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# Presence of the KPC Carbapenemase Gene in *Enterobacteriaceae* Causing Bacteremia and Its Correlation with In Vitro Carbapenem Susceptibility▽

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**During 6 months, we obtained *Enterobacteriaceae* isolates from patients with gram-negative bacteremia at a 1,250-bed teaching hospital in St. Louis, MO, and compared carbapenem susceptibilities with the presence of $bla_{KPC}$, a transferable carbapenemase gene. Three (1.2%) out of 243 isolates were $bla_{KPC}$ positive. Ertapenem nonsusceptibility had a low positive predictive value.**

The serine carbapenemase KPC (*Klebsiella pneumoniae* carbapenemase) has emerged as a beta-lactamase capable of inactivating carbapenem antibiotics. First identified in *Klebsiella pneumoniae* (21), KPC has since been detected in other *Enterobacteriaceae* (7). The gene encoding KPC, $bla_{KPC}$, is plasmid transmissible among *Enterobacteriaceae*, which has implications for infection control (3, 20). The presence of $bla_{KPC}$ may not always result in carbapenem resistance in vitro (19), thereby impeding detection during routine workup. KPC-producing bacteria have primarily been reported from the New York City area; however, $bla_{KPC}$ is present among *Enterobacteriaceae* isolates as far west as Arkansas (7). The aim of this study was to systematically screen *Enterobacteriaceae* bacteremia isolates for reduced susceptibility to carbapenems and to correlate results with the presence of $bla_{KPC}$.

(This work was presented in abstract form at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, September 2007 [12a].)

Microbiological and molecular analyses were performed on bacterial isolates from inpatients with *Enterobacteriaceae* bacteremia at Barnes-Jewish Hospital in St. Louis, MO. We included patients with bacteremia occurring between 1 August 2006 and 31 January 2007. Isolates were tested for susceptibility to the three carbapenem antibiotics (ertapenem, imipenem, and meropenem) and noncarbapenem antibiotics, using the disk diffusion method (6) (Sensi-Disc antibiotic disks; Becton Dickinson and Co., Sparks, MD).

Total DNA was extracted using the QIAamp DNA minikit (Qiagen, Valencia, CA). A real-time PCR assay of all available isolates ($n = 243$) was developed for initial screening for the presence of $bla_{KPC}$ using primers and cycle parameters as described previously (17). All isolates that were positive for the $bla_{KPC}$ gene by real-time PCR were confirmed with a conventional PCR assay as described previously (5). The three positive isolates were further characterized by DNA sequencing of the $bla_{KPC}$ PCR product using primers (forward, 5′-ATGTCACTGTATCGCCGTC-3′; reverse, 5′-CTCAGTGCTCTACAGAAAACC-3′) and thermocycling parameters described by Yigit et al. (21), with a BigDye Terminator cycle sequencing kit, v3.1 (Applied Biosystems Inc., Foster City, CA) in an MJ Research PTC-200 DNA Engine thermal cycler (Bio-Rad Laboratories, Waltham, MA). Sequencing reaction mixtures were purified by ethanol precipitation, separated, and analyzed using an ABI Prism 3100 genetic analyzer (ABI, Foster City, CA) following the manufacturer's protocols. Forward and reverse strands of two independent PCR products from each isolate were sequenced. Sequences were aligned and compared to published sequences for the $bla_{KPC-2}$ gene using Vector NTI v10.3.0 software (Invitrogen, Carlsbad, CA) and found to be identical to the $bla_{KPC-2}$ published sequence.

Patient data on demographics, comorbidities, treatment, and in-hospital mortality were abstracted from medical records. The Washington University Human Research Protection Office approved this study.

During the study period, 247 *Enterobacteriaceae* isolates were recovered from blood cultures at Barnes-Jewish Hospital. Four isolates were unavailable for testing, leaving 243 *Enterobacteriaceae* isolates from 223 patients. Ninety isolates (37.0%) were *Escherichia coli*, 79 (32.5%) were *Klebsiella pneumoniae*, 25 (10.3%) were *Enterobacter* spp., 13 (5.3%) were *Proteus mirabilis*, 11 (4.5%) were *Klebsiella oxytoca*, 7 (2.9%) were *Citrobacter* spp., 6 (2.5%) were *Serratia marcescens*, and 12 (4.9%) were other species. Seven (2.9%) isolates had reduced susceptibility to one or more carbapenems (Table 1). Two isolates were resistant to all carbapenems tested; both were $bla_{KPC}$ positive. Three isolates were nonsusceptible only to ertapenem; none of these were $bla_{KPC}$ positive.

Three (1.2%) isolates carried the $bla_{KPC}$ gene. These isolates infected three patients (Table 2) and included one *K. pneumoniae*, one *Enterobacter cloacae*, and one *P. mirabilis* isolate. The in vitro ertapenem nonsusceptibility assay detected $bla_{KPC}$ with high sensitivity (100% [three/three]) and high specificity (98.3% [236/240]), similar to results for imipenem (100% [three/three] and 100% [240/240], respectively) and meropenem (66.6% [two/three] and 99.6% [239/240], re-

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TABLE 1. Characteristics of *Enterobacteriaceae* strains exhibiting in vitro carbapenem nonsusceptibility and/or harboring the $bla_{KPC}$ gene

| Source patient | Organism | Disk diffusion test result for antibiotic[a]: | | | | | | $bla_{KPC}$ genotype |
|---|---|---|---|---|---|---|---|---|
| | | Carbapenem | | | Noncarbapenem | | | |
| | | Ertapenem | Imipenem | Meropenem | Cefepime | Ciprofloxacin | Gentamicin | |
| 1 | *K. pneumoniae* | R | R | R | R | R | S | + |
| | *K. pneumoniae* | R | S | R | | | | − |
| 2 | *E. cloacae*[b] | R | R | R | R | S | S | + |
| | *E. cloacae* | R | S | S | | | | − |
| 3 | *P. mirabilis* | I | R | S | S | S | S | + |
| | *E. coli* | R | S | S | | | | − |
| | *Citrobacter freundii* | I | S | S | | | | − |

[a] S, susceptible; I, intermediate; R, resistant. In additional susceptibility testing, the *K. pneumoniae* isolate recovered from patient 1 was intermediately susceptible to tigecycline and susceptible to colistin. Also, the *E. cloacae* isolate from patient 2 was intermediately susceptible to tigecycline and susceptible to colistin.
[b] Recovered from a patient with polymicrobial *Enterobacteriaceae* bacteremia.

spectively) (Table 1). The positive predictive value (PPV) of ertapenem nonsusceptibility for detecting $bla_{KPC}$ was 43% (three/seven) versus 100% (three/three) for imipenem and 66.6% (two/three) for meropenem. The PPV of ertapenem as sole carbapenem showing resistance was 0% (zero/three); the PPV of resistance to all three carbapenems for detecting $bla_{KPC}$ was 100% (two/two). One (33%) of the patients infected with a $bla_{KPC}^{+}$ isolate and 41 (18.6%) of the patients infected with a $bla_{KPC}^{-}$ isolate died.

KPC-positive bacteria were present in 1.3% (3/223) of bacteremia episodes in our study, which is relatively low. However, plasmid transfer and subsequent dissemination can occur (3, 21). In a study by Landman et al., the susceptibility of *K. pneumoniae* isolates to carbapenems decreased from 97% to 76% within 5 years, probably due to $bla_{KPC}$ (11). In a United States-wide surveillance study, the prevalence of $bla_{KPC}$ among various *Enterobacteriaceae* was 0.5% (7), whereas a study of Brooklyn hospitals reported 38% prevalence in *K. pneumoniae* (11). Our data confirm that $bla_{KPC}$ is not restricted to the northeastern United States and warrant surveillance of carbapenem susceptibilities among *Enterobacteriaceae*.

Ertapenem has been proposed as the carbapenem that most accurately detects the presence of $bla_{KPC}$ by disk diffusion (1, 4, 12). This may be because diameter cutoffs for inhibition zones were set more stringently for ertapenem than for other carbapenems (6). Ertapenem was the most frequently nonsusceptible carbapenem in our study; however, the PPV of ertapenem nonsusceptibility for identifying $bla_{KPC}$ was low (43%). This is possibly due to carbapenem resistance mediated by mechanisms other than $bla_{KPC}$ (16). Other studies (15, 19) have found that carbapenem susceptibility testing by the disk diffusion method is unreliable at predicting the presence of $bla_{KPC}$. Possible explanations for undetected $bla_{KPC}$ carriage are an unexpressed $bla_{KPC}$ gene, the inoculum effect (4), and misinterpretation of the resistance pattern to signify an extended-spectrum beta-lactamase producer (15). An MIC that is in the upper range of susceptibility may be the only indication of $bla_{KPC}$. Lowering the imipenem MIC breakpoints (13) or PCR-based screening (2, 9) might increase the chance of detecting resistance.

A limitation of our study is that we did not assess isolates for additional beta-lactamases other than $bla_{KPC}$, which is a constellation increasingly encountered (12, 14). We also had a relatively small sample size and a single-center design, and we restricted analysis to bacteremia isolates. We did not test gram-negative bacteria outside the *Enterobacteriaceae* family for $bla_{KPC}$ (18). In conclusion, our study is among the first prospective investigations into the endemic epidemiology of $bla_{KPC}$-positive bacteria, demonstrating that $bla_{KPC}$ is currently present at a low level in a major Midwestern city. Disk diffusion tests currently remain the simplest screening tests to

TABLE 2. Characteristics of patients with bacteremias caused by $bla_{KPC}$-positive *Enterobacteriaceae*[a]

| Source patient | Age (yrs) | Underlying disease | Location from which admitted | Location at time of blood culture | Source of infection | Type of bacteremia | Organism | Adequate empirical antibiotic treatment | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 61 | Primary biliary cirrhosis with hepatorenal syndrome | Home (central Illinois) | ICU | Respiratory tract | Hospital acquired | *K. pneumoniae* | No (cefepime + ciprofloxacin) | Died |
| 2 | 79 | Enterocutaneous fistula post-hernia repair | Long-term care facility (St. Louis, MO) | Non-ICU | Central venous catheter | Community acquired, health care associated | *E. cloacae*[b] | No (piperacillin-tazobactam) | Recovered |
| 3 | 53 | PVD/DM-associated gangrene | Long-term care facility (St. Louis, MO) | Non-ICU | Skin/soft tissue | Community acquired, health care associated | *P. mirabilis* | Yes (piperacillin-tazobactam) | Recovered |

[a] ICU, intensive care unit; PVD, peripheral vascular disease; DM, diabetes mellitus. A bacteremia was considered hospital acquired if it occurred >48 h after admission. Community-acquired infections were defined as health care associated by using published criteria (8). Inadequate empirical antibiotic treatment was defined as no antibiotic being given to which the bacteria were susceptible within 24 h of the positive blood culture being obtained (10).
[b] Recovered from a patient with polymicrobial *Enterobacteriaceae* bacteremia.

detect *bla*$_{KPC}$-positive bacteria in clinical microbiology laboratories.

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## REFERENCES

1. **Anderson, K. F., D. R. Lonsway, J. K. Rasheed, J. Biddle, B. Jensen, L. K. McDougal, R. B. Carey, A. Thompson, S. Stocker, B. Limbago, and J. B. Patel.** 2007. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in *Enterobacteriaceae*. J. Clin. Microbiol. **95:**2723–2725.
2. **Bratu, S., S. Brooks, S. Burney, S. Kochar, J. Gupta, D. Landman, and J. Quale.** 2007. Detection and spread of *Escherichia coli* possessing the plasmid-borne carbapenemase KPC-2 in Brooklyn, New York. Clin. Infect. Dis. **44:**972–975.
3. **Bratu, S., D. Landman, R. Haag, R. Recco, A. Eramo, M. Alam, and J. Quale.** 2005. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. Arch. Intern. Med. **165:**1430–1435.
4. **Bratu, S., M. Mooty, S. Nichani, D. Landman, C. Gullans, B. Pettinato, U. Karumudi, P. Tolaney, and J. Quale.** 2005. Emergence of KPC-possessing *Klebsiella pneumoniae* in Brooklyn, New York: epidemiology and recommendations for detection. Antimicrob. Agents Chemother. **49:**3018–3020.
5. **Bratu, S., P. Tolaney, U. Karumudi, J. Quale, M. Mooty, S. Nichani, and D. Landman.** 2005. Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, N.Y.: molecular epidemiology and *in vitro* activity of polymyxin B and other agents. J. Antimicrob. Chemother. **56:**128–132.
6. **Clinical and Laboratory Standards Institute.** 2006. Performance standards for antimicrobial susceptibility testing: 16th informational supplement, M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Deshpande, L. M., P. R. Rhomberg, H. S. Sader, and R. N. Jones.** 2006. Emergence of serine carbapenemases (KPC and SME) among clinical strains of Enterobacteriaceae isolated in the United States Medical Centers: report from the MYSTIC Program (1999–2005). Diagn. Microbiol. Infect. Dis. **56:**367–372.
8. **Friedman, N. D., K. S. Kaye, J. E. Stout, S. A. McGarry, S. L. Trivette, J. P. Briggs, W. Lamm, C. Clark, J. MacFarquhar, A. L. Walton, L. B. Reller, and D. J. Sexton.** 2002. Health care-associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann. Intern. Med. **137:**791–797.
9. **Iredell, J. R., and V. Sintchenko.** 2006. Screening for antibiotic resistant Gram-negative bacteria. Lancet Infect. Dis. **6:**316–317.
10. **Kang, C. I., S. H. Kim, W. B. Park, K. D. Lee, H. B. Kim, E. C. Kim, M. D. Oh, and K. W. Choe.** 2005. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli; risk factors for mortality and impact of inappropriate antimicrobial therapy on outcome. Antimicrob. Agents Chemother. **49:**760–766.
11. **Landman, D., S. Bratu, S. Kochar, M. Panwar, M. Trehan, M. Doymaz, and J. Quale.** 2007. Evolution of antimicrobial resistance among *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* in Brooklyn, N.Y. J. Antimicrob. Chemother. **60:**78–82.
12. **Lomaestro, B. M., E. H. Tobin, W. Shang, and T. Gootz.** 2006. The spread of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* to upstate New York. Clin. Infect. Dis. **43:**e26–e28.

12a. **Marschall, J., R. J. Tibbetts, W. M. Dunne, Jr., V. J. Fraser, and D. K. Warren.** 2007. Abstr. 47th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-1935.

13. **Moland, E. S., J. A. Black, J. Ourada, M. D. Reisbig, N. D. Hanson, and K. S. Thomson.** 2002. Occurrence of newer beta-lactamases in *Klebsiella pneumoniae* isolates from 24 U.S. hospitals. Antimicrob. Agents Chemother. **46:**3837–3842.
14. **Moland, E. S., S. G. Hong, K. S. Thomson, D. H. Larone, and N. D. Hanson.** 2007. *Klebsiella pneumoniae* isolate producing at least eight different beta-lactamases, including AmpC and KPC beta-lactamases. Antimicrob. Agents Chemother. **51:**800–801.
15. **Moland, E. S., N. D. Hanson, V. L. Herrera, J. A. Black, T. J. Lockhart, A. Hossain, J. A. Johnson, R. V. Goering, and K. S. Thomson.** 2003. Plasmid-mediated, carbapenem-hydrolysing beta-lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. J. Antimicrob. Chemother. **51:**711–714.
16. **Paterson, D. L.** 2006. Resistance in Gram-negative bacteria: *Enterobacteriaceae*. Am. J. Med. **119:**S20–S28.
17. **Tibbetts, R., J. G. Frye, J. Marschall, D. Warren, and W. M. Dunne.** 2008. Detection of KPC-2 in a clinical isolate of *Proteus mirabilis:* first reported description of carbapenemase resistance in this species caused by a KPC β-lactamase. J. Clin. Microbiol. **46:**3080–3083.
18. **Villegas, M. V., K. Lolans, A. Correa, J. N. Kattan, J. A. Lopez, J. P. Quinn, and the Colombian Nosocomial Resistance Study Group.** 2007. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing beta-lactamase. Antimicrob. Agents Chemother. **51:**1553–1555.
19. **Villegas, M. V., K. Lolans, A. Correa, C. J. Suarez, J. A. Lopez, M. Vallejo, J. P. Quinn, and the Colombian Nosocomial Resistance Study Group.** 2006. First detection of the plasmid-mediated class A carbapenemase KPC-2 in clinical isolates of *Klebsiella pneumoniae* from South America. Antimicrob. Agents Chemother. **50:**2880–2882.
20. **Woodford, N., P. M. Tierno, Jr., K. Young, L. Tysall, M. F. Palepou, E. Ward, R. E. Painter, D. F. Suber, D. Shungu, L. L. Silver, K. Inglima, J. Kornblum, and D. M. Livermore.** 2004. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3, in a New York medical center. Antimicrob. Agents Chemother. **48:**4793–4799.
21. **Yigit, H., A. M. Queenan, G. J. Anderson, A. Domenech-Sanchez, J. W. Biddle, C. D. Steward, S. Alberti, K. Bush, and F. C. Tenover.** 2001. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **45:**1151–1161.