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

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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.[1] Reports surrounding these outbreaks have been more focused on molecular epidemiology or *in vitro* susceptibilities, but not on specific antimicrobial regimens and patient outcomes.[2–4] Recently, the epidemiology and molecular genetics of KPCs have been elegantly reviewed.[1] 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.[5,6] 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.[7] Carbapenemases include enzymes from classes A, B and D.[6] This article will focus specifically on the KPC enzymes, which fall under Ambler class A and Bush functional group 2f enzymes.[8] 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.[6]

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*.[6–8] The identification of a KPC enzyme outside the Enterobacteriaceae family was first reported in 2007 in *Pseudomonas aeruginosa*[9] and most recently in an *Acinetobacter baumannii* strain from Puerto Rico.[10] The KPC

family has a great potential for spreading due to the location of KPC genes on plasmids.[6] In fact, transfer of the bla_{KPC-2} gene has been documented between two unrelated patients in the same US hospital.[11]

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.[10,12–14] Of note, re-sequencing of the bla_{KPC-1} gene revealed it to be identical to bla_{KPC-2}.[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_{KPC-1}, was found to reside on a large plasmid that was responsible for resistance to the carbapenems, 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.[9,20–27] Epidemic situations have also been reported in Israel and Greece.[3,28,29]

The rapid spread and growing list of pathogens in which the bla_{KPC} gene has been isolated is probably due to its carriage on plasmids. The gene is carried in a Tn*3*-based transposon, Tn*4401*.[30] Recently, a dominant strain, sequence type 258 (ST258), was found to account for 70% of the CDC's *K. pneumoniae* PFGE database.[12] 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.[17,32–34] 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_{KPC} 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.[45,46] 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.[47] 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.*[48] 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 carbapenemases.[37] 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.[11,49,50] 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.[9,14,18,46,50–53] 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).[3,4,54,55] Although most isolates are often reported as susceptible to the tetracyclines (i.e. doxycycline), it is important to note that MIC_{90} 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 glycylcycline shown to have potent *in vitro* activity against KPC bacteria, is not approved for the treatment of bloodstream infections.[56–58] 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.[59] Its use in urinary tract infections (UTIs) is also questionable due to low concentrations found in the urine.[60] 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.[61] However, caution should be exercised as selection of tigecycline-resistant isolates may be possible as a result of suboptimal drug concentrations.[62,63] In one case, a patient had pan-resistant *K. pneumoniae* isolated from multiple urine cultures.[64] 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.[65,66] 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,[67] while others have demonstrated higher clinical success rates similar to other first-line treatment options.[68,69] Different success rates reported could be attributed to poor drug penetration into the epithelial lining fluid of the lungs.[67,70]

Table 1. Selected antimicrobial susceptibility studies for agents with consistent *in vitro* activity against KPC-producing isolates

	Susceptible (%)		
Agent	Castanheira *et al.*[55] (*n*=60)	Bratu *et al.*[54] (*n*=96)	Bratu *et al.*[4] (*n*=62)
Tigecycline	100	100	NT
Tetracycline	66.7	66[a]	32[a]
Polymyxin B	93	91	73
Gentamicin	58.3	61	65
Amikacin	53.3	45	6

NT, not tested.
[a]Doxycycline.

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 as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/)]. 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, carbapenems 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

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).[35,71] 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.[35,71] 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).[19,35,72–75] Four patients were treated with gentamicin alone; three had clinical success. One patient was treated for pneumonia and the other two for bacteraemia. 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 bacteraemia 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 bacteraemia. Finally, the remaining patient with clinical success was treated with a combination of 'an aminoglycoside + tetracycline' for bacteraemia.

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.[18,21,35,49,51,72] 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 bacteraemia 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 bacteraemia, 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 bacteraemia) and four failed. Those with failure were treated for bacteraemia, tracheobronchitis and bacteraemia 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.[9,19,51,74,75] 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 bacteraemia, UTI, ventilator-associated pneumonia or unknown disease state.[9,19,75] 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.[76] 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.[77] The remaining two patients with treatment successes received either piperacillin/tazobactam followed by ciprofloxacin or ceftizoxime followed by imipenem.[78,79]

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.[80] 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.[81] 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.[82] Lastly, ACHN-409, a new-generation aminoglycoside (neoglycoside), appears to have potent *in vitro* activity against KPC-producing isolates.[83] When tested against 25 KPC-producing *K. pneumoniae* isolates, MIC_{50} and MIC_{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/).

References

1 Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis* 2009; **9**: 228–36.

2 Marchaim D, Navon-Venezia S, Schwaber MJ *et al*. Isolation of imipenem-resistant *Enterobacter* species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. *Antimicrob Agents Chemother* 2008; **52**: 1413–8.

3 Pournaras S, Protonotariou E, Voulgari E *et al*. Clonal spread of KPC-2 carbapenemase-producing *Klebsiella pneumoniae* strains in Greece. *J Antimicrob Chemother* 2009; **64**: 348–52.

4 Bratu S, Mooty N, Nichani S *et al*. Emergence of KPC-possessing *Klebsiella pneumoniae* in Brooklyn, New York: epidemiology and recommendations for detection. *Antimicrob Agents Chemother* 2005; **49**: 3018–20.

5 Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. *Clin Microbiol Rev* 2005; **18**: 657–86.

6 Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. *Clin Microbiol Rev* 2007; **20**: 440–58, table of contents.

7 Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. *Am J Infect Control* 2006; **34**: S20–8; discussion S64–73.

8 Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995; **39**: 1211–33.

9 Villegas MV, Lolans K, Correa A *et al*. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing β-lactamase. *Antimicrob Agents Chemother* 2007; **51**: 1553–5.

10 Robledo IE, Aquino EE, Sante MI *et al*. Detection of KPC in *Acinetobacter* sp. in Puerto Rico. *Antimicrob Agents Chemother* 2010; **54**: 1354–7.

11 Rasheed JK, Biddle JW, Anderson KF *et al*. Detection of the *Klebsiella pneumoniae* carbapenemase type 2 carbapenem-hydrolyzing enzyme in clinical isolates of *Citrobacter freundii* and *K. oxytoca* carrying a common plasmid. *J Clin Microbiol* 2008; **46**: 2066–9.

12 Kitchel B, Rasheed JK, Patel JB *et al*. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob Agents Chemother* 2009; **53**: 3365–70.

13 Wolter DJ, Kurpiel PM, Woodford N *et al*. Phenotypic and enzymatic comparative analysis of the novel KPC variant KPC-5 and its evolutionary variants, KPC-2 and KPC-4. *Antimicrob Agents Chemother* 2009; **53**: 557–62.

14 Yigit H, Queenan AM, Anderson GJ *et al*. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2001; **45**: 1151–61.

15 Yigit H, Queenan AM, Anderson GJ *et al*. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2008; **52**: 809.

16 Srinivasan A, Patel JB. *Klebsiella pneumoniae* carbapenemase-producing organisms: an ounce of prevention really is worth a pound of cure. *Infect Control Hosp Epidemiol* 2008; **29**: 1107–9.

17 Bratu S, Landman D, Haag R *et al*. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. *Arch Intern Med* 2005; **165**: 1430–5.

18 Lomaestro BM, Tobin EH, Shang W *et al*. The spread of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* to upstate New York. *Clin Infect Dis* 2006; **43**: e26–8.

19 Nadkarni AS, Schliep T, Khan L *et al*. Cluster of bloodstream infections caused by KPC-2 carbapenemase-producing *Klebsiella pneumoniae* in Manhattan. *Am J Infect Control* 2009; **37**: 121–6.

20 Naas T, Nordmann P, Vedel G *et al*. Plasmid-mediated carbapenem-hydrolyzing β-lactamase KPC in a *Klebsiella pneumoniae* isolate from France. *Antimicrob Agents Chemother* 2005; **49**: 4423–4.

21 Wei ZQ, Du XX, Yu YS *et al*. Plasmid-mediated KPC-2 in a *Klebsiella pneumoniae* isolate from China. *Antimicrob Agents Chemother* 2007; **51**: 763–5.

22 Cuzon G, Naas T, Demachy MC *et al*. Plasmid-mediated carbapenem-hydrolyzing β-lactamase KPC-2 in *Klebsiella pneumoniae* isolate from Greece. *Antimicrob Agents Chemother* 2008; **52**: 796–7.

23 Monteiro J, Santos AF, Asensi MD *et al*. First report of KPC-2-producing *Klebsiella pneumoniae* strains in Brazil. *Antimicrob Agents Chemother* 2009; **53**: 333–4.

24 Baraniak A, Izdebski R, Herda M *et al*. The emergence of *Klebsiella pneumoniae* ST258 with KPC-2 in Poland. *Antimicrob Agents Chemother* 2009; **53**: 4565–7.

25 Samuelsen O, Naseer U, Tofteland S *et al*. Emergence of clonally related *Klebsiella pneumoniae* isolates of sequence type 258 producing plasmid-mediated KPC carbapenemase in Norway and Sweden. *J Antimicrob Chemother* 2009; **63**: 654–8.

26 Woodford N, Zhang J, Warner M *et al*. Arrival of *Klebsiella pneumoniae* producing KPC carbapenemase in the United Kingdom. *J Antimicrob Chemother* 2008; **62**: 1261–4.

27 Akpaka PE, Swanston WH, Ihemere HN *et al*. Emergence of KPC-producing *Pseudomonas aeruginosa* in Trinidad and Tobago. *J Clin Microbiol* 2009; **47**: 2670–1.

28 Leavitt A, Navon-Venezia S, Chmelnitsky I *et al*. Emergence of KPC-2 and KPC-3 in carbapenem-resistant *Klebsiella pneumoniae* strains in an Israeli hospital. *Antimicrob Agents Chemother* 2007; **51**: 3026–9.

29 Navon-Venezia S, Chmelnitsky I, Leavitt A *et al*. Plasmid-mediated imipenem-hydrolyzing enzyme KPC-2 among multiple carbapenem-resistant *Escherichia coli* clones in Israel. *Antimicrob Agents Chemother* 2006; **50**: 3098–101.

30 Naas T, Cuzon G, Villegas MV *et al*. Genetic structures at the origin of acquisition of the β-lactamase bla_{KPC} gene. *Antimicrob Agents Chemother* 2008; **52**: 1257–63.

31 Deshpande LM, Jones RN, Fritsche TR *et al*. Occurrence and characterization of carbapenemase-producing Enterobacteriaceae: report from the SENTRY Antimicrobial Surveillance Program (2000-2004). *Microb Drug Resist* 2006; **12**: 223–30.

32 Tenover FC, Kalsi RK, Williams PP *et al*. Carbapenem resistance in *Klebsiella pneumoniae* not detected by automated susceptibility testing. *Emerg Infect Dis* 2006; **12**: 1209–13.

33 McGettigan SE, Andreacchio K, Edelstein PH. Specificity of ertapenem susceptibility screening for detection of *Klebsiella pneumoniae* carbapenemases. *J Clin Microbiol* 2009; **47**: 785–6.

34 Anderson KF, Lonsway DR, Rasheed JK *et al*. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in Enterobacteriaceae. *J Clin Microbiol* 2007; **45**: 2723–5.

35 Weisenberg SA, Morgan DJ, Espinal-Witter R *et al*. Clinical outcomes of patients with *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* after treatment with imipenem or meropenem. *Diagn Microbiol Infect Dis* 2009; **64**: 233–5.

36 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement M100-S19*. CLSI, Wayne, PA, USA, 2009.

37 Pasteran F, Mendez T, Guerriero L *et al*. Sensitive screening tests for suspected class A carbapenemase production in species of Enterobacteriaceae. *J Clin Microbiol* 2009; **47**: 1631–9.

38 Lee K, Chong Y, Shin HB *et al*. Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect* 2001; **7**: 88–91.

39 Moland ES, Hanson ND, Overman SB *et al*. Concerns about KPC screening and confirmatory tests. In: *Abstracts of the Forty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2009*. Abstract D-729. American Society for Microbiology, Washington, DC, USA.

40 Anderson KF, Patel JB, Wong B *et al*. Characterization of Enterobacteriaceae with a false-positive modified Hodge test. In: *Abstracts of the Forty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2009*. Abstract D-719. American Society for Microbiology, Washington, DC, USA.

41 Carvalhaes CG, Picao RC, Nicoletti AG *et al*. Cloverleaf test (modified Hodge test) for detecting carbapenemase production in *Klebsiella pneumoniae*: be aware of false positive results. *J Antimicrob Chemother* 2010; **65**: 249–51.

42 Beesley T, Gascoyne N, Knott-Hunziker V *et al*. The inhibition of class C β-lactamases by boronic acids. *Biochem J* 1983; **209**: 229–33.

43 Coudron PE. Inhibitor-based methods for detection of plasmid-mediated AmpC β-lactamases in *Klebsiella* spp., *Escherichia coli*, and *Proteus mirabilis*. *J Clin Microbiol* 2005; **43**: 4163–7.

44 Yagi T, Wachino J, Kurokawa H *et al*. Practical methods using boronic acid compounds for identification of class C β-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli*. *J Clin Microbiol* 2005; **43**: 2551–8.

45 Tsakris A, Kristo I, Poulou A *et al*. Evaluation of boronic acid disk tests for differentiating KPC-possessing *Klebsiella pneumoniae* isolates in the clinical laboratory. *J Clin Microbiol* 2009; **47**: 362–7.

46 Tsakris A, Kristo I, Poulou A *et al*. First occurrence of KPC-2-possessing *Klebsiella pneumoniae* in a Greek hospital and recommendation for detection with boronic acid disc tests. *J Antimicrob Chemother* 2008; **62**: 1257–60.

47 Tsakris A, Poulou A, Themeli-Digalaki K *et al*. Use of boronic acid disk tests to detect extended-spectrum β-lactamases in clinical isolates of KPC carbapenemase-possessing Enterobacteriaceae. *J Clin Microbiol* 2009; **47**: 3420–6.

48 Doi Y, Potoski BA, Adams-Haduch JM *et al*. Simple disk-based method for detection of *Klebsiella pneumoniae* carbapenemase-type β-lactamase by use of a boronic acid compound. *J Clin Microbiol* 2008; **46**: 4083–6.

49 Villegas MV, Lolans K, Correa A *et al*. First detection of the plasmid-mediated class A carbapenemase KPC-2 in clinical isolates of *Klebsiella pneumoniae* from South America. *Antimicrob Agents Chemother* 2006; **50**: 2880–2.

50 Endimiani A, Hujer AM, Perez F *et al*. Characterization of bla_{KPC}-containing *Klebsiella pneumoniae* isolates detected in different institutions in the Eastern USA. *J Antimicrob Chemother* 2009; **63**: 427–37.

51 Bradford PA, Bratu S, Urban C *et al*. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 β-lactamases in New York City. *Clin Infect Dis* 2004; **39**: 55–60.

52 Chmelnitsky I, Hermesh O, Navon-Venezia S *et al*. Detection of *aac(6′)-Ib-cr* in KPC-producing *Klebsiella pneumoniae* isolates from Tel Aviv, Israel. *J Antimicrob Chemother* 2009; **64**: 718–22.

53 Doumith M, Ellington MJ, Livermore DM *et al*. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. *J Antimicrob Chemother* 2009; **63**: 659–67.

54 Bratu S, Tolaney P, Karumudi U *et al*. Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, NY: molecular epidemiology and *in vitro* activity of polymyxin B and other agents. *J Antimicrob Chemother* 2005; **56**: 128–32.

55 Castanheira M, Sader HS, Deshpande LM *et al*. Antimicrobial activities of tigecycline and other broad-spectrum antimicrobials tested against serine carbapenemase- and metallo-β-lactamase-producing Enterobacteriaceae: report from the SENTRY Antimicrobial Surveillance Program. *Antimicrob Agents Chemother* 2008; **52**: 570–3.

56 Muralidharan G, Micalizzi M, Speth J *et al*. Pharmacokinetics of tigecycline after single and multiple doses in healthy subjects. *Antimicrob Agents Chemother* 2005; **49**: 220–9.

57 Muralidharan G, Fruncillo RJ, Micalizzi M *et al*. Effects of age and sex on single-dose pharmacokinetics of tigecycline in healthy subjects. *Antimicrob Agents Chemother* 2005; **49**: 1656–9.

58 Rodvold KA, Gotfried MH, Cwik M *et al*. Serum, tissue and body fluid concentrations of tigecycline after a single 100 mg dose. *J Antimicrob Chemother* 2006; **58**: 1221–9.

59 Peleg AY, Potoski BA, Rea R *et al*. *Acinetobacter baumannii* bloodstream infection while receiving tigecycline: a cautionary report. *J Antimicrob Chemother* 2007; **59**: 128–31.

60 Meagher AK, Ambrose PG, Grasela TH *et al*. The pharmacokinetic and pharmacodynamic profile of tigecycline. *Clin Infect Dis* 2005; **41** Suppl 5: S333–40.

61 Cunha BA. Pharmacokinetic considerations regarding tigecycline for multidrug-resistant (MDR) *Klebsiella pneumoniae* or MDR *Acinetobacter baumannii* urosepsis. *J Clin Microbiol* 2009; **47**: 1613.

62 Reid GE, Grim SA, Aldeza CA *et al*. Rapid development of *Acinetobacter baumannii* resistance to tigecycline. *Pharmacotherapy* 2007; **27**: 1198–201.

63 Curcio D. Treatment of recurrent urosepsis with tigecycline: a pharmacological perspective. *J Clin Microbiol* 2008; **46**: 1892–3.

64 Elemam A, Rahimian J, Mandell W. Infection with panresistant *Klebsiella pneumoniae*: a report of 2 cases and a brief review of the literature. *Clin Infect Dis* 2009; **49**: 271–4.

65 Wagner C, Sauermann R, Joukhadar C. Principles of antibiotic penetration into abscess fluid. *Pharmacology* 2006; **78**: 1–10.

66 Strausbaugh LJ, Sande MA. Factors influencing the therapy of experimental *Proteus mirabilis* meningitis in rabbits. *J Infect Dis* 1978; **137**: 251–60.

67 Levin AS, Barone AA, Penco J *et al*. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Clin Infect Dis* 1999; **28**: 1008–11.

68 Garnacho-Montero J, Ortiz-Leyba C, Jimenez-Jimenez FJ *et al*. Treatment of multidrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia (VAP) with intravenous colistin: a comparison with imipenem-susceptible VAP. *Clin Infect Dis* 2003; **36**: 1111–8.

69 Kallel H, Bahloul M, Hergafi L *et al*. Colistin as a salvage therapy for nosocomial infections caused by multidrug-resistant bacteria in the ICU. *Int J Antimicrob Agents* 2006; **28**: 366–9.

70 Montero A, Ariza J, Corbella X *et al*. Efficacy of colistin versus β-lactams, aminoglycosides, and rifampin as monotherapy in a mouse model of pneumonia caused by multiresistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2002; **46**: 1946–52.

71 Daly MW, Riddle DJ, Ledeboer NA *et al*. Tigecycline for treatment of pneumonia and empyema caused by carbapenemase-producing *Klebsiella pneumoniae*. *Pharmacotherapy* 2007; **27**: 1052–7.

72 Endimiani A, Carias LL, Hujer AM *et al*. Presence of plasmid-mediated quinolone resistance in *Klebsiella pneumoniae* isolates possessing bla_{KPC} in the United States. *Antimicrob Agents Chemother* 2008; **52**: 2680–2.

73 Bennett JW, Herrera ML, Lewis JS II *et al*. KPC-2-producing *Enterobacter cloacae* and *Pseudomonas putida* coinfection in a liver transplant recipient. *Antimicrob Agents Chemother* 2009; **53**: 292–4.

74 Maltezou HC, Giakkoupi P, Maragos A *et al*. Outbreak of infections due to KPC-2-producing *Klebsiella pneumoniae* in a hospital in Crete (Greece). *J Infect* 2009; **58**: 213–9.

75 Endimiani A, Depasquale JM, Forero S *et al*. Emergence of bla_{KPC}-containing *Klebsiella pneumoniae* in a long-term acute care hospital: a new challenge to our healthcare system. *J Antimicrob Chemother* 2009; **64**: 1102–10.

76 Lee J, Patel G, Huprikar S *et al*. Decreased susceptibility to polymyxin B during treatment for carbapenem-resistant *Klebsiella pneumoniae* infection. *J Clin Microbiol* 2009; **47**: 1611–2.

77 Marschall J, Tibbetts RJ, Dunne WM Jr *et al*. Presence of the KPC carbapenemase gene in Enterobacteriaceae causing bacteremia and its correlation with *in vitro* carbapenem susceptibility. *J Clin Microbiol* 2009; **47**: 239–41.

78 Tibbetts R, Frye JG, Marschall J *et al*. Detection of KPC-2 in a clinical isolate of *Proteus mirabilis* and first reported description of carbapenemase resistance caused by a KPC β-lactamase in *P. mirabilis*. *J Clin Microbiol* 2008; **46**: 3080–3.

79 Mendes RE, Bell JM, Turnidge JD *et al*. Carbapenem-resistant isolates of *Klebsiella pneumoniae* in China and detection of a conjugative plasmid (bla_{KPC-2} plus *qnrB4*) and a bla_{IMP-4} gene. *Antimicrob Agents Chemother* 2008; **52**: 798–9.

80 Stachyra T, Levasseur P, Pechereau MC *et al*. *In vitro* activity of the β-lactamase inhibitor NXL104 against KPC-2 carbapenemase and Enterobacteriaceae expressing KPC carbapenemases. *J Antimicrob Chemother* 2009; **64**: 326–9.

81 Paukner S, Hesse L, Prezelj A *et al*. *In vitro* activity of LK-157, a novel tricyclic carbapenem as broad-spectrum β-lactamase inhibitor. *Antimicrob Agents Chemother* 2009; **53**: 505–11.

82 Petersen PJ, Jones CH, Venkatesan AM *et al*. Efficacy of piperacillin combined with the penem β-lactamase inhibitor BLI-489 in murine models of systemic infection. *Antimicrob Agents Chemother* 2009; **53**: 1698–700.

83 Endimiani A, Hujer KM, Hujer AM *et al*. ACHN-490, a neoglycoside with potent *in vitro* activity against multidrug-resistant *Klebsiella pneumoniae* isolates. *Antimicrob Agents Chemother* 2009; **53**: 4504–7.