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

Michael S.M. Brouwer1\*#, Richard N. Goodman2\*, Arie Kant1, Dik Mevius1, Enas Newire3, Adam P. Roberts2, Kees T. Veldman1

Running title: MCR-1 encoded on IncI1 plasmids.

1 Wageningen Bioveterinary Research, Lelystad, The Netherlands

2 Liverpool School of Tropical Medicine, Liverpool, United Kingdom

3Institute of Systems, Molecular & Integrative Biology, Faculty of Health and Life Sciences, University of Liverpool, Liverpool, UK.

\* These authors contributed equally

# Corresponding author:

Michael Brouwer

Department of Bacteriology and Epidemiology

Wageningen Bioveterinary Research

Wageningen University and Research

Tel: 0031-320238327

Email: mike.brouwer@wur.nl

**Keywords**

Mobile colistin resistance, IncI1 plasmid, conjugation

**Abstract**

Objectives

Mobile colistin resistance genes (*mcr*) encoded on conjugative plasmids, although described only relatively recently, have been reported globally in both humans and livestock. The genes are often associated with IS*Apl1* which can transpose the genes to novel genetic locations. Since the first report, multiple variants of *mcr* have been discovered on 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 WGS 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 to previously reported IncI1 plasmids was performed.

Results

The genomic sequence has identified that *mcr-1* is present on a complete IncI1 plasmid. Comparison to previously reported ESBL-encoding plasmids from E. coli in the Netherlands from the same time period indicated a distinct lineage for this plasmid.

Conclusion

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.

**Main text**

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 these genes have been described in Enterobacteriaceae(2). The genetic context of *mcr-1* often includes two copies of IS*Apl1* through which the gene can be transposed between replicons and which 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 as prophylactic and therapeutic agent. Nowadays, colistin is still important for treatment of infections by Enterobacteriaceaein livestock, but it has now been elevated to critically important for human medicine by the World Health Organisation (5). Due to side effects of the drug, colistin is not commonly prescribed for humans but there is renewed attention to use it as a last resort drug for multi-resistant infections such as carbapenemase-producing Enterobacteriaceae (5, 6). The widespread use of colistin facilitated the spread of *mcr* genes in animals and humans.

Colistin resistant *Escherichia coli* from the culture collection of the Dutch national monitoring program for AMR in animals were tested by PCR for the presence of *mcr-1-5* as previously described, see table S1 (7, 8). Whole genome sequencing using Illumina short reads and Oxford Nanopore Technologies long reads were followed by hybrid assembly using Unicycler (v0.4.7) (9). Analysis of the genomes indicated that in one isolate, *mcr-1* is present on an IncI1 plasmid which was selected for further analysis. The *E. coli* is ST101 and was isolated from a sample of turkey meat collected at retail in the Netherlands in 2015. The sequence was deposited in Genbank, accession SAMN14826803. Genetic analysis of the plasmid demonstrated that pMCR-E2899 is an IncI1α plasmid and comparison to the IncI1 reference plasmid R64 shows 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 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 Figure 1. As the loss of the flanking IS*Apl1*-elements occurs over time in order to stabilize 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* pMLST indicated that the plasmid encodes a novel variant of *pilL* which was submitted to PubMLST and 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-matings were carried out as previously described (14). A sodium-azide resistant mutant of *E. coli* DH5-α (Thermo Scientific, Surrey, UK), referred to as *E. coli* DH5α-Azir was prepared by selecting for spontaneous mutants of sodium azide resistance *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-Azir were mixed at 1:1 ratios, spread on 0.45nm filter paper on non-selective LB Agar plates and incubated for 18 hours at 37 °C. Cells were resuspended in LB broth and plated onto MacConkey agar plates supplemented with colistin (2mg/L) and sodium azide (200mg/L). 4 putative transconjugants were subcultured and tested by PCR for presence of *mcr-1* and the recipient chromosomal locus NHR, see Table S1. One confirmed transconjugant containing pMCR-E2899 was subjected to hybrid sequence analysis, as described above, sequence deposited in Genbank, accession 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 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 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 to the novel pMCR-E2899 ST316 using BacCompare (17). A core-genome MLST (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 beta-lactamase encoding IncI1 plasmids and specifically, not with the *bla*TEM-1 encoding plasmids, although the plasmid also encodes this beta-lactamase.

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 EU. Continued monitoring for colistin resistance genes, despite the reduction of colistin use in agriculture in the Netherlands, is warranted as *mcr-1* is still likely to be circulating both intracellularly between plasmid replicons and intercellularly between bacteria on these different plasmids.

**Acknowledgements**

The authors would like to thank Ben Wit of the Netherlands Food and Consumer Product Safety Authority and Michel Rappalini and Bart Wullings of Wageningen Food Safety Research for contributing the *E. coli* isolated from food sources.

Funding for this research was received from the Dutch ministry of Agriculture, Nature and Food Quality (WOT-01-002-003), and the European Union’s Horizon 2020 research and innovation programme (grant agreement No 773830). RG was supported by the Medical Research Council via the LSTM-Lancaster Doctoral Training Partnership (grant number MR/N013514/1). EN received funding from the Schlumberger Foundation; Faculty of the Future Programme. AR would like to acknowledge funding from the AMR Cross-Council Initiative through a grant from the Medical Research Council, a Council of UK Research and Innovation, and the National Institute for Health Research. (Grant Numbers MR/S004793/1 and NIHR200632).

**Transparency declarations**

None to declare.

References

1. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, 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 16:161-8.

2. Wang C, Feng Y, Liu L, Wei L, Kang M, Zong Z.2020. Identification of novel mobile colistin resistance gene mcr-10. Emerg Microbes Infect 9:508-516.

3. Matamoros S, van Hattem JM, Arcilla MS, Willemse N, Melles DC, Penders J, Vinh TN, Thi Hoa N, Bootsma MCJ, van Genderen PJ, Goorhuis A, Grobusch M, Molhoek N, Oude Lashof AML, Stobberingh EE, Verbrugh HA, de Jong MD, Schultsz C.2017. Global phylogenetic analysis of Escherichia coli and plasmids carrying the mcr-1 gene indicates bacterial diversity but plasmid restriction. Sci Rep 7:15364.

4. 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.

5. Organization GWH. 2019. Critically important antimicrobials for human medicine, 6th revision.

6. Rhouma M, Beaudry F, Letellier A.2016. Resistance to colistin: what is the fate for this antibiotic in pig production? Int J Antimicrob Agents 48:119-26.

7. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA, Battisti A, Franco A, Alba P, Perrin-Guyomard A, Granier SA, De Frutos Escobar C, Malhotra-Kumar S, Villa L, Carattoli A, Hendriksen RS.2018. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Euro Surveill 23.

8. 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.

9. Wick RR, Judd LM, Gorrie CL, Holt KE.2017. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595.

10. Wang R, van Dorp L, Shaw LP, Bradley P, Wang Q, Wang X, Jin L, Zhang Q, Liu Y, Rieux A, Dorai-Schneiders T, Weinert LA, Iqbal Z, Didelot X, Wang H, Balloux F.2018. The global distribution and spread of the mobilized colistin resistance gene mcr-1. Nat Commun 9:1179.

11. Liu Y, Liu JH.2018. Monitoring Colistin Resistance in Food Animals, An Urgent Threat. Expert Rev Anti Infect Ther 16:443-446.

12. Jolley KA, Bray JE, Maiden MCJ.2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 3:124.

13. Garcia-Fernandez A, Chiaretto G, Bertini A, Villa L, Fortini D, Ricci A, Carattoli A.2008. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum beta-lactamases in Escherichia coli and Salmonella of human and animal origin. J Antimicrob Chemother 61:1229-33.

14. Roberts AP, Mullany P, Wilson M.2001. Gene transfer in bacterial biofilms. Methods Enzymol 336:60-5.

15. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, Mevius DJ, Hordijk J.2018. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother 73:1121-1137.

16. Smith H, Bossers A, Harders F, Wu G, Woodford N, Schwarz S, Guerra B, Rodriguez I, van Essen-Zandbergen A, Brouwer M, Mevius D.2015. Characterization of epidemic IncI1-Igamma plasmids harboring ambler class A and C genes in Escherichia coli and Salmonella enterica from animals and humans. Antimicrob Agents Chemother 59:5357-65.

17. Liu YY, Lin JW, Chen CC.2019. cano-wgMLST\_BacCompare: A Bacterial Genome Analysis Platform for Epidemiological Investigation and Comparative Genomic Analysis. Front Microbiol 10:1687.

18. Zhou Z, Alikhan NF, Sergeant MJ, Luhmann N, Vaz C, Francisco AP, Carrico JA, Achtman M.2018. GrapeTree: visualization of core genomic relationships among 100,000 bacterial pathogens. Genome Res 28:1395-1404.

Figure legends

Figure 1

Genetic structure of plasmid pMCR-E2899. A) The outer circle denotes the size of the circular DNA from 0 to 107,399bp. 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*.*

Figure 2

Minimum spanning tree based on cgMLST of complete IncI1 plasmids encoding *mcr-1*, ESBL, pAmpC or beta-lactamase genes. Nodes are coloured by host species from which the *E. 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 beta-lactamase. Green dashed lines indicate the isolates encoding *bla*TEM-1 or *bla*CTXM-1.

Supplementary

Table S1 Oligonucleotides

|  |  |  |  |
| --- | --- | --- | --- |
| Oligonucleotide | Sequence (5’-3’) | Target gene | Reference |
| *mcr1*\_320bp\_fw | AGTCCGTTTGTTCTTGTGGC | *mcr-1* | (7) |
| *mcr1\_*320bp*\_*rev | AGATCCTTGGTCTCGGCTTG |
| *mcr2*\_700bp\_fw | CAAGTGTGTTGGTCGCAGTT | *mcr-2* | (7) |
| *mcr2*\_700bp\_rev | TCTAGCCCGACAAGCATACC |
| *mcr3*\_900bp\_fw | AAATAAAAATTGTTCCGCTTATG | *mcr-3* | (7) |
| *mcr3*\_900bp\_rev | AATGGAGATCCCCGTTTTT |
| *mcr4*\_1100bp\_fw | TCACTTTCATCACTGCGTTG | *mcr-4* | (7) |
| *mcr4*\_1100bp\_rev | TTGGTCCATGACTACCAATG |
| *MCR5*\_fw | ATGCGGTTGTCTGCATTTATC | *mcr-5* | (8) |
| *MCR5*\_rev | TCATTGTGGTTGTCCTTTTCTG |
| NHR-fw | ATCGGGTTCTTGCCAGTGAG | *yjhU* | This study |
| NHR-rev | TGGAGTCATTACCGACCATGT |