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Short Communication

# A novel F type plasmid encoding *mcr-10* in a clinical *Enterobacter ludwigii* strain from a tertiary hospital in the Czech Republic

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ABSTRACT

*Objective:* Here we describe a novel IncFIA plasmid harbouring *mcr-10* gene in a clinical *Enterobacter ludwigii* strain isolated at the University Hospital in Pilsen in the Czech Republic.
*Methods:* The strain was subjected to antibiotic susceptibility testing. Whole genome sequencing was performed using Illumina for short-read sequencing and Oxford Nanopore Technologies for long-read sequencing followed by hybrid assembly. The resulting genome was used to detect species using average nucleotide identity, resistance genes, plasmid replicon and MLST (using centre for genomic epidemiology databases; ResFinder, PlasmidFinder and MLST, respectively) and virulence genes using VFDB.
*Results:* The strain showed susceptibility against tetracycline, cefuroxime and chloramphenicol, and it was susceptible to the second and third generation of cephalosporins, carbapenems and colistin. Genome analysis identified the strain as *E. ludwigii* sequence type ST20 and located the *mcr-10* gene on an IncFIA (HI1)/IncFII (Yp) plasmid (pI9455333_MCR10; 129 863 bp). Upon blasting the nucleotide sequence of pI9455333_MCR10 against the NCBI database, no similar plasmid sequence was detected, implying a novel plasmid structure. Nevertheless, it showed a partial similarity with pRHBSTW-00123_3 and FDAARGOS 1432, which were detected in *Enterobacter cloacae* complex (ECC) strains in wastewater samples in 2017 in UK and in 2021 in the United States, respectively, and pEC81-mcr, which was detected in a clinical *Escherichia coli* strain in 2020 in China. Moreover, I9455333cz genome carried virulence genes coding for curli fibers, fimbrial adherence determinants, siderophore aerobactin, iron uptake proteins and regulators of sigma factor.
*Conclusion:* In conclusion, we identified a novel IncF plasmid harbouring *mcr-10* gene in a clinical *Enterobacter ludwigii* strain. To our knowledge, this is the first clinical report of *mcr-10* in the Czech Republic.


## 1. Introduction

*Enterobacter* spp. belong to *Enterobacterales* and are classified into the 'ESKAPE' group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) [1]. Among the *Enterobacter* genus, one of the most significant pathogens that are encountered in clinical settings, and a member of the Enterobacter cloacae complex (ECC) is *Enterobacter ludwigii*. It is a fermentative, motile, rod-shaped Gram-negative bacterium that was recognized as a novel species in 2005 from a set of clinical samples [1,2]. *E. ludwigii* has the ability to adapt to hospital environments causing nosocomial infections and can evolve to multidrug resistant pathogen through acquisition of multiple antibiotic resistance genes [1]. All *Enterobacter* strains are intrinsically resistant to ampicillin, amoxicillin-clavulanic acid, first-generation cephalosporins and cefoxitin due to the presence of AmpC $\beta$-lactamases encoding genes. Moreover, with the increased incidence of multidrug-resistant Gram-negative bacteria, including *Enterobacter* spp. resistant to carbapenems [1],

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colistin (polymyxin E) remains the 'last resort' available treatment for complicated infections in humans [3]. Transmissible plasmid-mediated colistin resistance (*mcr*) genes have been recently discovered; currently, 10 types of *mcr* genes have been reported globally. Nine mobile colistin resistance genes (*mcr-1* to *mcr-9)* had been identified by May 2019, and the first *mcr-10* was reported in 2020 [4], carried by an *Enterobacter roggenkampii* strain that was isolated in 2016 from a patient with an ascites condition in China [5]. In the Czech Republic, cases on *mcr-1* and *mcr-9* in *Enterobacterales* isolates harboured on IncHI2 and IncX4 have been reported [6,7]. The emergence of these genes has raised a broad concern because their dissemination might hinder the efficiency of colistin in clinical treatments. Here, we describe a novel IncFIA (F-:A21:B-) plasmid harbouring *mcr-10* gene from a clinical *Enterobacter ludwigii* strain isolated from the University Hospital in Pilsen in the Czech Republic.

## 2. Materials and methods

### 2.1. Case study

In 2021, a 57-year-old female patient suffering from acute lymphoblastic leukaemia (ALL) was hospitalized in the University Hospital in Pilsen in the Czech Republic. A rectal swab was collected from the patient in the haematology/oncology ward in accordance with the hospital routine procedure for such cases. Due to an ongoing screening survey for *mcr* genes [6] in the Czech Republic, all strains admitted to the microbiology lab in the hospital were screened for the presence of *mcr* genes. PCR screening for *mcr* genes resulted positive.

### 2.2. Species identification and antibiotic susceptibility testing

Species identification was initially performed using the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) using MALDI Biotyper software (Bruker Daltonics, Bremen, Germany). Antibiotic susceptibility testing was performed using broth microdilution assay following EUCAST 2023 breakpoints (https://www.eucast.org/clinical_breakpoints/).

### 2.3. Whole genome sequencing (WGS) and data analysis

Genomic DNA of the isolate was extracted using a NucleoSpin microbial DNA kit (Macherey-Nagel, Duren, Germany). The genomic DNA was subjected to library preparation using Nextera XT DNA library preparation kit (Illumina, Inc., USA), and then it was subjected to short-read sequencing with NovaSeq 6000 (Illumina, Inc., USA) according to the manufacturer's guidelines. Data were analysed as described previously [8]. Raw reads acquired by Illumina sequencing were trimmed using Trimmomatic v0.39 to remove adaptor residues and discard low-quality read regions (Q ≤ 20), and high-quality trimmed reads were *de novo* assembled using SPAdes v3.13.1. Genomic DNA was subjected to long-read sequencing on MinION Mk1b platform (Oxford Nanopore Technologies, ONT, Oxford, UK). The Nanopore library was constructed using an SQK-RBK004 rapid barcoding 1D kit (Oxford Nanopore Technologies, ONT, Oxford, UK) according to the manufacturer's protocol. The barcoded library mix was loaded onto a flow cell (FLO-MIN106 R9.4 SpotON) and sequenced for 48 h. The raw electrical signals were converted to raw reads in fastq format by high accuracy basecalling using Guppy v6.0.1 (ONT). BBDuk (https://jgi.doe.gov/data-and-tools/software-tools/bbtools/bb-tools-user-guide/bbduk-guide/) and Porechop v0.2.4 (ONT) were used for adapter and quality trimming (Q ≤7) and for demultiplexing, respectively. Hybrid assembly was performed using Unicycler v0.4.8 using both short and long reads. The hybrid assembly generated complete circular chromosome and plasmids. Annotation of the genome was performed by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [9]. The pairwise average nucleotide identity (ANI) was used for precise species identification using the online web-based software, JSpeciesWS [10]. A ≥95%–96% ANI cutoff was used to define a bacterial species. Multilocus sequence types (MLST), plasmid replicons and antibiotic resistance genes were detected by uploading the assembled data to online databases found in the Center for Genomic Epidemiology (https://www.genomicepidemiology.org/) (MLST 2.0, PlasmidFinder and ResFinder respectively) as described elsewhere [7]. EasyFig v.2.2.2 was used to perform linear comparative genomic alignment [11]. We further investigated virulence-associated genes (VAGs) using the Virulence Factor Database (VFDB) [12].

## 3. Results and discussion

Plasmid-mediated *mcr* genes have disseminated around the world, addressing a public health threat. Recently, *mcr* genes have been broadly detected in animals, the environment and humans due to the extensive use of colistin in animal feed, agriculture and health sectors [4]. To our knowledge, this is the first report of a clinical *Enterobacter* strain producing MCR-10 in the Czech Republic.

This isolate expressed elevated MICs to cefuroxime (MIC, 8 mg/L), chloramphenicol (MIC, 8 mg/L) and tetracycline (MIC, 4 mg/L) but, nevertheless, was susceptible to multiple antibiotics such as cefprozil (MIC, <0.5 mg/L), cefotaxime (MIC, 0.125 mg/L), ceftazidime (MIC, 0.25mg/L), ciprofloxacin (<0.0063 mg/L) and amikacin (<0.5 mg/L). Moreover, the strain showed susceptibility against most of the clinically important antibiotics such as cefepime (MIC, <0.125), meropenem (MIC, <0.125 mg/L), ertapenem (MIC, <0.016) and colistin (MIC, 0.25 mg/L) (Supplementary Table S1).

WGS data from short-read sequencing of the strain I9455333cz were used to assemble the draft genome. The pairwise average nucleotide identity (ANI) identified I9455333cz strain as *E. ludwigii*. Moreover, MLST typing designated the strain as sequence type 20 (ST20). In 2022, Sarangi and colleagues also reported a clinical *E. ludwigii* ST20 strain in Japan, which was isolated from a hospitalized patient's blood culture [13].

The hybrid assemblies of Illumina and Nanopore reads of I9455333cz generated a complete circular chromosome of 5 247 533 bp in size. The chromosome carried the intrinsic antibiotic resistance genes $bla_{\text{ACT-12}}$ and *fos*A2 associated with AmpC beta-lactamase and fosfomycin resistance, respectively. Moreover, *marR* mutations (not reported previously with amino acid substitutions; E10D, E31D, A41T, A52E, A53V, V84I, L87S, T102S, G103B, I107M, D118T, Y137L, V142I) have been detected using the Comprehensive Antibiotic Resistance Database (CARD, https://card.mcmaster.ca/home). The *marR* gene is a repressor of the *mar* operon (*marRAB*), which regulates the expression of the activator (*marA*) of the efflux pump AcrAB. These mutations are associated with elevated MICs against ciprofloxacin and tetracycline (CARD accession number ARO:3003378) [14]. Furthermore, the I9455333cz complete genome included five complete circular plasmids: two COL plasmids, pI9455333_COL4401I (3771 bp) and pI9455333_COL (2495 bp), two un-typable plasmids pI9455333_1 and pI9455333_2 (6306 bp and 4307 bp, respectively) and an IncFIA(HI1) (F-:A21:B-) plasmid (pI9455333_ MCR10; 129 863 bp), harbouring the *mcr-10* gene as the sole antibiotic resistance gene (Table 1). There have been reports of *mcr-10*-carrying *Enterobacter* spp. being recovered from sewage water from hospitals and animals [15]. In 2020, *mcr-10* was discovered on an IncFIA plasmid from an *Enterobacter roggenkampii*

**Table 1**
WGS data of the MCR-10 producing *Enterobacter luwigii* strain isolated from a Czech hospital.

| ID | Species | ST | Replicons | Size (bp) | Inc group | Resistance genes |
|---|---|---|---|---|---|---|
| I9455333cz | *E. ludwigii* | 20 | Chromosome | 5 247 533 | NA | $bla_{ACT-12}$, *fos*A2 |
| | | | Plasmid pI9455333_ MCR10 | 129 863 | IncFIA(HI1) (F-:A21:B-) | *mcr-10* |
| | | | Plasmid pI9455333_COL4401I | 3771 | COL4401I | - |
| | | | Plasmid pI9455333_COL | 2495 | COL | - |
| | | | Plasmid pI9455333_1 | 6306 | - | - |
| | | | Plasmid pI9455333_2 | 4307 | - | - |

NA, not applicable.

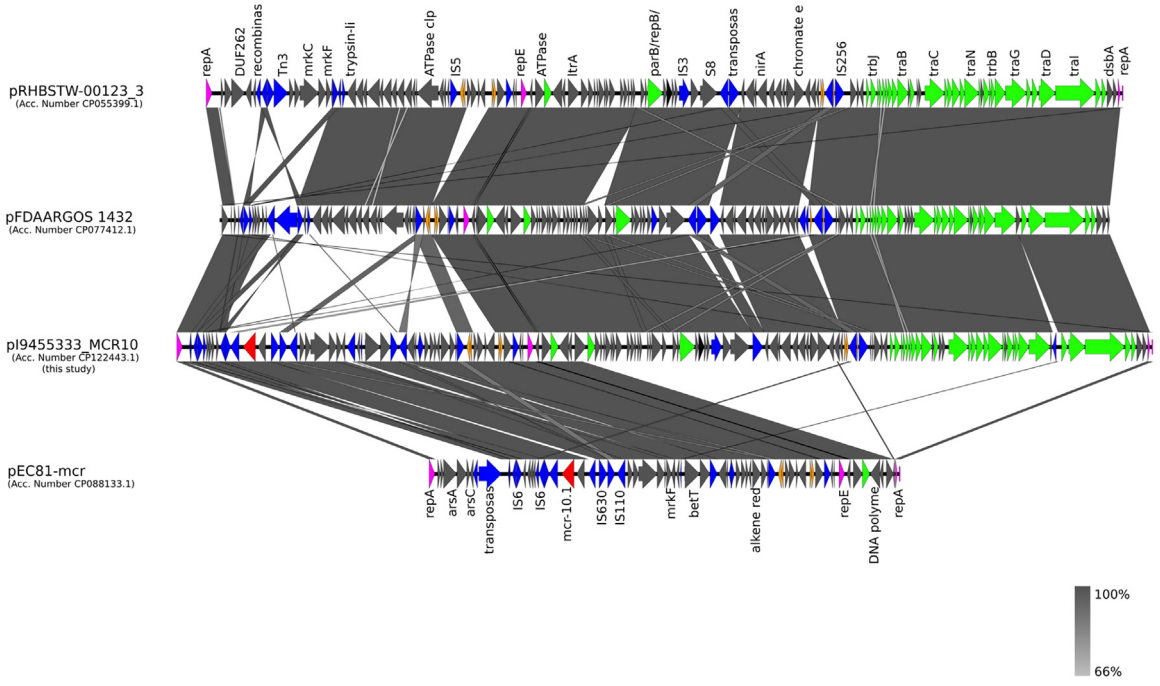


**Fig. 1.** Linear map of pI9455333_ MCR10 against pRHBSTW-00123_3, FDAARGOS 1432 and pEC81-MCR10. The green, purple, navy blue, orange, red, and grey colours correspond to conjugal transfer system, replication, transposases, toxin-antitoxin systems, maintenance system, *mcr* gene, and the rest of genes respectively. The figure was obtained with EasyFig v.2.2.2.

clinical strain from China, which imparts a 4-fold rise in colistin MIC [15]. Subsequently, *mcr-10* has been reported in other IncF type plasmids such as IncFIB, IncFII:IncFIA, IncFII:IncFIB and IncFIB:IncFIA carried by Salmonella in Asia, Europe, Oceania and North America [16]. According to previous studies, *mcr-10* genes were usually associated with colistin resistance or reduced susceptibility to colistin [1]. However, we found that our strain, which had *mcr-10* gene, belonged to colistin-susceptible *Enterobacter* spp. isolates. Recent studies have also shown the profusion of these genes in susceptible bacteria. Therefore, some *mcr* variants are incapable of conferring colistin resistance due to multiple factors that play a role in the decreased gene expression, lower plasmid copy number and increased plasmid fitness cost [17].

The backbone of pI9455333_ MCR10 carried genes coding for replication initiation protein (*repA* and *repE*), conjugative transfer proteins (*tra* genes), plasmid maintenance (*parB*/*parM*) and multiple toxin-antitoxin systems (*relB*/*relE, ccdB*/*ccdA* and *hok*). In addition, it also harboured other genes that are associated with various functions such as antibiotic tolerance, stress response, persister cell formation, intestinal colonization, bacterial virulence and biofilm formation [18].

Upon blasting the nucleotide sequence of pI9455333_ MCR10 against the NCBI database, no similar plasmid sequence was detected, implying a novel plasmid structure. Nevertheless, it showed partial similarity with three IncFIA(HI1) (F-:A21:B-) plasmids: (1) pRHBSTW-00123_3 (121 982 bp, accession no. CP055399), with a 76% query coverage and 99.99% sequence identity detected in *Enterobacter roggenkampii* isolated from wastewater influent samples in 2017 in the UK [19], and (2) FDAARGOS 1432 plasmid unnamed (118 421bp, accession no. CP077412.1) with a 75% query coverage and 100% sequence identity detected in an *Enterobacter asburiae* in 2021 in the United States. These plasmids showed sequence similarity with most of the plasmid backbone (Fig. 1). The third plasmid, pEC81-mcr (62 662 bp, accession no. CP088133) (41% query coverage and 99.98% sequence identity), which was detected in a clinical *Escherichia coli* strain (stool sample) in 2020 from China [15]. This plasmid showed sequence similarity in the region harbouring *mcr-10* and its genetic environment (Fig. 1). The genetic environment of the *mcr-10* shows that it has the well-conserved *xerC-mcr-10* sequence, which is detected in most of the reported *mcr-10* positive strains [15]. This well-conserved sequence suggests that it has a high rate of different mobile genetic elements' inser-

tions at the peripheries. The results suggest the plasmid backbone that was previously identified in ECC strains has acquired the *mcr-10* and its genetic environment, which was detected in a clinical *E. coli* strain through insertion or recombination driven by the transposases on one or both ends.

Detection of virulence genes was performed *in silico* using VFDB database. Results showed that the chromosome of the strain I9455333cz has genes coding for: (1) curli fibers (*csgA, csgD, csgF* and *csgG*); (2) biofilm formation (*adeG* and *pgaC*); (3) adhesin type 3 fimbriae (*mrkB*) (detected on the pI9455333_ MCR10 plasmid); (4) fimbrial adherence determinants (*fimA/C/D/F/H/I/Z*), which play a vital role in bacterial attachment. Interestingly, type 3 fimbriae (*mrkB*) is considered one of the most important virulence factors detected in *K. pneumoniae,* which significantly contributes to its pathogenicity [20], and (5) several siderophore biosynthesis pathways such as those for siderophore aerobactin (*iucC/D/A/B*), siderophore enterobactin (*fepD* and *fepC*) and iron uptake (*hemC/E/G/H/L/N)*. The production of siderophores is crucial for the acquisition and transport of iron across the bacterial cell membrane [21]. Furthermore, regulators of sigma factor (*RpoS and phoP/Q*) were also found, which are essential for all stress responses and which promote survival under environmental challenges [22].

In conclusion, we identified a novel plasmid harbouring *mcr-10* gene in a clinical *Enterobacter* strain. To our knowledge, this is the first report in the Czech Republic. Based on our genomic findings, the presence of the *mcr-10* on a plasmid that is well maintained by genes coding for further conservation, propagation and transfer through two TA systems and the intact conjugative transfer system, even though conferring low-level colistin resistance, warrants close monitoring.

## Nucleotide sequence accession numbers

The nucleotide sequences of the chromosome and plasmids of the strain I9455333cz have been deposited in GenBank under accession no. CP122442-CP122447 under the Bioproject number PRJNA954571.

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**Competing interests:** We have no conflicts to declare.

## Supplementary data

Supplementary material related to this article can be found in the online version, at doi: 10.1016/j.jgar.2024.03.015.

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