KPC-2 producing *Enterobacter cloaceae* and *Pseudomonas putida* co-infection in a liver transplant recipient

JASON W. BENNETT¹, MONICA L. HERRERA², JAMES S. LEWIS II³⁵, BRIAN W. WICKES³, and JAMES H. JORGENSEN²³⁴*¹

¹San Antonio Military Medical Center, Fort Sam Houston, Texas 78234
Departments of Microbiology², Medicine³, and Pathology⁴, University of Texas Health Science Center, San Antonio, Texas 78229
⁵Pharmacy Department, University Health System, San Antonio, Texas 78229

*Corresponding author

James H. Jorgensen, Ph.D.
Department of Pathology
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, Texas 78229-3900
PH 210-567-4088
Fax 210-567-2367
jorgensen@uthscsa.edu
Abstract

Carbapenemases are among the newest resistance mechanisms to emerge in some gram-negative bacteria. We describe bacteremia in a critically ill liver transplant recipient with KPC-2 producing \textit{Enterobacter cloacae} and \textit{Pseudomonas putida}. Although previously described in \textit{Enterobacter} spp., this is the first report of this enzyme in \textit{P. putida}.
Carbapenems are the broadest-spectrum β-lactam antibiotics, and retain activity against many antibiotic resistant organisms to include gram-negatives that produce extended spectrum or AmpC β-lactamases (11). However, reports of carbapenem hydrolyzing enzymes have become increasingly frequent in some locations in recent years (5). In the U.S., the most common carbapenemases to emerge have been the *Klebsiella pneumoniae* carbapenemases (KPCs) (5).

Since the first report of this plasmid-mediated carbapenemase in North Carolina, several outbreaks caused by KPC-producing isolates have been documented elsewhere, in particular in the Northeastern U.S. (5, 11). KPC-producing isolates have also emerged in states outside of the Northeastern U.S. to include Arkansas, Michigan, Missouri, Ohio, and Pennsylvania (12, 13).

Clinical microbiology laboratories are becoming increasingly aware of the emergence of carbapenemase-producing organisms, but identification of such isolates remains difficult (2). In 2008, the Clinical and Laboratory Standards Institute suggested that KPC-producing isolates may display elevated carbapenem MICs of 2 or 4 µg/ml (6). Although these isolates are still considered “susceptible” based upon current CLSI interpretive criteria, they may not respond to carbapenem therapy (6). At present, CLSI has not recommended a phenotypic test to confirm KPC production. In this report we describe simultaneous infection with KPC-2 producing *Enterobacter cloacae* and *Pseudomonas putida* in a critically ill liver transplant recipient.

A 54 year old female was admitted to our University Hospital to undergo an orthotopic liver transplant from an unrelated donor. Immediately following surgery, she required emergent surgical exploration due to hemorrhage. She experienced a complicated hospital course including acute renal failure requiring hemodialysis, pulmonary embolus, and right lobe liver infarct. She had an open post-surgical abdominal wound following biliary anastamosis, right hepatic lobectomy, and repair of jejuno-jejunostomy. Due to a persistently elevated white blood
cell count and the frequent need for vasopressor support, she received prolonged courses of broad spectrum antibiotics to include 5 weeks of empiric meropenem and 7 weeks of linezolid. She also received trimethoprim-sulfamethoxazole, valgancyclovir, and anti-fungal prophylaxis throughout her hospital course in accordance with the local transplant protocol.

On hospital day 45, multi-drug resistant *Enterobacter cloacae* and *Pseudomonas putida* with similar antibiotic susceptibility profiles grew from separate blood cultures (Table 1). Both isolates were susceptible to amikacin and the patient was treated with that agent. Ciprofloxacin was added to the antibiotic regimen to broaden coverage; the *E. cloacae* demonstrated only intermediate susceptibility to this antimicrobial agent. The colistin MIC of the *P. putida* was 2 µg/ml, but the *E. cloacae* had an MIC > 16 µg/ml based upon CLSI reference broth microdilution testing (7). CLSI does not have specific colistin breakpoints for the *Enterobacteriaceae* or for *P. putida*, but an MIC ≤ 2 µg/ml is considered susceptible for *P. aeruginosa* and *Acinetobacter* spp., and an MIC > 16 µg/ml would be resistant for both organisms (6). Subsequent to these blood isolates, both of these organisms grew from tissue cultures obtained during debridement of sacral and abdominal wounds. *Proteus mirabilis*, *Stenotrophomonas maltophilia*, and *E. coli* were also isolated from the same wound cultures. Despite aggressive broad spectrum antimicrobial therapy, the patient expired 12 days following her first episode of bacteremia.

The *E. cloacae* and *P. putida* isolates were both initially identified using the VITEK 2 instrument (bioMérieux, Hazelwood, MO). The identifications were confirmed by performing 16S rDNA sequencing (15). Because of resistance to meropenem and to all other β-lactams tested, both isolates were screened for the presence of a carbapenemase using the modified Hodge test (2). Both strains demonstrated carbapenem hydrolysis using imipenem as the test
substrate (Figure 1). PCR amplification of DNA extracts using previously described primers and test conditions for various extended-spectrum β-lactamases (ESBLs) and KPCs were performed followed by sequencing of PCR products (9, 12). This revealed the presence of \( \text{bla}_{\text{KPC-2}} \) in both isolates and \( \text{bla}_{\text{SHV-12}} \) in the \( \text{E. cloacae} \).

Our patient suffered from bacteremia due to KPC-2 producing \( \text{Enterobacter cloacae} \) and \( \text{Pseudomonas putida} \) recovered simultaneously from multiple cultures, and she eventually expired. Although the initial source of infection was undetermined, both organisms were isolated from wound cultures as well as blood. It is possible that transfer of the plasmid encoding the carbapenemase could have occurred in the milieu of the mixed wound infection. Prior to this patient’s infection, no KPC-producing isolates had been recovered in this hospital, and no others have been detected since this case.

In the United States, KPC enzymes have emerged as a major clinical concern amongst some members of the \( \text{Enterobacteriaceae} \), but these enzymes have rarely been described outside of that family (5, 11). Recently, three \( \text{Pseudomonas aeruginosa} \) clinical isolates from Colombia were found to express KPC-2; however, we believe that this represents the first report of KPC production in \( \text{P. putida} \) (14). Infections caused by \( \text{P. putida} \) are relatively rare and are generally restricted to immunocompromised patients and patients with invasive medical devices in place (4). While not previously recognized to produce KPC, this member of the fluorescent group of pseudomonads is often resistant to fluoroquinolones, aminoglycosides, and various β-lactams (1, 3, 8, 10). Previously described carbapenem-resistant \( \text{P. putida} \) isolates have been associated with production of IMP- or VIM-type metallo-β-lactamases (MBLs), but not KPCs (1, 3, 8, 10).

Non-susceptibility to colistin is described amongst the \( \text{Enterobacteriaceae} \) (5). When present in combination with a carbapenemase, therapeutic options are extremely limited. It is
particularly unusual that this isolate was non-susceptible given that this patient had not been
exposed to colistin during her hospital course. Susceptibility to colistin should not be assumed
and appropriate testing should be performed by when therapy with this potentially toxic
antimicrobial agent is contemplated.

These appear to represent the first KPC-producing clinical isolates in Texas as well as the
first occurrence of \textit{bla}_{KPC-2} in \textit{P. putida}. This extends the host range for the KPC-2 $\beta$-lactamase
into another \textit{Pseudomonas} species. Microbiologists and clinicians should be aware that
carbapenemases can appear in several different species and in different gram-negative bacterial
families. Practical phenotypic screening and confirmatory tests are needed to facilitate timely
detection of such strains by clinical microbiology laboratories.
References


Table 1. Antimicrobial agent susceptibility of the two clinical isolates

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>E. cloacae</th>
<th>P. putida</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefepime</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td></td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2</td>
<td>&gt;8</td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>2</td>
<td>&gt;16</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>16</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>&gt;16</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;4</td>
<td>&gt;4</td>
<td></td>
</tr>
</tbody>
</table>

bla enzymes detected  KPC-2, SHV-12  KPC-2
FIG. 1 The modified Hodge test using a 10 μg imipenem disk. Isolate A (K. pneumoniae, ATCC 700603) does not produce a carbapenemase and is negative by this test. Isolates B (P. putida), C (E. cloacae), and D (K. pneumoniae, CAP D05-07) all produce KPC-2 and are positive by the test.