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REVIEW

Overview of the epidemiology and the threat of *Klebsiella pneumoniae* carbapenemases (KPC) resistance

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Abstract: *Klebsiella pneumoniae* carbapenemases (KPCs) confer resistance to nearly all β-lactams. This broad-spectrum drug resistance mechanism has rapidly spread in the United States and is reportedly increasing elsewhere in the world. Thus, the emergence of KPC resistance is a major threat to global health. This article reviews the epidemiology and provides an overview of the dissemination of KPC-producing organisms.

Keywords: beta-lactam resistance, carbapenemase, drug resistance, epidemiology, treatment failure

Introduction

Carbapenems are often used to treat infections caused by Gram-negative bacteria that produce extended-spectrum β-lactamases (ESBL). A new class of bacterial enzymes capable of inactivating carbapenems, known as *Klebsiella pneumoniae* carbapenemases (KPCs), has rapidly spread in the United States and is increasing elsewhere in the world. KPCs are Ambler molecular class-A carbapenemases[1] that typically reside on transferable plasmids and can hydrolyze all penicillins, cephalosporins, aztreonam, and carbapenems. Infections caused by KPCs have very limited options for treatment and often require the use of polymyxins, which fell into disuse in the 1970s due to high rates of nephrotoxicity. The epidemiology and molecular characteristics of KPC-producing organisms are still evolving and are intensively studied by researchers. The following review describes the current understanding of the epidemiology, genetic diversity, and clinical implications of the drug resistance mediated by KPC-type β-lactamases.

Microbiologic and molecular characteristics of KPC-producing organisms

Klebsiella pneumoniae is the most common organism associated with KPC resistance determinants. Molecular studies have shown that many *K. pneumoniae* isolates with KPC enzymes belong to a single clonal complex, CC11, and predominantly to a single sequence type, ST 258.[2] However, KPCs are increasingly reported in other genera of the *Enterobacteriaceae* family, such as *Escherichia*,[3] *Proteus*,[4] *Serratia*,[5] *Salmonella*,[6] and *Citrobacter*.[7] Worse still, KPC resistance has been reported in inherently-resistant organisms such as *Acinetobacter baumannii* and *Pseudomonas* spp.[8,9] For example, Akpaka and colleagues from Trinidad studied an isolate of multidrug-resistant *P. aeruginosa* that harbored a novel KPC-6 gene.[10] Interestingly, the isolate was obtained from a patient who was hospitalized following a traumatic hip fracture who reported no

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recent history of travel, suggesting that the specific isolate of KPC-producing *Pseudomonas* emerged locally, and likely continued to circulate in that region of the world.

The KPC gene was first identified from an isolate in North Carolina and was named KPC-1.[11] Subsequent variants of the KPC gene were named in sequential numeric order from KPC-2 to KPC-11 (Table 1). However, a recent correction in the gene sequence of KPC-1 revealed that KPC-1 and KPC-2 were in fact identical enzymes; hence, the KPC-1 designation is no longer used.[12]

Evidence derived from minimal spanning tree analysis showed that KPC-2 was most likely the ancestral origin from which other KPC variants were derived. Currently identified KPC variants only differ from KPC-2 by minor nucleotide changes within four codons (nucleotides 147, 308, 716, and 814).[13] For example, KPC-3 differs from KPC-2 by only one amino acid substitution (814C to 814T); KPC-6 differs from KPC-2 by a different amino acid substitution (716T to 716G).[14]

KPC enzymes also differ in their kinetic properties and vary in their efficiency to hydrolyze different β-lactams. For example, KPC-2 appears to be a unique carbapenemase that is also not inhibited by currently available β-lactamase inhibitors such as clavulanic acid;[15] KPC-3, however, demonstrates greatly heightened hydrolytic activity against ceftazidime (approximately 30 times higher than KPC-2 enzyme).[16] Moreover, genetic changes associated with the KPC gene are stable in the wild and are increasingly common because antibiotic resistance conferred by KPC is adaptive for the organism against ongoing antibiotic selection pressure.

Of the 10 genetic variants of KPC identified thus far, KPC-2 and KPC-3 are the most common in clinical specimens and account for most epidemic outbreaks. KPC-2 appears to be more predominant worldwide, with outbreaks arising not only within the United States but also in Europe (especially Greece) and China. KPC-3 is mainly detected in the United States, Latin America, and Israel.

Table 1 *Klebsiella pneumoniae* carbapenemase (KPC) resistance genotypes listed by year of first identification and geographic distribution

blaKPC	Year of identification	Distribution
KPC-1	1996	USA (North Carolina)
KPC-2	1998–1999	USA, Israel, China, Greece, Italy, Brazil, France, Colombia, Taiwan
KPC-3	2000–2001	USA, Israel
KPC-4	2003	Puerto Rico, Scotland
KPC-5	2006	Puerto Rico
KPC-6	2003	Puerto Rico
KPC-7	2007–2008	USA
KPC-8	2008	Puerto Rico
KPC-9	2009	Israel
KPC-10	2009	Puerto Rico
KPC-11	2010	Greece

The rapid global dissemination of KPC genes is staggering. This observation can be attributed to a combination of three major social and microbiological mechanisms: international travel, patient-to-patient transmission of KPC-producing organisms, and interspecies transfer of KPC-resistant elements.[17–19] Horizontal transfer of KPC resistance is a fascinating microbiological phenomenon that has devastating public health implications. Horizontal transfer of KPC can occur between bacteria because KPC resistance elements are often flanked by transposons (particularly Tn*4401*) and are carried on transferable plasmids of Gram-negative organisms.[7,20] Worse still, many plasmids that carry KPC resistance elements concurrently carry other plasmid-mediated resistance elements, such as quinolone (QnrA and QnrB) and aminoglycoside (rmtB) resistance.[21,22]

Epidemiology

Carbapenem resistance due to KPC has evolved rapidly since 2001. The distribution of KPC resistance determinants now vary substantially by geography. Although KPC is reported in most countries around the world, two divergent epidemiological patterns are seen: regions that report very few KPC-producing isolates (Australasia and Africa) and areas where KPCs are now considered endemic (northeastern USA, Puerto Rico, Greece, Zhejiang Province of China, and Israel).

North America

The first isolate of KPC-producing bacteria was discovered through the Intensive Care Antimicrobial Resistance Epidemiology (ICARE) surveillance program in a clinical specimen of *K. pneumoniae* from North Carolina in 1996.[11] KPCs were infrequently isolated until 2001 when KPC-producing *Enterobacteriaceae* were reported in several extended clonal outbreaks in metropolitan hospitals located in the states of New York and New Jersey.[23–25] Soon after, KPC-producing organisms spread rapidly along the east coast and then became widely disseminated westward and throughout the country. KPC-producing organisms have now been reported in 38 out of the 50 states of the US.[26]

Puerto Rico is a small island territory of the United States but it is surprisingly overrepresented with the number of reports and unique clones of KPC-producing organisms. For example, first isolates of KPC-4, -5, -6, -8, and -10 were

identified in Puerto Rico.[27] Furthermore, an island-wide surveillance study of β-lactam resistance in 2009 showed widespread KPC dissemination. Of 10,507 isolates submitted to the study, 12% of isolates were categorized as resistant to multiple beta-lactams. Notably, KPC was detected in 43% of isolates with resistant to multiple beta-lactams, including isolates of *Escherichia coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii.*[28] KPC-producing organisms are truly endemic in Puerto Rico.

Canada, which shares a border with the United States, has only reported sporadic cases and limited outbreaks with KPC-producing organisms in hospitals in Ottawa,[18] Toronto,[29] and Montreal.[30] Although the true epidemiology of KPC resistance in Canada is not completely understood, the prevalence of KPC resistance is thought to be low.

Europe

The first KPC-producing organism detected outside of the United States occurred in an isolate of *K. pneumoniae* that produced KPC-2 in Paris, France.[17] Interestingly, the patient was hospitalized in a New York City hospital 2 months prior, suggesting that the resistant organism was imported from the USA. Subsequent surveillance data have shown that the prevalence of KPC organisms in Western Europe is generally low. However, there are several European regions with endemicity and outbreaks with KPC organisms.[31]

Greece has the highest prevalence of carbapenem-resistant *K. pneumoniae* in Europe. In 2010, it was estimated that approximately 49% of all *K. pneumoniae* isolates from Greece reported to the European Centre for Disease Control and Prevention were carbapenem-resistant. KPC (particularly KPC-2)-producers were believed to be responsible for approximately 53% of the isolates with carbapenem resistance; VIM-producing organisms were responsible for the remaining 47%.[31–33] Epidemiological data from Athens, the capital of Greece, has reported disproportionately high numbers of KPC organisms. The city is thought to be an epicenter of carbapenem-resistant *Enterobacteriaceae* (CRE) in Greece.

The same 2010 European Centre for Disease Control and Prevention report showed that 15.2% of *K. pneumoniae* isolates in Italy were resistant to carbapenems. It is believed that Italy now has low-level KPC endemicity following the first report of KPC-3-producing *K. pneumoniae* in Tuscany in 2008. Indeed, dissemination of KPC producers continued relentlessly in Italy. In 2010, identical strains of KPCs were detected from seven different regions around Italy.[34]

The prevalence of carbapenem-resistant organisms in other parts of Europe is generally confined to sporadic imported cases.[35] In the United Kingdom, the New Delhi metallo-β-lactamase 1 (NDM-1) seems to be the dominant carbapenemase among *Enterobacteriaceae*.[34] In France, outbreaks involving organisms producing KPC-2 enzymes have been reported; some of these French cases were imported from Greece or the USA.[17,36,37]

Middle East

The first report of KPC in Israel was in a returned traveler who received health care in New York in 2005. However, since the first reported case, both KPC-2 and KPC-3 have firmly established endemic status in Israel.[38] Similarly to the USA, a single clone (ST258) of KPC-producing *K. pneumoniae* is responsible for most isolates in Israel. Fortunately, the Israeli government and its health care systems recognized the threat posed by KPCs and implemented a nationwide reporting system. Following the implementation of wide-ranging control measures, the occurrence of KPCs in Israel had reduced and stabilized.[39]

South America

The first report of KPC in South America was detected in a pair of KPC-2-producing *K. pneumoniae* isolates in Colombia in 2006.[40] Since then, KPC-mediated resistance has become widespread in different parts of Colombia and has been reported in different clones of *Enterobacteriaceae* and *Pseudomonas* spp.[41] Similarly, Brazil and Argentina have reported sporadic cases and clusters of infections with KPC-mediated resistance in different types of Gram-negative pathogens, including *K. pneumoniae*, *E. coli*, and *Pseudomonas* spp.[42,43]

Asia

As a region, Asia has widely disseminated KPC resistance elements.[44] Both Korea and China have reported KPC in a variety of Gram-negative organisms, including *Pseudomonas* spp. and numerous genera of *Enterobacteriaceae*, including *Proteus mirabilis*, *Enterobacter* spp., *Morganella morganii*, *Serratia marcescens*, and *Citrobacter freundii*.[45,46] Interestingly, the predominant type of *K. pneumoniae* associated with KPC production in China is ST11, which is closely related to ST258 found more commonly elsewhere in the world.[47] Zhejiang, a southeastern province, appears to be the epicenter for KPC-producing organisms in China.[22,48] Taiwan has reported imported cases of KPC related to visitors to the Zhejiang province in China.[49]

Although the Indian subcontinent has been linked as the source for imported cases of KPC to other countries (eg, France), recent data indicate carbapenem-resistance in this region is predominantly due to NDM-1.[50,51]

Australasia

KPC-producing organisms have not been widely reported in Australia or New Zealand; sporadic cases have resulted from travel through KPC endemic areas. For example, the first isolate of KPC in Australia was detected in an isolate of *K. pneumoniae* from a returned traveler who was hospitalized during a holiday to Greece in the prior year.[52] Similarly, the first isolate of KPC in New Zealand was *K. pneumoniae* harboring KPC-2 resistance obtained from a patient repatriated from a Chinese hospital.[53]

Clinical and epidemiologic characteristics of KPC infections – lessons from outbreaks

KPC-producing organisms can colonize and cause infections similarly to their wild-type counterparts. Many infections are either systemic infections, occurring in patients with multiple invasive devices, or urinary tract infections without an indwelling catheter, particularly in immunocompromised patients.[54,55]

KPC-producing organisms rarely manifest as community-onset infections in nonendemic regions without any prior health care contact.[56] Indeed, risk factors for colonization or infection with KPC-producing bacteria are all associated with health care, including exposure to antibiotic therapy, prolonged hospitalization, intensive care unit stay, immunosuppression, and organ transplantation.[54,57,58]

Data from an outbreak investigation in Chicago showed that admission to long-term care facilities was a risk factor for KPC transmission and acquisition. The investigators aptly summarized that long-term care facilities can act as a "point of convergence of patients at high risk; as an amplifier, by cross-transmission of KPC-positive cases; and as a facilitator of regional dissemination through discharge of patients harboring KPC-producing *Enterobacteriaceae* to multiple nursing homes".[3,59]

Detection of KPC-producing organisms

The spread of KPC-producing organisms in health care settings represents a serious infection control issue. Detection of KPC-harboring organisms remains challenging due to several factors. First, KPC carbapenemases may not confer resistance to carbapenems but confer only reduced susceptibility.[25,60] Additionally, various bacteria express KPC-encoded β-lactam resistance differently. For example, a KPC-producing *E. coli* isolate may have an ertapenem minimal inhibitory concentration of 1 mg/L, whereas this value for a KPC-producing *K. pneumoniae* may be 8 mg/L. Thus, heterogeneous expression of KPC-derived resistance among different bacteria makes it extremely challenging to designate a uniform cut-off point for phenotypic detection of resistance. These problems may be reduced with revision of carbapenem breakpoints for *Enterobacteriaceae*.[61]

Second, some phenotypic tests may mistakenly label KPC producers as ESBL producers.[60] Third, imipenem- and meropenem-based automated susceptibility testing (AST) techniques may lack sensitivity for detecting KPC-producing organisms. A study from Israel showed that meropenem-based susceptibility missed 24% of KPC-producing *Enterobacter* spp.[55] In contrast, cumulative data showed that ertapenem is the most suitable substrate for screening of carbapenemase activity, even though ertapenem resistance itself did not consistently correlate with KPC expression. Other causes of phenotypic ertapenem resistance may include coexistence of ESBLs or AmpC β-lactamases and outer membrane protein alterations. In light of increasing supportive evidence, laboratories using AST should switch to ertapenem to improve screening for KPC-producing Gram-negative bacilli. Laboratories must remain vigilant regarding the pitfalls of many AST systems whereby KPC carbapenemases may not be detected if low inocula of the organism are used in automated testing.[25,54,62] Laboratories not using AST may consider a new CHROMagar (Paris, France) KPC screening technique that was shown to be 100% sensitive and 98.4% specific compared to KPC polymerase chain reaction (PCR).[63]

The final challenge in detecting KPC-producing organisms lies in the fact that isolates with positive results on screening tests still need to be confirmed as KPC producers. The modified Hodge test, a boronic acid-based test or a double-disc synergy test using β-lactamase inhibitors, such as clavulanic acid or tazobactam, have been used as confirmatory tests.[64] These tests have limitations; paramount amongst these are that they are not specific for KPC production. Molecular tools, such as PCR, are the only way to definitively confirm the presence of genes encoding KPC resistance elements.

These difficulties in KPC detection may lead to an underestimation of the true prevalence and incidence of KPC-producing organisms as well as the duration of a KPC epidemic.

Implications for therapy and clinical outcomes of KPC infection

KPC confers broad-spectrum resistance to β-lactams and carbapenems. Furthermore, KPC producers frequently carry additional genetic determinants, which confer resistance to other antibiotics, such as fluoroquinolones, aminoglycosides, and cotrimoxazole.[21,33,48]

Few safe and practical therapeutic options remain for patients infected with KPC producers. Many clinicians have resorted to the use of tigecycline, polymyxins, and the few remaining aminoglycosides for the treatment of KPC-producing organisms.[65]

Tigecycline, a glycylcycline antibiotic, consistently shows in vitro activity against most isolates of KPC-producing organisms. However, several shortcomings limit the use of tigecycline. For example, tigecycline does not achieve high serum or urinary concentrations after infusion.[66,67] A recent study placed additional uncertainty on the clinical efficacy of tigecycline as an antibiotic for hospital-acquired infections. Freire et al compared rates of curing hospital-acquired pneumonia in patients receiving tigecycline against those receiving imipenem–cilastatin. The multicenter, randomized, double-blind study showed that tigecycline was inferior to the comparator drug using a clinical evaluable study population. In addition, there were more gastrointestinal adverse effects (approximately twice as often as imipenem–cilastatin).[68] Furthermore, a separate pooled analysis of all Phase III and IV studies of tigecycline showed higher all-cause mortality (4.0%) in patients who received tigecycline compared to patients who received comparator drugs (3.0%) (95% confidence interval [CI]: 0.1–1.2). These findings have led to the issue of a black box warning from the US Food and Drug Administration.[69] Finally, treatment emergent resistance to tigecycline has been reported; thus, clinicians should remain vigilant for clinically refractory infections when tigecycline is being used.[70] Despite these limitations, tigecycline has been used to successfully treat and cure patients infected with KPC-producers.[71]

Polymyxins, such as colistimethate and polymyxin B, are another class of drugs that have been used successfully to treat KPC-producers. These drugs are active against most genera of Gram-negative aerobic bacilli with the exception of *Proteus* spp. Additionally, polymyxins have poor activity against *Serratia*, *Morganella*, *Providencia*, *Burkholderia*, *Vibrio*, *Brucella*, *Helicobacter*, *Moraxella*, *Aeromonas*, and *Edwardsiella*.

Polymyxin B and colistin have been increasingly used, but they are associated with high rates of nephrotoxicity and have been considered drugs of last resort. There are recent data to show that the polymyxins may not be as nephrotoxic as previously thought; however, optimal dosing of polymyxins is not known. The optimal dosing regimens for colistin are currently under evaluation.[72]

Fosfomycin has been used successfully to treat KPC-producing organisms that still showed in vitro susceptibility. A study showed that fosfomycin retained in vitro activity against 93% of KPC-producing isolates collected in the USA in 2009.[73] Notably, five out of six extremely drug-resistant KPC producers nonsusceptible to tigecycline and colistin were susceptible to fosfomycin. This drug causes very little toxicity and penetrates tissues readily; the concern with fosfomycin, however, is the propensity for resistance to rapidly develop when it is used in monotherapy. Thus, many investigators have proposed using fosfomycin in combination with other agents, such as aminoglycosides, since synergism has been demonstrated.[74]

Surveillance data show that the majority of KPC-producing isolates remain susceptible to polymyxins, and tigecycline. However, there are increasing worrisome reports of colistin-resistant and tigecycline-resistant isolates of KPC infections. For example, investigators in Greece reported that 18.6% of 301 KPC isolates they studied were resistant to colistin and that the prevalence of colistin resistance among KPC producers had increased from 3.5% in 2008 to 20.8% in 2010.[33] Furthermore, pan-resistant KPC-isolates have been sporadically reported around the world.[75]

Combination therapy of different antibiotics may improve the chances of cure in highly resistant infections. Separate analyses from researchers in Pittsburgh and from Greece demonstrated that combination therapy may be associated with improved survival and increased chance of cure of infection due to KPC-producers.[76,77]

Whether monotherapy or combination therapy is used, the outcomes associated with infections associated with KPC-producing organisms remain very poor compared to infections due to non-KPC-producing strains. For instance, the overall mortality in patients with KPC-associated infections has been estimated to be between 22% and 59%.[78,79]

A number of new antibiotics are under development for KPC producers. These include combinations of existing β-lactam antibiotics with new β-lactamase inhibitors able to inhibit KPC. These studies are now in Phase III clinical development. Additionally, neoglycosides (novel aminoglycosides) are under development and have activity against KPC producers.

Prevention and infection control

Containment of KPC-producing organisms is a key priority and has been a focus of intense study and policy making. The Centers for Disease Control and Prevention has updated its guidance for control of carbapenem-resistant *Enterobacteriaceae* (CRE). The interventions emphasize the need to develop region-wide activity and facility-level CRE prevention.[64] The updated guidance recommends a set of core measures for all acute and long-term care facilities, including: (1) enhancing performance of hand hygiene, (2) placing CRE-colonized or CRE–infected patients on contact precautions, (3) judicious use of patient and staff cohorting if required, (4) minimize use of invasive medical devices, (5) promotion of antibiotic stewardship, and (6) screening for patients with risks for CRE. Facilities with documented transmission of CRE can undertake two additional measures: (1) active surveillance for CRE and (2) use of chlorhexidine bath or wipes.

A good example of success in controlling KPCs came from Kochar et al[80] from the State University of New York Downstate Medical Center who used a combination of the above interventions to curtail an outbreak of multidrug-resistant bacteria that included KPCs in their intensive care unit. The authors performed rectal swab screening for all new admissions to the intensive care unit and repeated the surveillance cultures weekly. KPC-infected or colonized patients were cohorted and were assigned dedicated nurses to care for them. Daily environmental cleaning was performed with a quaternary ammonium compound on all work surfaces in clinical areas. As a result of these interventions, the number of KPC infections/colonizations significantly decreased over the following 12 months.

Conclusions

Carbapenems have long been a reliable last line of defense in the treatment of infections caused by antimicrobial-resistant *Enterobacteriaceae*. Thus, the emergence of KPC resistance is a major threat to global health. Recent results show that KPC genes are diverse, stable genetic elements, which can be difficult to detect. Furthermore, KPC-producing organisms can spread inside hospitals as well as outside in the community setting. Currently, few treatment options remain active against organisms that produce KPCs and have resulted in the increased use of tigecycline and polymyxins and intensified research on combination therapy. Until new effective drugs or combinations of drugs are found, detection, prevention, and containment are the keys to curtailing the spread of this dangerous antimicrobial resistance.

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