



# Complete Genome Sequence of *Kluyvera intestini* sp. nov., Isolated from the Stomach of a Patient with Gastric Cancer

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**ABSTRACT** We report here an update to the draft genome sequence of *Kluyvera intestini* sp. nov. strain GT-16, generated using MinION long-read sequencing technology. The complete genome sequence of the human-derived strain GT-16 measured 5,768,848 bp. An improved high-quality complete genome sequence provides insights into the mobility potential of resistance genes in this species.

*Kluyvera intestini* sp. nov. strain GT-16 is a newly described species that was isolated, using an original genomic workflow, from the stomach of a patient with gastric cancer (1–3). This strain is characterized by numerous virulence factors and antibiotic resistance genes (ARGs) that correspond to those in other clinically significant *Kluyvera* sp. pathogens (4–6). To update the draft genome sequence, we used a combination of Illumina short-read and Oxford Nanopore Technologies (ONT) MinION long-read sequencing technologies (7).

Whole-genome shotgun libraries for Illumina sequencing were prepared as follows: 500 ng of genomic DNA were sheared to 500-bp fragments using a Covaris E220 ultrasonicator, and a library was prepared using the Kapa low-throughput “with bead” preparation kit (Kapa Biosystems, catalog no. KK8231) without PCR amplification. The DNA was sequenced as 300-bp paired-end reads on the Illumina MiSeq platform. DNA was also sequenced using a flow cell system on the ONT MinION sequencer.

Base-calling of the initial ONT MinION FAST5 files was performed with the Metrichor service. The base-called FAST5 files were converted to FASTQ format using poretools (8). Assembly was performed using Canu (9), and nanopolish (10) was used to compute an improved consensus sequence.

*De novo* assembly, combined with manual integration of Nanopore data via LASTZ alignments, yielded two scaffolds of 5,520,555 bp and 248,293 bp, with an average coverage of 150× and a total of 6,098 coding sequences. The DNA-DNA hybridization value showed 99.5% similarity with *K. intestini* sp. nov. GT-16 (GenBank accession no. MKZW000000000), meaning that these genomes belong to the same species (11).

Numerous virulence-related genes, such as those coding hemolysin D, peptidases, permeases, chemotaxis regulators, and exonucleases, were found in *K. intestini* sp. nov. GT-16. High-confidence ARGs (containing over 75% amino acid identity) were identified using the NCBI and Comprehensive Antibiotic Resistance databases (12, 13).

Using complete genome sequencing, we evaluated the mobility potential of the identified ARGs. We identified all mobile genetic elements (MGEs), such as insertion and/or transposable elements, or phages, using the ISFinder, PHAST, and GypsyGenes algorithms (14, 15). The genome was shown to harbor 24 insertion sequence (IS) elements, 2 transposases, 8 resolvases, 11 integrases, and 5 Tn3 transposons. Next, we

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merged the outputs to identify all composite elements that were suggested as ARGs and colocalized within the MGEs. The composite elements were determined to be an ARG flanked by two ISs belonging to the same family within a 10-kb region, a transposon with an ARG within a 5-kb region, or a transposable phage.

Overall, the genome of *K. intestini* strain GT-16 harbored 18 ARGs associated with MGEs, including those encoding resistance to tellurium, quaternary ammonium compounds, arsenic, beta-lactams, copper, chloramphenicol, polymyxin, and multidrug resistance (MDR) transporters. This indicates that these ARGs can be involved in horizontal gene transfer (16). Follow-up studies of *K. intestini* and its harbored bacteriophages would enable us to understand its possible pathogenicity and role in cancer (17).

**Accession number(s).** This complete genome sequencing project has been deposited in GenBank under the accession no. [MKZW00000000](https://doi.org/10.1128/genomeA.01432-16). The version described in this paper is the second version, MKZW02000000.

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