The complete genome sequence of multidrug-resistant *Salmonella enterica* serovar monophasic Typhimurium (1,4,[5],12:i:-) isolate with the *mcr-1.1* gene

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Introduction

Monophasic *S*. Typhimurium (1,4,[5],12:i:-) is one of the leading *Salmonella* serovars causing human salmonellosis in Europe. It has been observed in Poland since 2008. This serovar is considered the one with the highest rate of *mcr* prevalence. We presented a sequence characteristic of multidrug-resistant (MDR) monophasic *S*. Typhimurium isolated from a pig fecal sample with the confirmed presence of the *mcr-1.1* gene.

Methodology

The isolate was confirmed to genus level on MALDI-TOF, then serotyped according to the White-Kaufmann-Le Minor scheme. DNA was extracted using Maxwell RSC (Promega). Quantity and quality of DNA were assessed by Qubit 3.0 (Thermo Fisher Scientific) and Fragment Analyzer (Agilent). Short- and long-fragment libraries were constructed using the KAPA HyperPlus Kit (Roche) and Ligation sequencing kits (SQK-LSK109; Oxford Nanopore Technologies), respectively. Whole genome sequencing was performed on the MiSeq (v3 2x300bp, Illumina) and MinION (Oxford Nanopore Technologies). The genome was assembled by the Unicycler and annotated using the RAST Server. MLST type was determine by MLST 2.0, plasmids replicons by PlasmidFinder 2.1, the resistance genes and the mobile genetic elements by CARD and MGE v1.0.3.

Results

The genome of the strain PIW95 was assembled into the complete chromosome and 4 plasmids: **ST4-IncHI2 (232 119 bp)** (Figure 1a), **IncFIB/IncFIC** (133 901 bp) (Figure 1d), **ColRNAI (6659 bp)** (Figure 1c), and **Col8282 (4066bp)** (Figure 1b). The total assembly size was 5 375 002 bp with the N50 value of 499 8257 bp and a GC content of 51.8%.

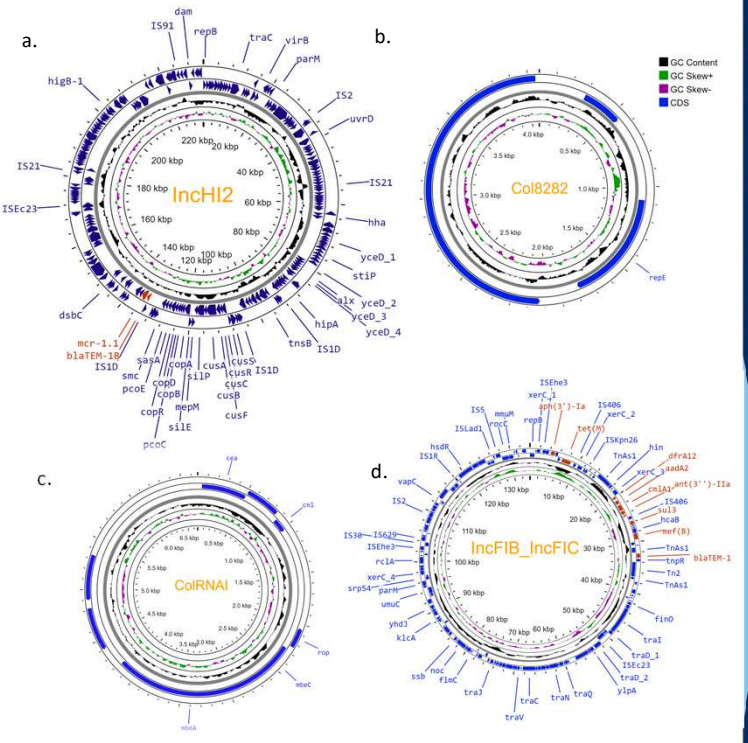

Figure 1. The gene composition in plasmids identified in PIW95 strain. Resistance genes were marked with red. The image was generated with Proksee.

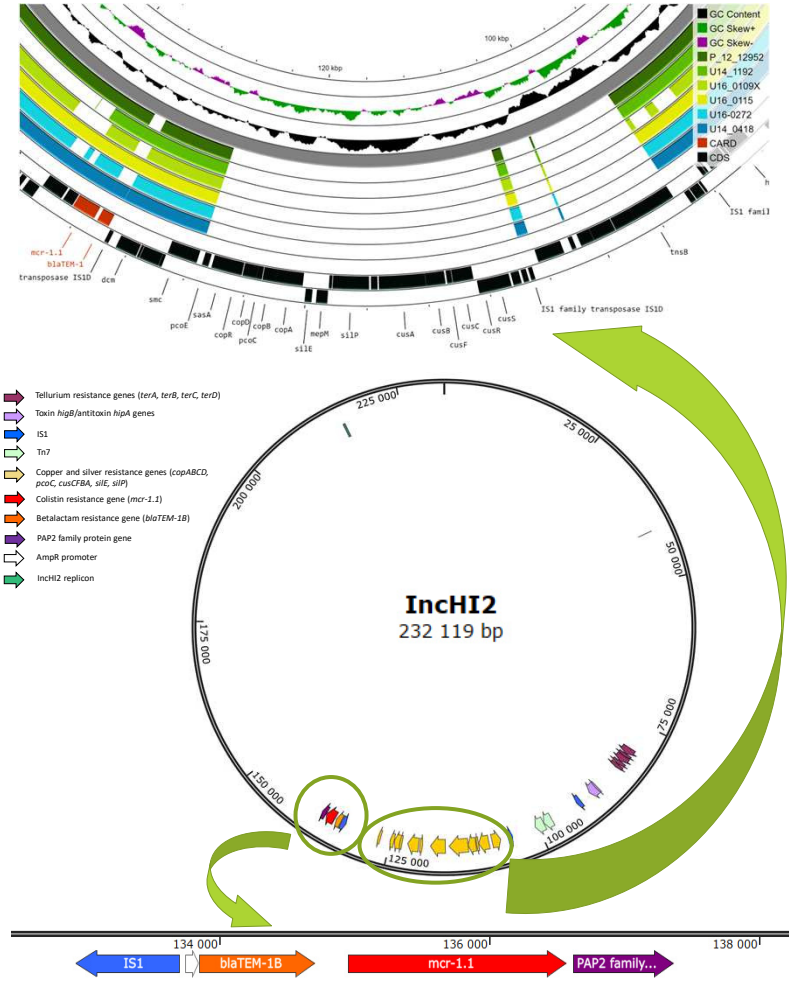

Figure 2. Detailed characterisation of IncHI2: a. characteristic of the genetic environment of the *mcr-1.1* gene, b. comparision with other IncHI2 plasmids indicating on presence of the resistance genes to heavy metals in the studied IncHI2 plasmid

The MLST analysis showed that the strain represented ST34. Multiple resistance genes were identified as followed: *mcr-1.1, blaTEM-1B, mef*(B), *aadA1, qacL, dfrA12, aadA2, cmlA1, sul3, tet*(M), and *tet*(B). BLAST analysis showed the presence of the gene cassette containing IS1, AmpR promoter, *bla*TEM-1B, and *mcr-1.1* gene followed by PAP2 family protein which was located on the IncHI2 plasmid (Figure 2). Moreover, multiple resistance genes to copper and silver were identified. Plasmid IncFIB/FIC(AP001918) carried the following resistance genes: *aadA1, aadA2, bla*TEM-1B, *cmlA1, dfrA12, mef*(B), *sul3,* and tet(M) (Figure 1d). The *tet*(B) gene was located on the chromosome.

GenBank accession

The genome was archived under the project number **PRJNA928386** and accession numbers **CP117033-CP117037**. The strain was included in the reference collection created in the CARE project. The information about the isolate and its availability is located on a website: https://cirmbp.bio-aware.com (strain ID CARE_00166).

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CONCLUSIONS

- The analyzed genome carried variable resistance genes mainly located on plasmids
- The IncHI2 plasmid, besides the *mcr-1.1* and *bla*TEM-1B genes, harbored genes coding tolerance to heavy metals which were not founded in other IncHI2 isolated from *E. coli* in Poland
- The genome content draws attention to the problem of multidrug-resistant pathogens isolated from livestock and a potential threat to human health.

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