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Short Communication

Mobile colistin resistance gene *mcr-1* detected on an IncI1 plasmid in *Escherichia coli* from meat

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ABSTRACT

Objectives: Mobile colistin resistance (*mcr*) genes encoded on conjugative plasmids, although described only relatively recently, have been reported globally both in humans and livestock. The genes are often associated with the insertion sequence IS*Apl1* that can transpose the genes to novel genetic locations. Since its first report, multiple variants of *mcr* have been discovered in a variety of genetic locations in *Escherichia coli*, in plasmids and integrated into the chromosome.
Methods: Using hybrid assembly of short-read and long-read whole-genome sequencing data, the presence of*mcr-1* was confirmed on an IncI1 plasmid in *E. coli*. In vitro conjugation assays were performed to determine the potential to transfer between strains. Genetic comparison with previously reported IncI1 plasmids was performed.
Results: The genomic sequence identified that*mcr-1* is present on a complete IncI1 plasmid. Comparison with previously reported extended-spectrum β-lactamase (ESBL)-encoding plasmids from *E. coli* in the Netherlands from the same time period indicated a distinct lineage for this plasmid.
Conclusions: The observation of*mcr-1* on an IncI1 plasmid confirms that the genetic region of this gene is actively transposed between genetic locations. This active transposition has consequences for the study of the epidemiology of *mcr* in populations.

Mobile colistin resistance was first reported in *Escherichia coli* isolated from a pig in China, where the *mcr-1* gene was encoded on an IncI2 plasmid [1]. Since then, a total of 10 variants of the *mcr* gene have been described in Enterobacteriaceae [2]. The genetic context of *mcr-1* often includes two copies of insertion sequence IS*Apl1* through which the gene can be transposed between replicons and that has led to insertion into the chromosome as well as various plasmid types, including IncI2, various types of IncF, IncHI1, IncHI2, IncX3, IncX4, IncY, IncP, IncK and colE [3,4].

Colistin has a long history of extensive use in animal production both as a prophylactic and therapeutic agent. Nowadays, colistin is still important for the treatment of infections by Enterobacteriaceae in livestock, but it has now been elevated to critically important for human medicine by the World Health Organisation (WHO) [5]. Due to side effects of the drug, colistin is not commonly prescribed for humans, but there is renewed attention in its use as a last-resort drug for multidrug-resistant infections such as carbapenemase-producing Enterobacteriaceae [5,6]. The widespread use of colistin has facilitated the spread of *mcr* genes in animals and humans.

Colistin-resistant *E. coli* from the culture collection of the Dutch national monitoring programme for antimicrobial resistance in animals were tested by PCR for the presence of the genes *mcr-1–5* as previously described (Supplementary Table S1) [7,8]. Whole-genome sequencing using Illumina short reads and Oxford Nanopore Technologies long reads were followed by hybrid assembly using Unicycler v.0.4.7 [9]. Analysis of the genomes indicated that in one *E. coli* isolate *mcr-1* was present on an IncI1 plasmid, and this isolate was selected for further analysis. The *E. coli* is sequence type 101 (ST101) and was isolated from a sample of turkey meat collected at retail in the Netherlands in 2015. The sequence was deposited in GenBank with accession no. **SAMN14826803**. Genetic analysis of the plasmid demonstrated

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that pMCR-E2899 is an IncI1α plasmid, and comparison with the IncI1 reference plasmid R64 showed that most of the sequence that is considered the backbone of these plasmids is present, including plasmid replication and stabilisation genes, transfer genes and pilus formation genes, suggesting that it may be conjugative. The genetic context of *mcr-1* contains flanking IS*Apl1* elements, as previously reported, which have been shown to enable transposition [10] (see Fig. 1). As loss of the flanking IS*Apl1* elements occurs over time in order to stabilise the genetic structure after integration, the complete presence of these elements here may suggest that the *mcr-1* gene was transposed into the IncI1 plasmid relatively recently before isolation [10,11]. In silico plasmid multilocus sequence typing (pMLST) indicated that the plasmid encodes a novel variant of *pilL*, which was submitted to PubMLST and was assigned *pilL-33* [12,13]. The other pMLST targets include *repI-1*, *ardA-38*, *trbA-16* and *sogS-2*, which was assigned ST316.

To determine mobility of the plasmid, filter mating was carried out as previously described [14]. A sodium-azide resistant mutant of *E. coli* DH5α (Thermo Scientific, Surrey, UK), referred to as *E. coli* DH5α-Azi^r, was prepared by selecting for spontaneous mutants of sodium-azide resistant *E. coli* DH5α following growth of 0.1 mL of an overnight DH5α culture on MacConkey agar supplemented with 200 mg/L sodium azide (Sigma-Aldrich, Poole, UK). Colonies were further subcultured twice on MacConkey agar supplemented with 200 mg/mL sodium azide to ensure the stability of the sodium azide resistance. Liquid cultures of donor E2899 and recipient *E. coli* DH5α-Azi^r were mixed at 1:1 ratios, spread on 0.45 nm filter paper on non-selective LB agar plates and incubated for 18 h at 37 °C. Cells were resuspended in LB broth and plated onto MacConkey agar plates supplemented with colistin (2 mg/L) and sodium azide (200 mg/L). Four putative transconjugants were subcultured and tested by PCR for the presence of *mcr-1* and the recipient chromosomal locus NHR (Supplementary Table S1). One confirmed transconjugant containing pMCR-E2899 was subjected to hybrid sequence analysis, as described above, and the sequence was deposited in GenBank (accession no. **SAMN14826402**). Comparison of the recipient and transconjugant sequences confirmed that the complete plasmid pMCR-E2899 had transferred and the element containing *mcr-1* had not transposed into the chromosome.

IncI1 plasmids encoding extended-spectrum β-lactamase (ESBL) genes are commonly isolated from humans, livestock and food products [15]. In a previous study, 31 IncI1 plasmids from the Netherlands and the UK were fully sequenced, indicating that the

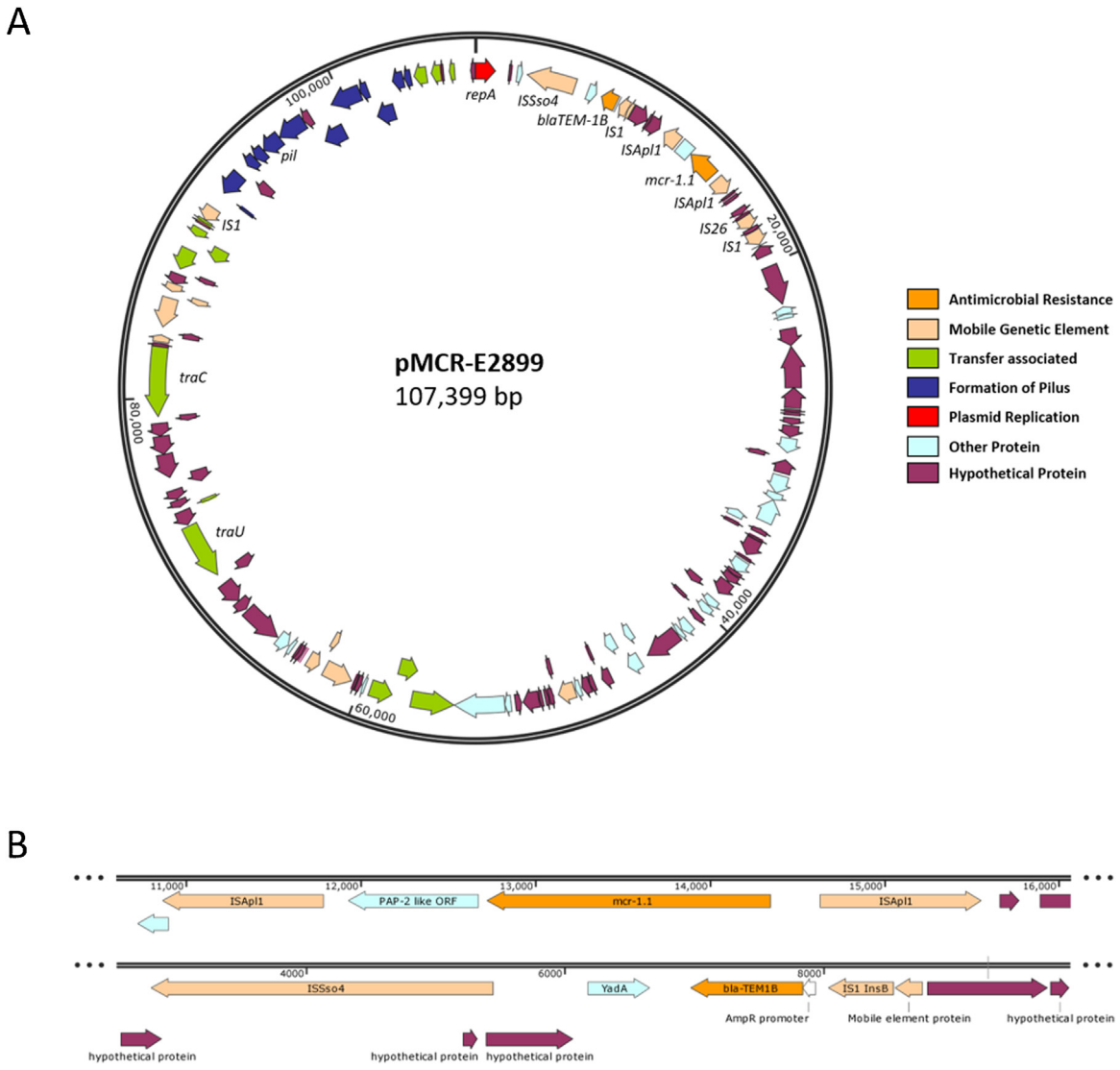

Fig. 1. Genetic structure of plasmid pMCR-E2899. (A) The outer circle denotes the size of the circular DNA from 0 to 107 399 bp. The genes are categorised by colour according to the function of the gene product. (B) Detailed structure of the genetic environment of *mcr-1.1* and *bla*$_{TEM-1B}$.

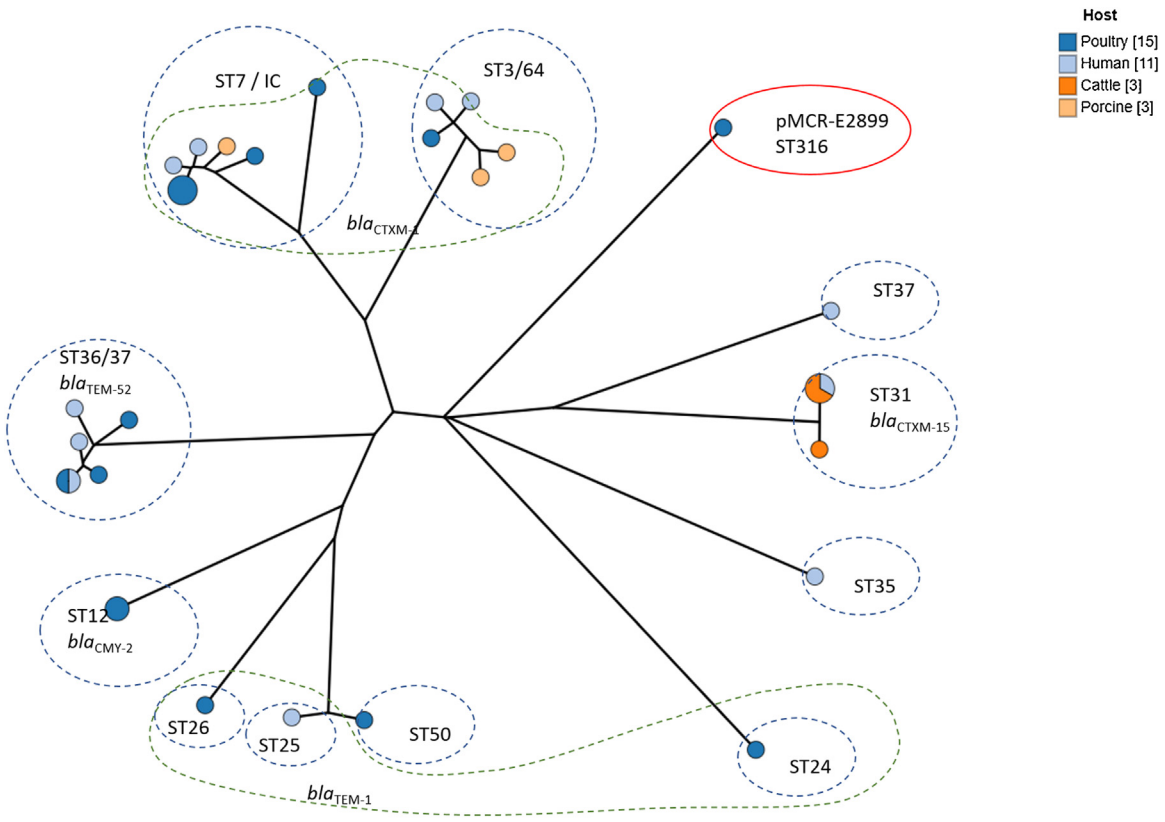

Fig. 2. Minimum spanning tree based on core genome multilocus sequence typing (cgMLST) of complete IncI1 plasmids encoding *mcr-1*, extended-spectrum β-lactamase (ESBL), plasmid AmpC (pAmpC) or β-lactamase genes. Nodes are coloured by host species from which the *Escherichia coli* was isolated (see labels in figure). The *mcr-1*-encoding plasmid is circled in red. ESBL- or pAmpC-encoding plasmids are circled by blue dashed lines to indicate clusters of identical or similar plasmid MLST and encoded β-lactamase. Green dashed lines indicate the isolates encoding $bla_{\text{TEM-1}}$ or $bla_{\text{CTX-M-1}}$.

type of ESBL that is encoded often correlates with specific genetic clades, demonstrating the circulation of specific successful plasmid–gene combinations [16]. The 31 IncI1 plasmids from this study were used to compare with the novel pMCR-E2899 ST316 using BacCompare [17]. A core genome MLST (cgMLST) (95% occurrence) was calculated and 39 discriminatory loci were used to build a tree. A minimum spanning tree was visualised using GrapeTree [18] in which the nodes are coloured by the host species from which the *E. coli* isolates originated. Based on the cgMLST, pMCR-E2899 does not cluster with any of the previously sequenced β-lactamase-encoding IncI1 plasmids, and specifically not with the $bla_{\text{TEM-1}}$ encoding plasmids, although the plasmid also encodes this β-lactamase (Fig. 2).

In summary, we have described a novel transferable plasmid type on which *mcr-1* is encoded. Although this *E. coli* was isolated from turkey meat at retail in the Netherlands, the origin of the meat could not be traced and, due to the low number of turkey farms in the Netherlands, the meat most probably originates from one of the neighbouring countries within the European Union. Despite the reduction of colistin use in agriculture in the Netherlands, continued monitoring for colistin resistance genes is warranted as *mcr-1* is still likely to be circulating both intracellularly between plasmid replicons and intercellularly between bacteria on these different plasmids.

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Conflict of interests

None declared.

Ethical approval

Not required.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2020.08.018.

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