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Surveillance and Characterization of Carbapenemase-Producing *Klebsiella pneumoniae* Recovered from Patient Stool Samples at a Tertiary Care Medical Center

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The enteric microbiota of hospitalized patients serves as one reservoir for carbapenem-resistant *Enterobacteriaceae* (CRE) infections (1), although it has not been well characterized. To better understand this potential reservoir of nosocomial carbapenem-resistant organisms, we estimated the frequency of carriage of coliform bacteria harboring carbapenemase resistance genes in patient enteric flora.

We screened diarrheic stool samples submitted for *Clostridium difficile* testing from patients of The Ohio State University Wexner Medical Center (OSUWMC) to estimate the frequency of carriage of carbapenemase-producing enteric bacteria. Submissions (n = 692) received at the OSUWMC Clinical Diagnostic Laboratory between July and December 2013 were deidentified, aliquoted to a transport swab, and taken by courier to our laboratory. Initially, each sample swab was inoculated to MacConkey broth modified with 2 μg/ml cefotaxime, incubated overnight at 37°C, and subsequently inoculated to MacConkey agar supplemented with 2 μg/ml meropenem. Resulting lactose-positive *Enterobacteriaceae* isolates were tested for their ability to reduce carbapenem antimicrobials using modified Hodge and Carba NP tests. Identification to species level was performed via matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker, Billerica, MA), and carbapenemase carriage was confirmed by both PCR and whole-genome sequencing (Illumina MiSeq; San Diego, CA).

From our selective culture, 13 samples (1.9%) produced *Enterobacteriaceae* or *Pseudomonas* spp. resistant to meropenem from the 692 total stool submissions (Table 1). Of these, two *Klebsiella pneumoniae* isolates (0.3%) produced both positive modified Hodge and Carba NP test results. Standard PCR confirmed one *K. pneumoniae* isolate, CRE-185, to harbor $bla_{\text{KPC-3}}$ while the second, CRE-626, carried $bla_{\text{NDM-1}}$.

Whole-genome sequencing identified CRE-185 as multilocus sequence type (MLST) ST258 and CRE-626 as ST1602. *K. pneumoniae* ST258 harboring $bla_{\text{KPC-3}}$ is a prominent CRE strain in the United States and worldwide (2). *K. pneumoniae* ST1602 isolates carrying $bla_{\text{NDM-1}}$ have not been previously reported.

K. pneumoniae isolates harboring bla_{KPC} have been previously recovered from patients of the OSUWMC by the Clinical Diagnostic Laboratory. As illustrated in the dendrogram (Fig. 1), BioNumerics (Applied Maths, Kortrijk, Belgium) analysis of pulsed-field gel electrophoresis (PFGE) banding patterns of CRE-185 and five bla_{KPC} *K. pneumoniae* isolates recovered from OSUWMC patient clinical diagnostic submissions during the 2 months prior to our recovery of CRE-185 revealed highly dissimilar strains, suggesting a diverse reservoir.

In addition to $bla_{\text{KPC-3}}$, *K. pneumoniae* CRE-185 carried a second β-lactam resistance gene, $bla_{\text{LEN-11}}$. Multiple aminoglycoside and quinolone resistance genes and single fosfomycin, phenicol, and sulfonamide resistance genes were also detected. Four plasmid replicon types were identified—FIB(K), FII(K), R, and ColE. *K. pneumoniae* ST258 isolates with similar plasmid content and harboring $bla_{\text{KPC-3}}$ have been previously recovered in Italy (3).

Sequencing of CRE-626 harboring $bla_{\text{NDM-1}}$ identified 3 additional β-lactam resistance genes—$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1A}}$, and $bla_{\text{OKP-B-3}}$. Multiple aminoglycoside and sulfonamide resistance genes were detected as well as individual genes conveying resistance to quinolones, tetracycline, and trimethoprim. CRE-626 carried the NDM-MAR plasmid, which represents incompatibility groups FIB(Mar) and HI1B. This plasmid with a highly similar resistance genotype was originally identified in *K. pneumoniae* recovered from hospitalized patients in Morocco and has been subsequently fully sequenced (4). Additionally, CRE-626 carried incompatibility group FIA(HI1), FII(K), and ColE plasmids.

We did not detect carbapenemase production by the remaining 11 meropenem-resistant isolates. This subset included 4 *Enterobacter cloacae*, 3 *K. pneumoniae*, 2 *Enterobacter aerogenes*, and 1 *Pseudomonas aeruginosa* isolate and a single isolate not identified to species level that could not be recovered from storage. Carbapenem resistance in these isolates may be due to several factors, including increased expression of efflux systems, reduced porin expression, increased chromosomal cephalosporinase activity, or some combination of these (5).

Although we know very little about the deidentified patient population from which these samples were obtained, it is reasonable to assume that these carbapenem-resistant isolates were recovered from antimicrobial-associated diarrhea cases because it is

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TABLE 1 *Enterobacteriaceae* and *Pseudomonas* spp. resistant to carbapenems recovered from 692 patient diarrheic stool submissions received at the OSUWMC Clinical Diagnostic Laboratory for *C. difficile* culture between July and December 2013

Enterobacteriaceae type and submission date (mo/day/yr)	Isolate identifier	Bacterial species	Resistance phenotype[a]	Cephalosporinase gene type
Carbapenemase producing				
8/5/2013	CRE-185	*Klebsiella pneumoniae*	Amp Aug2 Axo Cep Faz Fep Fot Fox Caz Chl Cip Fis Gen Imi Mer Nal Pod P/T4 Xnl	KPC-3, LEN11
11/1/2013	CRE-626	*Klebsiella pneumoniae*	Amp Aug2 Axo Cep Faz Fep Fot Fox Caz Fis Gen Imi Mer Nal Pod P/T4 Str Sxt Tet Xnl	NDM-1, CTX-M-15, TEM-1A, OKP-B-3
Reduced susceptibility to carbapenems				
7/8/2013	CRE-4[b]	Not determined	Not determined	CTX-M group 1
7/15/2013	CRE-53	*Klebsiella pneumoniae*	Amp Aug2 Axo Cep Faz Fep Fot Fox Caz Chl Cip Fis Imi Mer Nal Pod P/T4 Str Sxt Tet Xnl	CTX-M group 1
7/24/2013	CRE-123	*Klebsiella pneumoniae*	Amp Aug2 Axo Cep Faz Fep Fot Fox Caz Chl Cip Fis Imi Mer Nal Pod P/T4 Str Sxt Tet Xnl	CTX-M group 1
7/25/2013	CRE-126	*Enterobacter cloacae*	Amp Aug2 Axo Cep Faz Fot Fox Caz Pod P/T4 Xnl	
7/31/2013	CRE-150	*Enterobacter cloacae*	Amp Aug2 Axo Cep Faz Fep Fot Fox Caz Chl Imi Mer Pod P/T4 Xnl	
8/7/2013	CRE-209	*Enterobacter aerogenes*	Amp Aug2 Axo Cep Faz Fot Fox Caz Imi Mer Pod P/T4 Xnl	
8/8/2013	CRE-222	*Enterobacter aerogenes*	Amp Aug2 Axo Cep Faz Fot Fox Caz Pod P/T4 Xnl	
8/19/2013	CRE-266	*Enterobacter cloacae*	Amp Aug2 Axo Cep Faz Fep Fot Fox Caz Chl Cip Fis Imi Mer Nal Pod P/T4 Tet Xnl	
8/19/2013	CRE-279	*Klebsiella pneumoniae*	Amp Aug2 Axo Cep Faz Fep Fot Fox Caz Chl Cip Fis Gen Mer Nal Pod P/T4 Str Sxt Tet Xnl	CMY
9/16/2013	CRE-438	*Pseudomonas aeruginosa*	Amp Aug2 Axo Cep Faz Fep Fot Fox Caz Chl Fis Imi Mer Pod P/T4 Sxt Tet Xnl	CTX-M group 1
9/16/2013	CRE-447	*Enterobacter cloacae*	Amp Aug2 Axo Cep Faz Fot Fox Caz Chl Pod P/T4 Tet Xnl	

[a] Abbreviations: Amp, ampicillin; Aug2, amoxicillin-clavulanic acid, 2:1 ratio; Axo, ceftriaxone; Cep, cephalothin; Faz, cefazolin; Fep, cefepime; Fot, cefotaxime; Fox, cefoxitin; Caz, ceftazidime; Chl, chloramphenicol; Cip, ciprofloxacin; Fis, sulfisoxazole; Gen, gentamicin; Imi, imipenem; Mer, meropenem; Nal, nalidixic acid; Pod, cefpodoxime; P/T4, piperacillin-tazobactam, constant 4; Str, streptomycin; Sxt, trimethoprim-sulfamethoxazole; Tet, tetracycline; Xnl, ceftiofur.
[b] Following PCR confirmation of the carriage of a CTX-M group 1 gene and subsequent preservation on a nutrient slant, isolate CRE-4 became nonviable and was not available for additional characterization.

standard practice at the OSUWMC to test stool specimens by PCR for opportunistic *C. difficile* infection in patients who develop diarrhea while receiving antibiotics. Our results indicate that while the prevalence of CRE is very low in patient fecal flora, even in this high-risk population, the threat of nosocomial CRE infections disseminated from an enteric flora reservoir exists in health care settings.

bla_{NDM-1} has been detected in a clinical diagnostic submission to the OSUWMC Clinical Diagnostic Laboratory only once subsequent to our study (*K. pneumoniae*, December 2014), from a

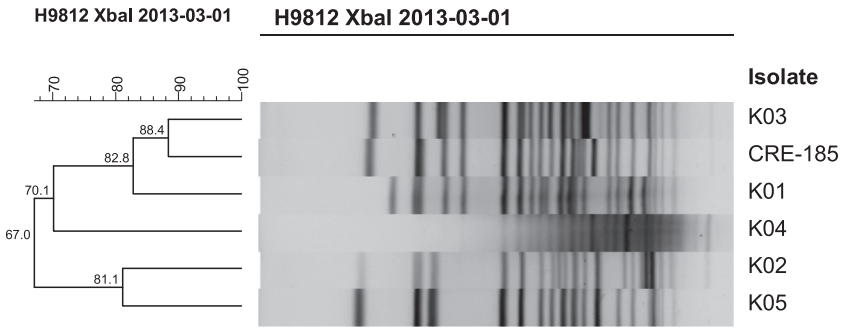

FIG 1 Dendrographic percent relatedness of *K. pneumoniae* CRE-185 recovered from an OSUWMC patient stool sample and 5 bla_{KPC}-bearing *K. pneumoniae* isolates recovered from OSUWMC patient clinical diagnostic submissions during the same month. After electrophoresis, banding patterns were compared and levels of similarity were assigned using generally accepted criteria (7). bla_{KPC}-bearing *K. pneumoniae* isolates were assessed using the Dice coefficient similarity index and the unweighted pair-group method with arithmetic averages with clustering settings of 1.00% optimization and 1.00% band position tolerance via BioNumerics software (Applied Maths, Kortrijk, Belgium).

patient referred to the OSUWMC who likely acquired the infection prior to admission. Our detection of carbapenemase-producing enteric bacteria in this population emphasizes the need for CRE surveillance and patient risk assessment in order to prevent the nosocomial dissemination of this important resistance genotype (6).

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