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Heterogeneous Genetic Location of *mcr-1* in Colistin-Resistant *Escherichia coli* Isolates from Humans and Retail Chicken Meat in Switzerland: Emergence of *mcr-1*-Carrying IncK2 Plasmids

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ABSTRACT We characterized the genetic environment of *mcr-1* in colistin-resistant *Escherichia coli* strains isolated in Switzerland during 2014 to 2016 from humans ($n = 3$) and chicken meat ($n = 6$). Whole-genome and plasmid sequencing identified the *mcr-1* gene integrated in IncX4 (of which, one strain carried the *mcr-1.2* variant), IncI2, IncHI2, and novel IncK2 plasmids (overall, $n = 7$), as well as in the bacterial chromosome ($n = 2$) in single or duplicate copies. Our study supports the easy mobilization of *mcr-1* across diverse genetic locations.

KEYWORDS colistin, chromosome, *E. coli*, *mcr-1*, food, IncK2, *Escherichia coli*, animals, chromosome organization, humans, meat, plasmids

In facing an era of multidrug-resistant *Enterobacteriaceae*, the recent discovery of plasmids carrying the colistin resistance *mcr-1* gene has raised considerable health concern (1, 22). To date, *mcr-1* has been identified in several plasmid types (IncI2, IncHI2, IncX4, IncP, IncY, and IncF plasmids) (2–5), as well as integrated in the bacterial chromosome primarily mobilized as IS*Apl1* composite transposon (6–8, 23). Herein, we investigated the *mcr-1* genetic environment in colistin-resistant *Escherichia coli* strains isolated in Switzerland from humans and retail chicken meat between 2014 and 2016 (Table 1).

Three human isolates of sequence type (ST) 10 and ST5 were isolated from the feces of two Swiss travelers and an HIV-positive individual (from totals of 38 and 101 subjects, respectively), as part of two previous studies (Table 1) (9, 10). Strains of retail chicken meat origin ($n = 6$) were isolated from meat imported from Germany within the framework of the National Surveillance Program of Antibiotic Resistance in Switzerland (11). In particular, 4 out of 234 (1.7%) and 2 out of 302 (0.7%) samples in 2014 and 2016, respectively, harbored *mcr-1*-carrying *E. coli* strains belonging to ST38, ST58, ST226, ST1049, and ST1775. In addition to colistin, seven strains showed resistance to other antibiotics (Table 1).

To characterize the genetic location of *mcr-1*, whole-genome sequencing of the strains was performed with the MinION (Oxford Nanopore) (10). The scaffolds were corrected with mapped Illumina reads and further characterized with an online platform (www.genomicepidemiology.org/). In human *E. coli* strains 100R and 19-M12, *mcr-1* was located on a multidrug resistance IncHI2 plasmid of 256 kb (p100R; KY689633) and 232 kb (p19-M12; KY689632), respectively. Both IncHI2 plasmids exhibited structural similarity with other previously described *mcr-1*-carrying IncHI2 plasmids (see Fig. S1 in the supplemental material). In particular, *mcr-1* was flanked by two copies of IS*Apl1* in opposite orientation, in which the putative *pap2* open reading frame (ORF) was disrupted (Fig. 1).

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TABLE 1 Overview of the plasmids, resistance genes, and colistin MICs of the *mcr-1*-positive *Escherichia coli* strains isolated in Switzerland included in this study

E. coli strain	Source[g]	ST[a]	Collection period	Antibiotic resistance[b]	Colistin MIC (μg/ml)[c]	Plasmid[d]	Plasmidic resistance gene(s)[e]	*mcr-1* location (plasmid length in kb)
100R	Human feces (traveler)	10	2015	CST, PMB, LVX, CIP, DOX, SXT	≥8	IncHI2	*aadA1*, *aadA2*, bla_{TEM-1}, *mcr-1*, *mph(A)*, *sul3*, *tetA*, *dfrA15*, *dfrA14*	IncHI2 (256)
						IncFII/FIB	bla_{TEM-1}, *aadA2*, *aadA24*, *aph(3)-Ia*, *qnrS1*, *cmlA1*, *sul3*, *tetA*, *dfrA12*	
						IncX1		
						ColRNAI		
19-M12	Human feces (traveler)	5	2015	CST, PMB, DOX, SXT	≥8	IncHI2	*aadA2*, bla_{TEM-1}, *mcr-1*, *mph(A)*, *cmlA1*, *sul3*, *tetA*, *dfrA12*, *dfrA14*	IncHI2 (232)
						IncFII/FIB	bla_{TEM-1}, *aadA1*, *qnrS1*, *tetA*, *dfrA1*	
31349	Human feces (HIV+ subject)	5	2015	CST, PMB	≥8	IncX4	*mcr-1.2*	IncX4 (33)
						IncL/M		
						IncFII		
						IncFIB		
Mcp0271	Retail chicken meat	58	2016	CST, AMP	4	IncX4	*mcr-1*	IncX4 (33)
						IncFII/FIB/FIA	bla_{TEM-1}	
Mcp0221	Retail chicken meat	1775	2016	CST	4	IncI2	*mcr-1*	IncI2 (65)
Mbl488	Retail chicken meat	38	2014	CST, SMX, TMP, NAL, CAZ, CTX, AMP, TET	8	IncK2	bla_{TEM-1}, *mcr-1*, *sul2*	IncK2 (101)
						IncFII/FIB	*aadA1*, bla_{TEM-1}, *sul1*, *tetA*, *dfrA1*	
						IncI1	*aadA5*, $bla_{CTX-M-1}$, *sul2*, *dfrA17*	
						po111		
Mbl536	Retail chicken meat	226	2014	CST, SMX, TMP, CIP, TET, NAL, AMP	4	IncK2	bla_{TEM-1}, *mcr-1*, *sul2*	IncK2 (101)
						IncI1	bla_{TEM-1}, *sul2*, *tetA*, *dfrA1*	
						IncX1	bla_{TEM-52}	
Mbl323	Retail chicken meat	38	2014	CST, CAZ, AMP, NAL, CTX	8	IncK2	bla_{CMY-2}	Chromosome
						IncN	*aadA22*, *lnuF*	
						IncFII/FIBColRNAI		
Mbl506[f]	Retail chicken meat	1049	2014	CST, TET, NAL, CTX, CHL, SMX, TMP, CAZ, AMP	8	IncFII/FIB	*tetA*	Chromosome[i]
						IncI1	*aadA1*, *erm(42)*	
						IncI2	*aad24*, bla_{TEM-1}	
						IncQ1[h]	*aad24*, *strA*, *strB*, *mph(B)*, *sul1*, *sul2*, *tetA*, *dfrA1*[h]	

[a]Sequence type (ST) based on multilocus sequence typing was determined with MLSTtool.
[b]Only antibiotics with a nonsusceptible phenotype according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria, except for TET, SMX, DOX, and minocycline for which Clinical and Laboratory Standards Institute (CLSI) criteria were used (20, 21). CST, colistin; PMB, polymyxin B; LVX, levofloxacin; CIP, ciprofloxacin; DOX, doxycycline; SXT, trimethoprim-sulfamethoxazole; SMX, sulfamethoxazole; AMP, ampicillin; CAZ, ceftazidime; TMP, trimethoprim; TET, tetracycline; CTX, cefotaxime; NAL, nalidixic acid; CHL, chloramphenicol.
[c]MIC values were obtained with microdilution Trek Diagnostic Systems panels GNX2F for human strains or EUVSEC panels for chicken meat strains; the two panels analyze different MIC ranges for colistin (0.25 to 4 μg/ml and 1 to 16 μg/ml, respectively).
[d]Incompatibility groups were determined with PlasmidFinder.
[e]Resistance genes were determined with ResFinder.
[f]Strain harboring a chromosomally integrated $bla_{CTX-M-32}$.
[g]Retail chicken meat was imported from Germany.
[h]Integrated into the chromosome based on whole-genome sequencing.
[i]Two *mcr-1* copies in different loci.

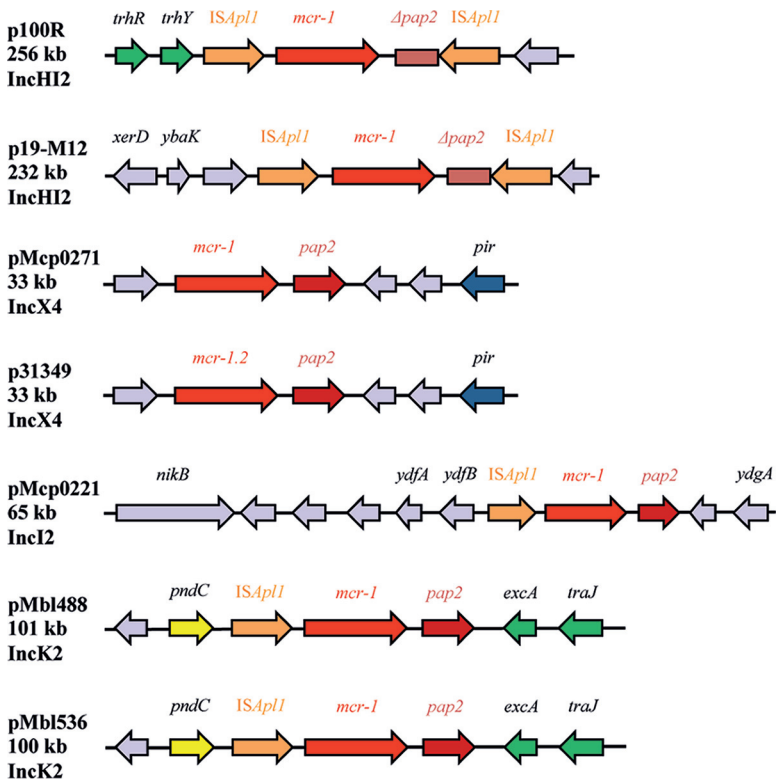

FIG 1 Schematic representation of the genetic environment of *mcr-1* in the *mcr-1*-carrying plasmids. The open reading frames (ORFs) are illustrated by arrows pointing toward their respective orientation. The color code is as follows: the transfer-associated genes are in green, *mcr-1* is in red, the putative *pap2* ORF is in brown, insertion sequence (IS) elements are in orange, replication initiation protein-encoding gene (*pir*) is in dark blue, partitioning genes and toxin/anti-toxin and other stabilization systems are in yellow, and other genes are in lavender. Important gene names are indicated above the sequence. The indications on the left-hand side are the respective plasmid profiles. Genes are not drawn to scale.

Two strains from human and chicken meat carried the *mcr-1* on 33 kb IncX4 plasmids (p31349 [KY689634] and pMcp0271 [KY565556], respectively) almost identical to others previously characterized, including the location of *mcr-1* lacking the upstream IS*Apl1* inserted downstream of *pir* (Fig. 1; see also Fig. S2 in the supplemental material). Plasmid p31349 harbored the *mcr-1.2* variant first detected in the IncX4 plasmid pMCR1.2-IT (KX236309) of a *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* strain isolated in Italy (12).

Strain Mcp0221 from chicken meat harbored *mcr-1* on a 65-kb IncI2 plasmid (pMcp0221; KY565557) showing structural similarity with other previously described *mcr-1*-carrying IncI2 plasmids (see Fig. S3 in the supplemental material). However, while *mcr-1* (with or without an upstream IS*Apl1*) was usually inserted immediately downstream of the *nikB* gene, the IS*Apl1-mcr-1* element in pMcp0221 was integrated five ORFs downstream of *nikB* (Fig. 1 and S3).

Sequence comparison based on average nucleotide identity (ANI) of pairwise sequence alignments performed with MUMmer (ANIm) (13) of complete sequences of IncHI2, IncX4, and IncI2 plasmids carrying the *mcr-1* available in NCBI (see Fig. S4A in the supplemental material) confirmed the similarity with the respective plasmids found in the present study. Clustering of the dissimilarity matrices performed with the neighbor-joining algorithm (BIONJ algorithm) (14) showed that the three IncHI2 plasmids clustered together, appearing also to be closely related to plasmid pS38 (KX129782) from an *E. coli* strain isolated in Switzerland from poultry meat imported from Italy (Fig. S4A). The analysis further confirmed the high sequence similarity among all members of the IncX4 plasmids (ANIm, >97.89%), suggesting a dissemination of this

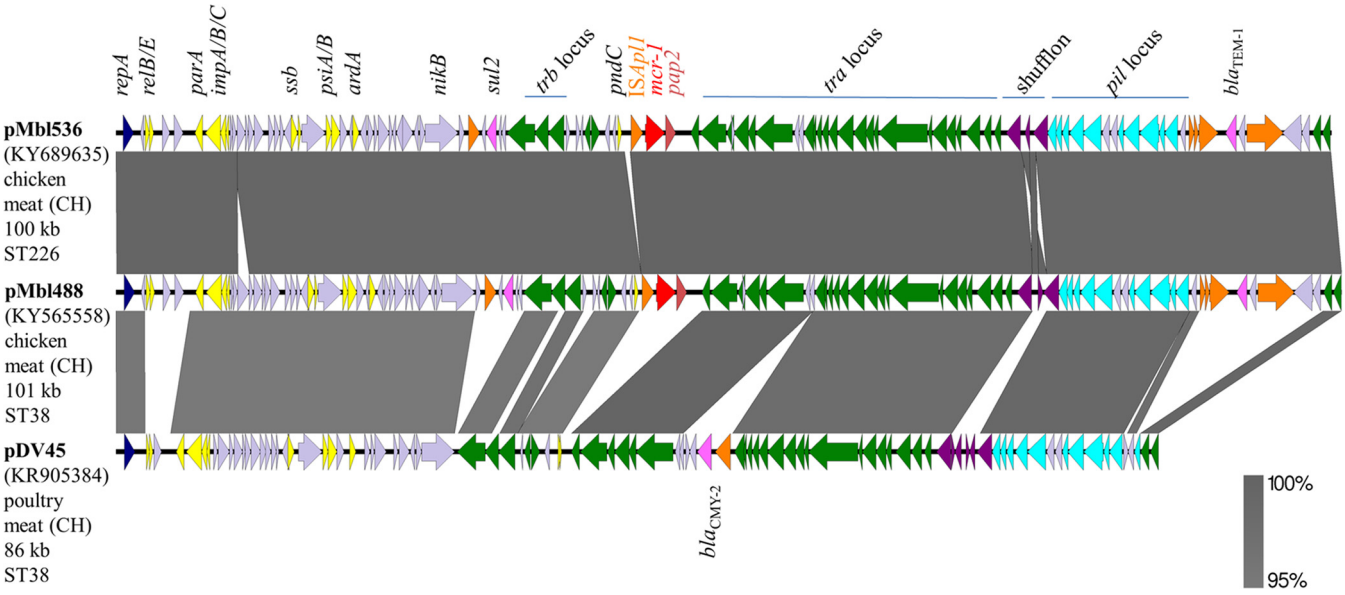

FIG 2 Linear comparison of IncK2 plasmids. The open reading frames (ORFs) are illustrated in arrows pointing toward their respective orientations. The color code is as follows: the transfer-associated genes are in green, pilus genes are in light blue, *mcr-1* is in red, the putative *pap2* ORF is in brown, other antibiotic resistance genes are in pink, insertion sequence (IS) elements are in orange, replicase genes are in dark blue, partitioning genes and toxin/anti-toxin and other stabilization systems are in yellow, shufflon-associated genes are in violet, and other genes are in lavender. Important genes and loci are indicated above or below the plasmid sequence. The blue lines above the figure indicate different loci and the shufflon region. The indications on the left-hand side are the respective isolate/plasmid profiles. CH, Switzerland.

plasmid type worldwide (Fig. S4B), whereas pMcp0221 did not closely cluster together with other IncI2 plasmid sequences (ANIm, ≤98.3%) (Fig. S4C).

Remarkably, in two strains from imported chicken meat (Mbl488 and Mbl536), *mcr-1* was found on novel 100-kb IncK2 plasmids, which also harbored bla_{TEM-1} and *sul2* (Table 1 and Fig. 2). Sequence comparison of both pMbl488 (KY565558) and pMbl536 (KY689635) with the recently described IncK2 bla_{CMY-2}-carrying plasmid pDV45 (KR905384) showed high sequence similarity (Fig. 2) (15). As in pDV45, the IS*Apl1-mcr-1* element was found integrated in the *tra* region, but in a different location compared to the IS*Ecp1*-bla_{CMY-2}-*blc-sugE1* element, namely, between *pndC* and *excA* (Fig. 1 and 2).

To assess transferability of the two IncK2 plasmids, conjugation experiments were performed by filter mating, using a rifampin-resistant derivative of the sodium azide-resistant *E. coli* strain J53. Transconjugants were selected on Luria-Bertani (LB) agar plus sodium azide (150 μg/ml) and colistin (2 μg/ml), and confirmed by counterselection on LB agar plus rifampin (50 μg/ml) and colistin (2 μg/ml) and *mcr-1* real-time PCR (16). Conjugation efficiencies at 37°C were 2.6×10^{-4} for pMbl488 and 6.3×10^{-6} transconjugants/donor for pMbl536, respectively, with the lower frequency observed for pMbl536 possibly due to an adenine insertion in *traE* (confirmed by Sanger sequencing) leading to a premature stop codon.

To the best of our knowledge, this is the first report of IncK2 plasmids carrying *mcr-1*. These plasmids were structurally highly similar to IncK2 $bla_{CMY-2/4}$-harboring plasmids that we recently described in *E. coli* (frequently of ST38) isolated in Switzerland from local chicken and chicken meat (15). Such *E. coli* strains carrying bla_{CMY-2}-IncK2 plasmids, including strains belonging to ST38, were also associated with broiler production in other European countries (17, 18). Furthermore, a recent Norwegian report strongly supports the hypothesis that clonal transfer of these ST38 *E. coli* strains from chicken meat to humans may occur (19). Thus, the additional acquisition of *mcr-1* in hyperepidemic extended-spectrum β-lactamase (ESBL)-producing ST38 *E. coli* (e.g., strain Mbl488 found in the present analysis) is concerning.

Notably, in the ST38 strain Mbl323 isolated from retail chicken meat, which also carried a 100-kb IncK2 plasmid harboring a bla_{CMY-2} gene, *mcr-1* was found on a

2.85-Mbp contig (i.e., integrated in the genome; see Table 1). Thus, besides confirming the continuous ongoing spread of such plasmid-mediated AmpC-producing ST38 *E. coli* hyperepidemic clones in broiler production in Switzerland and other European countries, our report further suggests that these strains can also acquire additional *mcr-1*-mediated colistin resistance.

Interestingly, *mcr-1* was also found on a 2.9-Mbp contig (i.e., in the chromosome of strain Mbl506) and sequence comparison of the *mcr-1* surrounding genetic regions of Mbl323 (KY689636) and Mbl506 (KY689637) revealed that the IS*Apl1-mcr-1* element was integrated in the same location (Fig. S5A). Additionally, a second IS*Apl1-mcr-1* copy in Mbl506 (KY689638) was found on a 2 Mbp contig in a completely different genomic region (Fig. S5B).

In conclusion, *mcr-1* was detected in a variety of plasmid types, including the newly identified IncK2-type plasmids, as well as integrated into the bacterial genome. Some of the plasmids were highly similar to others previously found in diverse *E. coli* lineages from different sources, suggesting horizontal spread. Nevertheless, direct colonization with *mcr-1*-carrying hyperepidemic *E. coli* clones from other reservoirs (e.g., food animals) cannot be excluded, as already suggested for ST38 *E. coli* carrying bla_{CMY-2}-IncK2 plasmids (17–19). Therefore, adequate measures should be taken, particularly in broiler production, to limit the acquisition of the *mcr-1* gene and further spread of such clones.

Accession number(s). The complete nucleotide sequence of the seven plasmids and 40-kb sequences of the three chromosomal regions surrounding *mcr-1* have been deposited into the GenBank database under accession numbers KY689633 (p100R), KY689632 (p19M12), KY565556 (pMcp0271), KY689634 (p31349), KY565557 (pMcp0221), KY565558 (pMbl488), KY689635 (pMbl536), KY689636 (Mbl323; chromosomal *mcr-1* insertion region), KY689637 (Mbl506; chromosomal *mcr-1* insertion region 1), and KY689638 (Mbl506; chromosomal *mcr-1* insertion region 2).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01245-17.

SUPPLEMENTAL FILE 1, PDF file, 0.8 MB.

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