


First Report of the Globally Disseminated IncX4 Plasmid Carrying the *mcr-1* Gene in a Colistin-Resistant *Escherichia coli* Sequence Type 101 Isolate from a Human Infection in Brazil

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A colistin-resistant *Escherichia coli* strain was recovered from a patient with a diabetic foot infection in Brazil. Whole-genome analysis revealed that the *E. coli* isolate belonged to the widespread sequence type (ST) 101 and harbored the *mcr-1* gene on an IncX4 plasmid that was highly similar to *mcr-1*-bearing IncX4 plasmids that were recently identified in *Enterobacteriaceae* from food, animal, and human samples recovered on different continents. These results suggest that self-transmissible IncX4-type plasmids may represent promiscuous plasmids contributing to the intercontinental spread of the *mcr-1* gene.

The plasmid-mediated colistin resistance mechanism MCR-1 has become a great challenge to public health worldwide. In fact, since its initial identification in *Enterobacteriaceae* strains (mostly *Escherichia coli*) isolated from animals, food, and humans in China (1), MCR-1 has also been reported in other countries in Asia, Africa, Europe, and North America (2). In South America, *E. coli* harboring the *mcr-1* gene has been present in food-producing animals since at least 2012 (3), being recently identified in human clinical samples from Argentina (4). We hereby report the first description (to our knowledge) of MCR-1 in a human *E. coli* isolate from Brazil.

In early 2016, a man in his late 60s with a medical history of type 2 diabetes mellitus, atrial fibrillation, obesity, dyslipidemia, and hypertension was admitted to a private hospital in northeastern Brazil with a 2-month history of a right calcaneal ulcer (informed consent was obtained from this patient). The patient underwent debridement with fasciotomy of necrosed tissue. Wound cultures taken during debridement were negative for aerobic bacteria, and empirical intravenous antibiotic therapy with ceftriaxone and clindamycin was initiated. Following 14 days of antibiotic therapy, the patient was discharged with clinical improvement. Nine days later, however, he returned to the hospital with worsened symptoms, and the combined therapy of ceftriaxone with clindamycin was restarted. As the patient's condition was deteriorating, the treatment was changed to piperacillin-tazobactam. Nevertheless, 10 days later the patient had developed recurrent episodes of fever associated with poor general condition. The antibiotic regimen was changed to meropenem, and the patient underwent another debridement, from which a fragment of soft tissue sent for culture yielded growth of colistin-resistant extended-spectrum β -lactamase (ESBL)-producing *E. coli* (ICBEC72H) and carbapenem-resistant *Citrobacter freundii*. Ciprofloxacin was added to the antibiotic regimen at that time, whereas meropenem was stopped after 3 days of combined therapy. The patient underwent lower-limb amputation due to foot necrosis; ciprofloxacin treatment was maintained for 14 days, and the patient was discharged after complete recovery.

The identification and antimicrobial susceptibility testing of the *E. coli* and *C. freundii* isolates were performed using the Mi-

croScan system (Beckman Coulter). While the *E. coli* strain presented resistance to ampicillin (>16 μ g/ml), ampicillin-sulbactam (>16/8 μ g/ml), aztreonam (>8 μ g/ml), ceftazidime (>8 μ g/ml), cefepime (>8 μ g/ml), cefotaxime (>16 μ g/ml), cefuroxime (>16 μ g/ml), cephalothin (>16 μ g/ml), and colistin (>4 μ g/ml), the *C. freundii* isolate showed resistance to aztreonam (>8 μ g/ml), ceftazidime (>8 μ g/ml), cefotaxime (>16 μ g/ml), ceftazidime (8 μ g/ml), ertapenem (>1 μ g/ml), imipenem (4 μ g/ml), meropenem (4 μ g/ml), piperacillin-tazobactam (>64 μ g/ml), tobramycin (8 μ g/ml), and trimethoprim-sulfamethoxazole (>2/38 μ g/ml) (5, 6). Both isolates remained susceptible to amikacin, ciprofloxacin, gentamicin, levofloxacin, and tigecycline. The presence of the *bla*_{KPC-2} gene in *C. freundii* was confirmed by PCR (7), whereas the total genomic DNA of *E. coli* ICBEC72H was used to construct a mate-paired library, which was sequenced using the MiSeq platform (Illumina, Inc.). Genome assembly was carried out using SPAdes v3.7.1 with the high-quality mate-pair option (8) and, after automatic annotation using Prokka (www.github.com/tseemann/prokka), the sequence was manually curated using the GenBank database and InterPro (www.ebi.ac.uk/interpro). Resistance genes were detected by BLASTn using the ResFinder 2.1 database (<https://cge.cbs.dtu.dk/services/ResFinder>), plasmid classification was carried out *in silico* by BLASTn using the PlasmidFinder 1.3 database (<https://cge.cbs.dtu.dk/services/PlasmidFinder>), and virulence

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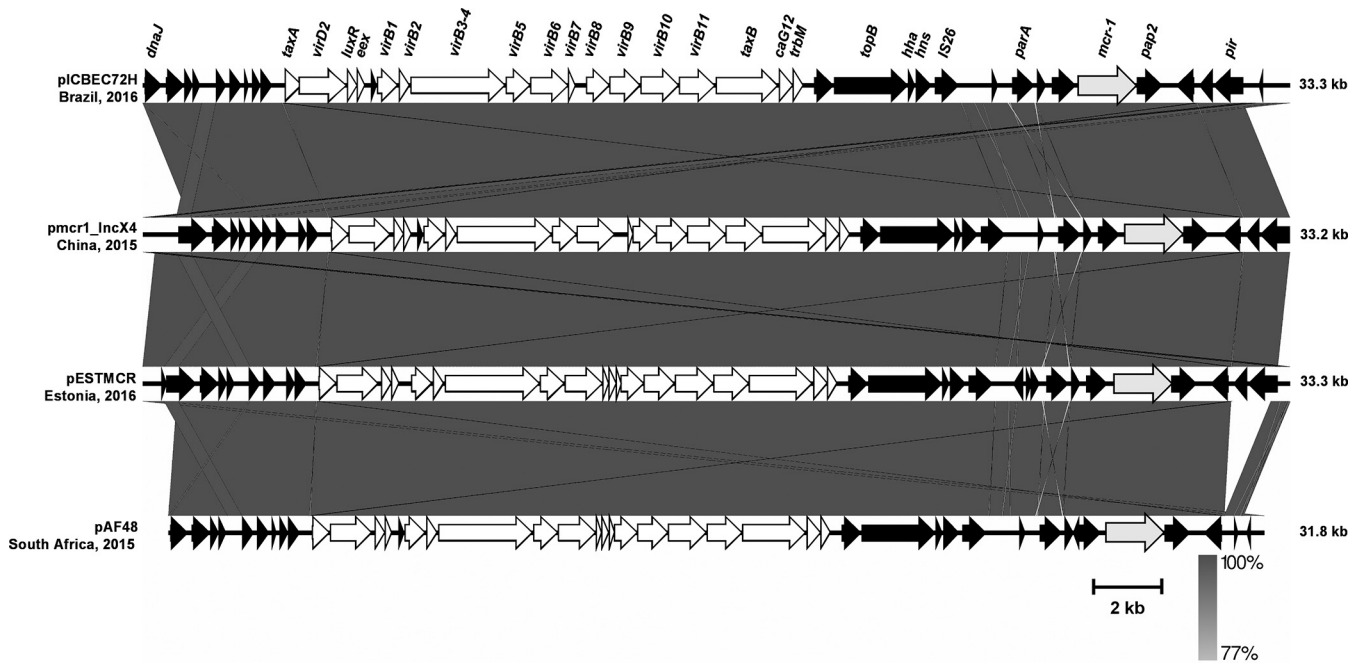


FIG 1 Backbone of pICBEC72Hmcr IncX4 plasmid (GenBank accession no. CP015977) carrying the *mcr-1* gene in a human *E. coli* strain isolated in Brazil. The pICBEC72H plasmid was compared with plasmid pmcr1_IncX4 (GenBank accession no. KU761327.1) from a MCR-1-positive ST25 *Klebsiella pneumoniae* strain isolated from a human clinical sample in China (12), pESTMCR (GenBank accession no. KU743383.1) from a MCR-1-positive *E. coli* strain isolated from pig slurry in Estonia, and pAF48 (GenBank accession no. KX032520.1) from a MCR-1-positive ST624 *E. coli* strain isolated from a human clinical sample in South Africa (13). Comparative analysis was performed using Easyfig version 2.2.2 for genome comparisons. White arrows, genes that are part of transfer modules.

genes were detected by BLASTn using the VirulenceFinder 1.5 database (<https://cge.cbs.dtu.dk/services/VirulenceFinder>).

Whole-genome sequencing revealed that *E. coli* ICBEC72H belonged to the widespread sequence type (ST) 101, which was found previously in Australia, Asia, Europe, and North America, where it was found to harbor *bla*_{NDM-1} and, less commonly, *bla*_{CTX-M} (9–11). The presence of the *iroN* (siderophore), *mcmA* (microcin), *mchB* (microcin), *mchC* (microcin), *mchF* (microcin), *lpfA* (fimbriae), and *iss* (increased serum survival) virulence genes found in ICBEC72H can be associated with the low-virulence B1 phylogenetic group. Further pulsed-field gel electrophoresis (PFGE) characterization, using XbaI restriction, showed that *E. coli* ICBEC72H was clonally unrelated to previously identified *mcr-1*-positive *E. coli* strains isolated from Brazilian livestock (3).

E. coli ICBEC72H carried the *bla*_{CTX-M-8} and *mcr-1* genes on two different plasmids, which were successfully transferred to *E. coli* strain EC600 by conjugation (broth mating method). Transconjugants were selected on MacConkey agar plates containing streptomycin (200 µg/ml) and colistin (2 µg/ml). Conjugative transfer of the plasmid mediating colistin resistance from *E. coli* strain ICBEC72H to recipient *E. coli* strain EC600 was accompanied by cotransfer of the compatible plasmid conferring third-generation cephalosporin resistance. Plasmid pICBEC72Hctx (92.2 kb in length) was classified as belonging to the IncI1 incompatibility group and carried only *bla*_{CTX-M-8}, whereas the plasmid carrying *mcr-1* (pICBEC72Hmcr) was 33.67 kb in length and belonged to the IncX4 incompatibility group.

Multiple MAFFT alignments between pICBEC72Hmcr and the other three IncX4 plasmids bearing *mcr-1* that are available in GenBank showed very high levels of architectural conservation among these plasmids, with pICBEC72Hmcr presenting 99.9%

nucleotide identity with pmcr1_IncX4 (GenBank accession no. KU761327.1), which was found in a ST25 *Klebsiella pneumoniae* strain isolated from a human clinical sample in China (12), and pESTMCR (GenBank accession no. KU743383.1), which was found in an *E. coli* strain isolated from pig slurry in Estonia. Plasmid pICBEC72Hmcr presented 96.3% identity with pAF48 (GenBank accession no. KX032520.1), which was found in an *E. coli* strain isolated from a human clinical sample in South Africa (13), because the latter plasmid presents an ~1,200-bp deletion that includes a partial deletion of the replication initiation protein PI and a few indels, mostly in noncoding sequences (Fig. 1). The insertion sequence IS*ApI1*, which was initially found to be associated with *mcr-1* in pHNSHP45 (1), was not present in the IncX4 plasmid (Fig. 1) (12, 13).

IncX plasmids are self-transmissible plasmids that include at least five subtypes (IncX1 to IncX5) and previously were thought to be of low prevalence (14, 15). Instead, IncX plasmids have been implicated in the spread of many resistance genes, including ESBL- and carbapenemase-encoding genes (15–18). Recently, IncX4 plasmids carrying the *mcr-1* gene were identified in *Salmonella enterica* (serovars Typhimurium, Paratyphi B, Java, Anatum, and Schwartzengrund) from human infections, ready-to-cook guinea fowl pie, and poultry meat in England, France, and Netherlands (19–21), respectively, and in *E. coli* isolated from imported chicken meat in Denmark (22), swine in Germany (23), livestock in the Netherlands (21), and human samples in China (12) and South Africa (13). What is surprising is the fact that the *mcr-1*-bearing IncX4 plasmids obtained from different bacterial species, belonging to different STs, isolated in different clinical contexts, and found on different continents are highly similar in the plasmid backbone sequences. This strongly suggests that self-

transmissible IncX4-type plasmids may represent promiscuous plasmids contributing to the intercontinental spread of the *mcr-1* gene.

Accession number(s). Sequences have been deposited in GenBank, and the accession numbers can be found via BioSample record number [PRJNA322664](https://www.ncbi.nlm.nih.gov/biosample/PRJNA322664).

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