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Presence of the KPC Carbapenemase Gene in *Enterobacteriaceae* Causing Bacteremia, and the Correlation with *in vitro* Carbapenem Susceptibility

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Abstract

During six months, we obtained *Enterobacteriaceae* isolates from patients with Gram-negative bacteremia at a 1250-bed teaching hospital in St. Louis, Missouri, and compared carbapenem susceptibility with the presence of bla_{KPC}, a transferable carbapenemase gene. Three (1.2%) out of 243 isolates were bla_{KPC}-positive. Ertapenem non-susceptibility had a low positive predictive value.

Note

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 since has been detected in other *Enterobacteriaceae* (7). The gene encoding KPC, bla_{KPC}, is plasmid-transmissible among *Enterobacteriaceae*, which has implications for infection control (20, 3). The presence of bla_{KPC} may not always result in carbapenem resistance *in vitro* (19), thereby impeding detection during routine work-up. 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}.

Microbiological and molecular analyses were performed on bacterial isolates from inpatients with *Enterobacteriaceae* bacteremia at Barnes-Jewish Hospital (BJH) in St. Louis, Missouri. We included patients with bacteremia occurring between August 1, 2006 and January 31, 2007. Isolates were tested for susceptibility to the three carbapenem antibiotics (ertapenem, imipenem, and meropenem) and non-carbapenem 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 mini kit (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

54 previously (17). All isolates that were positive for the *bla*$_{KPC}$ gene by real-time PCR were
55 confirmed with a conventional PCR assay as described previously (5). The three positive
56 isolates were further characterized by DNA sequencing of the *bla*$_{KPC}$ PCR product using
57 primers (F-5'-ATGTCACTGTATCGCCGTC-3'; R-5'-
58 CTCAGTGCTCTACAGAAAACC-3') and thermocycling parameters described by Yigit
59 et al. (21), with a BigDye® Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems
60 Inc., Foster City, CA) in a MJ Research PTC-200 DNA Engine thermal cycler (Bio-Rad
61 Laboratories, Waltham, MA). Sequencing reactions were purified by ethanol
62 precipitation, separated and analyzed using an ABI PRISM® 3100 Genetic Analyzer
63 (ABI, Foster City, CA) following the manufacturers protocols. Forward and reverse
64 strands of two independent PCR products from each isolate were sequenced. Sequences
65 were aligned and compared to published sequences for the *bla*$_{KPC-2}$ gene using Vector
66 NTI v10.3.0 software (Invitrogen, Carlsbad, CA) and found to be identical to the *bla*$_{KPC-2}$
67 published sequence.
68 Patient data on demographics, comorbidities, treatment, and in-hospital mortality
69 were abstracted from medical records. The Washington University Human Research
70 Protection Office approved this study.
71 During the study period, 247 *Enterobacteriaceae* isolates were recovered from
72 blood cultures at BJH. Four isolates were unavailable for testing, leaving 243
73 *Enterobacteriaceae* isolates from 223 patients. Ninety isolates were (37.0%) *Escherichia*
74 *coli*, 79 (32.5%) *Klebsiella pneumoniae*, 25 (10.3%) *Enterobacter spp.*, 13 (5.3%)
75 *Proteus mirabilis*, 11 (4.5%) *Klebsiella oxytoca*, 7 (2.9%) *Citrobacter spp.*, 6 (2.5%)
76 *Serratia marcescens*, and 12 others (4.9%). Seven (2.9%) isolates had reduced

susceptibility to ≥1 carbapenem (Table 1). Two isolates were resistant to all carbapenems tested; both were *bla*$_{KPC}$-positive. Three isolates were only non-susceptible 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 *E. cloacae*, and one *P. mirabilis*. *In vitro* ertapenem non-susceptibility detected *bla*$_{KPC}$ with high sensitivity [100% (3/3)] and high specificity [98.3% (236/240)], similar to imipenem [100% (3/3) and 100% (240/240), respectively] and meropenem [66.6% (2/3) and 99.6% (239/240)] (Table 1). The positive predictive value (PPV) of ertapenem non-susceptibility for detecting *bla*$_{KPC}$ was 43% (3/7) versus 100% (3/3) for imipenem, and 66.6% (2/3) for meropenem. The PPV of ertapenem as sole carbapenem showing resistance was 0% (0/3); the PPV of resistance to all three carbapenems for detecting *bla*$_{KPC}$ was 100% (2/2). One (33%) of the patients infected with a *bla*$_{KPC}$(+) isolate and 41(18.6%) 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 (21, 3). In a study by Landman et al., susceptibility of *K. pneumoniae* isolates to carbapenems decreased from 97% to 76% within 5 years, probably due to *bla*$_{KPC}$ (11). In a U.S.-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 U.S. and warrant surveillance of carbapenem susceptibilities among *Enterobacteriaceae*.

100 Ertapenem has been proposed as the carbapenem that most accurately detects the
101 presence of *bla*$_{KPC}$ by disk diffusion (12, 4). This may be because diameter cutoffs for
102 inhibition zones were set more stringently for ertapenem than other carbapenems (6).
103 Ertapenem was the most frequently non-susceptible carbapenem in our study; however,
104 the positive predictive value of ertapenem non-susceptibility for identifying *bla*$_{KPC}$ was
105 low (43%). This is possibly due to carbapenem resistance mediated by mechanisms other
106 than *bla*$_{KPC}$ (15). Other studies (19, 16) have found that carbapenem susceptibility testing
107 by the disk diffusion method is unreliable at predicting the presence of *bla*$_{KPC}$. Possible
108 explanations for undetected *bla*$_{KPC}$-carriage are an unexpressed *bla*$_{KPC}$ gene, the inoculum
109 effect (4), and misinterpretation of the resistance pattern to signify an ESBL-producer
110 (16). A minimal inhibitory concentration (MIC) that is in the upper range of susceptibility
111 may be the only indication of *bla*$_{KPC}$. Lowering the imipenem MIC breakpoints (13) or
112 PCR-based screening (9, 2) might increase the chance of detecting resistance.
113 A limitation of our study is that we did not assess isolates for additional beta-
114 lactamases other than *bla*$_{KPC}$, which is a constellation increasingly encountered (14, 12).
115 We also had a relatively small sample size, a single-center design, and restricted analysis
116 to bacteremia isolates. We did not test Gram-negative bacteria outside the
117 *Enterobacteriaceae* family for *bla*$_{KPC}$ (18). In conclusion, our study is among the first
118 prospective investigations into the endemic epidemiology of *bla*$_{KPC}$-positive bacteria,
119 demonstrating that *bla*$_{KPC}$ is currently present at a low level in a major midwestern city.
120 Disk diffusion tests currently remain the simplest screening tests to detect *bla*$_{KPC}$-positive
121 bacteria in clinical microbiology laboratories.

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References

1. Atkinson R.M., J.J. Lipuma, D.B. Rosenbluth, and W.M. Dunne Jr. 2006. Chronic colonization with *Pandoraea apista* in cystic fibrosis patients determined by repetitive-element-sequence PCR. J. Clin. Microbiol. **44**:833-836.
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, NY: 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: Sixteenth Informational Supplement, M100-S16, CLSI, Wayne, PA, USA.

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, NY. 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**:e26-8.

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. Paterson D.L. 2006. Resistance in Gram-negative bacteria: *Enterobacteriaceae*. Am. J. Med. **119**:S20-S28.
16. Smith Moland E., 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.
17. Tibbetts R., J.G. Frye, J. Marschall, D. Warren, 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. 2008. J. Clin. Microbiol. **46**:3080-3.
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, and J.P. Quinn; 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.

223 **Table 1. Characteristics of *Enterobacteriaceae* strains exhibiting *in vitro* carbapenem non-susceptibility and/or harboring the**

224 ***bla*$_{KPC}$ gene**

225

Source patient	Organism	Disk diffusion test results – Carbapenem antibiotics			Disk diffusion test results – Non-carbapenem antibiotics			*bla*$_{KPC}$ genotype
		Ertapenem	Imipenem	Meropenem	Cefepime	Ciprofloxacin	Gentamicin	
1	*K. pneumoniae*	R	R	R	R	R	S	+
-	*K. pneumoniae*	R	S	R				-
2	*E. cloacae* *	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				-
-	*C. freundii*	I	S	S				-

226

Note: S = susceptible, I = intermediate, R = resistant. In additional susceptibility testing, the *K. pneumoniae* recovered from Patient 1 was intermediately susceptible to tigecycline and susceptible to colistin. Also, the *E. cloacae* from Patient 2 was intermediately susceptible to tigecycline and susceptible to colistin.

* recovered from polymicrobial *Enterobacteriaceae* bacteremia.

Table 2. Characteristics of patients with bacteremias caused by *bla*$_{KPC}$-positive *Enterobacteriaceae*

Source patient	Age	Underlying disease	Admitted from	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, Missouri)*	Non-ICU	Central venous catheter	Community-acquired, healthcare-associated	*E. cloacae**	No (piperacillin/ tazobactam)	Recovered
3	53	*PVD/DM-associated gangrene*	*Long-term care facility (St. Louis, Missouri)*	Non-ICU	Skin/soft tissue	Community-acquired, healthcare-associated	*P. mirabilis*	Yes (piperacillin/ tazobactam)	Recovered

Note: ICU, intensive care unit; PVD, peripheral vascular disease; DM, diabetes mellitus. A bacteremia was considered hospital-acquired if it occurred >48 hours after admission. Community-acquired infections were defined as healthcare-associated using

published criteria (8). Inadequate empirical antibiotic treatment was defined as no antibiotic given to which the bacteria were susceptible within 24 hours of obtaining the positive blood culture (10)

* recovered from polymicrobial *Enterobacteriaceae* bacteremia.