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First Clinical Cases of OXA-48-Producing Carbapenem-Resistant *Klebsiella pneumoniae* in the United States: the "Menace" Arrives in the New World

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OXA-48 has emerged as a major carbapenemase associated with the *Enterobacteriaceae* in Europe, North Africa, and Asia. We report the first two clinical cases of OXA-48-type carbapenemase-producing *Enterobacteriaceae* in the United States from patients recently hospitalized in Saudi Arabia and India. Each is more carbapenem resistant than nearly all previously reported OXA-48-type-producing *Enterobacteriaceae*.

CASE REPORTS

Case 1. In April 2012, a 55-year-old woman with hepatitis C-related end-stage liver disease was admitted to the University of Virginia Medical Center (UVAMC) for evaluation for liver transplantation. Before admission, she was repeatedly hospitalized for cirrhosis, most recently at a tertiary care center in Riyadh, Saudi Arabia. In Saudi Arabia, she was treated for culture-negative peritonitis with 7 days of piperacillin-tazobactam before being transferred to UVAMC. She was initially placed in a double room without contact isolation. Upon admission she had evidence of ongoing peritonitis with a peritoneal fluid neutrophil count of 4,000 cells per cubic millimeter. No fluid culture was sent, and the piperacillin-tazobactam was continued. Four days into the hospitalization, a perirectal swab was obtained per hospital surveillance protocol, because another patient on the same unit was known to be colonized with *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacter cloacae*.

On hospital day 6 she developed acute kidney injury, required intubation due to uncompensated metabolic acidosis, and was transferred to the intensive care unit for mechanical ventilation and continuous renal replacement therapy. Vasopressors were initiated for blood pressure support. The following day, her perirectal swab was positive for a carbapenemase-producing *K. pneumoniae* isolate and she was placed on contact precautions. Antibiotics were changed to meropenem, tigecycline, amikacin, and fluconazole. Additional blood, urine, and peritoneal fluid cultures were sent, but all were negative. Despite improvement in her peritoneal fluid cell count, she continued to deteriorate in the setting of decompensated liver failure and septic shock. Cultures failed to reveal a definitive source of infection, and she expired on hospital day 10. An autopsy was not performed.

Case 2. In May 2012, a 55-year-old woman who was recently diagnosed with intrahepatic cholangiocarcinoma underwent left hepatectomy in a large hospital near New Delhi, India. Following discharge, the patient developed intrahepatic biliary dilatation and peritonitis. Percutaneous drains were placed after failed stenting. The patient traveled to the United States seeking further care. She was originally admitted to a Northern Virginia hospital in early July. Cholangiography revealed complete occlusion of the anterior intrahepatic ducts and transection of the right inferior intrahepatic duct with free extravasation of contrast. Her drains were exchanged, and she was discharged after completing 7 days of piperacillin-tazobactam. Despite some early improvement she developed intermittent fever, right upper quadrant pain, and drainage around her percutaneous drains. She presented in the last week of July to the UVAMC emergency department.

Fluid from her abdominal drain grew multidrug-resistant *K. pneumoniae* (Table 1). On hospital day 5, trimethoprim-sulfamethoxazole and amikacin were added to empirical piperacillin-tazobactam. She was placed in a single room on contact precautions. Due to ongoing biliary leak, the patient underwent Roux-en-Y repair. A perirectal swab obtained on hospital day 8 isolated *K. pneumoniae* with the same resistance pattern as her clinical isolate. At 1 month she is without evidence of ongoing biliary leak or infection.

Organism identification and susceptibility testing were performed by VITEK2 (bioMérieux, Durham, NC) and MicroScan (West Sacramento, CA) (Table 1). Both cases' perirectal swabs were originally placed in tryptic soy broth with a 10-μg ertapenem disk for overnight incubation and then plated on RambaCHROM agar KPC (CHROMagar, Paris, France). Both isolates (case 1 isolate, CAV1543; case 2 isolate, CAV1675) were identified as *K. pneumoniae* and demonstrated evidence of carbapenemase production with positive modified Hodge tests (MHT). Double-disk tests for metallo-β-lactamase production were negative (1). Interestingly, the *K. pneumoniae* strain from the biliary drain culture of case 2 (CAV1636) had a negative MHT despite having similar susceptibilities and a multilocus sequence type identical to those of the patient's perirectal isolate.

Plasmid transfer from the CAV1543 took place via mating with *Escherichia coli* J53 Rif^r as previously described (2). PCR of the *K.*

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TABLE 1 Features of clinical strains of *K. pneumoniae* and *E. coli* J53 and transconjugate description

Parameter	Result for strain			
	CAV1543	CAV1636	J53 p1543A	J53
Origin	Saudi Arabia	India	NA[d]	NA
MLST	ST199	ST43	NA	NA
Plasmid incompatibility group	IncL/M	IncHIIB	IncL/M	NA
Associated β-lactamase	OXA-48, TEM, SHV, CTX-M-9	OXA-181, TEM, SHV, CTX-M-15	OXA-48, CTX-M-9	NA
Susceptibility testing (μg/ml)[a]				
Ampicillin	>16	>16	>16	≤8
Aztreonam	16	≤8	≥16	≤8
Ceftazidime	>16	>16	4	≤1
Cefotetan	≤16	32	≤16	≤16
Cefepime	>16	>16	>16	≤2
Piperacillin-tazobactam	>64	>64	>64	
Ciprofloxacin	>2	>2	≤1	≤1
Amikacin	8	32	≤4	≤4
Gentamicin	≤1	>8	≤1	≤1
Tobramycin	8	>8	≤1	≤1
Trimethoprim-sulfamethoxazole	>2/38	≤2/38	≤2/38	≤2/38
Tetracycline[b]	≥16	8	≥16	≤1
Tigecycline	≥8	3	≤0.5	≤0.5
Ertapenem				
VITEK[b]	≥8	≥8	≥8	≤0.5
Diam (mm)[c]	10	8	18	33
Imipenem				
MicroScan	≤4	≤4	≤4	≤4
VITEK[b]	2	8	8	≤1
Diam (mm)[c]	17	19	20	30
Meropenem				
MicroScan	≤4	8	≤4	≤4
VITEK[b]	4	≥16	1	≤0.25
Diam (mm)[c]	14	13		32

[a] MICs determined with Clinical and Laboratory Standards Institute (CLSI) M100-S19 using MicroScan unless otherwise noted.
[b] MICs determined by VITEK2 automated system using ASTGN45.
[c] Diameters of inhibition by disk diffusion.
[d] NA, not applicable.

pneumoniae isolates demonstrated the presence of $bla_{\text{OXA-48}}$ family gene products. From case 1, bidirectional sequencing of the PCR product in both CAV1543 and its transconjugate J53p1543 demonstrated the presence of $bla_{\text{OXA-48}}$ (100% identity over 698 bp) (3). From case 2, sequencing of the PCR product from CAV1636 demonstrated the presence of a different allele in the same family of OXA-48-type enzymes: $bla_{\text{OXA-181}}$ (100% identity over 668 bp) (4). PCR screening for bla_{KPC}, bla_{VIM}, and bla_{NDM} failed to demonstrate the presence of other carbapenemases (5–7).

Both cases' isolates were found to have additional β-lactamases by multiplex PCR and sequencing (5). CAV1543 and J53p1543 were positive for $bla_{\text{CTX-M-9}}$ in addition to $bla_{\text{OXA-48}}$ by PCR and sequencing. The presence of $bla_{\text{CTX-M-9}}$ on the same plasmid as $bla_{\text{OXA-48}}$ was further established by Southern blotting of CAV1543 and J53p1543 (6). In addition, CAV1543 but not J53p1543 was found to harbor bla_{TEM} and bla_{SHV} by PCR. The *K. pneumoniae* isolate from case 2 (CAV1636) had bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M-15}}$ by PCR and sequencing; however, we were unable to generate a transconjugate for CAV1636.

Multilocus sequence typing (MLST) of *K. pneumoniae* using established primers revealed that the two patients' strains were different (Table 1) (8). The sequence type of CAV1543 has not been reported to carry a $bla_{\text{OXA-48}}$-type gene; however, the sequence type of CAV1363 was recently identified in an isolate from India carrying both $bla_{\text{OXA-181}}$ and $bla_{\text{CTX-M-15}}$ (9). PCR evaluation of the CAV1543 plasmid for elements conserved in the OXA-48 epidemic plasmid demonstrated amplicons of the predicted sizes for *parA*, *repA*, and *traU*. Furthermore, the CAV1543 plasmid, like the OXA-48 epidemic plasmid, belonged to the incompatibility group IncL/M (3).

Once $bla_{\text{OXA-48}}$-type carbapenemase-producing *Enterobacteriaceae* (CPE) were identified, perirectal sweeps were performed on 42 inpatients who had received care on the same unit as either case. No $bla_{\text{OXA-48}}$-type CPE were detected. In addition, for 30 days before and after each patient admission, all *Enterobacteriaceae* throughout the health system with a positive carbapenemase phenotype were negative for the $bla_{\text{OXA-48}}$-type gene by PCR.

The emergence and global spread of CPE are creating a worldwide health care crisis in the management of these infections. The surge in CPE infections has been driven by dissemination of the Ambler class A *K. pneumoniae* carbapenemase (KPC) and the class B New Delhi metallo-β-lactamase (NDM) (7). Until recently, class D β-lactamases, also termed oxacillinases (OXA), were pri-

marily associated with *Acinetobacter baumannii* but not the *Enterobacteriaceae*. However, over the last 3 years, OXA-48 and related variants have rapidly emerged in *Enterobacteriaceae* from the Middle East, North Africa, India, and Europe to become a major etiology of CPE (10).

To our knowledge, these are the first two case reports with clinical analysis of OXA-48-type CPE in the United States. Lascols et al. recently detected two bla_{OXA-48}-positive *K. pneumoniae* isolates from the United States in a retrospective analysis of archived isolates, but the origin of those strains is unclear and no clinical information was provided in the report (9). Prospective identification of two unique isolates at a single hospital within a 4-month period along with retrospective data showing presence in the United States since 2009 raises concern that other institutions may have unknowingly cared for patients harboring similar organisms (9). This is worrisome because poor recognition has facilitated OXA-48 to become the most frequently encountered carbapenemase produced by *Enterobacteriaceae* in some countries (10, 11). The negative MHT for the clinical isolate of case 2, despite the presence of $bla_{OXA-181}$, is notable. Indeed, the performance of the MHT for OXA-48 CPE is largely unknown. While an evaluation of 10 OXA-48-producing CPE isolates showed them all as positive by MHT in one study, this may not reflect the sensitivity of the MHT in a typical clinical microbiology laboratory (12). Exacerbating the problem of detection of OXA-48-producing CPE isolates is the possibility that detection may be dependent upon which susceptibility testing method is utilized. As seen in Table 1, the relative MICs and zone sizes for imipenem with isolates CAV1543, CAV1636, and J53 p1543A are inconsistent for the three methods used for evaluation: VITEK2, MicroScan, and disk diffusion. These disagreements strongly indicate the need for development of more-definitive detection methods that can be used by most laboratories.

The clinical implications of OXA-48-type CPE are being defined. The carbapenem MICs tend to be low for bla_{OXA-48}-type CPE, and the enzyme hydrolyzes extended-spectrum cephalosporins poorly. One might therefore predict better outcomes compared to other CPE types as seen with efficacy of ceftazidime in a murine model (13). However, thus far the mortality rates of OXA-48 CPE infection are 45 to 55%, similar to those for other CPE (10, 11, 14). In this report, patient 1 expired following a course consistent with uncontrolled sepsis; notwithstanding, the paucity of culture data limits our ability to make more-definitive conclusions. Patient 2 did well after surgical control of an ongoing intra-abdominal infection complemented with directed antibiotic therapy.

There are several novel features of the first isolate that highlight the rapidly evolving global epidemiology of OXA-48-producing CPE. Earlier reports of OXA-48-producing *K. pneumoniae* and *E. coli* from geographically diverse locations are associated with a single conserved, epidemic 62-kb IncL/M-type plasmid that harbors no other resistance determinants (2, 3, 10). While we found conservation of the epidemic plasmid backbone, we also found that the plasmid acquired $bla_{CTX-M-9}$. This likely accounts for the higher cephalosporin and carbapenem MICs seen here compared to those found in previously described OXA-48-producing CPE and OXA-48 J53 transconjugates (2).

The importance of medical tourism in dissemination of novel resistance mechanisms is being increasingly recognized. NDM, KPC, and OXA-48 have all spread as a consequence of this traffic (15). The Fr1ench Healthcare Safety Advisory Committee has recognized this risk and issued recommendations to isolate and screen patients recently hospitalized in other countries (15). As a result of these two cases, we have developed a similar policy at our institution. The impact of such a policy remains to be investigated.

In summary, these are the first two clinical, case-based descriptions of OXA-48-producing CPE in the United States. These sentinel strains were identified through a combination of phenotypic and genotypic screening paired with awareness of global CPE epidemiology. Both *K. pneumoniae* isolates were multidrug resistant, carried multiple β-lactamases (including bla_{CTX-M}), and were more carbapenem resistant than was indicated in many previous reports. As a response to this "menace," we now preemptively isolate and screen all patients admitted to our institution who were recently hospitalized in another country with endemic CPE, and we propose that other hospitals consider developing similar policies.

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