of the family are commonly associated with *Serratia* spp., *Pseudomonas aeruginosa*, *Providencia* spp., indole-positive *Proteus mirabilis/vulgaris*, *Citrobacter* spp., and *Enterobacter* spp., which collectively are often referred to as “SPICE” organisms. AmpC β-lactamase are generally resistant to penicillins, β-lactamase inhibitors, and cephalosporins such as cefoxitin, cefotetan, ceftriaxone and cefotaxime. In many Enterobacteriales, AmpC expression is low but inducible in response to β-lactam exposure, and resistance occurs through two primary mechanisms. Disruption of peptidoglycan (murein) synthesis by beta-lactam agents leads to an accumulation of *N*-acetylglucosamine-1,6-anhydro-*N*-acetylmuramic acid oligopeptides. Displacement of the UDP-*N*-acetylmuramic acid peptides signals a conformational change in AmpR, which activates the transcription of *ampC* regulatory genes. Additionally, many Enterobacteriales contain a cytoplasmic *N*-acetyl-muramyl-L-alanine amidase enzyme (AmpD) which removes peptides from the 1,6-anhydro-*N*-acetylmuramic acid and *N*-acetylglucosamine-1,6-anhydro-*N*-acetylmuramic acid oligopeptide derivatives. Under wild-type expression, AmpD aids in reducing their concentrations of the aforementioned enzymes, preventing the overexpression of AmpC. The

most common cause of AmpC overexpression in clinical isolates is a mutation in *ampD* leading to AmpC hyper-inducibility and/or constitutive hyperproduction [22]. AmpR mutations are less common but can also result in high-constitutive or hyper-inducible phenotypes. Infrequently, AmpC induction can also occur via mutations in AmpG, an inner membrane permease enzyme, involved in cell wall turnover and AmpC regulation into the cytosol [23, 23]. AmpC expression can often combine with other mechanisms of resistance often related to permeability such as porin loss and/or efflux pumps, resulting in clinically significant resistance [25].

Class D Serine Oxacillinases

Class D beta-lactamases, initially characterized as oxacillinases because of their hydrolytic activity against β -lactams have recently become a clinical concern. Class D beta-lactamases, and OXA variants, possess variable but significant carbapenemase activity and are generally not inhibited by clavulanic acid, tazobactam, and sulbactam (with some exceptions; e.g., OXA-2 and OXA-32 are inhibited by tazobactam but not by sulbactam and clavulanate, and OXA-53 is inhibited by clavulanate) [9]. Nearly one-third of OXA variants hydrolyze carbapenems. OXA-carbapenemases are predominantly isolated in *Acinetobacter* spp.; however, the OXA-48 carbapenemase was described in a MDR isolate of *K. pneumoniae* in 2004 in a patient with a UTI [26]. The initial cases of an OXA-48 producing *K. pneumoniae* presented as a highly drug-resistant strain because it co-expressed other beta-lactamases (class A extended-spectrum β -lactamase SHV-2a and the narrow-spectrum β -lactamases TEM-1 and OXA-47) as well as defects in outer membrane proteins [27]. While OXA-48 is a class D β -lactamase with the highest known catalytic efficiency for imipenem [28, 28], organisms containing the plasmid-mediated *bla*_{OXA-4} gene effectively hydrolyze penicillins, but hydrolyze carbapenems at low levels and show weak activity to extended-spectrum cephalosporins, and poor hydrolytic activity against ceftazidime and cefepime. Recently,

OXA-48-positive organisms have spread to over 30 different countries and within various bacteria of the Enterobacterales [27, 27, 27].

This review will focus on enzymatic forms of CRE, attributed to carbapenemase-producing bacteria. However, it should be noted that carbapenem-resistance is often regarded as a phenotypic designation and encompasses non-enzymatic forms of carbapenem resistance. Cell wall differences, external decreased membrane permeability, efflux pumps, and the presence of various broad-spectrum β -lactamases (e.g., AmpC cephalosporinase), often manifest in carbapenem resistance when measured phenotypically in laboratories.

EPIDEMIOLOGY OF CARBAPENEM RESISTANCE AND CRE IN THE UNITED STATES

Over a decade ago, the acronym ESKAPE pathogens was used to describe the names of six bacterial pathogens (*Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *A.baumannii*, *P. aeruginosa* and *Enterobacter* spp.) associated with declining clinical responses and rising rates of antimicrobial resistance [31]. Since this initial description, and plea for funding recognition to support antibiotic development, global expansion of carbapenem resistance among Gram-negative bacteria has steadily increased, prompting the US Centers for Disease Control and Prevention (CDC) to issue concerns over antibiotic resistance as an urgent public health threat [32].

Much of the increase in CRE is likely related to the emergence and spread of the genes coding for carbapenemases. In the US, the most commonly identified carbapenemase is *K. pneumoniae* carbapenemase (KPC). This enzyme was first described in 2001 in an isolate obtained from a patient in North Carolina [33]. Cases involving KPC harboring organisms have been described from every US state (Fig. 1.), and other carbapenemases produced by Enterobacterales, including NDM, VIM, IMP, and OXA-48, have been reported in patients hospitalized in the US. CRE producing a carbapenemase other than KPC are generally uncommon in the US and

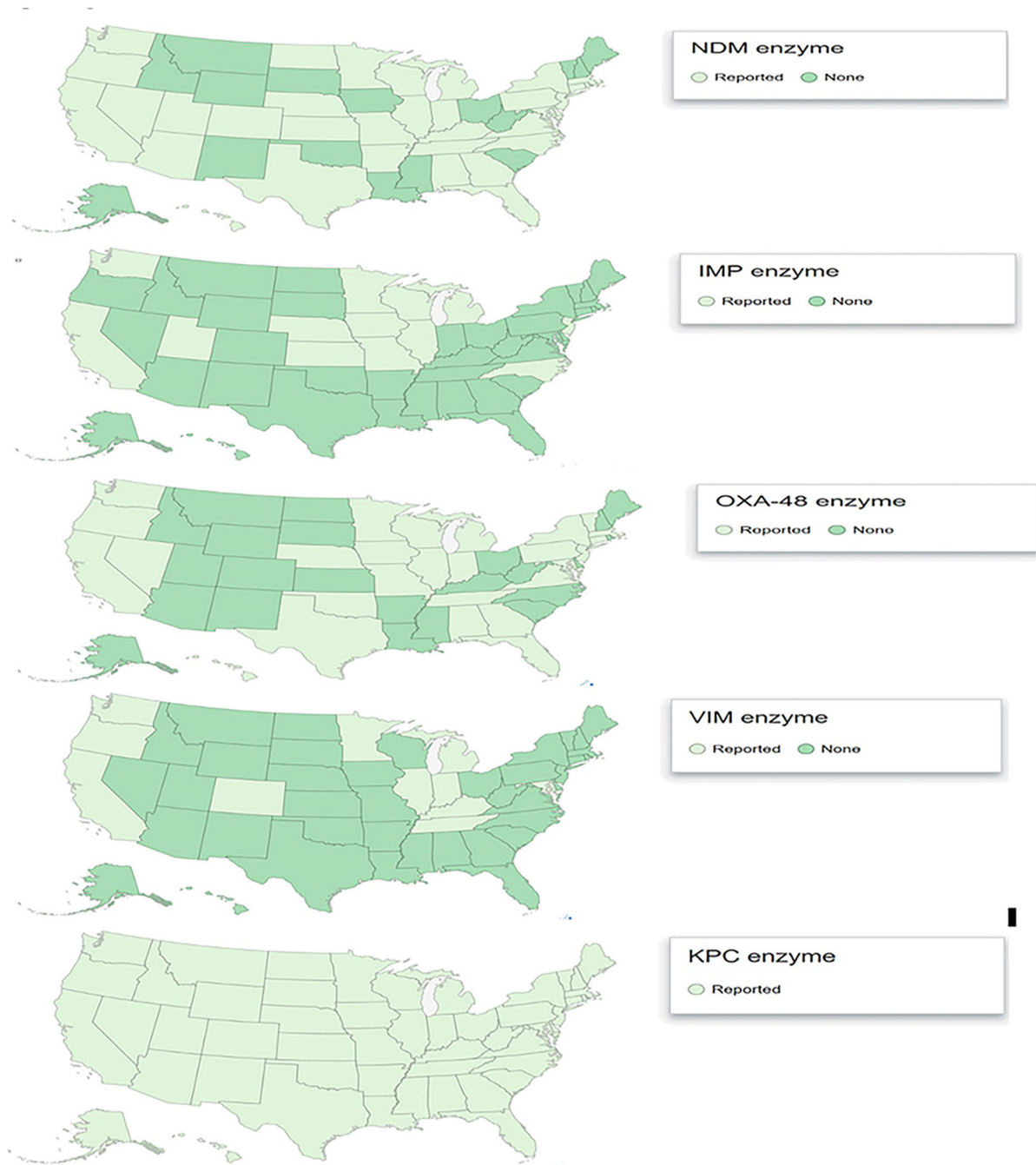


Fig. 1 CRE reported cases, Centers for Disease Control as of 2017, reported by geographic US state reporting [55]. CRE defined by 2015 CDC case definition for CRE; resistant to imipenem, meropenem, doripenem, or

ertapenem OR documentation that isolates possess a carbapenemase gene. Reference Source CDC [56]

often associated with healthcare exposures outside the US. However, recent reports suggest that patients may be colonized with (CR)

organisms without traditional associated risk factors such as foreign travel [34] (personal

communication, Minnesota Department of Health).

In response to concerns surrounding antibiotic resistance, the CDC launched the National Healthcare Safety Network (NHSN), a system which is used by the CDC, healthcare facilities, state health departments, and the Centers for Medicare and Medicaid Services, to provide surveillance of patient and healthcare safety, as well as a surveillance tool for tracking US antibiotic resistance trends. In 2006–2007, NHSN reported carbapenem resistance rates for *E. coli* and *K. pneumoniae* associated with central line-associated bloodstream infections (CLABSI) at 0.9% and 10.8%, respectively [35]. Two years later, the 2009–2010 NHSN data showed CLABSIs CRE resistance rates in all tested isolates had doubled to 2% for *E. coli*, and *K. pneumoniae* increased to 12.8% [36] (Fig. 2). Additionally, MDR among these *E. coli* isolates increased to 3.7% [36], mostly because of extended-spectrum cephalosporin resistance; this trend of MDR continued between 2011 and 2014 increasing from 11.1 to 14.1%, respectively [37].

By 2014, carbapenem resistance in CLABSIs was readily detected among *Klebsiella (pneumoniae/oxytoca*.9%), *E. coli* (1.9%), *Enterobacter* spp. (6.6%), *P. aeruginosa* (25.8%) and *Acinetobacter* spp. (46.6%) [37]. Although carbapenem resistance rates are higher in *P. aeruginosa* and *Acinetobacter* spp., compared to members of the Enterobacteriaceae, the overall incidence of infections with *P. aeruginosa* and *Acinetobacter* spp. are less than that observed for the Enterobacteriaceae. Additionally, non-enzymatic forms of carbapenem resistance, such as efflux and porins, are common in *P. aeruginosa*.

National US-surveillance programs have focused on reports of carbapenem resistance from urine, CSF, and bloodstream infections, which is often a consequence of surveillance programs that focus on reporting from sterile sites. Among patients with VAP, rates of carbapenem resistance have risen steadily over the past decade. The incidence of carbapenem resistance in *K. pneumoniae* and *E. coli* among VAP patients increased from 3.6 to 1.8% [35] to 11.2% to 10.1%, and 3.5% to 2.2% between 2013 and 2014 [36][36], respectively. Non-

fermenting Gram-negative species including *Acinetobacter* spp., and *P. aeruginosa* associated with VAP continue to be problematic with reported carbapenem resistance rates of 55% and 28, respectively [37].

The incidence of CRE is increasing globally. Cai et al. evaluated carbapenem resistance rates for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* across 9 regions in the US, using Premiere Health Database data from 2009–2013, finding the overall carbapenem resistance rate among the 4 selected pathogens was 4.5% out of 292,742 isolates tested (Fig. 3) [38]. *A. baumannii* had the highest carbapenem resistance rates: blood 40.1%, respiratory 50.4%, urine 42.0%, and other sites 42%. Carbapenem resistance rates for *P. aeruginosa* ranked second highest, but varied more by infection site: 10.3% in blood, 19.4% in respiratory, 11.7% in urinary, and 12.6% in other. In the US, more than 80% of all carbapenem-resistant infections were caused by non-fermenters, *A. baumannii* or *P. aeruginosa*, which was a consistent finding across the 9 US regions sampled, except for the middle Atlantic region where only 67% were caused by these pathogens [38].

Ceftazidime-avibactam-resistant strains of *K. pneumoniae* contain point mutations in KPC-2 and have now been described in the US. Nelson and colleagues, [39] phenotypically described a case in Los Angeles involving a resistant strain of *K. pneumoniae* isolated from blood cultures from a pancreatic cancer patient with an MIC of 32/4 µg/ml. While the authors failed to provide a genetic basis for ceftazidime-avibactam resistance, additional molecular testing for other known carbapenemase enzymes, and testing in the setting of an efflux pump inhibitor, phenyl-arginine β-naphthylamide, at 40 µg/ml, did not result in a decrease in the ceftazidime-avibactam MIC [40]. Follow-up subsequently revealed transposition of the Tn4401 transposon harboring *bla*_{KPC-3} into a second plasmid, pIncX3, which also harbored *bla*_{SHV-12}, ultimately resulting in a higher copy number of *bla*_{KPC-3} in the resistant isolate [39]. KPC-3 gene mutations, D179Y, T243M, D179Y/T243M, and EL165-166, impacting the KPC Ω-loop, have also been described in the US as additional resistance mechanisms leading to ceftazidime-avibactam

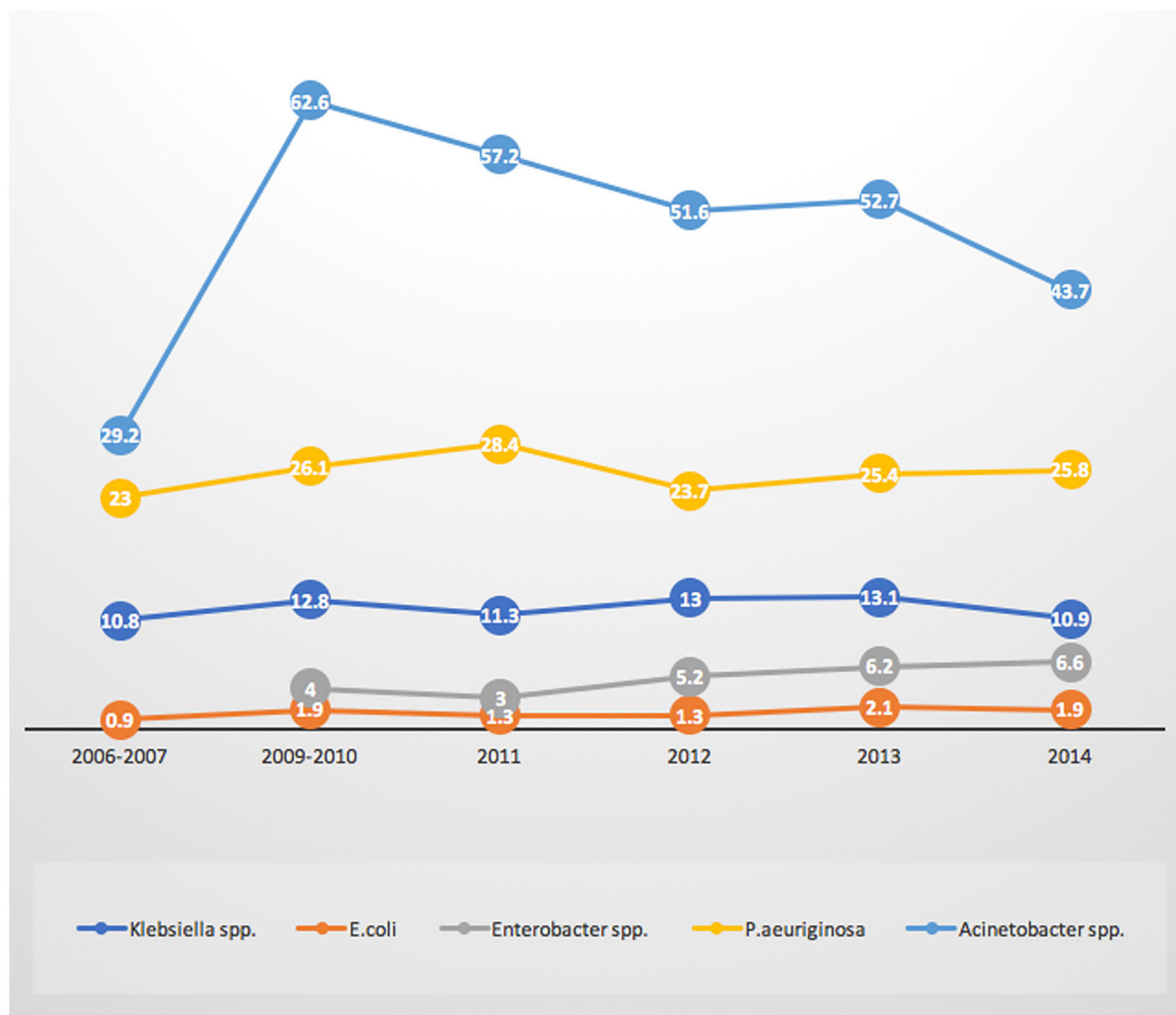


Fig. 2 Carbapenem resistance from Central Line–Associated Bloodstream Infections (CLABSIs). Figure adapted from Sievert et al. [32]; values represent percent carbapenem resistant for respective bacteria isolated from positive blood cultures representative of CLABSI infection. Carbapenem resistance, as defined in this report, included all applicable pathogens with a result of I or R to imipenem, meropenem, or doripenem. 2006–2007 Klebsiella data inclusive of *K. pneumoniae* isolates only.

resistance in *K. pneumoniae* [41], further indicating that resistance mutations in KPC-genes can account for clinical resistance in some global strains [41].

Carbapenem resistance rates by pathogen differ depending on the site of infection [33][34]. Rates for *P. aeruginosa* and *A. baumannii* are lower for bloodstream infections that have been reported from other types of infections [e.g., ventilator-associated pneumonia (VAP)] [32][34]. Thus, it is likely that studies reporting bloodstream and CLABSI infections may under-report true prevalence of carbapenem resistance rates

GLOBAL PERSPECTIVE ON THE EPIDEMIOLOGY OF CARBAPENEM RESISTANCE

Access to global travel has unquestionably led to the dissemination of antibiotic resistance throughout many countries [42]. The first

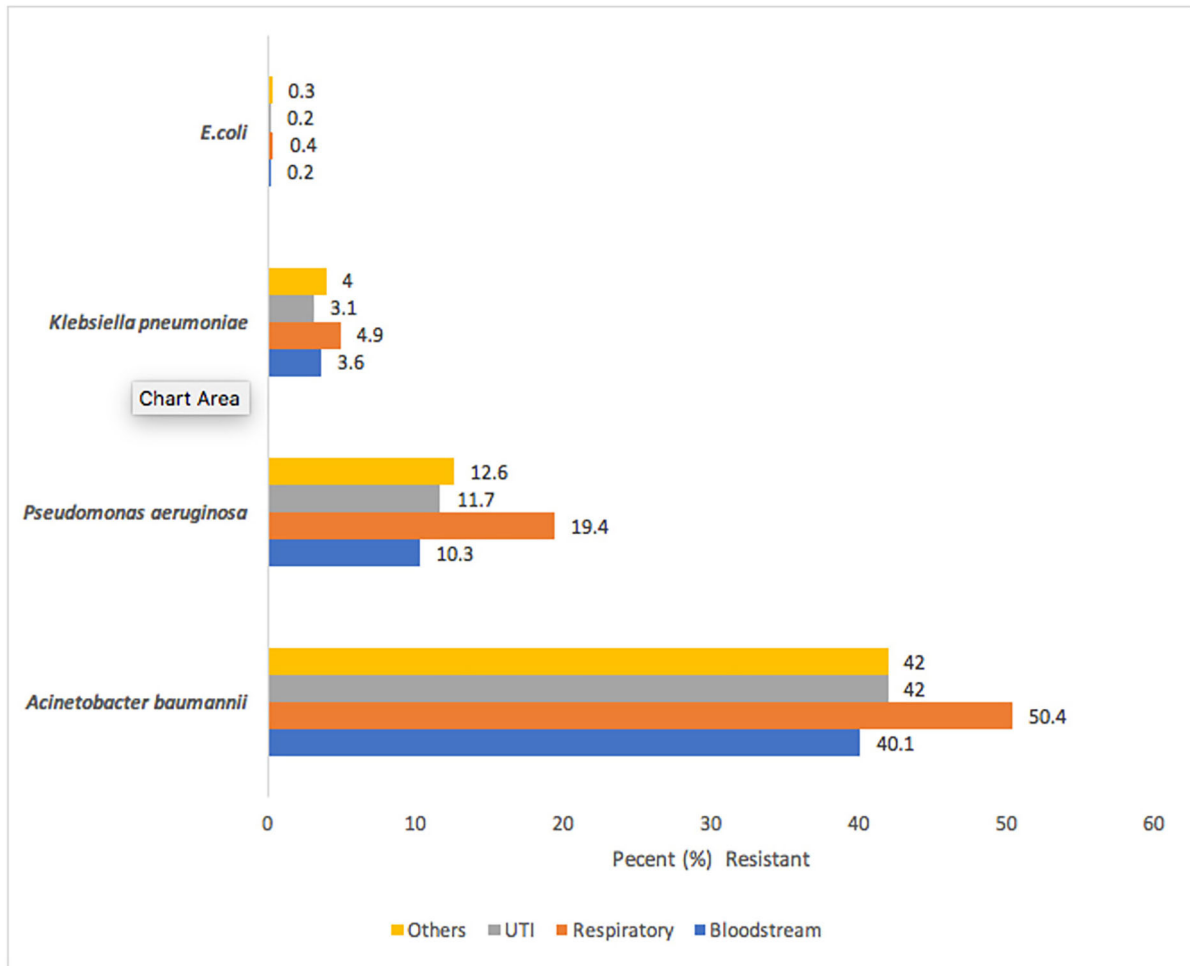


Fig. 3 Prevalence of carbapenem-resistant Gram-negative infections in the United States. Adapted from Cai et al. [38]. In the US, overall carbapenem resistance among the 4

selected pathogens (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*) was 4.5% out of 292,742 isolates tested

carbapenemase was reported in Japan from an *Aeromonas hydrophila* isolate in the 1980s, which was subsequently followed by a report describing the Seoul imipenemase (SME-1) from *Serratia marcescens*, imipenemases (IMI-1) in California (1984) and NMC-A in France (1990), both from *Enterobacter cloacae* [43].

Globally, clinical characteristics of carbapenem-resistant carrying organisms vary with local conditions and across geographical regions (Fig. 4). Table 2 details the global distribution of reported enzymatic carbapenem resistance rates and published outbreak series by geographical region. Countries such as Israel, Greece, and Colombia have all reported

significant outbreaks of CRE-associated infections [12]. No scenario better exemplifies the concerns surrounding the spread of carbapenem-resistant Gram-negative bacteria than the experience of Greece, which now has some of the highest carbapenem-resistance rates. Prior to 2001, the Greek System for the Surveillance of Antimicrobial Resistance reported a carbapenem-resistance prevalence of <1%, and that CRE resistance was largely attributable to VIM-1-type MBL. In 2008, CRE in Greece had dramatically increased to 30% in hospital wards and 60% in ICUs [44]. By 2014, the European Centre for Disease Prevention and Control, EARS-Net, revealed that 62.3% of all *K.*

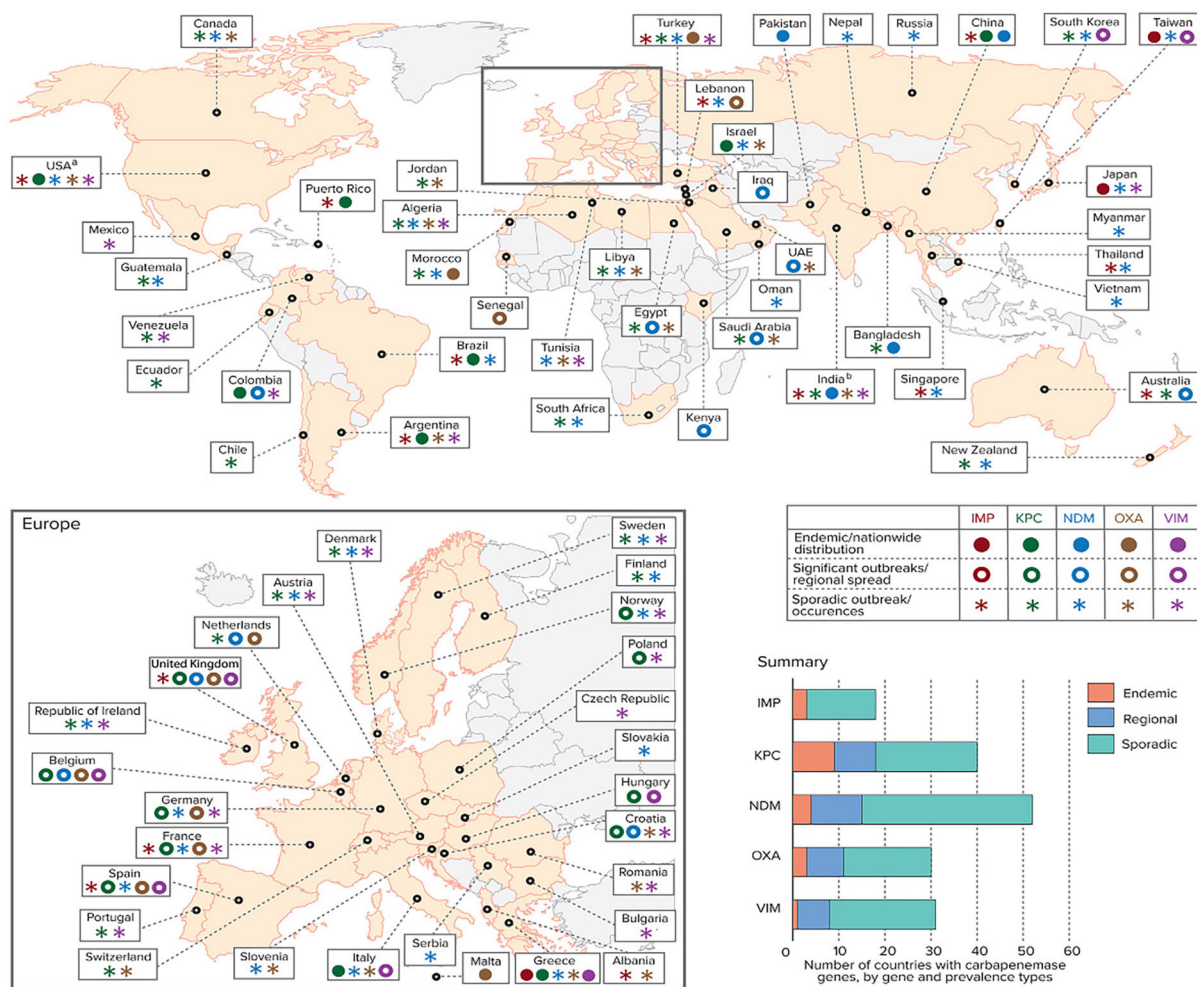


Fig. 4 Global distribution of Carbenapemase-producing bacteria: global distribution of carbapenemases in Enterobacteriaceae, by country and region. ^aKPCs are endemic in some US states; ^bOXA mainly refers to OXA-48, except in India, where it refers to OXA-181. *IMP* active on imipenem metallo-β-lactamase, *KPC* Klebsiella pneumoniae carbapenemase, *NDM* New Delhi metallo-β-lactamase, *OXA* oxacillinase-type carbapenem-hydrolyzing β-

lactamase, *VIM* Verona integron-encoded metallo-β-lactamase. Reprinted with permission from: Logan, K., Weinstein, R. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. *J of Infect Disease*. 2017;215(S1):S28–36. <https://doi.org/10.1093/infdis/jiw282>. (12)

pneumoniae isolates tested in Greece were now resistant to carbapenems [45, 45]. This spread in Greece is attributable to the introduction of the *bla*KPC-2 gene which spread through the country in 2007. Towards the end of the following year (2008), surveillance of 21 Greek hospitals revealed that 96% of carbapenem-resistant *K. pneumoniae* could be attributed to a single dominant sequence type (ST258 lineage) of *K. pneumoniae* harboring *bla*KPC-2 [44, 44].

Dissemination of carbapenemase genes and outbreak of CR infections in Europe are shown in Fig. 5. Following worldwide spread of *K. pneumoniae* ST258-type strain, including throughout the US, multi-loci sequence typing was used as a molecular tool to track the global epidemiology of CRE and the dissemination of KPC genes. *K. pneumoniae* ST258 continues to exist as a dominant global strain, which has been associated in outbreaks in [47] Poland,

Table 2 Global distribution of reported enzymatic carbapenem resistance rates and published outbreak series by geographical region

Region	Country	Carbapenemase genes described	Bacterial species implicated	Percent carbapenem resistance reported	Examples; Published outbreak	Reference
N. America	USA/ Canada	KPC, NDM, VIM, OXA, IMP	<i>Klebsiella pneumoniae</i>	10.3–58.5%	<i>K. pneumoniae</i> (KPC-2)	(57–60) (25) (30–34)
			<i>Escherichia coli</i>	0.2–4%		
			Enterobacterales ^a	2–4%		
			<i>Acinetobacter baumannii</i>	11–50%		
			<i>Pseudomonas aeruginosa</i>	10–59%		
Asia - Pacific	Japan Thailand Vietnam Singapore Taiwan S. Korea	NDM, VIM, IMP, KPC, NDM, NDM, NDM, NDM, NDM	<i>Klebsiella pneumoniae</i>	1.6–20%	<i>K. pneumoniae</i> (KPC-16))	(58, 61–65)
			<i>Escherichia coli</i>	0–%	<i>K. pneumoniae</i> (KPC-15)	
			Enterobacterales ^a	0–32%	<i>K. pneumoniae</i> (KPC-3)	
			<i>Acinetobacter baumannii</i>	25%–91%	<i>K. pneumoniae</i> (OXA-48)	
			<i>Pseudomonas aeruginosa</i>	10–47%		
E. Asia	China	KPC, NDM, VIM	<i>Klebsiella pneumoniae</i>	5–52%	<i>K. pneumoniae</i> (NDM-5)	(66–69, 70–71)
			<i>Escherichia coli</i>	^ID (insufficient data)	<i>K. pneumoniae</i> (NDM-15)	
			Enterobacterales ^a	^ID (insufficient data)	<i>E. cloacae</i> (NDM1,5)	
			<i>Acinetobacter baumannii</i>	10–90%	<i>E. cloacae</i> (KPC-2)	
			<i>Pseudomonas aeruginosa</i>	10–100%	<i>E. cloacae</i> (VIM-1) <i>A. baumannii</i> (OXA-23) <i>P. aeruginosa</i> (KPC-2)	
S. Asia	India/ Pakistan		<i>Klebsiella pneumoniae</i>	0–52%	<i>K. pneumoniae</i> (NDM-1)	(63, 72–76)
			<i>Escherichia coli</i>	0–34%	<i>K. pneumoniae</i> (NDM-2)	
			Enterobacterales ^a	3–21%		
			<i>Acinetobacter baumannii</i>	0–100%		
			<i>Pseudomonas aeruginosa</i>	^^ID (insufficient data)		
Europe			<i>Klebsiella pneumoniae</i>	0.2–59%	<i>K. pneumoniae</i> (NDM-1)	(16, 40, 42, 58, 77–79)
			<i>Escherichia coli</i>	0–7%	<i>K. pneumoniae</i> (KPC-1–2–3–4–16)	
			Enterobacterales ^a	^ID (insufficient data)	<i>K. pneumoniae</i> OXA-48	
			<i>Acinetobacter baumannii</i>	3–100%	<i>E. coli</i> OXA-48	
			<i>Pseudomonas aeruginosa</i>	0–56%		

Table 2 continued

Region	Country	Carbapenemase genes described	Bacterial species implicated	Percent carbapenem resistance reported	Examples; Published outbreak	Reference
Latin America			<i>Klebsiella pneumoniae</i>	1.3–20%	<i>K. pneumoniae</i> (NDM-1)	(21, 58, 61,62,80–83)
			<i>Escherichia coli</i>	0.5–9%	<i>K. pneumoniae</i> (KPC-2)	
			Enterobacterales ^a	0.5–9%	<i>P. aeruginosa</i> (KPC)	
			<i>Acinetobacter baumannii</i>	21–90%		
			<i>Pseudomonas aeruginosa</i>	14–65%		

VIM Verona integron–encoded metallo-β-lactamase, *NDM* New Delhi MBL, *KPC* *Klebsiella pneumoniae* carbapenemase, *IMP* active-on-imipenem, *OXA* Oxacillinase

^a Species not indicated

Columbia, and the United States, and is responsible for 90% of all *K. pneumoniae* infections in Israel [48].

KPC gene variants are the most commonly globally encountered carbapenemase, with the *bla*_{KPC-2} and *bla*_{KPC-3} gene variants being the most common. Countries with a high prevalence of KPC-producing *K. pneumoniae* include the US, Europe (Italy, Greece), the UK, and Asia (China), as well as some countries in the Middle East (Israel) and South America (Brazil, Colombia) [12].

The first identification of an OXA-48-producing *K. pneumoniae* occurred as part of a reported outbreak in May 2006 to January 2007 [27]. In subsequent years, OXA-48-producing organisms remained largely restricted to healthcare systems in Turkey [27]. Since these initial reports, outbreaks of OXA-48-producing isolates have spread from Turkey to countries in the Middle East (Lebanon, Oman, Saudi Arabia, and Kuwait), as well as to countries in North Africa (Morocco, Tunisia, Egypt, and Libya). While OXA-48 harboring bacteria were initially geographically restricted to areas surrounding Turkey, OXA-48 producers have now been described in several European countries, such as France, Germany, Netherlands, Italy, Belgium, the UK, Ireland, Slovenia, Switzerland, and Spain. The first description of isolates with OXA-48-like (*bla*_{OXA-48}-like) genes in the US was from a surveillance study in 2013, which incidentally reported 2 *K. pneumoniae* isolates [49]. This description was followed shortly

afterward by a report of 2 clinical *K. pneumoniae* isolates with *bla*^{OXA-48}-like genes in patients in a Virginia institution who had traveled to both India and Saudi Arabia [29, 29]. The rapid dissemination of OXA-48 producers among Enterobacterales species in addition to *K. pneumoniae*, including *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *K. oxytoca*, *Serratia marcescens*, and *Providencia rettgeri*, suggests that the clinical significance of OXA-48 on antibiotic resistance may be more important than originally perceived. The OXA-48 enzyme hydrolyzes penicillins efficiently, carbapenems slowly, and extended-spectrum cephalosporins poorly, often resulting in variable antibiotic resistance patterns. Thus, recognizing the production of OXA-48 in isolates recovered from infections, and lack of routine screening processes for enterobacterial isolates with MIC values of ertapenem ≥ 0.5 mg/L, or imipenem or meropenem ≥ 1 mg/L, may result in an underestimation of the true prevalence and incidence of OXA-48-harboring organisms.

Environmental vectors of CR transmission also substantiate this suggestion. There is recent evidence in the US of wild bird (gull) feces harboring both plasmid-encoded *bla*_{KPC-2} or chromosomally-encoded *bla*_{OXA-48} genes [50]. A recent report linking the association of CRE to livestock continues to inform the epidemiology of carbapenem-resistance. Kock et al. [51] describe the overall prevalence of CRE at < 1% among livestock and companion animals in Europe, but note that CRE can also be found in

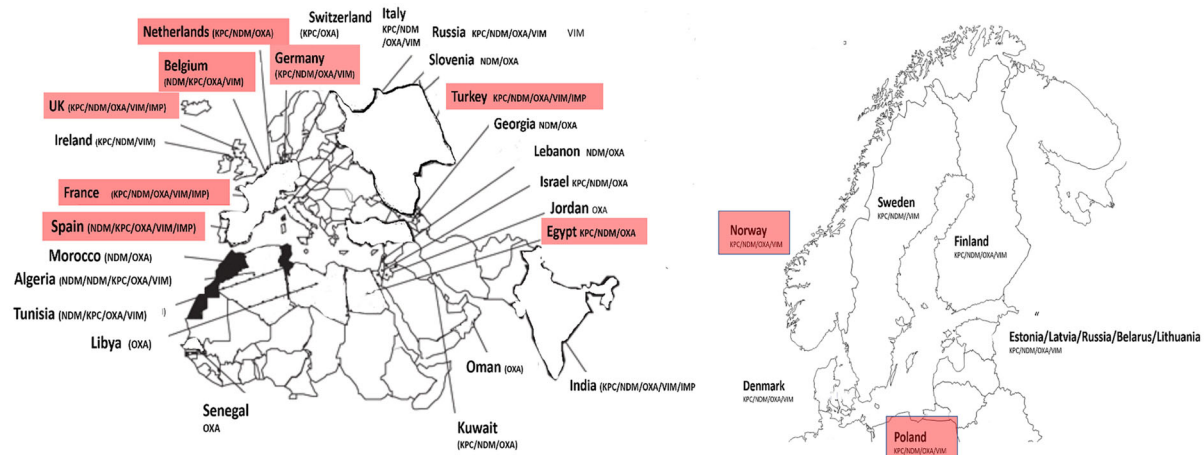


Fig. 5 Countries in which carbapenemase genes have been described are shown. Countries shown in red boxes represents cases in which outbreaks involving carbapenemase genes have been described. Figure of Europe adapted from [12, 12, 12, 12]

animals and livestock with a prevalence of 2–26% in Africa, and 1–15% in Asia. While wild birds (gulls) in Australia and Europe carried CRE in 16–19% [51], indicating that transmission of CRE between animals and humans may be an unknown reservoir and birds may become a vector for transmission of CRE, further research linking animal to human transmission is needed. Environmental sources of carbapenemases linked to recreational waters and sewage have also been reported as reservoirs for CRE [52]. Mahon and colleagues [52] identified NDM-producing *E. coli* and *K. pneumoniae* in recreational waters and sewers in Ireland. NDM-producing *E. coli* were indistinguishable between environment and human isolates, and *K. pneumoniae* isolated from sewage and waterways were closely related by pulsed-field gel [52].

Mani et al. [53] found KPC-producing Enterobacteriales in *Mytilus galloprovincialis* (a filter-feeding Mediterranean mussel) bought at a retail market in Tunisia, the *E. coli* isolate contained *bla*_{KPC} genes and was resistant to all β -lactams, ertapenem, meropenem, fluoroquinolones, sulphonamides, and trimethoprim. The isolate is most likely of human origin as the sampled mussels were grown in an area receiving hospital effluents; this is also supported by the global epidemiology of *bla*_{KPC} plasmids and KPC-producing *E. coli* clones, while carbapenems are not used in animals [53]. KPC-

producing *K. pneumoniae* have also been reported from wastewater in Austria and Brazil [54, 54]. These findings suggest that aquatic organisms may act as a secondary reservoir of KPC enzymes.

FUTURE PERSPECTIVES

Carbapenemases continue to evolve, and the global dissemination of mobile genes encoding for carbapenem-resistance results in infections that have increased morbidity and mortality [56].

Does carbapenem resistance foreshadow a return to a pre-antibiotic era? Carbapenemase-encoding genes are already widespread in certain parts of the world, with carbapenem-resistance rates in some European and Asian countries already exceeding 50%. In the US, infections with KPC-positive bacteria are a major health concern, validated by the reality that every state in the US has now reported infections with KPC-expressing *K. pneumoniae* (Fig. 1). However, unlike the pre-antibiotic era, newer therapies, based on carbapenemase inhibitors, such as vaborbactam, avibactam, and most recently relebactam, provide a response to the threat of carbapenemase-based resistance. Coupling carbapenemase inhibitors to existing antimicrobial backbone molecules, such as third-generation cephalosporins or

carbapenems, offer therapeutic options and extend the clinical lifespan of existing antibiotics.

It is likely that effective control and management of carbapenem-resistant infections will require multifaceted efforts rooted in active stewardship, ongoing surveillance, optimizing dosing, and rapid diagnostics. Rapid molecular testing is required to supplement phenotypic approaches to traditional susceptibility testing. The ability to quickly identify carbapenemase genes within Gram-negative bacteria, or direct from clinical specimens, decreases the time to appropriate therapy, which has been shown to decrease mortality [56, 56]. Coupling molecular testing with phenotypic testing allows the laboratory diagnosis to differentiate carbapenemases across different types, which is important, as novel beta-lactamases and beta-lactamase inhibitor combinations demonstrate differential activity against different classes of carbapenemases. While both Ehrlich and Fleming predicted the scourge of antibiotic resistance, contemporary tools and data can help to slow the insidious nature of carbapenem resistance.

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