Complete Genome Sequence of *Aeromonas caviae* Strain MS6064, a *mcr-3*-Carrying Clinical Isolate from Japan

Liansheng Yu,a,b,c Hiroki Kitagawa,c,d,e Shizuo Kayama,a,b,c Junzo Hisatsune,a,b,c Hiroki Ohge,c,e Motoyuki Sugai,a,b,c

aAntimicrobial Resistance Research Center, National Institute of Infectious Diseases, Tokyo, Japan
bDepartment of Antimicrobial Resistance, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
cProject Research Center for Nosocomial Infectious Diseases, Hiroshima University, Hiroshima, Japan
dDepartment of Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
eDepartment of Infectious Diseases, Hiroshima University Hospital, Hiroshima, Japan

ABSTRACT We report the complete genome sequence of *mcr-3*-carrying *Aeromonas caviae* strain MS6064, isolated from a blood sample from a Japanese patient. The strain carried *mcr-3* variant 3.38 and was borderline resistant to colistin (2 μg/ml).

*Aeromonas caviae* is the most frequently isolated causative pathogen of *Aeromonas* bacteremia (1, 2). *A. caviae* strain MS6064 was isolated in 2014 from a blood sample from a 60-year-old Japanese woman diagnosed with polycystic kidney disease (2).

The strain, which had been kept in a glycerol (20%) stock at −80°C, was grown overnight on a Luria-Bertani (LB) agar plate, and a single colony was picked and transferred into LB broth at 37°C. Genomic DNA was extracted from the pellet using the Qiagen Genomic-tip 20/G kit (Qiagen). For Illumina sequencing, a library was constructed using the QIAseq FX DNA library kit (Qiagen), and paired-end sequencing (2 × 300 bp) was performed using the MiSeq reagent kit v3 on the MiSeq platform. A total of 1,274,512 paired-end reads were obtained from the MiSeq run after adaptor trimming. Trimming of low-quality reads and assembly (average coverage of 36×) were performed with Shovill v1.0.9 (https://github.com/tseemann/shovill). Long-read library preparation for MinION (Oxford Nanopore Technologies [ONT]) sequencing was performed using the SQK-RBK004 rapid barcoding kit (ONT) without DNA size selection, and sequencing was performed with MinKNOW software using a FLO-MIN106 R9.4 flow cell (ONT). Fast5 read files were base called and demultiplexed with Guppy v2.3.1 (ONT). Hybrid assembly of Illumina short reads and MinION long reads was performed using the hybrid assembler Unicycler v0.4.7 (3) with default parameters. The Unicycler pipeline automatically identified and trimmed overlaps for circular genomes and rotated the genome to begin with the *dnaA* gene. Sequence annotation and analysis were performed using PATRIC (https://www.patricbrc.org), ResFinder v3.2, and MLST v2.0 software (http://www.genomicepidemiology.org). BLASTn was used for the alignment of MCR-3 variants. Default parameters were used for all software unless otherwise specified.

The complete genome sequence of MS6064 contained a circular 4,578,485-bp chromosome, with a GC content of 61.29%. A total of 4,306 protein-coding genes, including 31 rRNAs and 121 tRNAs, were predicted by PATRIC. Genomic analysis showed that MS6064 was in sequence type 368 and contained various antibiotic resistance genes, including aadA1, *bla*MOX-4, *mcr-3*, *cat*, *sul1*, and *tet(C)*. This novel *mcr-3* variant was designated *mcr-3.38*.

Some *mcr-3*-carrying isolates are susceptible or borderline resistant to colistin, but there was no clear relationship between colistin MICs and the phylogeny of MCR-3 variants. Among the four available *A. caviae mcr-3*-positive strains, only the *mcr-3.10*-positive strain showed a high colistin MIC (32 μg/ml) (4); all of the other isolates, which


Editor Vincent Bruno, University of Maryland School of Medicine

Copyright © 2021 Yu et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Motoyuki Sugai, sugai@niid.go.jp.

Received 10 September 2020
Accepted 5 February 2021
Published 4 March 2021
were mcr-3.13, mcr-3.18 (5), and mcr-3.38 positive, had a low MIC (0.5 or 2 μg/ml) (Fig. 1). Thirty-three amino acid substitutions in MCR-3.10 are present in at least one of MCR-3.13, MCR3.18, and MCR3.38, but none of them corresponded to potential zinc-interacting residues or possible phosphatidylethanolamine substrate-binding residues (6). Common variations of MCR-3.13, MCR3.18, and MCR3.38, compared with MCR-3.10, are clustered in the C-terminal region. The molecular mechanism of borderline colistin resistance of A. caviae carrying mcr-3.38 remains unknown.

**Data availability.** The sequence data for A. caviae MS6064 have been deposited in the DDBJ Sequence Read Archive (DRA) under accession number DRA010575. The nucleotide sequence of mcr-3.38 has been deposited in GenBank under accession number MT787344.
ACKNOWLEDGMENTS

We thank Editage for English language editing.
This work is supported by the Research Program on Emerging and Re-emerging Infectious Diseases from the Japan Agency for Medical Research and Development (grant 20fk0108132j0001).
We have no conflicts of interests to declare.

REFERENCES