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# Whole-Genome Sequencing Overrules a Suspected Case of Carbapenem-Resistant *Enterobacter cloacae* Transmission

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**KEYWORDS** *Enterobacteriaceae*, beta-lactamases, genomics, nosocomial transmission

*Enterobacter cloacae* is an important nosocomial bacterium that is prevalent in hospital intensive care units (ICUs) (1–4). The presence of carbapenem-hydrolyzing beta-lactamases, such as *Klebsiella pneumoniae* carbapenemase (KPC) encoded by the $bla_{KPC}$ gene, underlies multidrug resistance in *E. cloacae* and other pathogens (5–7).

Active microbial surveillance at the NIH Clinical Center, which tests patients on admission and at regular intervals thereafter, detected multidrug-resistant *E. cloacae* colonization in two patients with overlapping stays in the ICU (Fig. 1). Both patients were undergoing treatment for a malignancy. Salient features of these two cases included multiple negative cultures at and after admission, administration of broad-spectrum antibiotics, and subsequent positive surveillance culture for $bla_{KPC}$-positive *E. cloacae* leading to transfer to cohorted care (Fig. 1).

The timing and features of these two cases, along with shared carbapenem resistance, triggered an investigation. It was postulated that transmission of the *E. cloacae* strain from patient 1 to patient 2 might have occurred through a shared medical team. The patients did not share a room or nursing staff, and other potential sources of *E. cloacae* were negative, including: environmental samples (e.g., shared equipment, sinks, and surfaces) and perirectal swab cultures for all patients housed in the ICU in the weeks prior.

The isolates from patient 1 and 2 were both resistant to doripenem and ertapenem, but patient 2's isolate had increased resistance to imipenem and meropenem (see Table S1 in the supplemental material). Genomic sequencing, standard in our institute for all $bla_{KPC}$-positive organisms (8), demonstrated that both isolates shared the $bla_{KPC}$ gene flanked by an IS*26* element and the IS*Kpn6 tnpA* gene, a previously observed genetic context (9). However, major differences between the two patient *E. cloacae* isolates were identified, ruling out direct transmission (Fig. 2). Patient 1's isolate is a sequence type 191 (ST191) *E. cloacae* strain with a 4.8-Mbp chromosome, a 50-kb ST*6* IncN $bla_{KPC-2}$ plasmid related to pKPC-47e, and additional 80- and 51-kb plasmids (lengths estimated from reference-based scaffolding) (10). The isolate from patient 1 is similar to *E. cloacae* ECNIH4, which was cultured in 2012 from a sink drain in our institution; however, sequence differences (including 35 nucleotide differences distributed along the chromosomes and insertion/deletions within the plasmids) help distinguish between these two isolates. Patient 2's isolate is an ST53 *E. cloacae* strain with a 5.1-Mbp chromosome, a 21-kb plasmid (pKPC-98f) carrying $bla_{KPC-2}$, and additional plasmids of 79 and 144 kb.

In summary, genomic sequencing does not support nosocomial transmission of the $bla_{KPC}$-positive *E. cloacae* strain from patient 1 to patient 2. Our epidemiologic

**Accepted manuscript posted online** 28 June 2017

**Citation** Chen M, Conlan S, Lau AF, Dekker JP, Deming C, NISC Comparative Sequencing Program, Henderson DK, Frank KM, Palmore TN, Segre JA. 2017. Whole-genome sequencing overrules a suspected case of carbapenem-resistant *Enterobacter cloacae* transmission. J Clin Microbiol 55:2868–2870. https://doi.org/10.1128/JCM.00915-17.

**Editor** Daniel J. Diekema, University of Iowa College of Medicine



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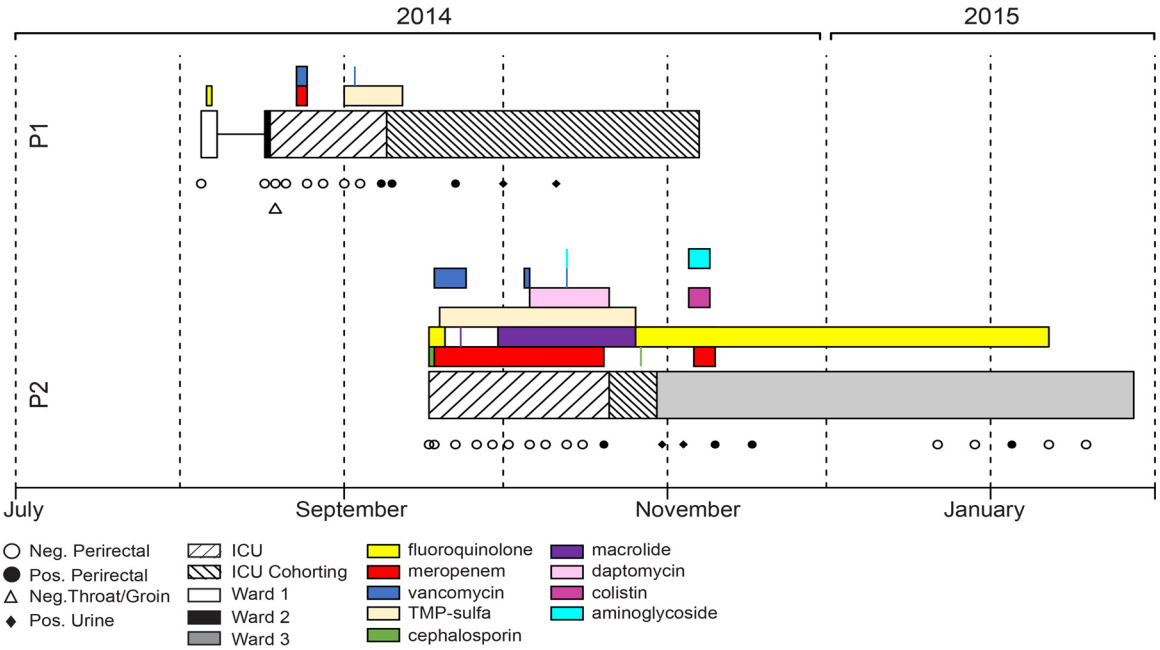


**FIG 1** Patient location, antibiotics received, and clinical culture results during the patients' hospital stay. The segment lengths indicate the duration of antibiotic receipt and stay in areas of the hospital or as an outpatient for each patient. Cultures are indicated as symbols below each patient trace (open symbols, negative; solid symbols, positive for $bla_{KPC}$-positive *E. cloacae*). TMP-Sulfa, trimethoprim-sulfamethoxazole; P1, patient 1; P2, patient 2.

and genomic results, including the absence of any matching isolates in a setting of extensive surveillance among a highly immunocompromised patient population, led us to postulate that these apparent acquisitions may rather represent rare transmission not detected by environmental surveillance or low-level gastrointestinal colonization on admission not detected by surveillance cultures. Administration of broad-spectrum antibiotics may have selected for the colonizing antimicrobial-resistant organisms, yielding a positive culture. These findings emphasize the importance of genomics for clarification of cases of suspected nosocomial transmission.

**Accession number(s).** The whole-genome sequencing data can be retrieved at NCBI BioProject no. PRJNA279652 and PRJNA279659.

**Availability of data.** Isolates can be obtained from K.M.F.; a material transfer agreement is necessary.

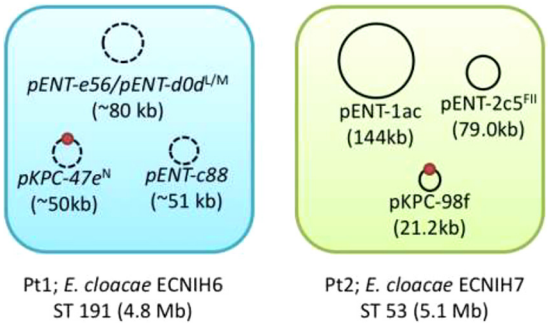


**FIG 2** ECNIH6 and ECNIH7 carry three nonoverlapping plasmids and belong to different sequence types. The ECNIH6 genome is a shotgun assembly scaffolded on the closest reference (ECNIH4). Plasmid names and sizes are inferred from the reference; ordered and oriented contigs are represented by a dashed plasmid backbone. The $bla_{KPC}$ gene is marked as a red circle for both strains. Plasmid incompatibility groups (PlasmidFinder version 1.3) are indicated by superscripted letters.

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00915-17.

**SUPPLEMENTAL FILE 1,** PDF file, 0.1 MB.

## ACKNOWLEDGMENTS

This research was supported by the National Human Genome Research Institute and NIH Clinical Center Intramural Research Programs.

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