Enterobacter cloacae Complex Sequence Type 171 Isolates Expressing KPC-4 Carbapenemase Recovered from Canine Patients in Ohio

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ABSTRACT Companion animals are likely relevant in the transmission of antimicrobial-resistant bacteria. *Enterobacter xiangfangensis* sequence type 171 (ST171), a clone that has been implicated in clusters of infections in humans, was isolated from two dogs with clinical disease in Ohio. The canine isolates contained IncHI2 plasmids encoding *bla*_{KPC-4}. Whole-genome sequencing was used to put the canine isolates in phylogenetic context with available human ST171 sequences, as well as to characterize their *bla*_{KPC-4} plasmids.

KEYWORDS antibiotic resistance, carbapenemase, plasmid-mediated resistance, veterinary microbiology

The plasmid-mediated *Klebsiella pneumoniae* carbapenemase (KPC) emerged in the late 1990s and has become the predominant carbapenemase in clinical carbapenem-resistant *Enterobacteriaceae* (CRE) isolates; these are primarily associated with the clonal dissemination of strains of *K. pneumoniae*, *Enterobacter* spp., and *Escherichia coli* (1, 2). While health care-associated infections (HAI) comprise the majority of all cases involving CRE, community-associated infections (CAI) comprise up to 10.8% of CRE cases in the United States (3).

The role of companion animals as reservoirs for the zoonotic transmission of CRE should be considered, particularly for CAI (4). In U.S. companion animals (e.g., dogs and cats), extralabel prescribing of carbapenems is permissible for veterinarians under the Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994 (5). While an assessment of the frequency of carbapenem usage in companion animals has not been reported, pharmacokinetic studies have been performed, and companion animal dosage recommendations for imipenem and meropenem are available in veterinary medical textbooks (6–8). Moreover, extended-spectrum β -lactamase (ESBL) and AmpC β -lactamase-mediated resistance among *Enterobacteriaceae* isolates obtained from dogs and cats is common, indicating a potential justification for carbapenem administration (9–14).

We identified two canine patients at The Ohio State University Veterinary Medical Center (OSUVMC) with infections involving *Enterobacter xiangfangensis* sequence type 171 (ST171) with IncHI2 plasmids encoding *bla*_{KPC-4}. Carbapenem-resistant ST171 has been responsible for major clusters of human CRE infections in the northeastern and upper midwestern United States (15–17). Patient 1, seen in July 2016 for cystitis, was a 13-year-old ovariohysterectomized (spayed) female Shetland sheepdog that had a

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2-month history of chronic kidney disease and a 2-year history of pyoderma. Patient 2, seen in September 2016, was a 6-year-old castrated mixed-breed male dog that presented with an infected bite wound inflicted by another dog. Patient 1 was an established patient of the hospital for the ongoing management of chronic health problems and had received doxycycline during the previous year; however, patient 2 had not presented previously to the facility and had no recent history of antimicrobial administration. The organisms, initially identified via matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS, Biotyper; Bruker Daltonics, Billerica, MA) as *E. cloacae*, were isolated in pure culture from urine collected from patient 1 and were coisolated with *Enterococcus faecium* from a culturette collected from patient 2.

Antimicrobial susceptibility testing (AST) via broth microdilution in accordance with the CLSI VET01A4 standard (CompGN1F, Sensititre; Thermo Fisher, USA) revealed the same phenotypes for both isolates, and the MIC results by agent were the following (in $\mu\text{g/ml}$): amikacin, ≤ 4 ; amoxicillin-clavulanic acid, > 8 ; ampicillin, > 8 ; cefazolin, > 32 ; cefovecin, > 32 ; cefpodoxime, > 8 ; ceftazidime, > 16 ; chloramphenicol, 8; doxycycline, > 8 ; enrofloxacin, > 4 ; gentamicin, 0.5; imipenem, ≤ 1 ; marbofloxacin, > 4 ; piperacillin-tazobactam, 16; pradofloxacin, > 2 ; tetracycline, > 16 ; and trimethoprim-sulfamethoxazole, > 4 (18). Notably, they were phenotypically susceptible to imipenem upon routine testing but produced carbapenemase, as determined by the Carba NP test (19). Both isolates were susceptible to meropenem, with an MIC of $\leq 1 \mu\text{g/ml}$ (CMV3AGNF, Sensititre; Thermo Fisher). However, the ertapenem Etest (bioMérieux, Durham, NC) revealed that the isolate from patient 1 was resistant (MIC, $2 \mu\text{g/ml}$) and the isolate from patient 2 was intermediate (MIC, $1 \mu\text{g/ml}$). KPC-4 can be difficult to detect with conventional phenotypic methods because of its low hydrolytic activity (20), which may be compounded by the weak promoter activity of *Tn4401b* (21). The identification of these isolates as CRE occurred by means of an institutional infection control program that includes an additional screening of third-generation cephalosporin-resistant *Enterobacteriaceae*, as well as restricted clinician access to carbapenems. Carba NP testing is not described in the CLSI VET01A4 document, but the CLSI M100 document for humans lists Carba NP test as optional for infection control programs (22).

Genomes were sequenced by both Illumina MiSeq and PacBio RSII platforms and assembled using Canu v1.4, followed by proofreading using Pilon (<https://github.com/broadinstitute/pilon>) (23). An analysis of the whole-genome sequences identified the isolates as *E. xiangfangensis* ST171 (24). Each contained two plasmids belonging to IncF1B and IncHI2, respectively (25). The IncHI2 plasmids (Fig. 1) were 351,806 bp (pOSUVMCKPC4-1) and 354,256 bp (pOSUVMCKPC4-2) from patients 1 and 2, respectively, and were each identified as ST1 via plasmid multilocus sequence typing (pMLST) (<http://pubmlst.org/plasmid>) (26); both contained *bla*_{KPC-4} in the context of *Tn4401b* (Fig. 2). A 21-kb region (nucleotide [nt] positions 94348 to 115575 in pOSUVMCKPC4-2), containing *strAB*, *aadA1*, *bla*_{OXA-129}, *dfra21*, and *sul1* flanked by two IS4321 elements, is inverted in comparison to pOSUVMCKPC4-1, identified as Inc524 (27). OXA-129 is an ESBL that does not have carbapenemase activity (28). In comparison with pOSUVMCKPC4-1, pOSUVMCKPC4-2 has two additional IS3 elements at the nt 62536 to 63760 and nt 180063 to 181287 positions, respectively. The IncF1B plasmids in the two isolates were identical in size, at 108,403 bp, and contained no identifiable resistance genes. Both isolates contained the S83L and D87N mutations in *gyrA*, which are known to confer fluoroquinolone resistance (29). Overall, the isolates' resistotypes corresponded with their complement of resistance genes (see Table S1 in the supplemental material).

Core single nucleotide polymorphism (SNP) phylogenetic analysis via kSNP3.0 (30) placed these two *E. xiangfangensis* strains in the previously described ST171 cluster II as a subclade, and they differ from each other by 14 core SNPs (Fig. 3) (15). Cluster II isolates, the majority of which harbor IncH plasmids with *bla*_{KPC-4}, have an average of

We also examined contemporaneous human clinical ESBL-positive *E. cloacae* isolates from The Ohio State University Medical Center collected between 2011 and 2016 ($n = 8$), which were tested via cefotaxime and ceftazidime double-disk

FIG 2 Schematic structure of Tn4401b containing *bla*_{KPC-4} present on large IncH12 plasmids in *E. xiangfangensis* ST171 isolates recovered from two canine patients of The Ohio State University Veterinary Medical Center in 2016. Genes and their corresponding transcription orientation are indicated by colored horizontal arrows. Corresponding insertion sequences (IS) that encode transposons are indicated at the top of the figure with a narrow blue line. Black triangles indicate inverted repeat right and left sequences associated with the *ISKpn6* and *ISKpn7* elements. Flanking parallelograms represent the two inverted repeat sequences associated with Tn4401 structures.

diffusion with clavulanate (32), modified Hodge test (MHT) (32), and ertapenem Etest. Two isolates were identified as ST171, MHT positive, and intermediate ($n = 1$) and resistant ($n = 1$) to ertapenem. Both isolates contained *bla*_{KPC-2}, suggesting that the *bla*_{KPC-4} canine strains may have been uniquely community associated during that time period. However, our SNP analysis comparing the canine isolates to other available ST171 whole-genome sequence data indicated that they are closely related to historical human clinical isolates from the northeastern United States, the United Kingdom, and Colombia (Fig. 3). Together, these observations indicate the diversity regarding the dissemination of ST171 strains harboring *bla*_{KPC}, including transcontinental movement and circulation in the community over multiple years, with sporadic HAI activity.

The presence of KPC-containing *E. cloacae* complex organisms in companion animal species provides additional evidence that there are biological reservoirs for zoonotic CRE transmission to humans in circulation. Additional surveillance of these reservoirs, as well as veterinary carbapenem use and resistance, is warranted.

Data availability. The genome sequences were deposited in GenBank under the accession numbers CP024908 to CP024910 and CP029246 to CP029248.

FIG 3 Heatmap of plasmids among *bla*_{KPC-4} bearing *E. xiangfangensis* ST171, with additional KPC-2- and KPC-3-containing ST171 isolates included as references. Left, core SNP phylogenetic tree generated by RaxML; center, metadata, including host, isolation location, year, Tn4401 isoform, and KPC allele; right, plasmid composition is illustrated by showing the BLASTn matches to each *E. xiangfangensis* ST171 genome across all of the genes on the pOSUVMCKPC4-2 plasmid.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.01161-18>.

SUPPLEMENTAL FILE 1, PDF file, 0.04 MB.

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