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Complete Genome Sequence of a Shiga Toxin-Producing *Enterobacter cloacae* Clinical Isolate

Michelle Qiu Carter,[a] Antares Pham,[a] Steven Huynh,[a] Xiaohua He[b]

Produce Safety and Microbiology Research Unit, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, California, USA[a]; Foodborne Toxin Detection and Prevention Research Unit, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, California, USA[b]

ABSTRACT *Enterobacter cloacae* strain M12X01451 was isolated from a patient with mild diarrhea. This strain produces a novel subtype of Shiga toxin 1, Stx1e. The Stx1e-converting prophage in strain M12X01451 is stable and can infect other bacteria following induction. Here we report the complete genome sequence and annotation of strain M12X01451.

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Address correspondence to Michelle Qiu Carter, michelle.carter@ars.usda.gov.

Enterobacter cloacae is ubiquitous in nature and occurs as normal intestinal microflora in humans and animals. Strains of *E. cloacae* are extremely diverse in genetic makeup and ecological function (1–4). *E. cloacae* recently emerged as a major nosocomial pathogen (5, 6).

Shiga toxins (Stxs) are cytotoxic proteins expressed mainly in the enteric pathogens *Shigella dysenteriae* serotype 1 and Shiga toxin-producing *Escherichia coli* (STEC). In 2014, an *E. cloacae* strain expressing a novel subtype of Shiga toxin 1 (Stx1e) was isolated from a patient with mild diarrhea (7). This Stx1e-converting prophage was stable and transduced *E. cloacae* and *E. coli* strains, including a STEC O157:H7 strain, following induction (8).

Illumina MiSeq libraries were prepared as described previously (9) with modifications. Briefly, bacterial genomic DNA was sheared using a Covaris M220 instrument at 50 peak power, 20 duty factor, 20°C, 200 cycles per burst, and 25-second duration. Adapter-ligated fragments were size selected to 700 to 800 bp. PCR was reduced to four cycles to minimize amplification bias. Pooled libraries were sequenced on an Illumina MiSeq instrument at 13.5 pM using 2 × 250-bp paired-end v2 kits. Single-molecule real-time (SMRT) sequencing was performed on a PacBio RSII instrument using 20-kb SMRTbell libraries with P6-C4 sequencing chemistry and the 360-min data collection protocols. A FASTQ file was generated from the PacBio reads using SMRT Analysis v2.3.0, and assembly was done with RS_HGAP_Assembly.3. The Illumina reads with a Phred quality score above 37 (Q score of >37) were used to assemble the polished PacBio sequences using the Geneious 10.2.2v assembler. The completed genome sequence was submitted to Rapid Annotation using Subsystem Technology (10) and Prokka (11) for annotation and PHASTER (12, 13) for prophages identification.

The *E. cloacae* M12X01451 genome is composed of a 4,918,273-bp chromosome and a 169,226-bp plasmid, encoding 4,726 coding sequences (CDSs), 25 rRNAs, and 88 tRNAs. The average GC contents of the chromosome and plasmid are 55.0% and 49.8%, respectively. *In silico* typing (https://pubmlst.org/ecloacae/) revealed that this strain belongs to sequence type 922 (ST922). Whole-genome-based phylogenetic analysis with other complete *E. cloacae* genomes in GenBank placed this strain in the same clade with strains ATCC 13047 (accession number CP001918), GGT036 (CP009756), SBP-8 (CP016906), NH52 (LT160614), and SDM (CP003678). PHASTER detected five

intact prophages on the chromosome, including the Stx1e-converting prophage that spans positions 1670381 to 1710590 (40.1 kb). This prophage is located between the nicotinate phosphoribosyltransferase gene and the aminopeptidase N gene, carrying 39 phage genes and 10 hypothetical genes. Similarly to *stx* phages 933W (14) and H-19B (15), the stx_{1e} gene is located in the late gene region, immediately downstream of the antiterminator Q gene. The stx_{1e} prophage genome is flanked by the direct repeat TTATACAAATGTAGCAA, annotated as *attL* (1670381 to 1670397) and *attR* (1710574 to 1710590). This integration site is present in genomes of other bacterial species, including STEC, implying a potential in acquisition of stx_{1e} by other bacterial strains. A partial stx_{1e} prophage genome was detected in strains of *Enterobacter hormaechel*, *Klebsiella aerogenes*, *Citrobacter freundii*, *and Citrobacter koseri*. To our knowledge, this is the first report of a complete genome sequence of Shiga toxin-producing *E. cloacae*.

Accession number(s). The *E. cloacae* strain M12X01451 genome sequences were deposited in GenBank under the accession numbers CP017473 and CP017475.

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