AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

Presence of an *mcr-3* Variant in *Aeromonas caviae*, *Proteus mirabilis*, and *Escherichia coli* from One Domestic Duck

Xiaoming Wang,[a] Weishuai Zhai,[b] Jiyun Li,[a] Dejun Liu,[c] Qidi Zhang,[b] Zhangqi Shen,[a] Shaolin Wang,[a] Yang Wang[c]

[a]Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agriculture University, Beijing, China

[b]College of Veterinary Medicine, Qingdao Agricultural University, Qingdao, Shandong, China

[c]Beijing Key Laboratory of Detection Technology for Animal-Derived Food Safety, College of Veterinary Medicine, China Agriculture University, Beijing, China

KEYWORDS *Aeromonas caviae*, *Proteus mirabilis*, *Escherichia coli*, *mcr-3.10*

The rapid rise and dissemination of multidrug-resistant (MDR) bacteria are a major threat to public health worldwide and have narrowed the treatment options for infections caused by these bacteria (1). Colistin is one of the last-resort drugs for the treatment of infections caused by MDR Gram-negative bacteria. However, within the past 2 years, shortly after the report of the first plasmid-mediated colistin resistance gene, *mcr-1*, in 2015, four other mobile colistin resistance genes (*mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5*) and multiple variants have been reported (2–6). Compared to *mcr-2*, *mcr-4*, and *mcr-5*, which have been detected solely in Europe so far, both *mcr-1* and *mcr-3* have been identified globally. Here, we report a novel *mcr-3* variant in *Aeromonas caviae*, *Proteus mirabilis*, and *Escherichia coli* from a single domestic duck.

A total of 15 cloacal samples were obtained from free-range domestic ducks near a river in a suburban area of Qingdao City, Shandong Province, China, in March 2017. Direct sample testing was then performed on all 15 samples to detect the *mcr-3* gene, and only one sample was identified as *mcr-3* positive. The sample positive for *mcr-3* was further isolated on CHROMagar orientation agar plates (bioMérieux, Lyon, France) containing 2 mg/liter colistin. Three *mcr-3*-positive strains, *A. caviae* 17AC, *P. mirabilis* 17PM, and *E. coli* 17EC, were obtained, and their sequences were confirmed by using 16S rRNA gene sequencing and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry analysis.

A. caviae 17AC, *P. mirabilis* 17PM, and *E. coli* 17EC were then subjected to 150-bp paired-end whole-genome sequencing (WGS) using the Illumina HiSeq 2500 platform (Annoroad, Beijing, China). The draft genomes were assembled using CLC Genomics Workbench 9.0 (CLC Bio, Aarhus, Denmark), and all contigs were searched for *mcr-3* by standalone BLAST analysis. WGS analysis identified *mcr-3*-carrying fragments in each genome, including a 26.2-kb contig from 17AC, a 17.8-kb contig from 17PM, and a 13.0-kb contig from 17EC. An *mcr-3* variant gene in three contigs showed 98.83% nucleotide sequence identity to *mcr-3* from porcine *E. coli*. The deduced protein sequence differed from MCR-3.1 by seven amino acid substitutions, one of which (V122G) was located in a putative transmembrane region, while the remaining six (R297L, I313V, E337K, H341Y, D358E, and Q468K) were in the catalytic domain. This novel *mcr-3* variant was designated *mcr-3.10*. *A. caviae* 17AC and *E. coli* 17EC belong to novel sequence types (STs), ST513 and ST457, respectively. *E. coli* of ST457 carrying *mcr-1* has been reported from humans in the United States and Vietnam (7, 8). Plasmid replicon typing revealed that the *mcr-3.10*-carrying plasmid in both 17EC and its transconjugant T-17EC belongs to the IncI2 replicon.

Accepted manuscript posted online 4 December 2017

Citation Wang X, Zhai W, Li J, Liu D, Zhang Q, Shen Z, Wang S, Wang Y. 2018. Presence of an *mcr-3* variant in *Aeromonas caviae*, *Proteus mirabilis*, and *Escherichia coli* from one domestic duck. Antimicrob Agents Chemother 62:e02106-17. https://doi.org/10.1128/AAC.02106-17.

Address correspondence to Shaolin Wang, shaolinwang@outlook.com, or Yang Wang, wangyang@cau.edu.cn.

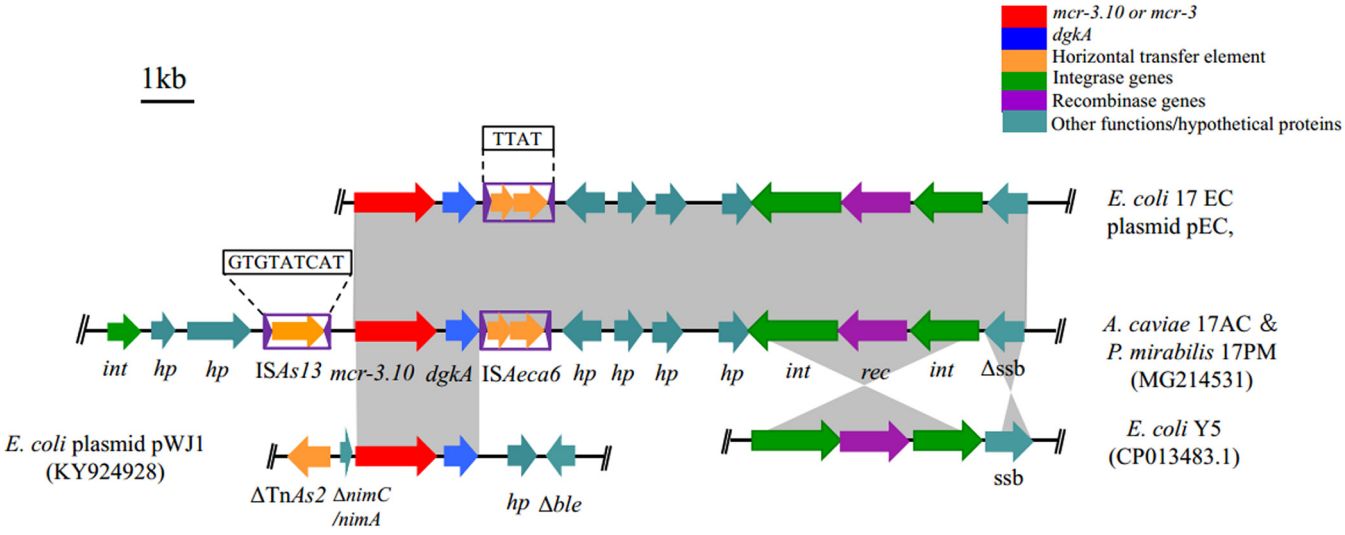

FIG 1 Genetic organizations of scaffolds containing *mcr-3.10* obtained from *A. caviae* 17AC, *P. mirabilis* 17PM, and *E. coli* 17EC in this study and structural comparison with plasmid pWJ1 from *E. coli* WJ1 (GenBank accession no. KY924928) and with the complete genome sequence of *E. coli* Y5 (GenBank accession no. CP013483.1). The positions and orientations of the genes are indicated by arrows, with the direction of transcription shown by the arrowhead. Gray shading indicates >90% nucleotide sequence identity. The 9-bp direct repeat (5′-GTGTATCAT-3′) of IS*As13* and the 4-bp direct repeats (5′-TTAT-3′) of IS*Aeca6* are boxed.

A conjugation experiment showed that *mcr-3.10* was able to be transferred from donor strain *E. coli* 17EC to recipient strain *E. coli* J53, and transconjugant T-17EC was obtained and its sequence confirmed by PCR targeting of *mcr-3.10* with Sanger sequencing. S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) and Southern blotting indicated that *mcr-3.10* is located on a chromosome in *A. caviae* 17AC and *P. mirabilis* 17PM and on an ~45-kb plasmid, named p17EC, in *E. coli* 17EC (see Fig. S1 in the supplemental material).

An antimicrobial susceptibility test was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The breakpoints for each antimicrobial agent were interpreted according to CLSI standards (9) and those of the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org) (10). All three *mcr-3.10*-carrying isolates showed resistance to most of the tested antimicrobial agents, including colistin (8 to 32 μg/ml), polymyxin B (8 to 32 μg/ml), gentamicin (16 to 512 μg/ml), chloramphenicol (128 to 512 μg/ml), ciprofloxacin (32 to 128 μg/ml), and tetracycline (16 to 128 μg/ml), and they remained sensitive to imipenem (0.13 μg/ml) and meropenem (0.06 μg/ml). Like the original *mcr-3* gene, the *mcr-3.10* gene resulted in an 8-fold increase in the MIC of colistin (2 mg/liter) for transconjugant T-17EC compared with that for the recipient *E. coli* J53 (0.25 mg/liter). It also exhibited resistance to gentamicin and tetracycline, which can be conferred by *ant(6)-Ia* and the *tet*(D) gene identified in p17EC, respectively (Table S1).

Genetic-environment analysis revealed that the 13.0-kb *mcr-3.10*-carrying contig in 17EC showed 100% nucleotide sequence identity to the corresponding regions of the 26.2-kb contig from 17AC and the 17.8-kb contig from 17PM. A complete insertion sequence (IS), IS*As13* (https://www-is.biotoul.fr/scripts/ficheIS.php?name=ISAs13), which originated from *Aeromonas salmonicida*, was located upstream of *mcr-3.10* in both 17AC and 17PM. In addition, an IS-like structure with 87% nucleotide identity to IS*As22* was located downstream of *mcr-3.10*. This IS-like element was 1,198 bp in length and contained two open reading frames flanked by imperfect putative inverted repeats (IRs) (right IR, 12 bp; left IR, 11 bp). This IS-like element was submitted to the ISfinder database (https://www-is.biotoul.fr/scripts/ficheIS.php?name=ISAeca6) and was assigned the designation IS*Aeca6*, which belongs to the IS*3* family (Fig. 1). Identical 13.0-kb *mcr-3.10*-carrying structures shared by three different bacterial species within one duck further confirm the dissemination of *mcr-3* between *Aeromonas* and *Enterobacteriaceae* in animal guts. Interestingly, the presence of *mcr-3.10* in *Proteus*

mirabilis indicated that even the naturally colistin-resistant bacterial species may serve as a reservoir of the *mcr-3* variant gene.

Accession number(s). The nucleotide sequence of the 26.2-kb contig carrying *mcr-3.10* in *A. caviae* 17AC determined in this study has been deposited in GenBank under accession number MG214531.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02106-17.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

ACKNOWLEDGMENTS

This study was supported by grants from the National Natural Science Foundation of China (31422055 and 81661138002) and the National Basic Research Program of China (2013CB127200).

REFERENCES

1. Wang Y, Zhang R, Li J, Wu Z, Yin W, Schwarz S, Tyrrell JM, Zheng Y, Wang S, Shen Z, Liu Z, Liu J, Lei L, Li M, Zhang Q, Wu C, Zhang Q, Wu Y, Walsh TR, Shen J. 2017. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. Nat Microbiol 2:16260. https://doi.org/10.1038/nmicrobiol.2016.260.
2. Liu Y, Wang Y, Walsh TR, Yi L, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu L, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu J, Shen J. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16:161–168. https://doi.org/10.1016/S1473-3099(15)00424-7.
3. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. 2016. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. Euro Surveill 21:30280. https://doi.org/10.2807/1560-7917.ES.2016.21.27.30280.
4. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, Zhang R, Walsh TR, Shen J, Wang Y. 2017. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. mBio 8:e00543-17. https://doi.org/10.1128/mBio.00543-17.
5. Carattoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppi A, Pezzotti G, Magistrali CF. 2017. Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Euro Surveill 22:30589. https://doi.org/10.2807/1560-7917.ES.2017.22.31.30589.
6. Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. 2017. Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in D-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. J Antimicrob Chemother 72:3317–3324. https://doi.org/10.1093/jac/dkx327.
7. McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R, Hinkle M, Whitman T, Lesho E, Schaecher KE. 2016. *Escherichia coli* harboring *mcr-1* and bla_{CTX-M} on a novel IncF plasmid: first report of *mcr-1* in the United States. Antimicrob Agents Chemother 60:4420–4421. https://doi.org/10.1128/AAC.01103-16.
8. Tada T, Nhung PH, Shimada K, Tsuchiya M, Phuong DM, Anh NQ, Ohmagari N, Kirikae T. 2017. Emergence of colistin-resistant Escherichia coli clinical isolates harboring mcr-1 in Vietnam. Int J Infect Dis 63:72–73. https://doi.org/10.1016/j.ijid.2017.07.003.
9. Clinical and Laboratory Standards Institute. 2013. VET01-A4. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 4th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
10. Bakthavatchalam YD, Veeraraghavan B. 2017. Challenges, issues and warnings from CLSI and EUCAST Working Group on Polymyxin Susceptibility Testing. J Clin Diagn Res 11:DL03–DL04.