AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

# Complete Genome Sequence of an *mcr-9*-Possessing *Enterobacter asburiae* Strain Isolated from a Cat in Japan

**Toyotaka Sato,[a] Masaru Usui,[b] Kazuki Harada,[c] Yukari Fukushima,[a,d] Chie Nakajima,[d,e] Yasuhiko Suzuki,[d,e] Shin-ichi Yokota[a]**

[a]Department of Microbiology, Sapporo Medical University School of Medicine, Sapporo, Japan
[b]Laboratory of Food Microbiology and Food Safety, Department of Health and Environmental Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan
[c]Department of Veterinary Internal Medicine, Tottori University, Tottori, Japan
[d]Division of Bioresources, Hokkaido University, Research Center for Zoonosis Control, Sapporo, Japan
[e]International Collaboration Unit, Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan

**ABSTRACT** We report the complete genome sequence of the *mcr-9*-possessing strain *Enterobacter asburiae* En30, isolated from a cat in Japan. The genome sequence was obtained by using long- and short-read sequencing.

*Enterobacter asburiae* is a bacterial species included in the *Enterobacter cloacae* complex (1) and isolated from the intestinal tracts and clinical specimens of humans and animals (2, 3). Some *E. asburiae* clinical isolates exhibit multidrug resistance (4), and the last-line drugs colistin and tigecycline are an option for treatment. *mcr-9*, like other *mcr* genes, encodes phosphoethanolamine transferase and reduces colistin susceptibility (5). *mcr-9*-harboring plasmids in the *Enterobacter cloacae* complex have been reported in human samples (6–8), while few have been reported in companion animals.

*E. asburiae* strain En30 was isolated by swabbing the nasal cavity of a cat in Japan in 2015, followed by isolation on CHROMagar *Escherichia coli* coliform (ECC) agar (Becton, Dickinson, NJ) and incubation overnight at 37°C. En30 was subcultured on the agar and kept at −80°C in 10% glycerol stock. Genomic DNA was extracted for short-read sequencing using the Wizard genomic DNA purification kit (Promega, Madison, WI). A DNA library was prepared using the Nextera XT kit and sequenced on the MiSeq platform (Illumina, San Diego, CA). The resulting 1,261,146 (300-bp, paired-end) reads were trimmed using fastp v0.20.1 (9).

Genomic DNA was extracted for long-read sequencing using the Genomic-tip 20/G and genomic DNA buffer set (Qiagen, Hilden, Germany). Library preparation was performed using the rapid barcoding sequencing kit SQK-RBK004 (Oxford Nanopore Technologies, Oxford, UK), following the manufacturer's protocol. Sequencing was performed on the MinION with a FLO-MIN-106 R9.4 flow cell (Oxford Nanopore Technologies) using MinKNOW software for 48 h with no alterations to any voltage scripts. The obtained 440,000 reads ($N_{50}$, 3,932 bp) were demultiplexed using Porechop v0.2.4 (https://github.com/rrwick/Porechop); then, the reads were adaptor trimmed and quality filtered using NanoFilt (Q score, 10; minimum length, 1,000 bp) (10).

The long reads were error corrected with the short reads using LoRDEC v0.6 (11). *De novo* assembly of the error-corrected long reads was performed using Flye v2.8 (12). The assembled contigs were error corrected with the short reads using Pilon v1.24 (13). Finally, the assembled sequences were annotated using DFAST v1.1.0 with standard settings (14). Software tools used default parameters unless otherwise indicated.

We obtained three different-sized complete genome sequences (Table 1). *mcr-9* was located on pEN30L (an IncHI2 plasmid) at nucleotide positions 160415 to 162034. pEN30L was 99.9% similar to the *mcr-9*-carrying plasmid, pIHI2-233, of *Enterobacter hormaechei* strain

**Citation** Sato T, Usui M, Harada K, Fukushima Y, Nakajima C, Suzuki Y, Yokota S-I. 2021. Complete genome sequence of an *mcr-9*-possessing *Enterobacter asburiae* strain isolated from a cat in Japan. Microbiol Resour Announc 10:e00281-21. https://doi.org/10.1128/MRA.00281-21.

**Editor** Catherine Putonti, Loyola University Chicago



Address correspondence to Toyotaka Sato, sato.t@sapmed.ac.jp.

**Received** 2 April 2021
**Accepted** 1 June 2021
**Published** 1 July 2021

**TABLE 1** Features of the En30 genome

| Genome component | Name | Length (bp) | Coverage (×) | G+C content (%) | Antimicrobial resistance genes |
|---|---|---|---|---|---|
| Chromosome | En30 chromosome | 4,722,066 | 82 | 55.9 | $bla_{ACT-10}$, *fosA* |
| Large plasmid | pEN30L | 284,167 | 51 | 46.8 | *mcr-9*, *aac(6')-Ib3*, *aph(6)-Id*, $bla_{TEM-1B}$, *dfrA19*, *aac(6')-Ib-cr*, *catA2*, *tetD* |
| Small plasmid | pEN30S | 85,172 | 161 | 52.7 | $bla_{DHA-1}$, *aac(3)-IIa*, *sul1*, *dfrA17*, *mph*(A), *qacE*, *qnrB4* |

Y233 (GenBank accession number CP049047.1), derived from a patient in China using BLAST (8). pEN30L possessed other antimicrobial resistance genes as revealed by ResFinder v4.1 (Table 1) (15).

**Data availability.** The whole-genome sequence has been deposited at DDBJ/ENA/GenBank under the accession numbers AP024498 (chromosome), AP024499 (pEN30S), and AP024500 (pEN30L). The Illumina and MinION sequence reads were deposited in the Sequence Read Archive (SRA) database under the accession numbers DRR283416 and DRR283417, respectively.

## ACKNOWLEDGMENTS

This research was supported by AMED (JP20ak0101118h0001) and JSPS KAKENHI (JP19K16648 and JP20H03488), the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan, and the Joint Research Program of the Research Center for Zoonosis Control, Hokkaido University.

## REFERENCES

1. Mezzatesta ML, Gona F, Stefani S. 2012. *Enterobacter cloacae* complex: clinical impact and emerging antibiotic resistance. Future Microbiol 7:887–902. https://doi.org/10.2217/fmb.12.61.
2. Brenner DJ, McWhorter AC, Kai A, Steigerwalt AG, Farmer JJ, III. 1986. *Enterobacter asburiae* sp. nov., a new species found in clinical specimens, and reassignment of *Erwinia dissolvens* and *Erwinia nimipressuralis* to the genus *Enterobacter* as *Enterobacter dissolvens* comb. nov. and *Enterobacter nimipressuralis* comb. nov. J Clin Microbiol 23:1114–1120. https://doi.org/10.1128/JCM.23.6.1114-1120.1986.
3. Harada K, Shimizu T, Mukai Y, Kuwajima K, Sato T, Kajino A, Usui M, Tamura Y, Kimura Y, Miyamoto T, Tsuyuki Y, Ohki A, Kataoka Y. 2017. Phenotypic and molecular characterization of antimicrobial resistance in *Enterobacter* spp. isolates from companion animals in Japan. PLoS One 12:e0174178. https://doi.org/10.1371/journal.pone.0174178.
4. Yuan S, Wu G, Zheng B. 2019. Complete genome sequence of an IMP-8, CTX-M-14, CTX-M-3 and QnrS1 co-producing *Enterobacter asburiae* isolate from a patient with wound infection. J Glob Antimicrob Resist 18:52–54. https://doi.org/10.1016/j.jgar.2019.05.029.
5. Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO, Wiedmann M. 2019. Identification of novel mobilized colistin resistance gene *mcr-9* in a multidrug-resistant, colistin-susceptible *Salmonella enterica* serotype Typhimurium isolate. mBio 10:e00853-19. https://doi.org/10.1128/mBio.00853-19.
6. Chavda KD, Westblade LF, Satlin MJ, Hemmert AC, Castanheira M, Jenkins SG, Chen L, Kreiswirth BN. 2019. First report of $bla_{VIM-4}$- and *mcr-9*-coharboring *Enterobacter* species isolated from a pediatric patient. mSphere 4:e00629-19. https://doi.org/10.1128/mSphere.00629-19.
7. Lin M, Yang Y, Yang Y, Chen G, He R, Wu Y, Zhong LL, El-Sayed Ahmed MAEG, Feng S, Shen C, Wen X, Huang J, Li H, Zheng X, Tian GB. 2020. Co-occurrence of *mcr-9* and $bla_{NDM-1}$ in *Enterobacter cloacae* isolated from a patient with bloodstream infection. Infect Drug Resist 13:1397–1402. https://doi.org/10.2147/IDR.S248342.
8. Zhu X, Li P, Qian C, Liu H, Lin H, Zhang X, Li Q, Lu J, Lin X, Xu T, Zhang H, Hu Y, Bao Q, Li K. 2020. Prevalence of aminoglycoside resistance genes and molecular characterization of a novel gene, *aac(3)-IIg*, among clinical isolates of the *Enterobacter cloacae* complex from a Chinese teaching hospital. Antimicrob Agents Chemother 64:e00852-20. https://doi.org/10.1128/AAC.00852-20.
9. 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.
10. De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. Bioinformatics 34:2666–2669. https://doi.org/10.1093/bioinformatics/bty149.
11. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol 37:540–546. https://doi.org/10.1038/s41587-019-0072-8.
12. Salmela L, Rivals E. 2014. LoRDEC: accurate and efficient long read error correction. Bioinformatics 30:3506–3514. https://doi.org/10.1093/bioinformatics/btu538.
13. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.
14. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039. https://doi.org/10.1093/bioinformatics/btx713.
15. Bortolaia V, Kaas RF, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AR, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 75:3491–3500. https://doi.org/10.1093/jac/dkaa345.