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Letter to the Editor

Characterisation of a porcine *Escherichia coli* strain from Switzerland carrying *mcr-1* on a conjugative multidrug resistance IncHI2 plasmid

Sir,

The discovery of plasmids carrying phosphoethanolamine transferase-encoding genes (*mcr-1* to *mcr-8)* conferring resistance to colistin in Enterobacteriaceae represents a major public-health issue. The spread of *mcr*-containing plasmids among pathogenic Gram-negative bacteria poses the risk of jeopardising treatment success with colistin, an antibiotic of second-line in animals and of last-resort in humans [1]. In Switzerland, *mcr* genes have so far only been identified in *Escherichia coli* isolates from human, imported food and environmental sources [1–3]. Here we characterise the genetic background of the first colistin-resistant porcine strain of *E. coli* carrying *mcr-1* on a conjugative multidrug resistance (MDR) plasmid in Switzerland.

Screening of 257 caecum samples from slaughtered pigs collected in Switzerland in 2015 using selective enrichment broth and selective plates [3] identified two colistin-resistant *E. coli* strains [RDB2 (15/140262) and RDB9 (15/142900)] in pigs raised on different farms (prevalence, 0.8%; 95% confidence interval 0.2–3%). Only strain RDB9 tested positive for *mcr-1* when targeting *mcr-1* to *mcr-5* by multiplex PCR [4]. The *mcr*-negative strain presented one amino acid substitution in PmrA (Gly29Ser) and two in PmrB (Ala159Val; Gln213Leu). Whilst the substitution Ala159Val has already been linked to colistin resistance in *E. coli* [1], the association of the other two amino acid substitutions with colistin resistance remains to be explored.

Antimicrobial susceptibility testing using broth microdilution EUVSEC plates (Thermo Fisher Scientific) showed that RDB9 was resistant to ampicillin (64 μg/mL), chloramphenicol (64 μg/mL), colistin (8 μg/mL), gentamicin (>32 μg/mL), kanamycin (>64 μg/mL), streptomycin (>32 μg/mL), sulfamethoxazole (>1024 μg/mL), tetracycline (>64 μg/mL) and trimethoprim (>32 μg/mL) according to Clinical and Laboratory Standards Institute (CLSI) guidelines and interpretation criteria (document M100, 27TH ed).

Whole-genome sequencing of strain RDB9 was performed using MinION (Oxford Nanopore Technologies) and MiSeq (Illumina Inc.) sequencing platforms as performed previously [2]. Subsequent in silico analysis with tools available at the Center for Genomic Epidemiology online platform (http://www.genomicepidemiology.org/) revealed that strain RDB9 belonged to sequence type 10 (ST10) and carried a 116-kb IncFIB plasmid and a 288-kb IncHI2 plasmid (pRDB9; GenBank accession no. **MH924589**). Only pRDB9 carried antimicrobial resistance genes (ARGs), which were associated with resistance to ampicillin ($bla_{\text{TEM-1}}$), chloramphenicol (*cmlA1*), colistin (*mcr-1*), gentamicin [*aac(3)-IIa*], kanamycin [*aph(3′)-Ia*], sulfamethoxazole (*sul1*, *sul2*, *sul3*), trimethoprim (*dfrA1*) and tetracycline [*tet*(A)] (Fig. 1). Except for *mcr-1* that was detected on the composite transposon Tn*6330* (IS*Apl1*–*mcr-1*–IS*Apl1*), all other ARGs in pRDB9 were found on a large MDR mosaic region (ca. 60 kb). Within this region, most ARGs were located on several modules, which included a Tn*6029D*-like transposon (*strA*, *strB*), integron In*369* (*dfrA1*, *aadA1*, *qacE*Δ*1*, *sul1*), ΔTn*1721* [*tet*(A)], a class 1 integron (*aadA2*, *cmlA*, *aadA1*, *qacH*, *sul3*) and ΔTn*5393* (*strA*, *strB*). Moreover, the plasmid carried heavy metal resistance modules such as transposon ΔTn*21* (*merTPCADE*), a tellurium resistance region (*terZABCDEF* and *terWYXY*), and a multi-heavy metal (copper, cobalt, nickel and silver) resistance gene cluster.

The transferability of pRDB9 was assessed by filter-mating conjugation assays at room temperature and at 37 °C using sodium azide-resistant *E. coli* strain J53 as recipient [2]. Transconjugants were selected on Luria–Bertani agar supplemented with ampicillin (50 μg/mL) and sodium azide (100 μg/mL) and were confirmed by PCR amplification of *mcr-1*, by identical repetitive element palindromic PCR (rep-PCR) profile as the recipient and antimicrobial susceptibility testing. Plasmid pRDB9 was transferred to *E. coli* J53 at a frequency of 2.72×10^{-2} at room temperature and 2.26×10^{-5} at 37 °C. Transconjugants carrying pRDB9 displayed the same antimicrobial resistance phenotype of the donor, which is in accordance with the genotype present in pRDB9. Sequence comparisons with other plasmids from the GenBank database showed that pRDB9 shared similar regions with other *mcr-1*-harboring IncHI2 plasmids such as p19-M12 (*E. coli* strain from a Swiss traveller; GenBank accession no. **KY689632** [2]) and pMCR_WCHEC050613 (sewage *E. coli* strain from China; GenBank accession no. **CP019214**) (Fig. 1). These plasmids shared a conserved IncHI2 backbone and mostly differed in the antimicrobial/heavy metal resistance genes carried.

Co-localisation of *mcr-1* with additional ARGs in the conjugative IncHI2 MDR plasmid pRDB9 is concerning since all of these ARGs conferred resistance to several antibiotics commonly used in pig husbandry (sulfonamides, trimethoprim, tetracyclines, penicillins and colistin). Plasmid pRDB9 could be selected if any of these antibiotics is used and it could also spread to other commensal or pathogenic *E. coli* strains, as suggested by the high conjugation frequencies obtained. Plasmid pRDB9 did not co-carry any genes encoding extended-spectrum β-lactamases (ESBLs), as found in other IncHI2 plasmids carrying *mcr-1* [1]. This probably reflects the low selective pressure with cephalosporins in the pig population in Switzerland. In addition, the presence of *mcr-1* within a full Tn*6330* denotes the possibility of transposition of the element itself into other genetic backgrounds [5]. Overall, the fact that other IncHI2 plasmids similar to pRDB9 were found in different niches confirms that these MDR plasmids can efficiently spread among animal,

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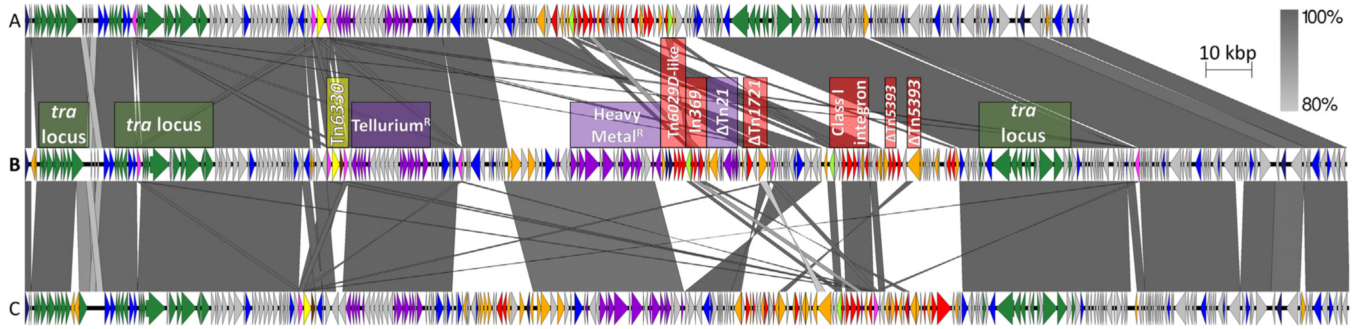

Fig. 1. Linear comparison of *mcr-1*-carrying IncHI2 plasmids: (A) p19-M12 (**KY689632**, *E. coli* isolated from Swiss traveller); (B) pRDB9 (**MH924589**, *E. coli* from pig caecum); and (C) pMCR_WCHEC050613 (**CP019214**, *E. coli* from sewage, China). Open reading frames (ORFs) are illustrated by arrows pointing towards their respective orientation. The figure was generated with Easyfig 2.1. The colour code is as follows: replicase genes are in dark blue; partitioning genes, toxin/antitoxin, stabilisation and modification systems are in blue; transfer-associated genes are in green; insertion sequence IS*Apl1* is in pink, while other transposase genes are in orange; *pap2* is in brown; heavy metal and biocide resistance-associated genes are in purple; *mcr-1* is in yellow, while the other antimicrobial resistance genes are in red; *intI1* is in lime green; finally the remaining genes (with other functions) are in grey. Coloured boxes depict the different loci of plasmid pRDB9; the colour was attributed depending on the content of each locus (e.g. tellurium^R regarding tellurium resistance).

environmental and human isolates, with the consequent risk of transmission to humans along the food chain. Despite the low proportion of *mcr*-carrying *E. coli* isolates found in healthy pigs at slaughterhouse in Switzerland, these results emphasise the importance of implementing monitoring and surveillance programmes of commensal and pathogenic colistin-resistant Enterobacteriaceae strains in pigs and advocate for careful use of antibiotics along with preventive measures to limit the risk of the spread of such MDR plasmids in livestock.

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Competing interests

None declared.

Ethical approval

Not required.

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