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
*USDA*

Victoria J. Fraser
*Washington University School of Medicine in St. Louis*

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Recommended Citation
https://digitalcommons.wustl.edu/open_access_pubs/2414

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact engeszer@wustl.edu.
Presence of the KPC Carbapenemase Gene in Enterobacteriaceae Causing Bacteremia and Its Correlation with In Vitro Carbapenem Susceptibility


Published Ahead of Print 19 November 2008.

Updated information and services can be found at: http://jcm.asm.org/content/47/1/239

These include:

**REFERENCES**

This article cites 20 articles, 14 of which can be accessed free at: http://jcm.asm.org/content/47/1/239#ref-list-1

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: http://journals.asm.org/site/misc/reprints.xhtml
To subscribe to another ASM Journal go to: http://journals.asm.org/site/subscriptions/
Presence of the KPC Carbapenemase Gene in Enterobacteriaceae Causing Bacteremia and Its Correlation with In Vitro Carbapenem Susceptibility

Jonas Marschall,1,∗ Robert J. Tibbetts,2 W. Michael Dunne, Jr.,2 Jonathan G. Frye,3 Victoria J. Fraser,1 and David K. Warren1

Division of Infectious Diseases, Washington University School of Medicine, St. Louis, Missouri;1 Medical Microbiology, Division of Laboratory Medicine, Washington University School of Medicine, St. Louis, Missouri;2 and Bacterial Epidemiology and Antimicrobial Resistance Research Unit, USDA, Agricultural Research Service, Athens, Georgia3

Received 5 November 2008/Accepted 8 November 2008

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 blaKPC, a transferable carbapenemase gene. Three (1.2%) out of 243 isolates were blaKPC 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, blaKPC, is plasmid transmissible among Enterobacteriaceae, which has implications for infection control (3, 20). The presence of blaKPC 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, blaKPC 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 blaKPC.

(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 Dickenson, 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 blaKPC using primers and cycle parameters as described previously (17). All isolates that were positive for the blaKPC 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 blaKPC PCR product using primers (forward, 5′-ATGTCA CTGTATCGCCGTC-3′; reverse, 5′-CTCAATGCTCCTACAG AAAACC-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 blaKPC-2 gene using Vector NTI v10.3.0 software (Invitrogen, Carlsbad, CA) and found to be identical to the blaKPC-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 blaKPC positive. Three isolates were nonsusceptible only to ertapenem; none of these were blaKPC positive.

Three (1.2%) isolates carried the blaKPC gene. These isolates infected three patients (Table 2) and included one K. pneumoniae, one Enterobacter cloaceae, and one P. mirabilis isolate. The in vitro ertapenem nonsusceptibility assay detected blaKPC 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-
spective) (Table 1). The positive predictive value (PPV) of ertapenem nonsusceptibility for detecting \( \text{bla}_{\text{KPC}} \) 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 \( \text{bla}_{\text{KPC}} \) was 100% (two/two). One (33%) of the patients infected with a \( \text{bla}^{+}_{\text{KPC}} \) isolate and 41 (18.6%) of the patients infected with a \( \text{bla}^{+}_{\text{KPC}} \) isolate died.

KPC-positive bacteria were present in 1.3% (3/232) 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 \( K. \text{pneumoniae} \) isolates to carbapenems decreased from 97% to 76% within 5 years, probably due to \( \text{bla}_{\text{KPC}} \) (11). In a United States-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 \( K. \text{pneumoniae} \) (11). Our data confirm that \( \text{bla}_{\text{KPC}} \) is not restricted to the northeastern United States and warrant surveillance of carbapenem resistances among \( \text{Enterobacteriaceae} \).

Ertapenem has been proposed as the carbapenem that most accurately detects the presence of \( \text{bla}_{\text{KPC}} \) 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 \( \text{bla}_{\text{KPC}} \) was low (43%). This is possibly due to carbapenem resistance mediated by mechanisms other than \( \text{bla}_{\text{KPC}} \) (16). Other studies (15, 19) have found that carbapenem susceptibility testing by the disk diffusion method is unreliable at predicting the presence of \( \text{bla}_{\text{KPC}} \). Possible explanations for undetected \( \text{bla}_{\text{KPC}} \) are an unexpressed \( \text{bla}_{\text{KPC}} \) 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 \( \text{bla}_{\text{KPC}} \). 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 \( \text{bla}_{\text{KPC}} \), 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 \( \text{Enterobacteriaceae} \) family for \( \text{bla}_{\text{KPC}} \) (18). In conclusion, our study is among the first prospective investigations into the endemic epidemiology of \( \text{bla}_{\text{KPC}} \)-positive bacteria, demonstrating that \( \text{bla}_{\text{KPC}} \) is currently present at a low level in a major Midwestern city. Disk diffusion tests currently remain the simplest screening tests to identify \( \text{bla}_{\text{KPC}} \)-positive bacteria.

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

<table>
<thead>
<tr>
<th>Source patient</th>
<th>Organism</th>
<th>Carbenem</th>
<th>Noncarbenem</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>( K. \text{pneumoniae} )</td>
<td>R</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>( K. \text{pneumoniae} )</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>( E. \text{cloacae} )</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>( P. \text{mirabilis} )</td>
<td>I</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>( E. \text{coli} )</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>Citrobacter freundii</td>
<td>I</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

* S: susceptible; I: intermediate; R: resistant. In additional susceptibility testing, the \( K. \text{pneumoniae} \) isolate recovered from patient 1 was immediately susceptible to tigecycline and susceptible to colistin. Also, the \( E. \text{cloacae} \) isolate from patient 2 was immediately susceptible to tigecycline and susceptible to colistin.

* Recovered from a patient with polymicrobial \( \text{Enterobacteriaceae} \) bacteremia.

### Table 2. Characteristics of patients with bacteremias caused by \( \text{bla}_{\text{KPC}} \)-positive \( \text{Enterobacteriaceae} \)

<table>
<thead>
<tr>
<th>Source patient</th>
<th>Age (yrs)</th>
<th>Underlying disease</th>
<th>Location from which admitted</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>( K. \text{pneumoniae} )</td>
<td>No (cefeplime + 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, MO)</td>
<td>Non-ICU</td>
<td>Central venous catheter</td>
<td>Community acquired, health care associated</td>
<td>( E. \text{cloacae} )</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, MO)</td>
<td>Non-ICU</td>
<td>Skin/soft tissue</td>
<td>Community acquired, health care associated</td>
<td>( P. \text{mirabilis} )</td>
<td>Yes (piperacillin-tazobactam)</td>
<td>Recovered</td>
</tr>
</tbody>
</table>

* 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).

* Recovered from a patient with polymicrobial \( \text{Enterobacteriaceae} \) bacteremia.
detect blaKPC-positive bacteria in clinical microbiology laboratories.

We thank Joan Hoppe-Bauer from the Microbiology department of Barnes-Jewish Hospital for her invaluable help in coordinating the retrieval of isolates and Cheerie Hill for her assistance in data management. We also thank Jennifer Bauer-Turpin for technical assistance.

J.M. received a research grant from the Swiss National Science Foundation (PBBSB-113014). D.K.W. (K23 AI050585) and V.J.F. (IK24 AI 06779401) are funded through the NIH. D.K.W. and V.J.F. also receive funding through the CDC Prevention Epicenter Program (CDC 1U1CI000033-01).

D.K.W. receives research support from Sage Products Inc. and 3M Healthcare and is a consultant for Enturia Inc., Novabay Pharmaceuticals, and 3M Healthcare. W.M.D. is a consultant for bioMérieux. V.J.F. is a consultant for Steris and Verimetrix and a member of the speakers’ bureau for Pfizer, Merck, and Cubist Pharmaceuticals. None of the following three authors have a conflict of interest: J.M., R.J.T., and J.G.F.

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

REFERENCES


