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Letter to the Editor

Chromosome-mediated *mcr-1* in *Escherichia coli* strain L73 from a goose

Sir,

Colistin is regarded as the drug of last resort to treat infections caused by multidrug-resistant (MDR) Gram-negative bacteria. Plasmid-mediated colistin resistance gene *mcr-1* was discovered in China in 2015 [1]. Since then, the following *mcr* genes have been described: *mcr-2* (LT598652), *mcr-3* (KY924928), *mcr-5* (KY807921), *mcr-6* (MF176240), *mcr-7* (MG267386) and *mcr-8* (MG736312). Mobile colistin resistance genes have received wide public attention; however, chromosome-mediated colistin resistance genes should not be overlooked. Although *mcr-1* has been reported in different sources, including in animals and humans, there is no report of *mcr-1* in the goose, which is an important farming animal in Jiangsu, China. In this study, we report a colistin-resistant *Escherichia coli* encoding a chromosomal *mcr-1* from a goose anus swab and characterize the genome with MinION long reads.

A total of 96 samples were collected from geese with no manifestation of disease from a goose farming center in Yangzhou, Jiangsu Province, China in 2017. A total of 162 Enterobacteriaceae isolates were obtained from these samples via screening on MacConkey Agar plates. *mcr* genes were identified by the multiplex polymerase chain reaction (PCR) method reported previously [2]. One isolate, L73, identified as *E. coli* by 16S rDNA sequencing, was positive for *mcr-1*. Antimicrobial susceptibility testing was performed on the *mcr-1*-positive *E. coli* using the broth microdilution method and interpreted according to the EUCAST breakpoints for Enterobacteriaceae. *E. coli* ATCC 25922 was the quality control. *E. coli* L73 was resistant to colistin (4 mg/L) and exhibited low susceptibility to streptomycin, doxycycline, amoxicillin, and florfenicol. Conjugation assay failed to transfer the colistin resistance phenotype to the azide-resistant *E. coli* J53 strain, which indicated the *mcr-1* in L73 was not in conjugative elements. Whole genome sequencing (WGS) was performed to genetically characterize *mcr-1*.

The genomic DNA of *E. coli* strain L73 was extracted using a TIANamp Bacteria DNA Kit. WGS was performed via Illumina and ONT MinION platforms. De novo assembly was performed by short-read and long-read data [3]. The Rapid Annotation using Subsystem Technology (RAST) was used to annotate the genome. Sequence analysis revealed that L73 harbored one chromosome (ST48) and two plasmids. *mcr-1* in L73 is located on the chromosome. A chromosomal segment containing *mcr-1* was extracted and BLASTn analysis was performed. The gene *yjbs* had been disrupted by insertion of the IS*Apl1*-*mcr-1*-*orf*-IS*Apl1* (Tn*6330*) exhibiting 100% identity to the Tn*6330* in the *E. coli* HS30-1 chromosome (Figure 1a). The surrounding sequences of Tn*6330* in L73 exhibited 99% identity (97% coverage) to the chromosome of *E. coli* str. K-12 (CP032667.1). This indicated that insertion of Tn*6330* occurred previously. A pair of reverse primers targeting *mcr-1* was used to test the ability of Tn*6330* to generate circular intermediate as previously described [4]. Primers Tn-F (TTTCTGATTGACCAGCCCCC) and Tn-R (CACCTTTAACCTCCTCGGCA) targeting the surrounding sequences of Tn*6330* in L73 were utilized to investigate whether the intact Tn*6330* was consistently present in the bacterial population. The sequences of PCR products were obtained by Sanger sequencing. Gel electrophoresis showed there were two PCR products (~5 kb and ~1.4 kb) (Figure 1c), which indicated Tn*6330* was unstable and still active, as described previously [4]. Tn*6330* in *E. coli* L73 could generate a circular intermediate (IS*Apl1*-*mcr-1*-*orf*) (Figure 1d), which was detectable in the form of a ~2.5 kb amplicon (Figure 1e). A previous study indicated the IS*Apl1* upstream of *mcr-1* participated in the formation of the *mcr-1*-*orf*-IS*Apl1* circular intermediate on its own or via recombination with the IS*Apl1* downstream of *mcr-1* [5]. Based on the genetic context of *mcr-1*, the *mcr-1* was likely mobilized into this chromosome via the IS*Apl1* element.

WGS showed there were two circular plasmids, pL73-2 and pL73-3, in *E. coli* L73. pL73-2 was a 167 170-bp, IncN plasmid sharing 99% identity (41% coverage) to the sequence of *E. coli* strain WCHEC050613 plasmid pP_WCHEC050613 (CP019216.2) and 99% identity (41% coverage) to the sequence of *E. coli* strain AR_0157 plasmid (CP029729.1), and with an overall GC content of 50.48%. Multiple resistance genes, including *qnrS1*, $bla_{\text{TEM-1B}}$, *aph(6)-Id*, *aph(3'')-Ib*, *sul2*, *dfrA14*, *floR* and two *tet*(A) genes, were observed in pL73-2 (Figure 1f). No resistance gene was found in plasmid pL73-3.

In conclusion, a chromosomal *mcr-1*-positive *E. coli* strain in the form of a composite transposon was identified from the goose. Nanopore sequencing can be utilized to accelerate the analysis of resistance genes in genomes without S1-pulsed-field gel electrophoresis (S1-PFGE) and Southern blot. To the best of our knowledge, this is the first report of an *mcr-1*-bearing *E. coli* from the goose. Active surveillance is essential to prevent further dissemination of *mcr-1*.

Accession numbers. The complete genome of *E. coli* strain L73 has been deposited in GenBank (CP033378, CP033379 and CP033380).

Declarations

None to declare.

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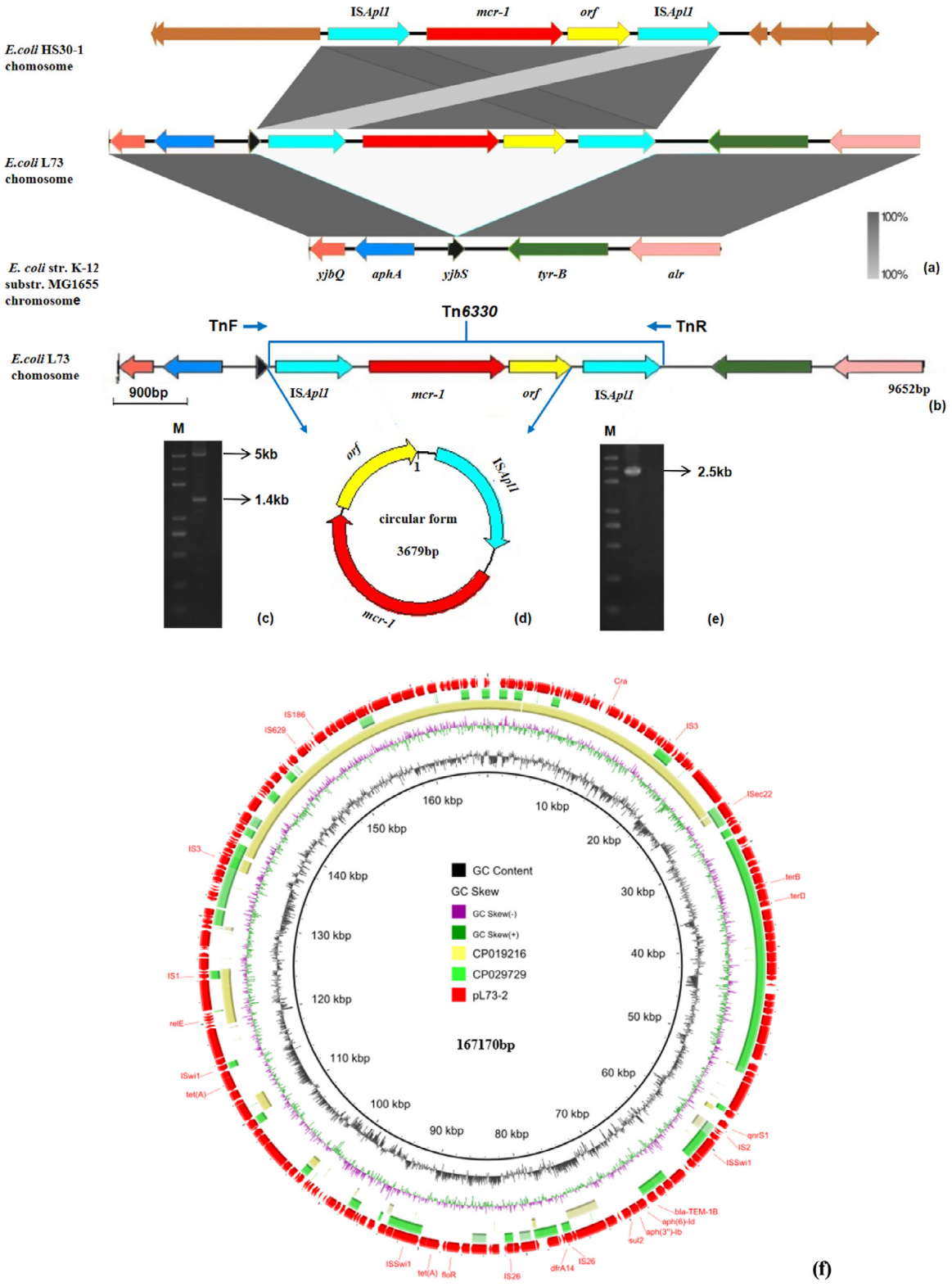

Fig. 1. (a) Sequence alignment between the *E. coli* L73 chromosome and two structurally similar *E. coli* chromosomes. Arrows indicate the direction of the genes. The regions in gray represent the two linked areas with high similarity. (b) A 9652-bp chromosomal segment containing *mcr-1* in the *E. coli* L73 chromosome. (c) Gel electrophoresis of PCR products generated by primers Tn-F and Tn-R targeting surrounding sequences of Tn*6330*. (d) Circular intermediate of Tn*6330* harboring the *mcr-1* gene generated by reverse primers. (e) Gel electrophoresis of the PCR product generated by primers MCR1-R and MCR1-RC-F [4]. (f) Sequence alignment between pL73-2 and two structurally similar plasmids.

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Competing Interests

None to declare.

Ethical Approval

None to declare.

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