Mobilization of Carbapenemase-Mediated Resistance in Enterobacteriaceae

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ABSTRACT There has been a dramatic increase in the last decade in the number of carbapenem-resistant Enterobacteriaceae, often leaving patients and their providers with few treatment options and resultant poor outcomes when an infection develops. The majority of the carbapenem resistance is mediated by bacterial acquisition of one of three carbapenemases (Klebsiella pneumoniae carbapenemase [KPC], oxacillinase-48-like [OXA-48], and the New Delhi metallo-β-lactamase [NDM]). Each of these enzymes has a unique global epidemiology and microbiology. The genes which encode the most globally widespread carbapenemases are typically carried on mobile pieces of DNA which can be freely exchanged between bacterial strains and species via horizontal gene transfer. Unfortunately, most of the antimicrobial surveillance systems target specific strains or species and therefore are not well equipped for examining genes of drug resistance. Examination of not only the carbapenemase gene itself but also the genetic context which can predispose a gene to mobilize within a diversity of species and environments will likely be central to understanding the factors contributing to the global dissemination of carbapenem resistance. Using the three most prevalent carbapenemase genes as examples, this chapter highlights the potential impact the associated genetic mobile elements have on the epidemiology and microbiology for each carbapenemase. Understanding how a carbapenemase gene mobilizes through a bacterial population will be critical for detection methods and ultimately inform infection control practices. Understanding gene mobilization and tracking will require novel approaches to surveillance, which will be required to slow the spread of this emerging resistance.

INTRODUCTION

After decades of worry, extreme drug resistance has become an unfortunate reality in many hospitals around the world. Rather than arriving in the form of vancomycin-resistant Staphylococcus aureus or pan-drug-resistant tuberculosis, extreme drug resistance has emerged in enteric Gram-negative bacilli. Essentially unheard of prior to 2003, in 2011 11% of Klebsiella pneumoniae isolates from intensive care units in the United States were resistant to carbapenems, with an attributable mortality rate of 40% from associated invasive infections (1–3). From a recent World Health Organization report, carbapenem resistance in Klebsiella pneumoniae had been seen in almost all countries that had data, and some countries reported carbapenem resistance rates of more than 50% (4). The Centers for Disease Control and Prevention (CDC) listed carbapenem-resistant Enterobacteriaceae as one of the three most urgent groups of drug-resistant microbes threatening human health in the United States (5).

The epidemiology of carbapenem resistance in Enterobacteriaceae around the globe has been dominated by the dissemination of three distinct Ambler classes of...
β-lactamases with carbapenem-hydrolyzing activity, the carbapenemases. In almost all cases, the genes for carbapenem resistance are carried not on the chromosome of a successful lineage of bacteria but rather on mobile elements of DNA which can be shared and spread between bacteria (6). This means that a previously susceptible member of the Enterobacteriaceae can acquire a single gene and become resistant to the majority of β-lactam antibiotics, including carbapenem.

Interestingly, the epidemiology around the globe has been slightly different, with unique carbapenemases having a different pattern of dissemination. This may be driven largely by the distinct mobile genetic location of the carbapenemase gene. The unique mobile elements surrounding the different carbapenemase genes may be best suited for specific bacterial host ranges and environments. The concept of tracking a gene of resistance rather than a strain or species of bacteria may be central to slowing the spread of resistance. In this chapter, I explore carbapenemase-mediated resistance in Enterobacteriaceae, including clinical context, epidemiology, and detection. I also examine the potential impact mobile elements have on the epidemiology of the extreme drug resistance and why understanding modes of genetic mobility may need to be considered in understanding the spread of these extremely antibiotic-resistant organisms.

**CLINICAL CONTEXT OF CARBAPENEMASES**

Enterobacteriaceae (e.g., Escherichia coli, Klebsiella, Enterobacter, Citrobacter, Serratia, and Proteus) are among the most frequently identified agents for a variety of serious bacterial infections. They account for 21% of all nosocomial infections (e.g., sepsis, ~30%; pneumonia, 15 to 20%; urinary tract infections, ~90%; and intra-abdominal infections, ~90%) (7–12). Carbapenem has long been held as the last-line agents against extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae. In general, carbapenem resistance negatively impacts patient outcomes (1, 13–15). In a large case-control study, carbapenem resistance increased overall mortality (48% versus 20%; *P* < 0.001) and attributable mortality (38% versus 12%; *P* < 0.001) for invasive *K. pneumoniae* infections matched with carbapenem-susceptible isolates (1).

Dissemination of carbapenemase genes is the primary cause of carbapenem resistance around the globe. A carbapenemase is a β-lactamase which has high affinity for carbapenem hydrolysis. Acquisition of a carbapenemase gene by Enterobacteriaceae may then result in the destruction and often clinical failure of this previously effective agent. Often these Enterobacteriaceae are multiderg resistant and other classes of antimicrobials are also ineffective, leaving the patient with few treatment options.

Carbapenemases can be placed into different classes depending on their biochemical and molecular characteristics, including metallocarbapenemase (e.g., New Delhi metallo-β-lactamase [NDM-1]), serine carbapenemase (e.g., *K. pneumoniae* carbapenemase [KPC]), and a group of penicillinases typically associated with carbapenem resistance in *Acinetobacter baumannii* (oxacillinase [OXA]) (16–18). In Enterobacteriaceae, the genetic elements encoding carbapenemases are typically contained within replicative transposons carried by plasmids that frequently harbor additional drug resistance determinants, thus enabling rapid horizontal transmissibility of multidrug resistance (19–24). Understanding the differences between the primary enzymes can be challenging but important in determining the antibiotic agents which may be effective. This will likely become more relevant as an increasing number of therapeutics which target specific carbapenemases become clinically available. For example, the traditional β-lactamase inhibitors, clavulanic acid, tazobactam, and sulbactam, do not have clinically relevant activity for metallo-β-lactamases, oxacillinases, or KPC, whereas the newly approved drug avibactam has strong affinity to inhibit KPC and therefore could be an effective therapeutic (25). The general classification of carbapenemases is shown in Table 1.

**TABLE 1** Ambler class β-lactamases with efficient carbapenem hydrolysis found in Enterobacteriaceae

<table>
<thead>
<tr>
<th>Class</th>
<th>β-Lactamase type</th>
<th>Frequent enzyme(s)</th>
<th>Obscure enzyme(s)</th>
<th>Spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Penicillinases</td>
<td>KPC</td>
<td>IMI, SME, GES</td>
<td>PCNs, cephalosporins, aztreonam, carbapenem</td>
</tr>
<tr>
<td>B</td>
<td>Metallo-β-lactamases</td>
<td>NDM, IMP, VIM, SIM, AIM, GIM</td>
<td>Same as above except aztreonam</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Oxacillinases</td>
<td>OXA-48, -181</td>
<td>OXA-163</td>
<td>+aztreonam, +cephalosporin</td>
</tr>
</tbody>
</table>

Abbreviations: KPC, Klebsiella pneumoniae carbapenemase; IMI, imipenemase; SME, Serratia marcescens enzyme; GES, Guiana extended spectrum; PCN, penicillin; NDM, New Delhi metallo-β-lactamase; IMP, active on imipenem; VIM, Verona integron-encoded metallo-β-lactamase; SIM, Seoul imipenemase; AIM, Adelaide imipenemase; GIM, German imipenemase; OXA, oxacillinase.

AMB class C cephalosporinases do not demonstrate efficient carbapenem hydrolysis and therefore are not listed.

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DISTINCT EPIDEMIOLOGIES OF THE MAJOR CARBAPENEMASES

The focus of this chapter is on the three most widely seen and rapidly emergent carbapenemases: NDM, KPC, and oxacillinase-48-like (OXA-48-like). The genes (bla) that encode these three carbapenemases all demonstrate unique epidemiological stories.

KPC has been the most frequently reported carbapenemase, with the highest number of clinical cases around the globe. KPC was first described in North Carolina in a case in 1996 (26). There were no further reports or identified isolates until 2001, when a few carbapenem-resistant K. pneumoniae isolates were seen in New York City. By 2005, KPC was carried by half of the K. pneumoniae organisms in some intensive care units in multiple New York City hospitals (27). Around the same time, KPC-producing Enterobacteriaceae were described in the Caribbean, South America, China, and Israel (28–30). Initial outbreak descriptions were dominated by a highly related strain of K. pneumoniae. This high-risk clone, multilocus sequence type 258 (ST258), has been successfully transmitted around the globe and has dominated almost all locations where KPC has been described. The majority of clinical cases are still confined to patients who have hospital exposure, complex medical histories, and receipt of antibiotics (31, 32). The regions of endemicity have increased in number and now include Italy and Greece (Fig. 1A). Israel, which undertook a national effort to eliminate the spread, has been the only country with a decrease in the number of infected patients. However, KPC-producing ST258 K. pneumoniae still persists (33).

The OXA-48-like enzymes present a different challenge for understanding transmission and gene movement as several of the alleles likely arose independently and should likely be considered several different outbreaks with unique epidemiology. The focus here is on blaOXA-48 and blaOXA-181. The first case of a blaOXA-48-positive K. pneumoniae isolate was seen in Turkey in 2001 (34). It then spread to Western Europe and escaped detection for a period of time, as the enzyme does not always confer full carbapenem resistance or cephalosporin resistance, and was found in multiple species (35). This enzyme has now been described as the dominant carbapenemase in Enterobacteriaceae in Northern Africa, the Gulf States of the Persian Gulf, and some countries in Western Europe (35, 36) (Fig. 1B). It does appear that in most described outbreaks, patients have had health care exposure (37). The other major class D carbapenemase in Enterobacteriaceae is blaOXA-181. This gene has been described for several isolates from India and is often coproduced with another carbapenemase, such as NDM (38, 39). Less is known about the full epidemiology of this enzyme, but it has been described in multiple genetic contexts and likely arose relatively independently of blaOXA-48 (40).

NDM is the most recent carbapenemase to be identified after a description of isolates in India, Pakistan, and the United Kingdom in 2008 (22). The epidemiology of NDM differs from that of the non-metallo-β-lactamases, as it has been seen in multiple species and epidemiologic locations. This enzyme has been found in drinking water in India, and although most clinical descriptions of infection involve nosocomial transmission, it appears that the barrier for community acquisition is likely lower and the enzyme does not remain confined to health care-exposed patients only (41). Microbiologically, NDM has also been seen in multiple Enterobacteriaceae species and even within the same host has a high degree of species and strain variability (42). The global epidemiology of this enzyme has largely dominated India, Pakistan, and Southeast Asia, but the enzyme has also been frequently imported, with resultant outbreaks (43) (Fig. 1C). It appears too early in the outbreak to understand completely if this enzyme will have global dominance similar to that of KPC; however, there is no barrier to this occurring.

CHALLENGES OF DETECTION OF A CARBAPENEMASE IN A CLINICAL MICROBIOLOGY LABORATORY

Determination of antibiotic sensitivities in a clinical microbiology laboratory is most often done by assessing the MIC against the antibiotic tested. The MIC at which an organism is considered susceptible in the United States is governed by the Clinical Laboratory and Standards Institute (CLSI) and Food and Drug Administration (FDA). The MIC cutoff for susceptibility is based largely on drug levels achievable in serum and, when possible, animal model and/or clinical outcome data (44). When PCR-confirmed KPC-producing bacteria began to spread, it was soon recognized that many isolates were in the range of susceptible (45–47). Reports of clinical failures soon followed, describing infections with KPC-producing Enterobacteriaceae treated with a carbapenem alone despite a MIC within the range achievable in serum (48–51). The inability of a MIC alone to predict clinical success in the setting of carbapenemases compelled the CLSI to generate an alternate approach (52).

In 2009, the CLSI recommended that clinical laboratories perform a phenotypic carbapenemase test for...
Enterobacteriaceae suspicious for carbapenemase production but falling within a range of previously determined susceptibility (53). However, phenotypic tests can give false-positive and false-negative results as to the presence of a carbapenemase, and results vary depending on the experience of the laboratory technician. In

**FIGURE 1** Global epidemiology of three major carbapenemase enzymes in Enterobacteriaceae: (A) Distribution of KPC; (B) distribution of OXA-48; (C) distribution of NDM. Legend colors (darkest to lightest): endemic, multiple outbreaks, sporadic, unknown.

*Figure 1 continues on next page*
June 2010, the CLSI amended their former recommendations and effectively replaced phenotypic testing for carbapenemase production with lower breakpoints (54). The lower breakpoints have better sensitivity for carbapenemase detection in clinical isolates and also make it possible for testing to be done in all clinical laboratories. However, this creates a problem of understanding the dissemination of carbapenemases within a hospital and/or within a region. Another issue which arises occurs because not all carbapenem resistance in Enterobacteriaceae is due to acquisition of a carbapenemase gene. The other major mechanism of carbapenem resistance in Enterobacteriaceae is through a combination of decreased outer membrane permeability and increased nonspecific β-lactamase activity (55–58). However, carbapenemase-producing Enterobacteriaceae are thought to be at higher risk for nosocomial dissemination, as maintaining porin loss confers fitness cost to the bacteria, making this mechanism a lower risk for causing a sustained outbreak (56). Therefore, it is ideal to understand when a carbapenemase is present and apply strict epidemiologic interventions to prevent nosocomial spread (59, 60). If continued carbapenemase inhibitors with a specific spectrum become therapeutics (e.g., avibactam), understanding the enzyme present may be relevant in clinical decision making.

Other challenges with detection arise due to the bacterial species variability of isolates which carry a carbapenemase. For example, in my institution, investigators have seen multiple strains and species with various degrees of phenotypic resistance from multiple mechanisms, and therefore, the task of identification for a clinical laboratory is often overwhelming (61, 62). Guidance from the CDC on clinical microbiology screening has recommended that laboratories focus on K. pneumoniae and E. coli; however, depending on the outbreak, a carbapenemase gene can move among different species and strains, and this could miss many isolates depending on the outbreak (63). This is in direct contrast to resistance in some Gram-positive organisms, such as Staphylococcus aureus, in which the presence of mecA is responsible for almost all methicillin resistance seen globally. Also, even with the lowered breakpoints, not all of the isolates that carry a carbapenemase will demonstrate full phenotypic resistance to all carbapenems by susceptibility testing (62). It is very difficult for laboratory technicians to notice all variability across species with carbapenem resistance and know when an isolate needs further investigation, especially at small community hospital laboratories.

Screening with a molecular diagnostic could address many of these issues, but to run a PCR on all isolates would be extremely costly for laboratories where there are finite resources (64). This therefore would lead back to labs having the ability to identify high-risk isolates to target expensive molecular diagnostics. Reporting the molecular information also implies the presence of clinicians and infection control practitioners who can then interpret the data and this expertise may not be present in all hospitals around the world.
The final issue which is related to detection of carbapenemase-producing *Enterobacteriaceae* arises from our current approaches to molecular epidemiology of outbreaks and transmission. Most of the tools of molecular epidemiology relate to tracking a group of highly related bacteria based on chromosomal DNA. However, the carbapenemase gene rarely associates with the chromosomal DNA and can mobilize to different strains even during an outbreak (24). Even with the high resolution of whole-genome sequencing, tracking mobile elements through a bacterial population takes a great effort and requires costly long-read sequencing to fully resolve structures. Even when the mobile elements from outbreak strains can be fully analyzed, if there are gaps in the strains collected, it can be difficult to apply molecular epidemiology to string together relatedness and evolution to understand routes of transmission.

**THE IMPACT OF THE GENETIC CONTEXT ON MOBILITY OF DIFFERENT CARBAPENEMASE GENES**

As described above, the three most globally relevant carbapenemase genes have very different epidemiologies. This may be driven largely by the genetic environment surrounding the gene rather than the carbapenemase gene or enzyme it produces. *Enterobacteriaceae* have an incredible ability to evolve through horizontal gene transfer (Fig 2). With antibiotics widely used in health care and agriculture, acquisition of a plasmid which encodes antibiotic resistance can provide a selective advantage, but there may be other, unknown selective advantages for particular environments which are not as readily apparent. KPC, NDM, and OXA-48 are most frequently described to occur on plasmids which can mobilize between bacteria via conjugation. The range of species that will maintain any given plasmid is thought to be dependent on interplay between the host range of the plasmid and the bacterial host species. In addition, all three carbapenemase genes discussed at length in this chapter are located within a mobile transposon within a plasmid. A transposon can also mobilize to new genetic locations within a bacterial cell and integrate into DNA, which can provide the potential for further benefit to the bacterial host.

As described above, the majority of KPC outbreak descriptions detail a highly successful strain of *K. pneumoniae* as being introduced and disseminating among patients in a health care setting. This occurs even though the KPC gene is carried on a mobile plasmid which could disseminate among other species. Plasmid transfer via conjugation occurs at a surprisingly low rate clinically.

**Figure 2** Schematic of mechanisms of mobility of carbapenemase genes in *Enterobacteriaceae*.
It appears that the most common \( \text{bla}_{\text{KPC}} \) plasmid, pKpQIL, may provide additional benefit to the \( K. \ pneumoniae \) strain \( (65) \). When the \( \text{bla}_{\text{KPC}} \) plasmid is lost, the ST258 strains do not appear as well suited for success in a health care environment \( (66) \). The plasmids that carry \( \text{bla}_{\text{KPC}} \) in the ST258 \( K. \ pneumoniae \) are all also narrow-host-range type plasmids, which may also provide insight into why \( \text{bla}_{\text{KPC}} \) plasmids do not frequently mobilize to other species from ST258 \( K. \ pneumoniae \) \( (63) \).

The epidemiology of OXA-48 has also been potentially driven by the genetic environment rather than the gene itself. Original descriptions of \( \text{bla}_{\text{OXA-48}} \) were in differing strains of \( E. \ coli \) and \( K. \ pneumoniae \) with a conserved broad-host-range IncL/M, 62.5-kb plasmid \( (67) \). In contrast to the initial KPC descriptions, there were multiple strains and species involved as the outbreak evolved, while the plasmid remained relatively conserved, indicating likely plasmid mobilization and maintenance in multiple bacterial backgrounds. The OXA-48 gene is also located within a mobile composite transposon, Tn1999. Interestingly, in the widely spread \( \text{bla}_{\text{OXA-48}} \) IncL/M plasmid, Tn1999 is inserted into a transfer inhibition \( (\text{tir}) \) gene, which functionally decreases conjugation rates \( (68, 69) \). This tir gene disruption likely also contributes to the high rate of plasmid mobility which has promoted the multispecies epidemiology of the \( \text{bla}_{\text{OXA-48}} \) outbreak seen in Europe and Northern Africa.

Finally, \( \text{bla}_{\text{NDM}} \) has been identified in the largest number of different bacterial contexts and on multiple different plasmids, indicating that the mobility of the transposon associated with \( \text{bla}_{\text{NDM}} \) has been the most promiscuous from the outset. For example, one review found at least eight different incompatibility plasmid types and 22 different \( \text{bla}_{\text{NDM}} \) plasmid sizes in \( E. \ coli \) and \( K. \ pneumoniae \) from around the world \( (70) \). The immediate genetic environment adjacent to \( \text{bla}_{\text{NDM}} \) consists of an insertion sequence (ISAba125) and a bleomycin resistance gene. The origin of \( \text{bla}_{\text{NDM}} \) mobility may have developed in \( Acinetobacter baumannii \) with a composite transposon, Tn1225, where \( \text{bla}_{\text{NDM}} \) is located between two copies of ISAba125. Several truncated versions of the Tn1225 have been described in almost all worldwide descriptions of \( \text{bla}_{\text{NDM}} \) \( (71) \), indicating that it was likely truncated over time after originating in \( A. \ baumannii \). NDM has been identified with a much higher degree of epidemiologic and genetic diversity than in the majority of descriptions of OXA-48 and KPC. A recent account described NDM in \( Pseudomonas aeruginosa \), \( Acinetobacter \) spp., and \( Aeromonas \) spp. as well as \( Enterobacteriaceae \) in multiple locations in the environment in Dhaka \( (72) \). Some of the environmental success has been attributed to the bleomycin gene, which may be advantageous for an environmental organism, as there could be low-level bleomycin-like compounds which result in bacterial toxicity \( (70) \). The high degree of horizontal gene transfer associated with \( \text{bla}_{\text{NDM}} \) is not confined to the environment, as this diversity has also been described clinically with a recent report of 11 different bacterial strains with multiple different plasmid types all carrying \( \text{bla}_{\text{NDM}} \) isolated from just four patients in Pakistan \( (42) \).

**CONCLUSIONS**

A recent World Health Organization report states that antimicrobial resistance is “a problem so serious that it threatens the achievements of modern medicine” \( (4) \). Ultimately, the medical community faces many new challenges posed by the wide dissemination of very successful genes of drug resistance between bacteria. Approaching this new type of clinically relevant resistance as gene-based outbreaks will challenge our current models of surveillance, molecular tracking, and detection. We will be required to realize the factors which contribute to horizontal gene transfer to understand the epidemiology and therefore the approach to slow the spread of these genes. The spread of organisms that carry multiple antimicrobial resistance mechanisms is anticipated to be more widely seen around the globe in the coming years, likely with significant clinical consequences. Carbapenemase-producing \( Enterobacteriaceae \) have all the qualities of an emerging infectious pathogen, with the ability to evolve and adapt central to their global success. Our ability to control the threat of continued spread will present new frontiers for the microbiology community and require that we adapt our methods of detection, molecular epidemiology, and infection control in ways that we have not yet fully realized but will likely bring new challenges.

**REFERENCES**


