F1000Prime REPORTS

Published: 04 September 2014

Carbapenemase-producing *Klebsiella pneumoniae*

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F1000Prime Reports 2014, **6**:80 (doi:10.12703/P6-80)

Abstract

The continuing emergence of infections due to multidrug resistant bacteria is a serious public health problem. *Klebsiella pneumoniae*, which commonly acquires resistance encoded on mobile genetic elements, including ones that encode carbapenemases, is a prime example. *K. pneumoniae* carrying such genetic material, including both bla_{KPC} and genes encoding metallo-β-lactamases, have spread globally. Many carbapenemase-producing *K. pneumoniae* are resistant to multiple antibiotic classes beyond β-lactams, including tetracyclines, aminoglycosides, and fluoroquinolones. The optimal treatment, if any, for infections due to these organisms is unclear but, paradoxically, appears to often require the inclusion of an optimally administered carbapenem.

Introduction

The following experience is a stark expression of the epidemiologic and therapeutic problems presented by carbapenem-resistant *K. pneumoniae* (CR-KP).

A 43-year-old woman with pulmonary alveolar proteinosis was discharged from the National Institutes of Health (NIH) Clinical Center on July 15, 2012 after 32 days of hospitalization [1]. Initially transferred from a facility in New York City and known to be colonized with a CR-KP, she was immediately placed into enhanced isolation. The organism was not detected again until three weeks after discharge of this index case when it was recovered from a tracheal aspirate specimen of a mechanically ventilated patient and was eventually recovered from a total of 17 patients. The index isolate was resistant to all antibiotics tested, with the exception of gentamicin, tigecycline, and colistin. As the outbreak progressed, however, further resistance emerged to those three antibiotics as well, so that there were no antibiotics with *in vitro* activity against the organism available for treatment of some patients. Of the 17 affected patients 10 died and the outbreak organism was responsible for death in 6 of the 10.

The experience at the NIH Clinical Center exemplifies the problem highlighted in a 2013 report on antibiotic resistance in which the US CDC identified carbapenem-resistant *Enterobacteriaceae* (CRE) among the top three "urgent (antibiotic resistance) threats" to US public health. In fact, of the 9300 healthcare facility-associated CRE infections and 600 deaths annually in the US, a preponderance are due to *Klebsiella pneumoniae* carbapenemases (KPCs) [2], while other carbapenemases are often more prevalent outside the US. The proportion of US acute care hospitals reporting at least one hospital-acquired infection due to CRE to the CDC's National Healthcare Safety Network (NHSN) increased from 1.2% to 4.2% between 2001 and 2011, with the largest share of the increase occurring among *Klebsiella* species [3]. KPCs, named for the species from which these enzymes were first isolated in 1996, consist of at least a dozen subtypes (KPC2-13) with varying substrate specificities [4-6]. Despite the name, KPCs have now appeared in a variety of other *Enterobacteriaceae*, as well as in other Gram-negative bacilli, including *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [7]. Invasive infections due to organisms producing KPCs are associated with mortality rates approaching 50% [1,4,8-10]. The effect

of these carbapenemases may be difficult to detect in the laboratory, and what constitutes optimal antibiotic therapy remains uncertain. Isolates may phenotypically appear susceptible to carbapenems, delaying both time to administration of appropriate antibiotic therapy and implementation of infection control policies, leading to transmission within an institution.

Laboratory identification

The US CDC, for surveillance purposes, currently defines CRE as Enterobacteriaceae isolates that are nonsusceptible to doripenem, imipenem, and/or meropenem, together with resistance to ceftriaxone, cefotaxime, and ceftazidime [11]. Phenotypic detection of carbapenem resistance, and the presence of a carbapenemase by standard *in vitro* susceptibility testing, especially with some automated systems, can be problematic [7]. This results, at least in part, from variable carbapenemase expression, as well as the frequent presence of additional resistance mechanisms. This observation led both the European Committee on Antimicrobial Susceptibility (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) to lower minimum inhibitory concentration (MIC) breakpoints in order to improve their sensitivity in the detection of CRE (Table 1). The CDC currently recommends the use of these new interpretive criteria for screening for the possible presence of a carbapenemase. As always, however, improved sensitivity is accompanied by reduced specificity. Thus, while the presence of reduced susceptibility to ertapenem may be the most sensitive indicator of the presence of a carbapenemase, it is the least specific indicator since full resistance to this antibiotic may result from the simultaneous presence of other mechanisms such as altered porin proteins together with derepressed AmpC or an extended spectrum beta-lactamase (ESBL). High level resistance to carbapenems in *K. pneumoniae* carrying KPCs is associated with increased bla_{KPC} copy number and/or non-functional outer membrane protein (Omp) K35 or Omp K36 [12]. In addition to these issues, some automated susceptibility testing methods may have difficulty detecting isolates carrying KPC [7].

The CDC recommends that, in order to detect the presence of a carbapenemase, isolates with reduced susceptibility to one or more carbapenems undergo further testing with the modified Hodge test (MHT) [13]. The CLSI indicates that testing by MHT is not necessary when the isolate is found to be intermediate or resistant to all carbapenems tested, since use of the recently reduced breakpoints should preclude the possibility of misclassification of CR-KP as carbapenem susceptible [14]. They do, however, suggest its use for epidemiological investigations.

Several selective agars, such as CHROMagar KPC and ChromID CARBA may be of use in screening for carbapenem resistance [15,16]. The presence of a carbapenemase may also be detected by phenotypic methods, such as inhibition of its activity by ethylenediaminetetraacetic acid (EDTA) and phenylboronic acid [6,17,18]. Direct phenotypic methods of carbapenemase detection include the identification of hydrolytic products of imipenem by UV spectrophotometry [19], by the Carba NP test [16], or by matrix-assisted laser desorption time-of-flight mass spectrometry [20].

The most sensitive method for establishing the presence of known carbapenemases is the detection of genes encoding these enzymes, and the CDC has published a protocol for multiplex real-time PCR detection of KPC and New Delhi metallo-β-lactamase (NDM)-1 genes [13]. A variety of test systems are in use [15]. Among the newer ones is the Biofire FilmArray, which is a multiplex PCR system that detects the gene encoding KPC as well as two other resistance genes and 24 pathogens (19 bacteria and 5 yeasts) in positive blood cultures [21]. The Verigene Gram Negative Blood Culture Test is a microarray system that detects, in addition to nine genus/species target pathogens, the genes encoding KPC, NDM, oxacillinase class D β-lactamase (OXA), verona integrin-encoded metallo-β-lactamase (VIM) and imipenemase metallo-β-lactamase (IMP), as well as CTX-M (β-lactamase showing preferential hydrolytic activity for cefotaxime [CTX] first identified in Munich [M]) [22]. The GeneXpert multi-drug resistant organism (MDRO) test, under development, detects genes encoding KPC, NDM and VIM [23]. Identification of the gene responsible may be of importance in epidemiological investigations, but is not currently of value in designing therapeutic regimens.

Table 1. Change in the Clinical and Laboratory Standards Institute interpretive carbapenem breakpoints

Carbapenem	Previous Breakpoints (M100-S19) MIC (μg/ml)			Current Breakpoints (M100-S22) MIC (μg/ml)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Doripenem	-	-	-	≤1	2	≥4
Ertapenem	≤2	4	≥8	≤0.5	1	≥2
Imipenem	≤4	8	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4

However resistance is detected, it remains important that clinical laboratories test isolates with reduced susceptibility to carbapenems for susceptibility to tigecycline, fosfomycin, and colistin or polymyxin B, without requiring a specific request from a clinician, in order to reduce any delay in appropriate treatment.

Epidemiology

Initially identified in 1996, the first description of CRE was not published until 2001[4]. Early reports of CREs were concentrated in and around the New York metropolitan area and primarily involved KPC-producing Klebsiella species [24,25]. In the US, KPCs (which are Ambler class A serine proteases) are the dominant carbapenemases and have also become important in several other countries, such as Israel and Greece [26]. A single sequence type, ST258, which belongs to clonal complex 11, is the predominant strain of *K. pneumoniae* carrying *KPC-2* in the US and Europe [27]. Since the early 2000s there have been several reported CRE outbreaks that have shaped our epidemiologic understanding, including a 2013 outbreak of CRE related to endoscopic retrograde cholangiopancreatography that was notably due to NDM-producing organisms, rather than KP-KPC organisms [28]. As of February 2014, CRE had been identified in all states in the US except Alaska, Maine, and Idaho, but its distribution is geographically heterogeneous with varying causes of resistance identified in different regions of the country [29,30]. In many countries outside the US, a number of metallo-β-lactamases, including NDM, VIM, and IMP are the dominant carbapenemases.

Genetics

While some carbapenemases are chromosomally encoded, most are present on mobile genetic elements that have allowed dissemination to a number of different Gram-negative bacteria [31]. As an example, the KPC-encoding gene, *bla*KPC, is present on a Tn3-based transposon, Tn*4401*, and carried on plasmids, many of which also carry *QnrA* and/or *QnrB*, which elevate fluoroquinolone MICs, and *rmtB*, which methylates the 16S rRNA target of many aminoglycosides [7,32]. Carbapenemases may coexist with other β-lactamases, such as ESBLs, and more than one carbapenemase may be simultaneously present, as in a *K. pneumoniae* isolate identified in China that carried both *blaKPC-2* and the metallo-β-lactamase gene, IMP-4 [33].

Infection control and public health

Rapid detection of CRE producing organisms, followed by aggressive infection prevention tactics, forms the basis of treatment and control of these organisms. The CDC has recently asked infection preventionists to enhance their vigilant surveillance for CRE and they have published a "toolkit" for clinicians and infection preventionists to assist in the task [11]. According to the CDC, institutions should, at a minimum, be aware of whether or not they have circulating CREs, at least among *Klebsiella* and *Escherichia coli* species. Additionally, the toolkit reinforces core measures that all hospitals should follow to reduce the CRE threat. Bundled interventions to disrupt CRE transmission among susceptible patients were evaluated in a recent review [34]. Given the multi-faceted nature of infection prevention bundles, the individual contribution of each intervention has not clearly been established, but the authors conclude that traditional targeted interventions (such as contact isolation), and systems approaches (including hand hygiene compliance and feedback) are essential to CRE mitigation. The role of these infection control practices and their inclusion in several national guidelines is largely based on outbreak experience rather than clinical trial data and was recently reviewed by Kruse and colleagues [35]. Clinicians involved in the NIH Clinical Center CRE outbreak echoed the need for diligent compliance with hand hygiene and other infection prevention protocols in a recent editorial on the future of CRE management [36]. They also highlighted the need to incorporate molecular diagnostics, rational antimicrobial utilization, new drug development and administrative leadership on patient safety issues as essential factors in effective CRE control. Countries other than the US have directed attention toward infection prevention to stop the spread of CRE. In Israel, for example, expanded national oversight of strategic infection control interventions has been successfully employed to control the spread of nosocomial CREs since 2007 [37].

Emerging therapies

In 2009, we stated in this journal regarding KP-KPC that "The optimal therapy for infections due to these multidrug-resistant pathogens is not well defined and depends upon the susceptibilities of individual isolates, and the choices are often severely limited" [38]. This assessment, which applies to CRE in general, unfortunately, remains largely unchanged [7,39]. The antibiotics most likely to be active *in vitro* against CR-KP, including KP-KPC, are gentamicin, tigecycline, fosfomycin, colistin, and polymyxin B. Therapy with a single antibiotic to which the pathogen is susceptible *in vitro* appears to be inferior to combination therapy with two or three active antibiotics and, furthermore, monotherapy is associated with an increased likelihood of emergence of resistance [7,39–41]. Daikos and colleagues in Athens, Greece, provide details of a large and highly instructive experience with the problem of treatment of infections due to CRE [41]. Of 205 patients with CR-KP bloodstream infection seen at

their institution during 2009 and 2010, 163 (79.3%) were infected with KPC-KP, with 36 also producing VIM-1. Another 42 produced VIM-1 alone. Despite the fact that all isolates contained carbapenemases, the proportions of isolates resistant to imipenem, meropenem, and doripenem were only 53.7%, 52.7%, and 57.1%, respectively, with application of EUCAST resistance breakpoints then in use (>8 μg/ml for imipenem and meropenem, >4 μg/ml for doripenem). Resistance to gentamicin was seen in 31.2% and to amikacin in 68.3%, while 97.6% were resistant to ciprofloxacin. The mortality rate in patients who received monotherapy was 44.4%, while it was only 27.2% with combination therapy, with the lowest mortality in those who received a carbapenem-containing combination regimen. Among patients who received a carbapenem together with another agent with *in vitro* activity against the etiologic pathogen, the mortality varied depending on the carbapenem MIC (19.3% with an MIC ≤8 μg/ml and 35.5% with MIC >8 μg/ml). None of the 11 patients who received a carbapenem together with tigecycline and either an aminoglycoside or colistin died. Combination therapy was an independent predictor of survival. In agreement with this experience, Tumbarello and colleagues found improved survival in patients with KPC-KP bloodstream infections who received two or more drugs with *in vitro* activity against the pathogen [42]. Once antimicrobial susceptibility data was available, the lowest mortality was documented in those receiving the combination of tigecycline, meropenem, and colistin.

This experience suggests that the optimal therapy for treatment of CR-KP involves combination therapy that, seemingly paradoxically, includes a carbapenem (imipenem, meropenem, or doripenem) together with tigecycline and either an aminoglycoside (usually gentamicin) or colistin, with at least two of the antibiotics being active against the pathogen *in vitro*. The doses and administration should be optimized, taking into account relevant pharmacokinetic and pharmacodynamic principles.

Fosfomycin

In a sample of 68 clinical KPC-KP isolates collected in the eastern US, 93% were susceptible to fosfomycin based on CLSI breakpoints for *E. coli* urinary tract infections, the only infection for which CLSI fosfomycin breakpoints currently exist [43]. Analysis of a subset of 23 isolates, each resistant to both tigecycline and colistin, found that 87% were susceptible to fosfomycin. In Germany, the MIC50 of 50 isolates of CR-KP (all but four produced one or more carbapenemase) was 16 μg/ml and the MIC90 was 256 μg/ml; 16 (32%) were resistant by EUCAST criteria (susceptible: ≤32 μg/ml; resistant: >32 μg/ml) [44]. Fosfomycin is not recommended as a monotherapy because of the likelihood of rapid emergence of on-treatment resistance to this agent; it should always be used as part of a combination regimen. Unfortunately, this strategy is not always successful and resistance may nonetheless emerge during combination therapy [45].

Published clinical experience with fosfomycin therapy of infections due to CR-KP is limited. In 11 Greek intensive care units (ICUs), 68 patients with infections due to multidrug resistant Gram-negative bacilli, 41 (60.3%) of which were due to CR-KP, received fosfomycin intravenously in a median total daily dose of 24 g for a median duration of 10 days, usually in combination with tigecycline or colistin [46]. In the entire evaluable cohort, bacterial eradication was achieved in 56.3% of cases and the 28 day crude mortality was 37.5%. The most frequently encountered adverse event was hypokalemia; resistance emerged in three cases. In the US, fosfomycin is only available in an oral formulation, making the achievement of systemic antibiotic exposure comparable to that seen with a 24 gram/day intravenous dose highly problematic, if not impossible.

Rifampin

In vitro time-kill experiments with a small number of NDM-producing *K. pneumoniae* evaluated several two and three drug combinations and found that the most active was the combination of rifampin with meropenem and fosfomycin [47]. The combination of rifampin, doripenem and colistin had previously been found to be bactericidal against KPC-KP isolates [48], and dual combinations of rifampin with colistin have been reported to exhibit synergy against this organism [49,50], as has rifampin with polymyxin B [51]. Clinical data are, however, lacking.

Double-carbapenems

The combination of doripenem and ertapenem was more active than either drug alone in both an in vitro chemostat model and a murine thigh infection model [35].

A limited number of case reports describe the use of double-carbapenem combination therapy. Giamarellou *et al*. successfully treated three patients with pan-resistant KPC-KP infections (two with bacteremia, one with urinary tract infection) with the combination of doripenem or meropenem (with prolonged infusion) plus ertapenem [52]. In another report, three patients with KPC-KP infections were successfully treated with a combination of meropenem and ertapenem and *in vitro* time-kill assay demonstrated bactericidal synergy against one of the isolates [53]. One proposed mechanism of action is as follows: the least potent carbapenem against

carbapenemase-producing *Enterobacteriaceae*, ertapenem, binds the carbapenemase with greater affinity, thereby protecting the more potent carbapenem from hydrolysis. This effect, if it exists at all, may not be universal across KPC subtypes, however, as doripenem has been found to have approximately threefold greater affinity than ertapenem for KPC-6 [54].

Aztreonam

Aztreonam, combined with meropenem and colistin, demonstrated *in vitro* synergistic or bactericidal activity against VIM and NDM-1 producing *K. pneumoniae*, despite the presence of high level resistance to the monobactam and non-susceptibility to meropenem [47]. It is speculated that this may result from the fact that aztreonam is a competitive inhibitor of metallo-β-lactamases, while meropenem is an inhibitor of ESBLs and AmpC enzymes, which may be co-produced by some organisms.

Glycopeptides

Potent synergy, with reduction of the MIC of vancomycin from 256 μg/ml to 1 μg/ml with each of six isolates (four *E. coli*, two *K. pneumoniae*) carrying NDM in combination with one or more ESBL, was observed when this glycopeptide was combined with colistin *in vitro* [55]. A retrospective review examining therapy with this combination in critically ill patients with Gram-negative bacillary infections included 24 patients with CR-KP infection [56]. Of these, 15 received colistin alone, 5 received it with a glycopeptide, 5 with another antibiotic targeting Gram-negative bacilli, and one received the combination with another anti-Gram-negative antibiotic. The number of patients was too small to identify significant differences in outcome. Furthermore, while similar synergy of vancomycin with colistin has been demonstrated *in vitro* against isolates of *Acinetobacter baumanii*, a retrospective study of patients with ventilator-associated pneumonia due to this organism failed to identify evidence of benefit from the addition of this glycopeptide to colistin [57–59].

Avibactam

Avibactam is an investigational non-β-lactam β-lactamase inhibitor being studied in combination with several β-lactam antibiotics [60]. It inhibits enzymes of Ambler classes A (including KPC) and C, as well as some in class D. It has, however, been reported to be slowly hydrolyzed by KPC [61]. In a US national survey, the combination of avibactam with ceftazidime inhibited all ESBL and KPC producing *Enterobacteriaceae* at a ceftazidime MIC <4 μg/ml [62]. Of 112 meropenem non-susceptible (MIC ≥2 μg/ml) *K. pneumoniae*, 29 (85.2%) had an MIC to the combination of ≤1 μg/ml.

BAL30072

BAL30072 is an investigational siderophore monosulfactam that, like aztreonam, is stable to metallo-β-lactamases of Ambler class B and is not hydrolysed by VIM or IMP. It is believed to be resistant to KPC, but its activity against KPC-KP is often poor because of the frequent simultaneous presence of SHV and/or derepressed *AmpC* [63,64].

Other novel agents

Several novel antibiotics have been reported to have *in vitro* activity against some CRE. These include the fluorocycline, eravacycline [65-67] and the neoglycoside sisomicin derivative, plazomicin (ACHN-490) [68].

Conclusion

The emergence of multidrug resistant *K. pneumoniae* is a perfect storm of antibiotic resistant threats — pervasive, transmissible and deadly. With the emergence of resistance to what many consider antibiotics of last resort — carbapenems — the problem is reaching critical proportions. Mortality associated with infections due to these organisms remains high and the optimal therapy uncertain. In some instances, no antibiotics with *in vitro* activity against the pathogen are available. Effective infection control and aggressive antimicrobial stewardship are imperative to prevent infections by these organisms. In the absence of the development of new antibiotics effective against these pathogens, however, the future may prove to be bleak. At present, the keys to dealing with the problem are prevention through antimicrobial stewardship and infection control, swift laboratory detection, and combination therapy with an existing carbapenem (imipenem, meropenem or doripenem) and at least one other antibiotic with *in vitro* activity against the pathogen. It goes without saying that these drugs need to be administered in the optimal dose and, in the case of β-lactams, optimal duration of infusion.

Abbreviations

CLSI, Clinical and Laboratory Standards Institute; CR-KP, carbapenem-resistant *Klebsiella pneumoniae*; CRE, carbapenem-resistant *Enterobacteriaceae*; ESBL, extended spectrum β-lactamase; EUCAST, European Committee on Antimicrobial Susceptibility Testing; NIH, National Institutes of Health; IMP, imipenemase metallo-β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; MHT, modified Hodge test; MIC, minimum inhibitory concentration; NDM, New Delhi metallo-β-lactamase; Omp, outer membrane protein; OXA, oxacillinase class D β-lactamase; VIM, Verona integrin-encoded metallo-β-lactamase.

Disclosures

The authors declare that they have no disclosures.

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