Key features of mcr-1-bearing plasmids from *Escherichia coli* isolated from humans and food

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Abstract

**Background:** *Mcr-1*-harboring Enterobacteriaceae are reported worldwide since their first discovery in 2015. However, a limited number of studies are available that compared full-length plasmid sequences of human and animal origins.

**Methods:** In this study, *mcr-1*-bearing plasmids from seven *Escherichia coli* isolates recovered from patients (n = 3), poultry meat (n = 2) and turkey meat (n = 2) in Switzerland were further analyzed and compared. Isolates were characterized by multilocus sequence typing (MLST). The *mcr-1*-bearing plasmids were transferred by transformation into reference strain *E. coli* DH5α and MCR-1-producing transformants were selected on LB-agar supplemented with 2 mg/L colistin. Purified plasmids were then sequenced and compared.

**Results:** MLST revealed six distinct STs, illustrating the high clonal diversity among *mcr-1*-positive *E. coli* isolates of different origins. Two different *mcr-1*-positive plasmids were identified from a single *E. coli* ST48 human isolate. All other isolates possessed a single *mcr-1* harboring plasmid. Transferable IncI2 (size ca. 60–61 kb) and IncX4 (size ca. 33–35 kb) type plasmids each bearing *mcr-1* were found associated with human and food isolates. None of the *mcr-1*-positive IncI2 and IncX4 plasmids possessed any additional resistance determinants. Surprisingly, all but one of the sequenced *mcr-1*-positive plasmids lacked the IS*Apl1* element, which is a key element mediating acquisition of *mcr-1* into various plasmid backbones.

**Conclusions:** There is strong evidence that the food chain may be an important transmission route for *mcr-1*-bearing plasmids. Our data suggest that some "epidemic" plasmids rather than specific *E. coli* clones might be responsible for the spread of the *mcr-1* gene along the food chain.

**Keywords:** Colistin, *Mcr-1*, Plasmid, IS*Apl1*

Background

The increasing number of multidrug-resistant Gram-negative bacteria and the lack of novel antimicrobials has led to the reintroduction of polymyxins as last-resort antimicrobials in human medicine, although once avoided because of its nephro- and neurotoxicity [1, 2]. By contrast, in veterinary medicine, colistin is still widely used for the treatment of diarrhea in food-producing animals such as calves and pigs in most countries [3]. Until late 2015, only chromosomally-encoded mechanisms of resistance to polymyxins were known [4]. The mobile colistin resistance gene, *mcr-1*, was first described on a conjugative IncI2 plasmid from Chinese isolates. It encodes a phosphoethanolamine transferase that adds phosphoethanolamine to the lipid A [5]. Retrospective studies performed worldwide revealed that the gene had been circulating undetected for at least twenty years and animals have been suggested to be its main reservoir [6]. The dissemination of *mcr-1* is associated with a large variety of plasmids including incompatibility groups IncI2, IncX4, IncF, IncHI1, IncHI2, IncP and IncY [7–10]. Most of these groups are well
known to be involved in the spread of a diversity of antibiotic resistance genes in Enterobacteriaceae.

The aim of this study was to characterize mcr-1-bearing plasmids from *E. coli* originating from humans and food isolated at the same location (Switzerland) in order to improve the understanding of the epidemiology and spreading potential of the mcr-1 gene.

**Methods**

In total, seven *E. coli* isolates harboring mcr-1 plasmids were used in the present study, including one uropathogenic *E. coli* (UPEC) isolate recovered from human urinary tract infection (CDF8), two isolates from humans with diarrhea and history of travel to Asia (ColR598 and ColR644SK1), and two isolates respectively from retail poultry meat (PC11 and PF11) and retail turkey meat (PF52 and PF91). UPEC strain CDF8 was obtained from a patient hospitalized in Switzerland in 2016 (unpublished) and food isolates PC11, PF11, PF52 and PF91 had been isolated in 2016 from food imported from Germany and sold in retail stores in Switzerland [11].

ColR598 and ColR644SK1 were obtained from a stool sample screening from patients with diarrhea during the June to December 2016 period. Briefly, a total of 320 non-duplicate samples were screened for the presence of colistin-resistant Enterobacteriaceae by enriching one loopful of stool in 5 ml *Enterobacteriaceae* enrichment (EE) broth (BD, Franklin Lakes, NJ, USA) for 24 h at 37 °C, followed by streaking one loopful onto LB agar plates containing 4 mg/L colistin, 10 mg/L vancomycin and 5 mg/L amphotericin B for selection of colistin-resistant Gram-negative bacteria. The isolates were identified using API ID 32 E (bioMérieux, Marcy l’Etoile, France) and analyzed for the presence of mcr-1 by PCR as described previously [5]. Minimal inhibitory concentration of colistin was determined for mcr-1-positive isolates using broth dilution tests as recommended by CLSI protocols and evaluated according to CLSI criteria [12].

Multilocus sequence typing (MLST) was performed as described previously [13], and isolates were assigned to sequence types (ST) and clonal complexes (CC) according to the Achtman scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli/).

The mcr-1-positive plasmids were extracted using the Qiagen Midi kit (Qiagen, Hombrechtikon, Switzerland) and transferred by transformation using electroporation into *E. coli* DH5α. Colistin-resistant transformants were selected on LB-agar supplemented with 2 mg/L colistin (Sigma-Aldrich, Buchs SG, Switzerland). The mcr-1 plasmids were extracted using the Large-Construct Kit (Qiagen, Hombrechtikon, Switzerland) according the manufacturer’s protocol and sequenced on a PacBio RS2 device (Pacific Biosciences, Menlo Park, USA) with a 10 kb size-selected insert library and P6/C4 chemistry. De novo assembly (using the HGAP3 algorithm) was performed using SMRTanalysis version 2.3.0 (Pacific Biosciences). The HGAP3 settings were kept at the defaults, except for the expected genome size, which was set between 50 kb and 100 kb. The plasmid sequence was automatically annotated using the online Rapid Annotation Subsequencing Technology (RAST) [14] and CLC Main Workbench Version 7.8.1 (CLC bio, Aarhus, Denmark). Automated annotation was manually refined using the BLASTn and BLASTp programs (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Results and discussion**

The results of this study analyzing seven distinct mcr-1-harbouring isolates of different sources are summarized in Table 1. Noticeably, all mcr-1-harbouring isolates were *E. coli* that correspond to the most important reservoir of MCR-1 producers identified so far. MLST analysis did not show any close clonal relationship between the seven mcr-1-positive *E. coli* isolates, suggesting that the dissemination of the mcr-1 gene is so far not primarily associated with any specific clonal lineage.

From the seven *E. coli* isolates, a total of eight mcr-1-bearing plasmids was recovered, with the human isolate ColR598 yielding two distinct mcr-1-positive plasmids. Li and colleagues showed in a recent study that coexistence of two mcr-1-bearing plasmids seems to be common [15]. Nevertheless, the MICs for colistin were not affected by the number of mcr-1-bearing plasmids present in one isolate [15]. The eight plasmids belonged to two plasmid types that have been often shown to be involved in the spread of mcr-1 [16]. Three IncI2 plasmids (pPC11, pCoR598_2 and pCoR644SK1) were ca. 60 kb in-size, and were similar to pHNSHP45 (Fig. 1), the original sequenced mcr-1 plasmid published in 2015 [5]. The three IncI2 plasmids shared a common plasmid backbone, however, in the case of pPC11 the mcr-1 cassette [17] was located in an inverted orientation compared to the others (data not shown). Additionally, the IncI2 plasmids from human isolates (pCoR598_2 and pCoR644SK1) varied greatly compared to the plasmid pPC11 (poultry isolate) in the shufflon region, which is a clustered inversion region encoding components of the pilV protein involved in plasmid transmission [18]. The components are rearranged by Rci, a recombinase encoded by the rci gene (Fig. 1). This observation is in accordance with recently sequenced IncI2 plasmids carrying mcr-1 detected in *E. coli* from swine and cattle in Japan [19].

The other five plasmids (pPF11, pPF52, pPF91, pCDF8 and pCoR598_1) all belonged to the plasmid incompatibility group IncX4 and were ca. 33 kb in-size. Their
### Table 1 Features of the eight mcr-1-harboring plasmids from seven E. coli isolated from humans and from food

| Host strain ID | Origin | Source | ST (CC) | MIC colistin [mg/L] | Resistance profile | mcr-1 harbouring plasmid | Plasmid size (bp) | Inc- group | mcr-1 gene cassette | Additional resistance genes on the mcr-1 harbouring plasmid | Reference |
|----------------|--------|--------|---------|---------------------|--------------------|--------------------------|---------------------|-----------|---------------------|-------------------------------------------------|----------|
| PC11           | Chicken | Meat   | ST1251  | 8                   | AM, CF             | pPC11                   | 59.830              | IncI2     | IS4401-mcr-1-orf    | none                                            | [11]     |
| PF11           | Chicken | Meat   | ST156   (CC156) | 8   | AM, CF, NA, CIP, TE | pPF11                  | 33.308              | IncX4     | mcr-1-orf           | none                                            | [11]     |
| PFS2           | Turkey  | Meat   | ST58    (CC155) | 4   | AM, CF, SMZ         | pPFS2                  | 33.300              | IncX4     | mcr-1-orf           | none                                            | [11]     |
| PF91           | Turkey  | Meat   | ST1431  | 8                   | AM, CF, TE, C, SMZ | pPF91                  | 33.310              | IncX4     | mcr-1-orf           | none                                            | [11]     |
| CDF8           | Human   | UTI    | nd      | 4                   | AM, CZ, CTX, FEP, NA, CIP, S, K | pCDF8                   | 33.660              | IncX4     | mcr-1-orf           | none                                            | this study |
| ColRS98        | Human   | Diarrhea | ST48   (CC10) | 4   | NA, CIP, TE        | pColRS98_1              | 33.299              | IncX4     | mcr-1-orf           | none                                            | this study |
| ColRS98        | Human   | Diarrhea | ST48   (CC10) | 4   | NA, CIP, TE        | pColRS98_2              | 60.939              | IncI2     | mcr-1-orf           | none                                            | this study |
| ColR644SK1     | Human   | Diarrhea | ST117  | 4                   | AM, CF, TE, C, SMZ, TMP | pColR644SK1             | 60.952              | IncI2     | mcr-1-orf           | none                                            | this study |

**Abbreviations:** CC clonal complex; Inc. plasmid incompatibility group; ST sequence type; UTI urinary tract infection, not determined; MIC minimal inhibitory concentration; AM ampicillin; CF cephalothin; CZ cefazolin; CIP ciprofloxacin; NA nalidixic acid; K kanamycin; S streptomycin; SMZ sulfamethoxazole; TMP trimethoprim; TE tetracycline; C chloramphenicol

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**Fig. 1** Sequencing alignment of IncI2-type mcr-1-harboring plasmids. The first mcr-1-harboring plasmid, pHNSHP45 (Accession-Nr. KP347127), which was isolated in China, was used as reference plasmid (black circle). The outmost circle in grey arrows shows the annotations of the reference plasmid. The insertions element and the mcr-1 gene were highlighted in orange and red arrows, respectively. Gaps indicate regions that were missing in the respective plasmid compared to the reference plasmid.
sequences varied only by very few nucleotides (≥99% homology), mostly located in non-coding regions (Fig. 2). In the case of pDCF8 the mcr-1 cassette was located in an inverted orientation compared to the others (data not shown). Of note, those almost identical IncX4 plasmids originate from humans, poultry and turkey meat, illustrating their wide dissemination throughout multiple sources, and providing further evidence of the likely association of mcr-1-mediated colistin resistance through food-producing animals.

An open reading frame (orf) encoding an hypothetical protein with similarities to a PAP2 superfamily protein was detected immediately downstream of the mcr-1 gene (both together hereafter referred to as mcr-1 cassette) was identified on all eight plasmids.

The insertion sequence ISApl1, has been shown to play a key role in the mobilization of mcr-1 [20], but was absent upstream of mcr-1 in most of our isolates. Further evidence for the importance of ISApl1 in the mobilization of mcr-1 was the presence of ISApl1 next to the mcr-1 gene on the chromosome of an E. coli veal calf isolate from Netherland [21]. Moreover, transposition of the mcr-1 gene by an ISApl1-made composite transposon was recently demonstrated [17]. Highly similar inverted repeat (IRR) and direct repeat (DR) sequences were identified on IncX4, IncHI1 and IncHI2 backbones immediately downstream of the mcr-1 cassette, resembling the target insertion site resulting from the ISApl1-mediated transposition [16], although the IS element itself was not always present. In a recent study, no putative inverted repeat sequences were identified at the extremities of the mcr-1 cassette [22]. Furthermore, Snesrud and colleagues [20] proposed the loss of one or both ISApl1 elements as an explanation for the minor variations (mismatches and deletions) at the 3’end of the mcr-1 element. Accordingly, in this study, ISApl1 was present only on a single IncI2 plasmid (pPC11) and was located upstream of the mcr-1 gene.

![Fig. 2 Sequencing alignment of IncX4-type mcr-1-harboring plasmids. The mcr-1-harboring plasmid, pmcr-1_X4 (Accession-Nr. KU761327), which was obtained from two Klebsiella pneumoniae isolates and one Escherichia coli isolate from patients in eastern China and which was one of the first sequenced IncX4 mcr-1 positive plasmids, was used as reference plasmid (black circle). The outmost circle in grey arrows shows the annotations of the reference plasmid. The insertions element and the mcr-1 gene were highlighted in orange and red arrows, respectively. The figure indicates the high degree of homology of the mcr-1 harboring IncX4 plasmids independently of their isolation source and geographical origin](image-url)
Notably, none of the sequenced plasmids carried additional antibiotic resistance determinants. This is in agreement with other observations [5, 9, 22] and appears to be quite a specificity to the mcr-1 gene, considering that most antibiotic resistance plasmids often carry multiple resistance genes. It is therefore tempting to speculate that this specificity is related to selection of those MCR-1 determinants by treatment containing polymyxins in animals. Moreover, the food samples described in this study originated from Germany, a country with high use of colistin in animal husbandry, and both humans with diarrhea had visited countries in Asia, where colistin is applied widely to treat animals [3]. However, the extended-spectrum β-lactamase gene bla⁹₆₄⁴ has recently been detected on an mcr-1-harboring IncI2 plasmid [23]. There are some further data were the mcr-1 gene was located on large multidrug resistance plasmids for example in combination with extended-spectrum beta-lactamase genes [9, 15, 24]. In these studies mcr-1 was mainly harboured on IncH12 or IncF plasmids. It is to be expected that mcr-1 harboring plasmids co-harboring resistances to antimicrobials crucial to human treatment become more frequent in future.

Conclusion
Transferable IncI2 and IncX4 type plasmids harbouring mcr-1 were found in E. coli of different clonal backgrounds isolated from humans and from food. The high similarity between the plasmids belonging to the same incompatibility groups shows that these “epidemic” plasmids may be responsible for the spread of the mcr-1 gene along the food chain and humans, rather than single specific E. coli clones. A single strain may even contain more than one mcr-1-harboring plasmid. Further studies are needed in order to determine the mechanisms that lead to the acquisition or even accumulation of mcr-1-harboring plasmids within Enterobacteriaceae.

Abbreviations
bla: β-lactamase gene; CC: Clonal complex; EE: Enterobacteriaceae enrichment; Inc: Plasmid incompatibility group; MLST: Multilocus sequence type; NENT: National Centre for Enteropathogenic Bacteria and Listenia; ST: Sequence type; UPEC: Uropathogenic E. coli; UTI: Urinary tract infection

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Availability of data and materials
The GenBank accession numbers for pPF91, pPF52, pPF11, pPC11, pColR664K1, pColRS98_2, pColRS98_1 and pCDF8 are MF175184, MF175185, MF175186, MF175187, MF175188, MF175189, MF175190 and MF175191 respectively.

Authors’ contributions
RS, LP and PN designed the study. KZ carried out the microbiological and molecular biological tests. KZ and JK did the bioinformatic analysis. KZ, MNI and RS analyzed and interpreted the data. KZ and MNI drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This type of study is approved by the local ethics committee of Zürich (BASEC Nr. Req-2016-00374).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

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