Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multidrug-resistant infection

Elizabeth B. Hirsch and Vincent H. Tam

1University of Houston College of Pharmacy, Houston, TX, USA; 2St Luke’s Episcopal Hospital, Houston, TX, USA

*Corresponding author. Tel: +1-713-795-8316; Fax: +1-713-795-8383; E-mail: vtam@uh.edu

Bacteria producing *Klebsiella pneumoniae* carbapenemases (KPCs) are rapidly emerging as a cause of multidrug-resistant infections worldwide. Bacterial isolates harbouring these enzymes are capable of hydrolysing a broad spectrum of β-lactams including the penicillins, cephalosporins, carbapenems and monobactam. Detection of isolates harbouring carbapenemases can be inconsistent using automated systems, often requiring subsequent confirmatory tests. Phenotypic methods utilizing boronic acid disc tests have demonstrated promising results and appear practical for use in clinical microbiology laboratories. Treatment of infection caused by KPC bacteria is particularly worrisome as the carbapenems are often agents of the last resort for resistant Gram-negative infections. The optimal treatment of infections caused by KPC bacteria is not well established and clinical outcome data remain sparse. We reviewed the current literature regarding clinical outcomes following KPC infections, with a specific effort to summarize the clinical data available for specific antimicrobial agents. A total of 15 papers involving 55 unique patient cases were reviewed. While the total number of patients is relatively small, some useful insights could still be gathered to guide clinicians in the management of KPC infections. Tigecycline and the aminoglycosides were associated with positive outcomes in the majority of cases. Clinical success rates were low when the polymyxins were used as monotherapy, but were much higher when they were used in combination. Studies examining combination therapy and well-controlled clinical trials are needed to ascertain the optimal treatment of infections caused by KPC bacteria.

**Keywords:** carbapenems, susceptibility, β-lactamases, plasmids

Introduction

In the last 5 years, the spread of isolates producing *Klebsiella pneumoniae* carbapenemases (KPCs) has become a significant problem. These β-lactamases are able to hydrolyse the carbapenems and confer resistance to a broad spectrum of antibiotics; treatment of infection caused by these pathogens is thus a considerable challenge for clinicians. The optimal treatment of KPC infections has yet to be determined and few clinical data are available on which to base antibiotic recommendations. In areas such as the north-eastern USA, Israel, Columbia, Greece and Puerto Rico, where KPCs are now considered endemic, many outbreaks have occurred. Reports surrounding these outbreaks have been more focused on molecular epidemiology or in vitro susceptibilities, but not on specific antimicrobial regimens and patient outcomes. Recently, the epidemiology and molecular genetics of KPCs have been elegantly reviewed. The purpose of this review is to provide practical information regarding detection and treatment of KPC infection that may be useful to clinicians at the bedside.

Characterization of carbapenemases

The Ambler classification scheme separates β-lactamases into four major classes (A–D) based on amino acid sequence homology. Classes A, C and D are β-lactamases with serine at their active site, while class B (also known as metallo-β-lactamases) have zinc at their active site. Carbapenemases include enzymes from classes A, B and D. This article will focus specifically on the KPC enzymes, which fall under Ambler class A and Bush functional group 2f enzymes. KPC enzymes differ from the other 2f enzymes by two specific characteristics: (i) they are found on transferable plasmids; and (ii) they are able to hydrolyse the aminothiazoleoxime cephalosporins such as cefotaxime.

KPCs are predominantly found in *K. pneumoniae*; however, they have also been found in many other Enterobacteriaceae including *Escherichia coli*, *Enterobacter* species, *Salmonella enterica*, *Proteus mirabilis* and *Citrobacter freundii*. The identification of a KPC enzyme outside the Enterobacteriaceae family was first reported in 2007 in *Pseudomonas aeruginosa* and most recently in an *Acinetobacter baumannii* strain from Puerto Rico. The KPC...
family has a great potential for spreading due to the location of KPC genes on plasmids. In fact, transfer of the *bla*<sub>KPC-2</sub> gene has been documented between two unrelated patients in the same US hospital.

To date, nine different variants (KPC-2–KPC-10) of the KPC enzyme have been reported, with KPC-2 and KPC-3 reported the most frequently. Of note, re-sequencing of the *bla*<sub>KPC-1</sub> gene revealed it to be identical to *bla*<sub>KPC-2</sub>.15

Epidemiology

The rapidly increasing prevalence of Enterobacteriaceae harbouring carbapenemases is alarming. Data on healthcare-associated infections reported to the CDC from 2007 indicated that 8% of all *Klebsiella* isolates were carbapenem-resistant *K. pneumoniae* (CRKP), in comparison with <1% in 2000.16 The first isolate harbouring the KPC β-lactamase was collected in 1996 and reported in 2001.14 The gene conferring resistance, *bla*<sub>KPC-1</sub>, was found to reside on a large plasmid that was responsible for resistance to the carbapenemases, extended-spectrum cephalosporins and aztreonam. Just 3 years later, outbreak reports from New York City began to appear citing the KPCs as an emerging cause of multidrug-resistant infections.4,17,18 Currently, many areas in the USA have been affected, with the highest density seen in the north-eastern states.17–19 KPC-producing isolates have now been reported from several countries outside the USA, France, China, Sweden, Norway, Colombia, Brazil, Scotland, Trinidad and Tobago, and Poland have all identified pathogens harbouring KPCs.20–22 Epidemic situations have also been reported in Israel and Greece.23

The rapid spread and growing list of pathogens in which the *bla*<sub>KPC</sub> gene has been isolated is probably due to its carriage on plasmids. The gene is carried in a Tn3-based transposon, Tn4401.30 Recently, a dominant strain, sequence type 258 (ST258), was found to account for 70% of the CDC's K. pneumoniae PFGE database.22 The 13 related (ST258) organisms were isolated from 10 different states and from an outbreak in Israel. KPC-producing ST258 has also been identified in patients from Norway and Sweden who had prior hospitalization in Greece and Israel. These results suggest international dissemination of ST258. Additional data supporting the high mobility of KPC genes was seen in a 2006 report from the SENTRY Antimicrobial Surveillance Program, which demonstrated the appearance of KPC-2/3 within and between genera.31 It is anticipated that KPC-mediated resistance will be a prominent mechanism of multidrug resistance in Gram-negative bacilli in the near future.

Challenges in laboratory detection

Identification of isolates harbouring KPCs has proved to be especially challenging in clinical microbiology laboratories. The presence of a KPC does not always result in high-level resistance to the carbapenems, but may cause MIC elevations that remain within the susceptible or intermediate range. These increased MICs may go unnoticed by the laboratory personnel unless phenotypic confirmatory tests are employed. Factors known to interfere with their detection include inadequate inocula used in susceptibility testing and day-to-day variability in MICs. When comparing MIC determination methods, the broth microdilution reference method appears to have the highest sensitivity (>90%), while automated systems provide the most variable results.32–34 Inaccurate detection of KPCs may lead to inappropriate treatment, resulting in compromised patient outcomes.35

Phenotypic methods for detection

The gold standard to confirm the presence of a KPC is the spectrophotometry assay (to detect hydrolysis of a carbapenem) followed by PCR of the *bla*<sub>KPC</sub> gene. This genotypic method, however, is time consuming for a clinical microbiology laboratory and usually requires isolates to be sent to reference laboratories for verification.

Several phenotypic tests for the detection of KPCs have been developed. The method currently endorsed by the CLSI is the modified Hodge test (MHT).36 This carbapenem inactivation assay has acceptable sensitivity and specificity for carbapenemase production;34 however, it may not be the ideal phenotypic confirmatory test for KPCs since interpretation can be difficult for some isolates and false positives have been reported.36–38 False-positive results seem to be most common in isolates producing CTX-M extended-spectrum β-lactamases (ESBLs) and those hyperproducing AmpC β-lactamase.37,39–41 Thus, in geographical areas where ESBL-producing isolates are prevalent, an alternative method may prove to be more useful.

A second phenotypic method shown to be promising for identification of KPCs utilizes boronic acid (BA)-based compounds. BA was originally described in the 1980s as a reversible inhibitor of class C β-lactamases and has been used in combination disc tests for the identification of AmpC-producing isolates.42–44 Recently, several disc tests combining BA compounds, phenylboronic acid and 3-aminophenyl boronic acid (APB), have proved to be highly sensitive and specific for the detection of KPC production. Tsakris et al.45 tested discs containing 400 μg of phenylboronic acid as an inhibitor and several β-lactams as the antibiotic substrates against 57 KPC-producing isolates. They found significantly increased (≥ 5 mm) inhibition zone diameters when used in combination with cefepime and all carbapenems (imipenem, meropenem and ertapenem) compared with zones produced by the β-lactam discs alone.46–48 Meropenem, imipenem and cefepime were the most sensitive and specific (100% for all), while meropenem demonstrated the largest difference in inhibition zone diameters. Due to the high prevalence of KPC-producing strains that also carry ESBL genes, the same research group investigated BA-based double-disc synergy tests (DDSTs) for the detection of ESBL genes in KPC producers.57 They found that a modified CLSI ESBL confirmatory test containing BA and clavulanate as inhibitors was the most accurate (100% sensitive and specific) for the 118 strains harbouring ESBLs. Based on their results, the authors proposed a modification of the current CLSI confirmatory test based on BA in order to detect ESBLs in isolates harbouring KPCs. Additionally, Doi et al.49 found that the addition of APB to ertapenem or meropenem (but not imipenem) discs resulted in an increased zone diameter ≥5 mm for 10 KPC-producing isolates when compared with the carbapenem disc alone. Optimal sensitivity and specificity was found using 300 μg of APB with a cut-off of a 5 mm difference in zone diameter. A third group investigated the utility of APB for detection of other class
A carbapenemase. They found BA-based MIC tests utilizing imipenem–APB to have 100% sensitivity and specificity to differentiate class A carbapenemase-producing bacteria from non-carbapenemase-producing bacteria when using a cut-off of ≥3-fold reduction in MIC compared with imipenem alone. In summary, these BA-based methods have shown promising results and appear practical for use in a clinical laboratory setting as a similar methodology/algorithm is currently recommended for the phenotypic confirmation of ESBLs.

**In vitro susceptibility**

As previously stated, KPCs are able to hydrolyse almost all β-lactam classes, rendering them ineffective. Unfortunately, the addition of commercially available β-lactamase inhibitors (clavulanic acid, sulbactam or tazobactam) only results in a negligible reduction in the MIC for most isolates, probably ruling out their clinical application. Additional resistance mechanisms are commonly found on the same plasmid in KPC isolates (i.e. multiple enzymes), conferring cross-resistance to other antimicrobial classes including the fluoroquinolones and aminoglycosides. As a result of the broad-spectrum antimicrobial resistance, treatment options are very limited.

Agents consistently shown to have in vitro activity against isolates harbouring KPCs include tigecycline and the tetracyclines, the polymyxins and the aminoglycosides. Two susceptibility studies showed similar results for the majority of agents tested, while other studies showed considerably different results for amikacin and doxycycline (Table 1). Although most isolates are often reported as susceptible to the tetracyclines (i.e. doxycycline), it is important to note that MIC₉₀ values are often at or near the CLSI susceptibility breakpoint (4 mg/L). Clinically achievable drug concentrations at the site of infection should be taken into account before using this class of agents.

**Pharmacokinetic/pharmacodynamic considerations**

When initiating antibiotic therapy for KPC infections, clinicians must also consider antibiotic pharmacokinetics and the site of infection, in addition to in vitro potency. Of importance, tigecycline, a glycyclcline shown to have potent in vitro activity against KPC bacteria, is not approved for the treatment of bloodstream infections. In view of the low serum concentrations achieved, breakthrough bloodstream infections caused by A. baumannii while on tigecycline treatment for other infections have been reported. Its use in urinary tract infections (UTIs) is also questionable due to low concentrations found in the urine. Reports of successful treatment of UTIs caused by multidrug-resistant isolates utilizing off-label ‘high-dose’ tigecycline (200 mg for one dose, then 100 mg every 12 h) have been published. However, caution should be exercised as selection of tigecycline-resistant isolates may be possible as a result of suboptimal drug concentrations. In one case, a patient had pan-resistant K. pneumoniae isolated from multiple urine cultures. She received 10 days of treatment with tigecycline and eventually had spontaneous resolution of symptoms even though her last available urine culture continued to show >100,000 cfu/mL of the pan-resistant K. pneumoniae more than a year later. The aminoglycosides may not be optimal for the treatment of abscesses or intra-abdominal infections caused by KPC bacteria due to their low penetration in acidic environments. Finally, it is unclear whether systemic polymyxins should be used for the treatment of nosocomial pneumonia. One study demonstrated poorer clinical outcomes when systemic polymyxins were used as monotherapy for the treatment of multidrug-resistant nosocomial pneumonia, while others have demonstrated higher clinical success rates similar to other first-line treatment options. Different success rates reported could be attributed to poor drug penetration into the epithelial lining fluid of the lungs.

**Treatment options**

Clinical data on the treatment of KPC infections are very limited and consist mainly of small case series and brief reports. In an effort to summarize the data associated with specific antimicrobial agents, we examined the pertinent literature for individual patient cases reporting both specific treatment and clinical outcomes. A total of 15 studies/reports containing 55 unique patient cases (57 treatment courses) were reviewed (Table S1, available online). Antibiotic regimens were divided into seven different categories. Patient cases where more than two antibiotics were used were excluded since a clear association with outcome could not be ascertained. Treatment with aminoglycosides (75%), polymyxin combinations (73%) and tigecycline (71%) appeared to have higher success rates. In contrast, carbapenem (40%) and polymyxin (14%) monotherapy had much lower associated success rates. While the total number of patients per treatment category is small, carbapenemers and polymyxins should probably not be used as monotherapy for infections caused by KPC bacteria, until more data are available. A limitation that should be recognized is that many of the papers were single case reports or small series where precise definitions were not given. In particular, criteria used to diagnose infection (versus colonization) and definitions for clinical success (versus failure) were not always detailed, nor were the antibiotic initiation times with regard to the index cultures. Additionally, all infection types were combined for the assessment of the success rates.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Susceptible (%)</th>
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<tbody>
<tr>
<td>Castanheira et al. (n=60)</td>
<td>Bratu et al. (n=96)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>66.7</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>93</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>58.3</td>
</tr>
<tr>
<td>Amikacin</td>
<td>53.3</td>
</tr>
</tbody>
</table>

NT, not tested.

aDoxycycline.

**Table 1.** Selected antimicrobial susceptibility studies for agents with consistent in vitro activity against KPC-producing isolates.
success rates. One should also consider that these data were gathered from the current literature and clinicians who have treated large numbers of patients in endemic areas may have additional unpublished experience in the treatment of KPC infections (potential for publication bias).

**Tigecycline**

Tigecycline was used in a total of seven patients with a 71% success rate (5/7 patients). Of the five patients with clinical success, two were treated for pneumonia, one for clinically significant tracheobronchitis, one for urosepsis and one for shunt-related meningitis (combined with gentamicin given intravenously and intrathecally). Of the two patients who failed, one was being treated for urosepsis and the other received a lengthy treatment for nosocomial pneumonia and empyema. Although the nosocomial pneumonia was successfully treated, the empyema recurred and was associated with a tigecycline MIC increase from 0.5 to 2 mg/L. This patient subsequently died after multiple hospitalizations.

**Aminoglycosides**

The aminoglycosides were used (alone or in combination) in a total of eight patients with a 75% success rate (6/8 patients). Four patients were treated with gentamicin alone; three had clinical success. One patient was treated for pneumonia and the other two for bacteremia. The fourth patient experienced a relapse of a UTI after 6 days of gentamicin followed by 9 days of colistin. In addition, three patients were treated with amikacin alone or in combination. One patient was treated successfully with amikacin alone for a wound infection. The other two patients were treated with amikacin plus ciprofloxacin for bacteremia following solid organ transplantation; one failed and the other had success. The patient who failed had both KPC-producing Pseudomonas putida and Enterobacter cloacae isolated from multiple blood cultures following liver transplantation and died 12 days after the first episode of bacteremia. Finally, the remaining patient with clinical success was treated with a combination of ‘an aminoglycoside + tetracycline’ for bacteremia.

**Carbapenems**

As a result of misleading susceptibility testing from automated systems, a total of 19 patients were treated with a carbapenem alone or in combination with another agent. Of the four patients treated with combination therapy, three experienced clinical success (75%). Two were treated for UTIs: one with imipenem plus piperacillin/tazobactam and the other with imipenem plus polymyxin B. The patient who failed therapy for bacteremia was treated with imipenem and tigecycline and subsequently died. In contrast, the 15 patients treated with carbapenem monotherapy had only a 40% success rate (6/15 patients). Four out of nine patients treated with imipenem had clinical success. Those with success were treated for bacteremia, pyelonephritis, urosepsis and pneumonia. The five who failed therapy were treated for tracheobronchitis (n=2), UTI, pneumonia and lower respiratory tract infection. A total of six patients were treated with meropenem. Two experienced success (both bacteremia) and four failed. Those with failure were treated for bacteremia, tracheobronchitis and bacteremia plus pneumonia. The fourth patient had the KPC isolated from a sputum culture and was switched to tigecycline therapy 1 day prior to death.

**Polymyxins**

The polymyxins [polymyxin B and polymyxin E (colistin)] were used in a total of 18 patients alone or in combination. The success rate was low (14%) when used alone, but much higher (73%) when used in combination. A total of seven patients received monotherapy; only one had clinical success after treatment with polymyxin B. The six patients who failed therapy were treated for bacteremia, UTI, ventilator-associated pneumonia or unknown disease state. When the polymyxins were used in combination therapy, clinical success was 73% (8/11 patients). Colistin was used in combination with tigecycline in a total of six patients. Four of these patients had clinical success: three cases were pneumonia and one was a surgical site infection. Both patients who failed this combination were treated for pneumonia. Three patients were successfully treated with a combination of colistin and gentamicin for pneumonia. Of the two patients treated with polymyxin B combinations, one patient experienced failure, while the other had clinical success. Interestingly, a report of 12 patients being treated with polymyxin B monotherapy documented decreased susceptibility during therapy in three (25%) of the patients. The initial source of the isolates was the peritoneal fluid, CSF and blood; the source for all subsequent isolates was the blood. The MICs significantly increased from 1.5 to 32, 0.75 to 12 and 0.75 to 1024 mg/L, respectively. The mean duration of treatment for the three patients with increased MICs did not differ from the other nine patients whose isolates did not have an increased MIC. The authors postulated that combination therapy could have prevented the emergence of resistance. More research is needed to further explore this postulation and whether or not poor clinical outcomes seen are related to polymyxin monotherapy.

**Cephalosporins and β-lactam/β-lactamase inhibitors**

While the success rate appears high (80%; 4/5 patients) for these agents, one paper provided only empirical (first 24 h) treatment for three of the patients. The remaining two patients with treatment successes received either piperacillin/tazobactam followed by ciprofloxacin or ceftizoxime followed by imipenem.

**Novel agents in development**

Several new β-lactamase inhibitors that are able to withstand hydrolysis by ESBLs and class A carbapenemases are currently in development. These include NXL104, LK-157 and BLI-489. NXL104 was shown to restore the activity of several β-lactam antibiotics against six different KPC-producing isolates. The MICs for all six isolates were reduced to below susceptibility breakpoints in the presence of NXL104. LK-157 is a novel tricyclic carbapenem shown to have potent activity against class A and class C β-lactamases. The activity of BLI-489, a bicyclic penem molecule, was shown against a wide variety of
enzymes, but has not been accurately assessed against KPC-producing organisms.\textsuperscript{82} Lastly, ACHN-409, a new-generation aminoglycoside (neoglycoside), appears to have potent in vitro activity against KPC-producing isolates.\textsuperscript{83} When tested against 25 KPC-producing \textit{K. pneumoniae} isolates, MIC\textsubscript{50} and MIC\textsubscript{90} values (0.5 and 1 mg/L, respectively) were much lower than those of comparator aminoglycosides. Given the current limited options, these new agents look promising for the treatment of KPC infections.

Conclusions

The optimal treatment of infections caused by KPC-producing isolates is unknown. Their evolving resistance mechanism(s) and the lack of agents with Gram-negative activity in the development pipeline represent a major treatment dilemma for clinicians. Currently, very limited data are available from in vitro infection models or animals, and research into these avenues is necessary. Observational studies and clinical outcome data are urgently needed in order to determine the optimal treatment for KPC infections. Lastly, infections caused by KPC-producing organisms further emphasize the need to study combination therapy and rational treatment strategies.

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Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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