





# Complete Genome Sequence of the Extensively Drug-Resistant Extended-Spectrum $\beta$ -Lactamase-Producing *Proteus mirabilis* Isolate HK294, Obtained from Poultry Feces in Hong Kong

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**ABSTRACT** Here, we report the complete genome sequence of *Proteus mirabilis* isolate HK294, recovered from pooled poultry feces in Hong Kong in 2022. The chromosome contained 32 antimicrobial resistance genes, including the extended-spectrum  $\beta$ -lactamases *bla*<sub>CTX-M-65</sub> and *bla*<sub>CTX-M-3</sub>. Almost all resistance genes were part of either an integrative conjugative element or a Tn7-like transposon.

*Proteus mirabilis* is a frequent cause of nosocomial infections and widely distributed throughout the natural environment and intestines of humans and animals. *P. mirabilis* is naturally resistant to various antimicrobial drugs but is usually sensitive to ampicillin, cephalosporins, and aminoglycosides (1, 2).

HK294 was isolated in June 2022 from a pooled fecal sample from a poultry flock in Hong Kong. The sample (0.3 g) was enriched in 2.7 mL brain heart infusion (BHI) broth (BD) with cefotaxime (3.5  $\mu$ g/mL) and vancomycin (32  $\mu$ g/mL) at 37°C for 24 h. One loopful of the broth was spread on Brilliance extended-spectrum  $\beta$ -lactamase (ESBL) agar (Oxoid) and incubated at 37°C for 24 h. Matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics) was used for species identification. Disk diffusion susceptibility testing was performed for 16 antimicrobial agents and interpreted according to CLSI protocols (3).

DNA was isolated from subcultures obtained from single colonies grown for 24 h at 37°C on sheep blood agar. For short-read sequencing, genomic DNA was extracted using the DNeasy blood and tissue kit (Qiagen). Libraries were prepared using the Nextera DNA Flex library preparation kit (Illumina) and sequenced on the Illumina MiniSeq platform (2  $\times$  150 bp). Read trimming and quality control were performed using fastp v0.20.1 (4). For long-read sequencing, DNA was extracted using the MasterPure kit (Lucigen) (no size selection/shearing). Libraries prepared using the SQK-LSK112 and EXP-NBD114 barcoding kits were sequenced on a MinION FLO-MIN112 flow cell (Oxford Nanopore). Base calling was performed using Guppy v6.1.1 (<https://community.nanoporetech.com>) and quality assessed using nanoq v0.9.0 (5). A hybrid assembly was generated from 373 Mbp short-read (1,254,711 paired reads; coverage, 93 $\times$ ) and 105 Mbp long-read data (15,165 reads; read *N*<sub>50</sub>, 23 kb; coverage, 20 $\times$ ) using the Unicycler v0.5 pipeline (6), which includes circularization and rotation. The assembly was annotated using PGAP v2022-12-13 (7). Resistance genes were detected using AMRFinder v3.10.24 (8).

The genome of HK294 consisted of a 4,003,686-bp chromosome (GC content, 39.1%) and contained 27 distinct resistance genes, some (*arr-3*, *catB3*, *sul1*, and *sul2*) in more than one copy. Most (31/32) resistance genes were on a putative integrative conjugative element (ICE) or a Tn7-like transposon. The putative ICE contained *aac(6')-Ib-cr5* (fluoroquinolone/aminoglycoside resistance), *arr-3* (rifamycin resistance), *bla*<sub>CTX-M-65</sub> (cephalosporin resistance), and *fosA3* (fosfomycin resistance), among others (Fig. 1A). It

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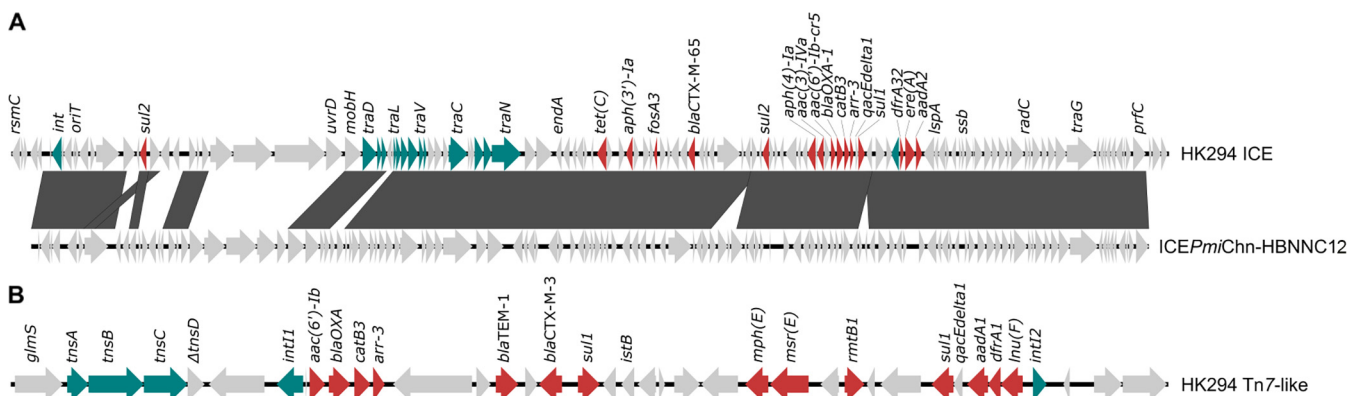
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**FIG 1** Genetic environment of antimicrobial resistance genes identified in the chromosome of HK294. (A) Comparison of the *bla*<sub>CTX-M-65</sub>-containing region in HK294 to the integrative conjugative element ICEPmiChn-HBNNC12 (GenBank accession number [MZ277865.1](https://doi.org/10.1093/nar/gkz569)). Gray shading indicates homologous regions. (B) Structure of the Tn7-like transposon harboring *bla*<sub>CTX-M-3</sub>. Antimicrobial resistance genes are shown in red. Integrases, *tra* genes, and the transposition genes *tnsABC* are shown in teal. The figures were generated using Easyfig v2.1 (10).

further contained an origin of transfer and transfer (*tra*) genes. An NCBI BLASTn analysis (nucleotide sequence collection) (9) revealed high homology to ICEPmiChn-HBNNC12 (GenBank accession number [MZ277865.1](https://doi.org/10.1093/nar/gkz569)).

The Tn7-like transposon was integrated downstream of *glmS* and comprised the transposase genes *tnsABC* and a nonfunctional class 2 integron integrase (*int1*) at the opposite end. The transposon contained *bla*<sub>CTX-M-3</sub> (cephalosporin resistance), *lnu(F)* (lincosamide resistance), and *mph(E)* and *msr(E)* (both conferring macrolide resistance), among others (Fig. 1B). HK294 was resistant to azithromycin (macrolide), ampicillin and cefazolin ( $\beta$ -lactams), nalidixic acid, ciprofloxacin (fluoroquinolone), streptomycin, kanamycin, and gentamicin (aminoglycosides), chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, and fosfomycin. *P. mirabilis* resides in the intestinal tract of chickens. Fecal contamination during slaughter may hence be a transmission route of multidrug-resistant *P. mirabilis* to the consumer.

**Data availability.** Sequencing data were deposited in the NCBI Sequence Read Archive (SRA) under accession numbers [SRR23693645](https://www.ncbi.nlm.nih.gov/sra/SRR23693645) (short reads) and [SRR23693644](https://www.ncbi.nlm.nih.gov/sra/SRR23693644) (long reads) and BioProject accession number [PRJNA935533](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA935533). The assembly is available under GenBank accession number [GCA\\_029201285.1](https://www.ncbi.nlm.nih.gov/genbank/GCA_029201285.1).

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## REFERENCES

- Schaffer JN, Pearson MM. 2015. *Proteus mirabilis* and urinary tract infections. *Microbiol Spectr* 3:3.5.10. <https://doi.org/10.1128/microbiolspec.UTI-0017-2013>.
- Girlich D, Bonnin RA, Dortet L, Naas T. 2020. Genetics of acquired antibiotic resistance genes in *Proteus* spp. *Front Microbiol* 11:256. <https://doi.org/10.3389/fmicb.2020.00256>.
- Clinical and Laboratory Standards Institute. 2020. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.
- Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
- Steinig E, Coin L. 2022. Nanoq: ultra-fast quality control for Nanopore reads. *J Open Source Softw* 7:2991. <https://doi.org/10.21105/joss.02991>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, Hoffmann M, Pettengill JB, Prasad AB, Tillman GE, Tyson GH, Klimke W. 2021. AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. *Sci Rep* 11:12728. <https://doi.org/10.1038/s41598-021-91456-0>.
- Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. *J Comput Biol* 7:203–214. <https://doi.org/10.1089/10665270050081478>.
- Sullivan MJ, Petty NK, Beatson SA. 2011. Easyfig: a genome comparison visualizer. *Bioinformatics* 27:1009–1010. <https://doi.org/10.1093/bioinformatics/btr039>.