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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*

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

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

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

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Corresponding author and reprint requests to:

Jonas Marschall, MD

Division of Infectious Diseases, Washington University School of Medicine, 660 S. Euclid, St. Louis 63110, MO, USA

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

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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.
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, \( \text{bla}_{KPC} \), is plasmid-transmissible among *Enterobacteriaceae*, which has implications for infection control (20, 3). The presence of \( \text{bla}_{KPC} \) 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, \( \text{bla}_{KPC} \) 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 \( \text{bla}_{KPC} \).

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 \( \text{bla}_{KPC} \) using primers and cycle parameters as described.
previously (17). All isolates that were positive for the \textit{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 \textit{bla}_{KPC} PCR product using primers (F-5’-ATGTCAGTGCTCTACAGAAAACC-3’; R-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 a MJ Research PTC-200 DNA Engine thermal cycler (Bio-Rad Laboratories, Waltham, MA). Sequencing reactions were purified by ethanol precipitation, separated and analyzed using an ABI PRISM® 3100 Genetic Analyzer (ABI, Foster City, CA) following the manufacturers 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 \textit{bla}_{KPC-2} gene using Vector NTI v10.3.0 software (Invitrogen, Carlsbad, CA) and found to be identical to the \textit{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 \textit{Enterobacteriaceae} isolates were recovered from blood cultures at BJH. Four isolates were unavailable for testing, leaving 243 \textit{Enterobacteriaceae} isolates from 223 patients. Ninety isolates were (37.0%) \textit{Escherichia coli}, 79 (32.5%) \textit{Klebsiella pneumoniae}, 25 (10.3%) \textit{Enterobacter} spp., 13 (5.3%) \textit{Proteus mirabilis}, 11 (4.5%) \textit{Klebsiella oxytoca}, 7 (2.9%) \textit{Citrobacter} spp., 6 (2.5%) \textit{Serratia marcescens}, and 12 others (4.9%). Seven (2.9%) isolates had reduced
susceptibility to $\geq 1$ carbapenem (Table 1). Two isolates were resistant to all carbapenems tested; both were $\text{bla}_{\text{KPC}}$-positive. Three isolates were only non-susceptible to ertapenem; none of these were $\text{bla}_{\text{KPC}}$-positive.

Three (1.2%) isolates carried the $\text{bla}_{\text{KPC}}$ 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 $\text{bla}_{\text{KPC}}$ 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 $\text{bla}_{\text{KPC}}$ 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 $\text{bla}_{\text{KPC}}$ was 100% (2/2). One (33%) of the patients infected with a $\text{bla}_{\text{KPC}}$-positive isolate and 41 (18.6%) infected with a $\text{bla}_{\text{KPC}}$-negative 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 $\text{bla}_{\text{KPC}}$ (11). In a U.S.-wide surveillance study, the prevalence of $\text{bla}_{\text{KPC}}$ among various $\text{Enterobacteriaceae}$ was 0.5% (7), whereas a study of Brooklyn hospitals reported 38% prevalence in $K.\ pneumoniae$ (11). Our data confirm that $\text{bla}_{\text{KPC}}$ is not restricted to the northeastern U.S. and warrant surveillance of carbapenem susceptibilities among $\text{Enterobacteriaceae}$. 

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Ertapenem has been proposed as the carbapenem that most accurately detects the presence of $bla_{KPC}$ by disk diffusion (12, 4). This may be because diameter cutoffs for inhibition zones were set more stringently for ertapenem than other carbapenems (6). Ertapenem was the most frequently non-susceptible carbapenem in our study; however, the positive predictive value of ertapenem non-susceptibility for identifying $bla_{KPC}$ was low (43%). This is possibly due to carbapenem resistance mediated by mechanisms other than $bla_{KPC}$ (15). Other studies (19, 16) 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 ESBL-producer (16). A minimal inhibitory concentration (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 (9, 2) 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 (14, 12). We also had a relatively small sample size, a single-center design, and 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 detect $bla_{KPC}$-positive bacteria in clinical microbiology laboratories.
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Neither of the following three authors have a conflict of interest: J Marschall, RJ Tibbetts, and JG Frye.

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

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References


Table 1. Characteristics of Enterobacteriaceae strains exhibiting in vitro carbapenem non-susceptibility and/or harboring the \( \text{bla}_{\text{KPC}} \) gene

<table>
<thead>
<tr>
<th>Source (patient)</th>
<th>Organism</th>
<th>Disk diffusion test results – Carbapenem antibiotics</th>
<th>Disk diffusion test results – Non-carbapenem antibiotics</th>
<th>( \text{bla}_{\text{KPC}} ) genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ertapenem</td>
<td>Imipenem</td>
<td>Meropenem</td>
</tr>
<tr>
<td>1</td>
<td>\textit{K. pneumoniae} &amp; R &amp; R &amp; R &amp; R &amp; R &amp; S &amp; +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>\textit{K. pneumoniae} &amp; R &amp; S &amp; R &amp; -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>\textit{E. cloacae} &amp; R &amp; R &amp; R &amp; R &amp; S &amp; S &amp; +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>\textit{E. cloacae} &amp; R &amp; S &amp; S &amp; -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>\textit{P. mirabilis} &amp; I &amp; R &amp; S &amp; S &amp; S &amp; S &amp; +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>\textit{E. coli} &amp; R &amp; S &amp; S &amp; -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>\textit{C. freundii} &amp; I &amp; S &amp; S &amp; -</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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*<sub>KPC</sub>-positive *Enterobacteriaceae*

<table>
<thead>
<tr>
<th>Source patient</th>
<th>Age</th>
<th>Underlying disease</th>
<th>Admitted from</th>
<th>Location at time of blood culture</th>
<th>Source of infection</th>
<th>Type of bacteremia</th>
<th>Organism</th>
<th>Adequate empirical antibiotic treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>Primary biliary cirrhosis with hepatorenal syndrome</td>
<td>Home (central Illinois)</td>
<td>ICU</td>
<td>Respiratory tract</td>
<td>Hospital-acquired</td>
<td><em>K. pneumoniae</em></td>
<td>No (cefepime + ciprofloxacin)</td>
<td>Died</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>Enterocutaneous fistula post-hernia repair</td>
<td>Long-term care facility (St. Louis, Missouri)</td>
<td>Non-ICU</td>
<td>Central venous catheter</td>
<td>Community-acquired, healthcare-associated</td>
<td><em>E. cloacae</em>&lt;sup&gt;*&lt;/sup&gt;</td>
<td>No (piperacillin/tazobactam)</td>
<td>Recovered</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>PVD/DM-associated gangrene</td>
<td>Long-term care facility (St. Louis, Missouri)</td>
<td>Non-ICU</td>
<td>Skin/soft tissue</td>
<td>Community-acquired, healthcare-associated</td>
<td><em>P. mirabilis</em></td>
<td>Yes (piperacillin/tazobactam)</td>
<td>Recovered</td>
</tr>
</tbody>
</table>

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.