Emergence of KPC-2-Producing *Raoultella ornithinolytica* Isolated from a Hospital Wastewater Treatment Plant

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Bacterial carbapenem resistance is a threat to the public health worldwide (1). *Klebsiella pneumoniae* carbapenemase (KPC) is the most common carbapenemase and can be obtained from carbapenem-resistant *Enterobacteriaceae* (CRE) that are distributed globally (2, 3). Although *bla*_{KPC} genes are usually recovered from samples from patients and healthy humans, transmission of *bla*_{KPC} genes between environmental isolates remains largely unknown (4). In this study, we isolated *Raoultella ornithinolytica* SRO1810 carrying *bla*_{KPC-2} from a wastewater treatment plant (WWTP) located at the First Affiliated Hospital of Zhejiang University (FAHZU). Comprehensive analysis of *R. ornithinolytica* SRO1810 was performed to understand the resistant profiles and molecular characteristics of this isolate.

R. ornithinolytica SRO1810 was isolated from the WWTP in July 2017 by using selective medium as described previously (5). The carbapenemase gene was identified using PCR and DNA sequencing. The plasmid was characterized using S1-pulsed-field gel electrophoresis (S1-PFGE), and the location of *bla*_{KPC-2} was identified with Southern hybridization. A plasmid conjugation experiment was carried out using *Escherichia coli* J53 as the recipient strain (5). MICs were established using a broth dilution method and were interpreted according to the EUCAST guidelines (<http://www.eucast.org/>). Genomic DNA was extracted using a DNA kit (Omega Bio-tek, Norcross, GA, USA). The DNA was subsequently sequenced using Illumina-HiSeq 4000-PE150 (Illumina, San Diego, CA, USA) and PacBio RS II platforms (Pacific Biosciences, Menlo Park, CA, USA). After sequencing, the complete genomic sequence of *R. ornithinolytica* SRO1810 was generated using Unicycler (6) by combining the sequencing results. Bioinformatic analysis was conducted as described previously (7–9).

As shown in Table S1 in the supplemental material, SRO1810 was resistant to all β -lactams tested but was susceptible to aminoglycosides, fluoroquinolones, tigecycline, and colistin (Table S1). Another β -lactamase gene, *bla*_{TEM-1B}, was further identified using the ResFinder database (<https://cge.cbs.dtu.dk/services/ResFinder/>). Interestingly, a variant of *fosA7*, which encodes fosfomycin resistance via a glutathione transferase, was detected in the genome via BLAST search analysis (Fig. S1A and B). Antimicrobial susceptibility tests confirmed that SRO1810 was resistant to fosfomycin. *bla*_{TEM-1B} and *bla*_{KPC-2} were located in the plasmid, while *fosA7* was located on the chromosome. In addition, we investigated the genetic environment surrounding *fosA7*, and the results suggest that mobile genetic elements may promote the transmission of the *fosA7* gene. The insertion sequences ISL3 and IS3 are located upstream of the *fosA7* gene, and IS110 and IS6 are located downstream of the *fosA7* gene (Fig. S1C). Southern blot analysis demonstrated that SRO1810 carried at least five plasmids with sizes of approximately 150 kb, 120 kb, 80 kb, 40 kb, and 30 kb. KPC-2 was located on an ~42-kb plasmid (designated pSRO1810-KPC; Fig. 1B and C). Whole-genome sequencing results indicate

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FIG 1 Comparison of genomic and plasmid profiles of *R. ornithinolytica* from clinical and sewage isolates. (A) PFGE patterns generated by XbaI digested total DNA of two isolates. (B) Plasmid profiles of two KPC-producing *R. ornithinolytica* isolates. (C) Southern blot hybridization of (Continued on next page)

that plasmid pSRo1810-KPC is an IncN-type plasmid with a length of 42,848 bp and contains 80 protein-coding genes with a GC content of 49.7% (Table S2). The PFGE profiles of SRo1810 were distinct from the PFGE profiles of Ro24724 (Fig. 1A). In addition, comparative genomics analysis indicates that there are no isolates similar to this strain in the database (Fig. 1F).

By comparison, we found that pSRo1810-KPC is an IncN3-type plasmid and matches the 42.85-kb plasmid pH5062105-3 (99.5% query coverage, 99.9% identity, GenBank accession number [KF623109](#)) from clinical KPC-2-producing *K. pneumoniae* in China. These two plasmids were found to share a similar backbone with a 41.3-kb plasmid, pKPC-ECN49, which was recovered from clinical *Enterobacter cloacae* in China (Fig. 1E). Compared to pH5062105-3, the Tn3-*tnpA-tnpR* element downstream of *bla*_{KPC-2} was missing from pKPC-ECN49. These three plasmids were classified as IncN3-type plasmids and were found to share a similar 14-kb P-type type IV secretion system, which usually encodes a rigid pilus and is found on virulence plasmids (10); they also possess an unknown maintenance mechanism. All these plasmids lacked a *tra* module which encodes a primary pilus for conjugation, which explains the failure to transfer pSRo1810-KPC by conjugation.

After annotating the contig with the carbapenem resistance gene, we compared SRo1810 with three similar gene sequences for genetic environmental analysis. The *bla*_{KPC-2} gene was found to be located on an 11-kb Tn3 transposon element in pSRo1810-KPC with the following linear structure: Tn3-ISKpn8-*bla*_{KPC-2}-ΔISKpn6-*korC-klcA*-unknown ORF-ΔrepB-Tn1721 (Fig. 1D). It is worth noting that SRo1810 shares the same genetic environment with pH5062105-3 from a clinical *K. pneumoniae* isolate. The Tn1721-based transposon is one of the most common elements carrying *bla*_{KPC-2} and is widespread among *Enterobacteriaceae* tested at different locations in China (11). The backbone of pSRo1810-KPC was distinct from that of pRo24724. pRo24724 is approximately 450 kb long and belongs to the IncU incompatibility group (12). In addition, PFGE results indicate that SRo1810 is not directly related to the clinical strain Ro24724. There are few studies of *R. ornithinolytica* carrying KPC-2. However, an *R. ornithinolytica* sample isolated from wastewater from a hospital in Spain in 2013 carried an IncP-6-type plasmid with KPC-2; *bla*_{KPC-2} was also located on the Tn3 transposon.

In summary, we report for the first time a KPC-2-producing *R. ornithinolytica* strain, SRo1810, isolated from a WWTP. To our knowledge, this is the first study to obtain an environmental *R. ornithinolytica* isolate carrying the carbapenemase gene. The clinical and sewage isolates of *R. ornithinolytica* obtained from the hospital may have distinct origins, but they share the same *bla*_{KPC-2} gene and carbapenem resistance mechanism. This work may have important implications regarding the transmission of the *bla*_{KPC-2} gene in hospitals. We emphasize the importance of improved multisectoral surveillance for carbapenemase-producing isolates in WWTP effluent, which may contribute to the spread of antimicrobial resistance genes in clinical settings and communities.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. [VRLU00000000](#). The version described in this paper is version VRLU00000000.

FIG 1 Legend (Continued)

S1-nuclease digested DNA using a *bla*_{KPC-2} specific probe. Lane1, sewage isolate (SRo1810); lane2, clinical isolate (Ro24724); M, XbaI digested total DNA of *Salmonella enterica* serotype Braenderup H9812 as a size marker. (D) Comparative analysis of genetic structures of *bla*_{KPC-2} in pSRo1810-KPC with other plasmids. Arrows represent open reading frames (ORFs) and their direction of transcription. The shaded areas indicate regions of synteny between adjacent schematics; the matching percentage nucleotide sequence identity for each such region is indicated in the scale bar. Gene labels identify the locations of genes on pH5062105-3. The GenBank accession numbers of pH5062105-3 and pKPC-ECN49 are [KF623109](#) and [KP726894](#), respectively. (E) Sequence comparison of scaffolds (portions of genome sequences reconstructed from whole-genome sequencing [WGS] data) identified in pSRo1810-KPC with 2 *bla*_{KPC-2}-bearing plasmids, pH5062105-3 and pKPC-ECN49, in BRIG (<http://brig.sourceforge.net/>). Arrows indicate positions and direction of transcription of genes. Reference plasmid pH5062105-3 is indicated in pink in the inner circle. The color intensity in each ring represents the BLAST match identity. (F) Comparative genomics analysis of *R. ornithinolytica* isolates based on single-nucleotide polymorphism (SNP) analysis shown as a maximum likelihood-based phylogenetic tree built from core genome SNPs of 45 *R. ornithinolytica* isolates mapped to the reference genome of *R. ornithinolytica* strain 10-5426.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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