

Genetic variety of plasmid mediated colistin resistance in Gram-negative bacteria from raw meat products

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Introduction:

Currently, there are multiple variants of *mcr*-mediated colistin resistance in Gram-negative bacteria from different sources around the world. The most prevalent gene in *Enterobacteriaceae mcr-1* has been located on several types of plasmids (Wang *et al.*, 2018).

Results:

- The most prevalent plasmid type carrying *mcr-1* was IncX4 detected in *E. coli* (n=8) and *K. pneumoniae* (n=2) isolated from meat of various country origin (Czech Republic, Poland, Germany, Brazil).
- The *mcr-3.8* and *mcr-7*-like genes from *Aeromonas* spp. isolated from a quail meat sample (France) were found to be located in the chromosome. The *mcr-7*-like gene was found twice in the chromosome and had 86% identity (96% coverage) and 82% identity (98% coverage) with the *mcr-7.1* gene described in the NCBI database. The *Aeromonas* isolate did not contain any plasmids.

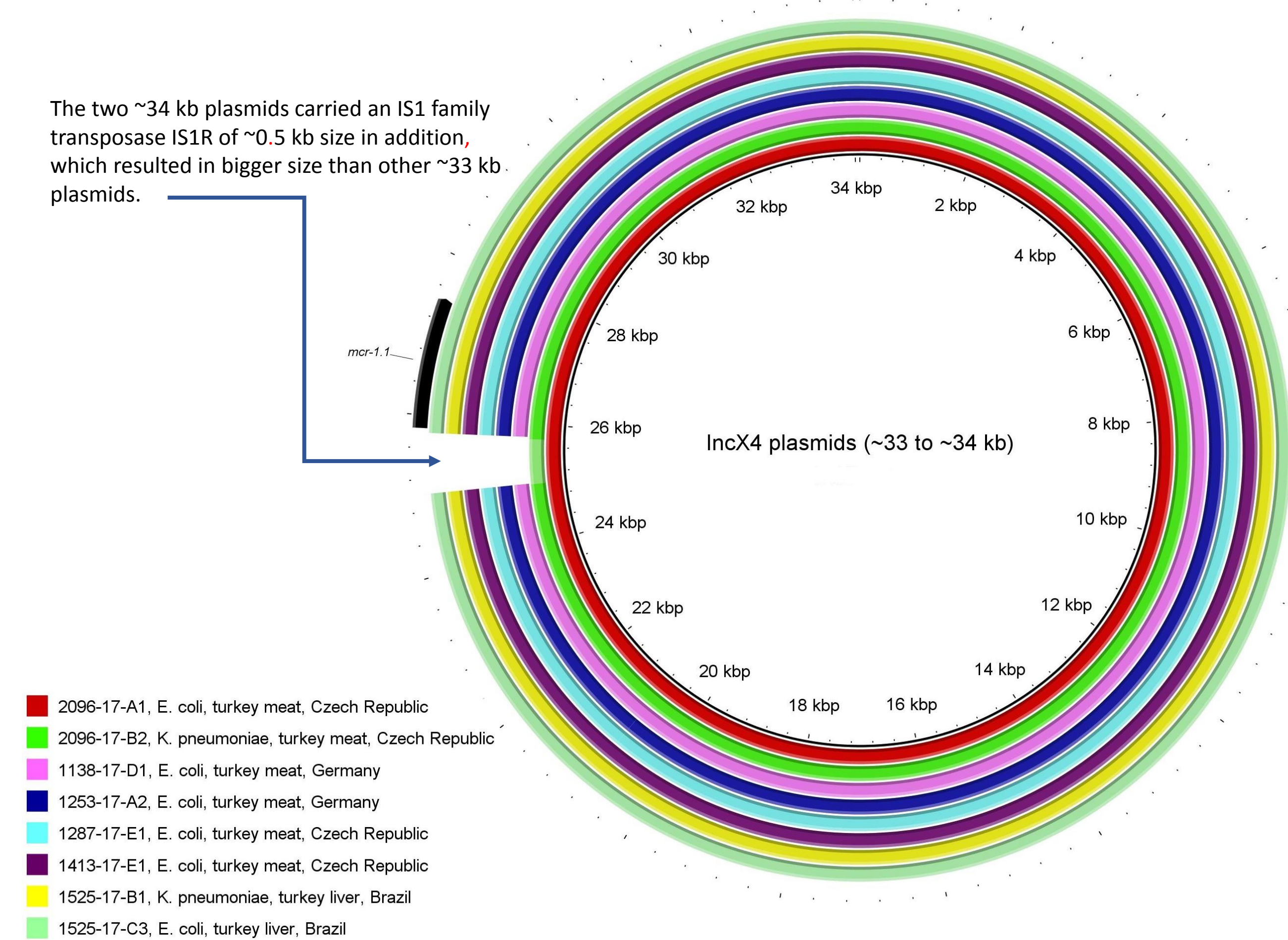


Fig. 1: Comparative analysis of IncX4 plasmids of size ~33 to ~34 kb

Gaps in the plasmid sequences represent missing sequences when compared to the reference plasmid (2096-17-A1). Genes encoding resistance to antibiotics are labelled by black arrows.

- Eight IncX4 plasmids varied in size (~33 to ~34 kb) and were highly conserved. They did not carry any other antimicrobial resistance genes except for *mcr-1*.

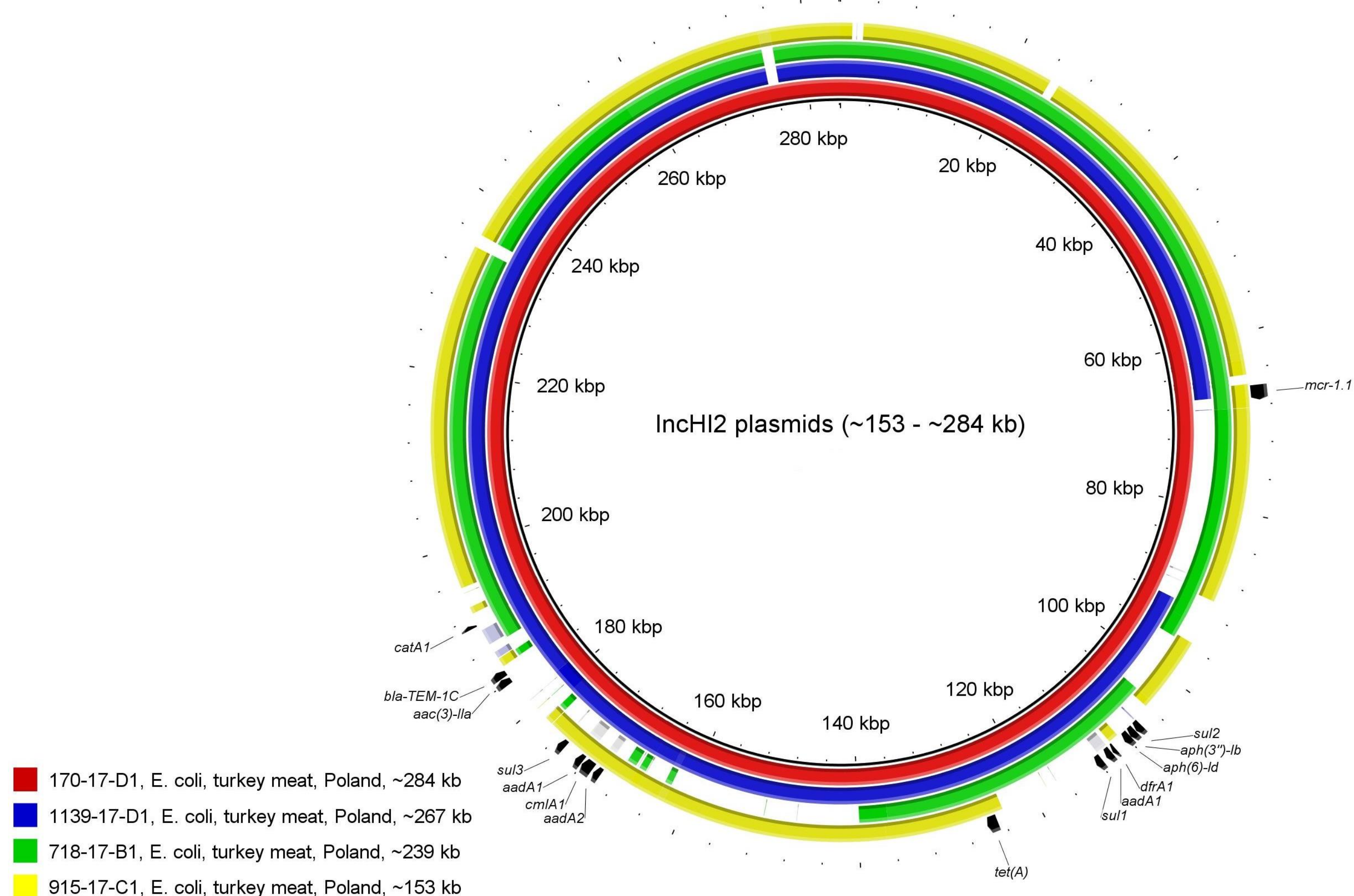


Fig. 3: Comparative analysis of IncHI2 plasmids of size ~153 kb to ~284 kb

Gaps in the plasmid sequences represent missing sequences when compared to the reference plasmid (170-17-D1).

- IncHI2 plasmid sizes varied between ~153 and ~284 kb. Plasmids carried different resistance genes.
- Three out of four IncHI2 plasmids contained the previously described structure IS*Apl1*-*mcr-1*-*orf-ISApl1* which is thought to be a transposon Tn6330 responsible for translocation of the *mcr-1* gene cassette into various plasmid backbones through formation of a circular intermediate (Li *et al.*, 2016).

Material and methods:

- 17 colistin resistant isolates carrying *mcr* genes originating from 12 meat samples were sequenced: 13 *Escherichia coli* (MIC_{CT} 4-8 mg/L), 2 *Klebsiella pneumoniae* (MIC_{CT} 8-64 mg/L), 1 *Citrobacter braakii* (MIC_{CT} 4 mg/L) and 1 *Aeromonas* spp. (MIC_{CT} 64 mg/L).
- Sequencing was performed by both Illumina Miseq and Oxford Nanopore MinION technologies.
- Hybrid assembly of long and short reads using Unicycler v0.4.4 resulted in circular contigs of the plasmids.
- The acquired antimicrobial resistance genes and plasmid types were detected using ResFinder v3.1 and PlasmidFinder v2.0 (<https://www.cge.cbs.dtu.dk/services/>).
- Annotation of the genomes was performed by Prokka v1.13 available at <https://usegalaxy.org/>.
- Visualization and comparison of plasmid sequences was done using BRIG (BLAST Ring Image Generator).

Aim:

This study was aimed at plasmid characteristics of *Enterobacteriaceae* isolates containing *mcr-1* gene and one *Aeromonas* spp. isolate carrying both *mcr-3* and *mcr-7*-like genes, all obtained from retail raw meat products.

Conclusions:

- IncX4 plasmids carrying *mcr-1* gene were found to be conserved among *Enterobacteriaceae* species isolated from raw meat products of various country origin.
- While most of the tested IncX4 and IncI2 plasmids had similar size and carried only *mcr-1* gene, the IncHI2 plasmids carrying *mcr-1* gene were diverse in size and in the content of resistance genes.
- The presence of *mcr-3* and *mcr-7*-like genes on plasmids in the colistin resistant *Aeromonas* spp. isolate was not confirmed.

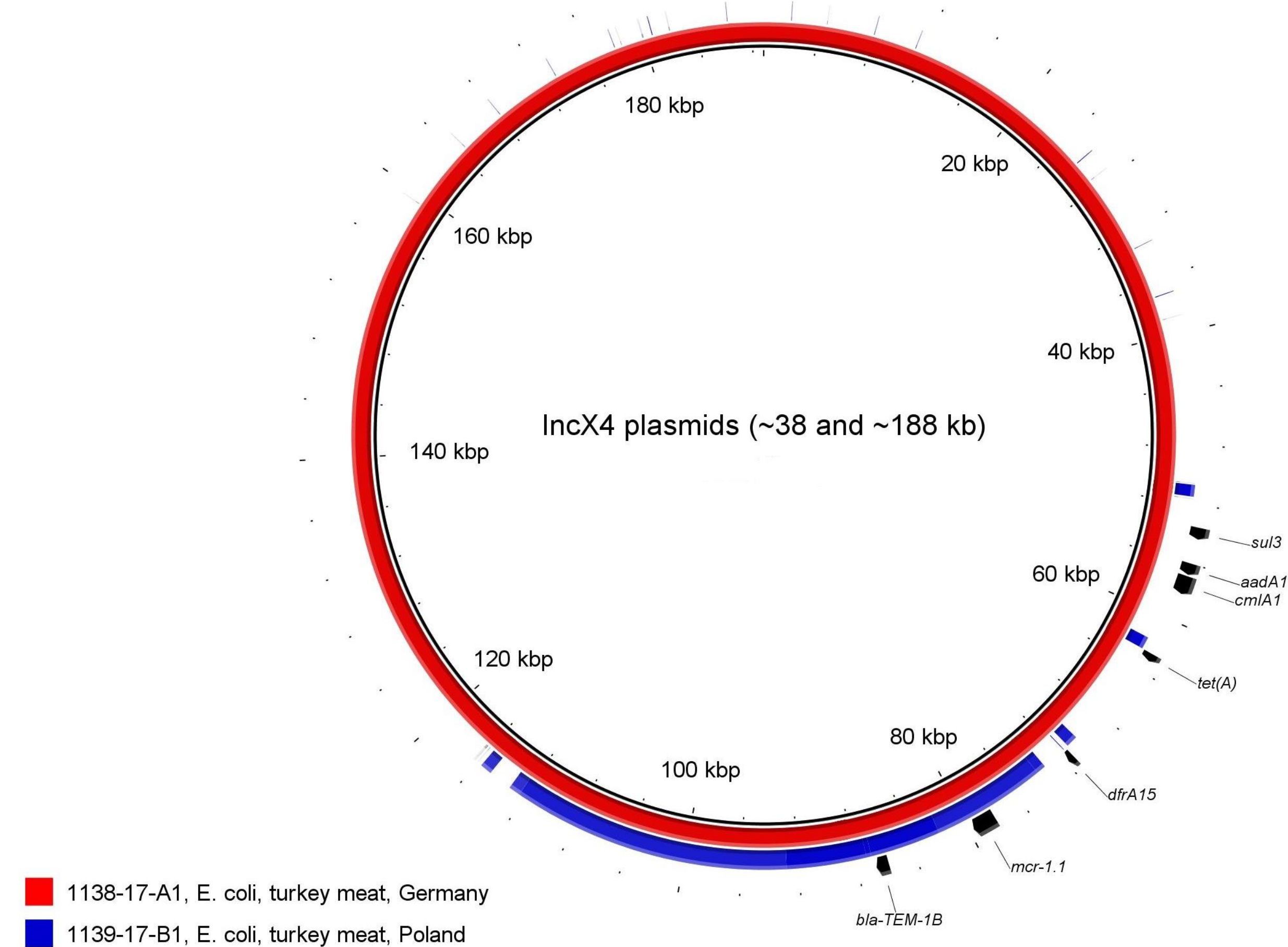


Fig. 2: Comparative analysis of IncX4 plasmids of size ~38 kb and ~188 kb

Gaps in the plasmid sequences represent missing sequences when compared to the reference plasmid (1138-17-A1).

- One IncX4 plasmid of size ~38 kb carried *bla*_{TEM-1B} resistance gene but the ~33 kb part was conserved in the rest of IncX4 plasmids.
- One IncX4 plasmid of size ~188 kb carried several resistance genes and was found to contain two replication regions, one related to IncX4 plasmid types and the other one related to IncFII plasmid types. This plasmid could be a cointegrate of two replicons.

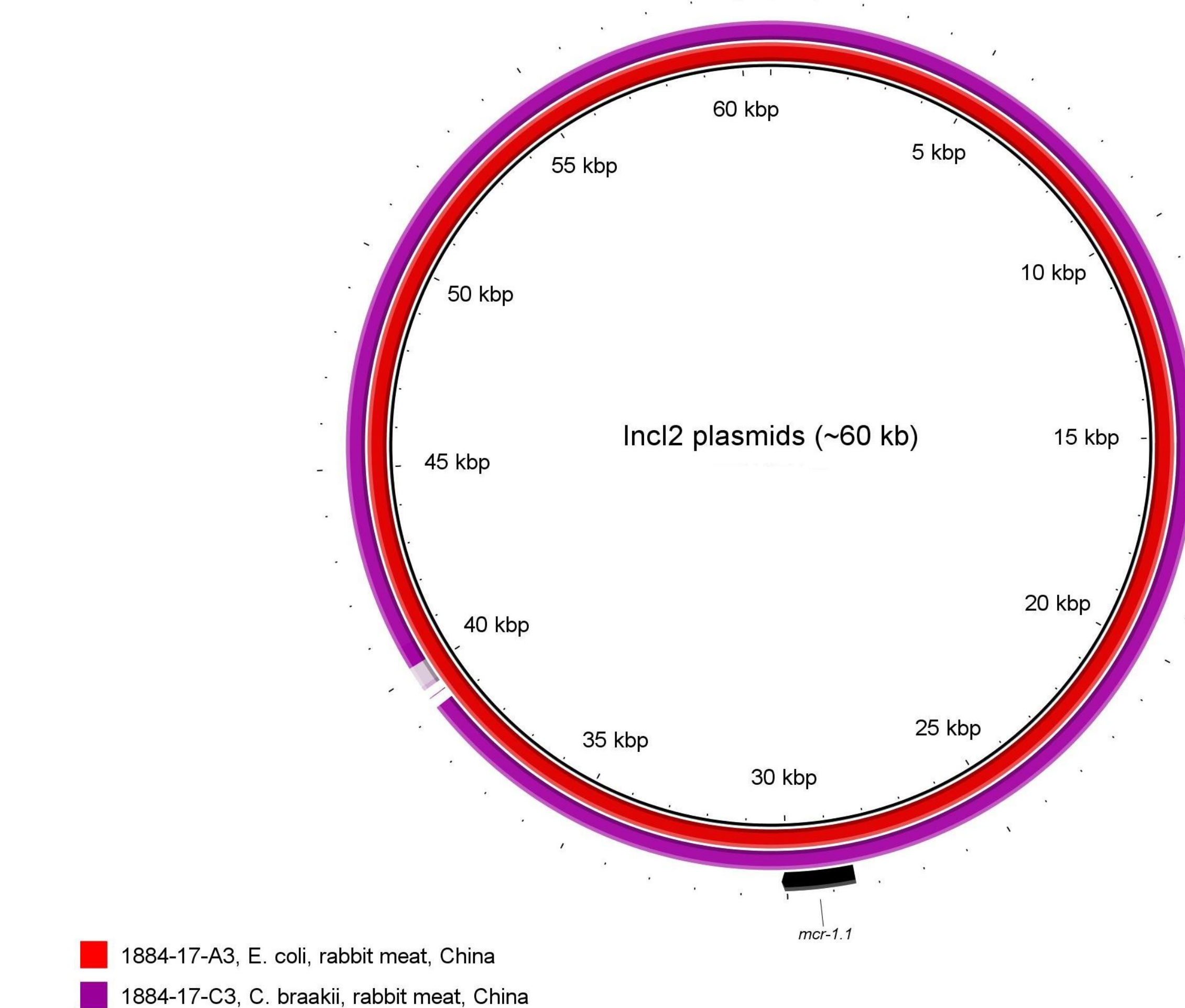


Fig. 4: Comparative analysis of IncI2 plasmids of size ~60 kb

Gaps in the plasmid sequences represent missing sequences when compared to the reference plasmid (1884-17-A3).

- IncI2 plasmids carried by *E. coli* and *C. braakii* isolated from a rabbit meat sample (China) were highly conserved with size ~60 kb (Fig. 4). They did not carry any other resistance genes except for *mcr-1*.

References:

- Li, R., Xie, M., Zhang, J. *et al.* Genetic characterization of *mcr-1*-bearing plasmids to depict molecular mechanisms underlying dissemination of the colistin resistance determinant. *Journal of Antimicrobial Chemotherapy*. 2017;72:393-401.
- Wang, R., van Dorp, L., Shaw, L.P. *et al.* The global distribution and spread of the mobilized colistin resistance gene *mcr-1*. *Nature Communications*. 2018;9:1179. Doi: 10.1038/s41467-018-03205-z.

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