

2009

Presence of the KPC carbapenemase gene in Enterobacteriaceae causing bacteremia and its correlation with in vitro carbapenem susceptibility

Jonas Marschall

Washington University School of Medicine in St. Louis

Robert J. Tibbetts

Washington University School of Medicine in St. Louis

W. Michael Dunne, Jr.

Washington University School of Medicine in St. Louis

Jonathan G. Frye

Bacterial Epidemiology and Antimicrobial Resistance Research Unit, USDA, Agricultural Research Service, Athens, Georgia

Victoria J. Fraser

Washington University School of Medicine in St. Louis

See next page for additional authors

Follow this and additional works at: http://digitalcommons.wustl.edu/icts_facpubs

 Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

Marschall, Jonas; Tibbetts, Robert J.; Dunne, Jr., W. Michael; Frye, Jonathan G.; Fraser, Victoria J.; and Warren, David K., "Presence of the KPC carbapenemase gene in Enterobacteriaceae causing bacteremia and its correlation with in vitro carbapenem susceptibility".

Journal of Clinical Microbiology, 47, 1, 239-241. 2009. Paper 19.

http://digitalcommons.wustl.edu/icts_facpubs/19

This Article is brought to you for free and open access by the Institute of Clinical and Translational Sciences at Digital Commons@Becker. It has been accepted for inclusion in ICTS Faculty Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact engeszer@wustl.edu.

Authors

Jonas Marschall; Robert J. Tibbetts; W. Michael Dunne, Jr.; Jonathan G. Frye; Victoria J. Fraser; and David K. Warren

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

4

5 Jonas Marschall, MD^{1*}, Robert J. Tibbetts, PhD², W. Michael Dunne Jr, PhD², Jonathan
6 G. Frye, PhD³, Victoria J. Fraser, MD¹, David K. Warren, MD, MPH¹

7 ¹ Division of Infectious Diseases, Washington University School of Medicine, St. Louis,
8 MO; ² Medical Microbiology, Division of Laboratory Medicine, Washington University
9 School of Medicine, St. Louis, MO; ³ Bacterial Epidemiology and Antimicrobial
10 Resistance Research Unit, USDA, Agricultural Research Service, Athens, GA.

11

12 **Keywords:** KPC, carbapenemase, bacteremia, Gram-negative bacteria,
13 *Enterobacteriaceae*

14

15 **Running title:** KPC in *Enterobacteriaceae* bacteremia

16

17 **Corresponding author and reprint requests to:**

18 Jonas Marschall, MD

19 Division of Infectious Diseases, Washington University School of Medicine,

20 660 S. Euclid, St. Louis 63110, MO, USA

21 Phone: (314) 454-8044, Fax: (314) 454-8294, E-mail: jmarscha@im.wustl.edu

22

23 **Word count:** Text: 994 (≤1000) Abstract: 50 (≤50) References: 21 (≤40) Tables: 2

24 **Abstract**

25

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

31 **Note**

32

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

44 Microbiological and molecular analyses were performed on bacterial isolates
45 from inpatients with *Enterobacteriaceae* bacteremia at Barnes-Jewish Hospital (BJH) in
46 St. Louis, Missouri. We included patients with bacteremia occurring between August 1,
47 2006 and January 31, 2007. Isolates were tested for susceptibility to the three carbapenem
48 antibiotics (ertapenem, imipenem, and meropenem) and non-carbapenem antibiotics,
49 using the disk diffusion method (6) (Sensi-disc™ antibiotic disks; Becton, Dickinson and
50 Co., Sparks, MD).

51 Total DNA was extracted using the QIAamp DNA mini kit (QIAGEN, Valencia,
52 CA). A real-time PCR assay of all available isolates (n=243) was developed for initial
53 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

77 susceptibility to ≥ 1 carbapenem (Table 1). Two isolates were resistant to all carbapenems
78 tested; both were *bla*_{KPC}-positive. Three isolates were only non-susceptible to ertapenem;
79 none of these were *bla*_{KPC}-positive.

80 Three (1.2%) isolates carried the *bla*_{KPC} gene. These isolates infected three
81 patients (Table 2) and included one *K. pneumoniae*, one *E. cloacae*, and one *P. mirabilis*.
82 *In vitro* ertapenem non-susceptibility detected *bla*_{KPC} with high sensitivity [100% (3/3)]
83 and high specificity [98.3% (236/240)], similar to imipenem [100% (3/3) and 100%
84 (240/240), respectively] and meropenem [66.6% (2/3) and 99.6% (239/240)] (Table 1).
85 The positive predictive value (PPV) of ertapenem non-susceptibility for detecting *bla*_{KPC}
86 was 43% (3/7) versus 100% (3/3) for imipenem, and 66.6% (2/3) for meropenem. The
87 PPV of ertapenem as sole carbapenem showing resistance was 0% (0/3); the PPV of
88 resistance to all three carbapenems for detecting *bla*_{KPC} was 100% (2/2). One (33%) of
89 the patients infected with a *bla*_{KPC}(+) isolate and 41(18.6%) infected with a *bla*_{KPC}(-)
90 isolate died.

91 KPC-positive bacteria were present in 1.3% (3/223) of bacteremia episodes in our
92 study, which is relatively low. However, plasmid transfer and subsequent dissemination
93 can occur (21, 3). In a study by Landman et al., susceptibility of *K. pneumoniae* isolates
94 to carbapenems decreased from 97% to 76% within 5 years, probably due to *bla*_{KPC} (11).
95 In a U.S.-wide surveillance study, the prevalence of *bla*_{KPC} among various
96 *Enterobacteriaceae* was 0.5% (7), whereas a study of Brooklyn hospitals reported 38%
97 prevalence in *K. pneumoniae* (11). Our data confirm that *bla*_{KPC} is not restricted to the
98 northeastern U.S. and warrant surveillance of carbapenem susceptibilities among
99 *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.

122 We thank Joan Hoppe-Bauer from microbiology for her invaluable help in coordinating
123 the retrieval of isolates, and Cherie Hill for her assistance in data management. We also
124 thank Jennifer Bauer-Turpin for technical assistance.

125

126 J.M. received a research grant from the Swiss National Science Foundation (PBBSB-
127 113014). D.K.W. (K23 AI050585) and V.J.F. (IK24 AI 06779401) are funded through
128 the NIH. D.K.W. and V.J.F. also receive funding through the CDC Prevention Epicenter
129 Program (CDC 1U1CI000033301).

130 DK Warren receives research support from Sage Products Inc. and 3M Healthcare, and is
131 a Consultant for Enturia Inc., Novabay Pharmaceuticals, and 3M Healthcare. WM Dunne
132 Jr is a Consultant for bioMérieux. VJ Fraser is a Consultant for Steris and Verimetrix,
133 and Member of the Speakers Bureau for Pfizer, Merck, and Cubist Pharmaceuticals.

134 Neither of the following three authors have a conflict of interest: J Marschall, RJ
135 Tibbetts, and JG Frye.

136 Note: The mention of trade names or commercial products in this manuscript is solely for
137 the purpose of providing specific information and does not imply recommendation or
138 endorsement by the U.S. Department of Agriculture.

139

140 This work was presented in abstract form at the 47th ICAAC, September 2007, Chicago,
141 IL (Abstract # C2-1935).

142 **References**

143

- 144 1. Atkinson R.M., J.J. Lipuma, D.B. Rosenbluth, and W.M. Dunne Jr. 2006. Chronic
145 colonization with *Pandora* *apista* in cystic fibrosis patients determined by
146 repetitive-element-sequence PCR. *J. Clin. Microbiol.* **44**:833-836.
- 147 2. Bratu S., S. Brooks, S. Burney, S. Kochar, J. Gupta, D. Landman, and J. Quale.
148 2007. Detection and spread of *Escherichia coli* possessing the plasmid-borne
149 carbapenemase KPC-2 in Brooklyn, New York. *Clin. Infect. Dis.* **44**:972-975.
- 150 3. Bratu S., D. Landman, R. Haag, R. Recco, A. Eramo, M. Alam, and J. Quale.
151 2005. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York
152 City: a new threat to our antibiotic armamentarium. *Arch. Intern. Med.* **165**:1430-
153 1435.
- 154 4. Bratu S., M. Mooty, S. Nichani, D. Landman, C. Gullans, B. Pettinato, U.
155 Karumudi, P. Tolaney, and J. Quale. 2005. Emergence of KPC-possessing
156 *Klebsiella pneumoniae* in Brooklyn, New York: Epidemiology and
157 recommendations for detection. *Antimicrob. Agents Chemother.* **49**:3018-3020.
- 158 5. Bratu S., P. Tolaney, U. Karumudi, J. Quale, M. Mooty, S. Nichani, and D.
159 Landman. 2005. Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn,
160 NY: molecular epidemiology and *in vitro* activity of polymyxin B and other
161 agents. *J. Antimicrob. Chemother.* **56**:128-132.
- 162 6. Clinical and Laboratory Standards Institute. 2006. Performance Standards for
163 Antimicrobial Susceptibility Testing: Sixteenth Informational Supplement, M100-
164 S16, CLSI, Wayne, PA, USA.

- 165 7. Deshpande L.M., P.R. Rhomberg, H.S. Sader, and R.N. Jones. 2006. Emergence
166 of serine carbapenemases (KPC and SME) among clinical strains of
167 Enterobacteriaceae isolated in the United States Medical Centers: report from the
168 MYSTIC Program (1999-2005). *Diagn. Microbiol. Infect. Dis.* **56**:367-372.
- 169 8. Friedman N.D., K.S. Kaye, J.E. Stout, S.A. McGarry, S.L. Trivette, J.P. Briggs,
170 W. Lamm, C. Clark, J. MacFarquhar, A.L. Walton, L.B. Reller, and D.J. Sexton.
171 2002. Health care--associated bloodstream infections in adults: a reason to change
172 the accepted definition of community-acquired infections. *Ann. Intern. Med.*
173 **137**:791-797.
- 174 9. Iredell J.R., and V. Sintchenko. 2006. Screening for antibiotic resistant Gram-
175 negative bacteria. *Lancet Infect. Dis.* **6**:316-317.
- 176 10. Kang C.I., S.H. Kim, W.B. Park, K.D. Lee, H.B. Kim, E.C. Kim, M.D. Oh, and
177 K.W. Choe. 2005. Bloodstream infections caused by antibiotic-resistant Gram-
178 negative bacilli; risk factors for mortality and impact of inappropriate
179 antimicrobial therapy on outcome. *Antimicrob. Agents Chemother.* **49**:760-766.
- 180 11. Landman D., S. Bratu, S. Kochar, M. Panwar, M. Trehan, M. Doymaz, and J.
181 Quale. 2007. Evolution of antimicrobial resistance among *Pseudomonas*
182 *aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* in Brooklyn,
183 NY. *J. Antimicrob. Chemother.* **60**:78-82.
- 184 12. Lomaestro B.M., E.H. Tobin, W. Shang, and T. Gootz. 2006. The spread of
185 *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* to upstate New
186 York. *Clin. Infect. Dis.* **43**:e26-8.

- 187 13. Moland E.S., J.A. Black, J. Ourada, M.D. Reisbig, N.D. Hanson, and K.S.
188 Thomson. 2002. Occurrence of newer beta-lactamases in *Klebsiella pneumoniae*
189 isolates from 24 U.S. hospitals. *Antimicrob. Agents Chemother.* **46**:3837-3842.
- 190 14. Moland E.S., S.G. Hong, K.S. Thomson, D.H. Larone, and N.D. Hanson. 2007.
191 *Klebsiella pneumoniae* isolate producing at least eight different beta-lactamases,
192 including AmpC and KPC beta-lactamases. *Antimicrob. Agents Chemother.*
193 **51**:800-801.
- 194 15. Paterson D.L. 2006. Resistance in Gram-negative bacteria: *Enterobacteriaceae*.
195 *Am. J. Med.* **119**:S20-S28.
- 196 16. Smith Moland E., N.D. Hanson, V.L. Herrera, J.A. Black, T.J. Lockhart, A.
197 Hossain, J.A. Johnson, R.V. Goering, and K.S. Thomson. 2003. Plasmid-
198 mediated, carbapenem-hydrolysing beta-lactamase, KPC-2, in *Klebsiella*
199 *pneumoniae* isolates. *J. Antimicrob. Chemother.* **51**:711-714.
- 200 17. Tibbetts R., J.G. Frye, J. Marschall, D. Warren, W.M. Dunne. 2008. Detection of
201 KPC-2 in a clinical isolate of *Proteus mirabilis*: First reported description of
202 carbapenemase resistance in this species caused by a KPC β -lactamase. 2008. *J.*
203 *Clin. Microbiol.* **46**:3080-3.
- 204 18. Villegas M.V., K. Lolans, A. Correa, J.N. Kattan, J.A. Lopez, J.P. Quinn; and the
205 Colombian Nosocomial Resistance Study Group. 2007. First identification of
206 *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-
207 hydrolyzing beta-lactamase. *Antimicrob. Agents Chemother.* **51**:1553-1555.
- 208 19. Villegas M.V., K. Lolans, A. Correa, C.J. Suarez, J.A. Lopez, M. Vallejo, and J.P.
209 Quinn; Colombian Nosocomial Resistance Study Group. 2006. First detection of

210 the plasmid-mediated class A carbapenemase KPC-2 in clinical isolates of
211 *Klebsiella pneumoniae* from South America. *Antimicrob. Agents Chemother.*
212 **50**:2880-2882.

213 20. Woodford N., P.M. Tierno Jr., K. Young, L. Tysall, M.F. Palepou, E. Ward, R.E.
214 Painter, D.F. Suber, D. Shungu, L.L. Silver, K. Inghima, J. Kornblum, and D.M.
215 Livermore. 2004. Outbreak of *Klebsiella pneumoniae* producing a new
216 carbapenem-hydrolyzing class A beta-lactamase, KPC-3, in a New York Medical
217 Center. *Antimicrob. Agents Chemother.* **48**:4793-4799.

218 21. Yigit H., A.M. Queenan, G.J. Anderson, A. Domenech-Sanchez, J.W. Biddle,
219 C.D. Steward, S. Alberti, K. Bush, and F.C. Tenover. 2001. Novel carbapenem-
220 hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of
221 *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* **45**:1151-1161.
222

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			<i>bla</i> _{KPC} genotype
		Ertapenem	Imipenem	Meropenem	Cefepime	Ciprofloxacin	Gentamicin	
		1	<i>K. pneumoniae</i>	R	R	R	R	
-	<i>K. pneumoniae</i>	R	S	R				-
2	<i>E. cloacae</i> *	R	R	R	R	S	S	+
-	<i>E. cloacae</i>	R	S	S				-
3	<i>P. mirabilis</i>	I	R	S	S	S	S	+
-	<i>E. coli</i>	R	S	S				-
-	<i>C. freundii</i>	I	S	S				-

226

227 Note: S = susceptible, I = intermediate, R = resistant. In additional susceptibility testing, the *K. pneumoniae* recovered from Patient 1
228 was intermediately susceptible to tigecycline and susceptible to colistin. Also, the *E. cloacae* from Patient 2 was intermediately
229 susceptible to tigecycline and susceptible to colistin.
230 * 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	<i>K. pneumoniae</i>	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	<i>E. cloacae</i> *	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	<i>P. mirabilis</i>	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.

