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Letter to the Editor

High occurrence of colistin- and multidrug-resistant strains carrying *mcr-1* or an underestimated *mcr-1.26* allelic variant along a large Brazilian river

Editor: Dr Marisa Haenni

Letter to the editor

Plasmid-mediated colistin resistance genes (*mcr*) are widely dispersed in different Enterobacterales species and niches, raising concerns about colistin use. The *mcr-1.1* allele is globally distributed at the expense of an epidemic IncX4 plasmid circulating efficiently between humans and foodborne animals [1]. Much less is known regarding the contribution of natural environments to *mcr* spread, especially in countries with poor sewage treatment systems and high rates of antimicrobial-resistant bacteria such as Brazil [2]. We hereby performed a detailed whole genome analysis on colistin-resistant strains recovered along one of the largest rivers in the Ribeirão Preto mesoregion in Brazil.

In February 2020, we collected eight water samples along the course of the long (~240 km) Sapucaí and Sapucaizinho rivers in São Paulo, Brazil (Table S1; Fig. S1). Colistin-resistant *Escherichia coli* and *Klebsiella* spp. strains were isolated (one presumptive colony/species) by previously established procedures. Antimicrobial susceptibility profiles were determined by disk diffusion, agar dilution, and/or broth microdilution methods according to the Clinical and Laboratory Standards Institute (CLSI; M100, 30th, 2020) and Brazilian Committee on Antimicrobial Susceptibility Testing/European Committee on Antimicrobial Susceptibility Testing (BrCast/EUCAST; v.10.0, 2020) guidelines; *mcr* (*mcr*-1 to *mcr*-9) genes were screened by polymerase chain reaction (PCR) [3]. Colistin-resistant strains were submitted to whole-genome sequencing using Illumina NovaSeq (Illumina Inc., San Diega, CA), followed by detailed characterization using tools available at the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/), EnteroBase (https://enterobase.warwick.ac.uk/), and Kleborate (https://github.com/katholt/Kleborate; Table S2). Mutations in colistin resistance determinants were detected in-house using appropriate reference strains and Geneious v.2021.0.3 (Biomatters Ltd., New Zealand), and predicted as neutral or deleterious by PROVEAN (http://provean.jcvi.org/index.php).

Eleven strains (eight *E. coli*, two *Klebsiella pneumoniae*, and one *Klebsiella quasipneumoniae* subsp. *similipneumoniae*) were resistant to colistin with a minimum inhibitory concentration (MIC) of 4 mg/L. Six of them were positive for the *mcr-1* gene (*mcr-1.1* [n = 4] and *mcr-1.26* [n = 2] alleles), highlighting the leakage of *mcr-1*-carrying bacteria to the environment. All *E. coli* strains and one *K. pneumoniae* strain (EW714) were multidrug-resistant and harboured diverse antimicrobial resistance, biocides, and metal tolerance genes (Tables S1, S3, and S4).

E. coli strains belonged mainly to phylogenetic group A and CC10 (ST10, ST744, and the new ST12841) with *fimH*54. Other diverse and uncommon sequence types (STs) (D-ST349, B1-ST1720, and F-ST6157) were identified. Interestingly, one *E. coli* strain (EW658) belonged to the high-risk clone of foodborne origin, ST131-*H*22. It was classified as ExPEC and carried a high-pathogenicity island including yersiniabactin and invasin clusters. The detection of MDR high-risk *E. coli* clones (ST131-*H*22 and ST10 clonal complex) at the human-animal–environment interface is of concern due to the risk of human exposure. Of highlight, strains EW655 and EW715 (both ST744/cgST-29209) were recovered from distant sites. *Klebsiella* spp. strains were assigned to STs (ST30, ST889, and ST5569) that have been occasionally reported in humans and animals in different world regions (https://bigsdb.pasteur.fr/; Tables S3 and S4).

Chromosomal mutations potentially linked to colistin resistance were detected in MCR-1 or non MCR-1 producers, described in detail in Tables S5 and S6. Mutations in *mgrB, phoQ, pmrBCD*, and *qseC* (*E. coli*), and *pmrBC, rstB*, and *crrB* (*K. pneumoniae*) were predicted as deleterious, or have been previously described in colistin-resistant strains. These mutations alone or in combination explain the resistance phenotype in all strains except *K. quasipneumoniae* [4]. In fact, the vast majority of mutations appear to be neutral, reflecting the phylogenetic distance to the reference strains used and the need of reliable genotypic-phenotypic correlations to explain colistin resistance patterns.

Both *mcr-1.1* and *mcr-1.26* were identified in highly similar IncX4 plasmids, confirming the distribution of this epidemic plasmid in different niches in Brazil and worldwide [3]. A total of six IncX4 plasmids with 33,304 bp in length were assembled (GenBank accession no. OM735812-OM735817). We report a new variant of the *mcr-1.26* allele, with the substitution Ile (ATA) in the first Met (ATG) codon that results in a shorter MCR-1 protein (1623 aa vs. 1626 aa), resulting in a MIC of 4 mg/L. The firstly described *mcr-1.26* allele (*E. coli*, Germany, 2020) carried a Thr (ACG) instead of the first Met (ATG) codon [5] (Tables S3 and S7). A comparative analysis of public *mcr-1.26*-bearing isolates revealed that IncX4 and IncI2 plasmids carrying *mcr-1.26* have been circulating since at least 2016 in diverse niches (humans, foodborne/companion animals, meat) and countries, often misidentified as *mcr-1.1*. To our knowledge, this is the first description of the *mcr-1.26* gene in environmental samples.

Despite the high nucleotide identity (>99.9%) observed between all genetic platforms (Fig. 1A), different adjacent regions of the *mcr-1.26* gene (*mcr-1* cassette) were identified with variable distribution: (i) *mcr-1.26* with the Thr substitution in the Netherlands and Czech Republic; (ii) *mcr-1.26* with the Ile substitution and deletions downstream *mcr-1* from China; and (iii) *mcr-1.26* with the Ile substitution and upstream/downstream deletions from

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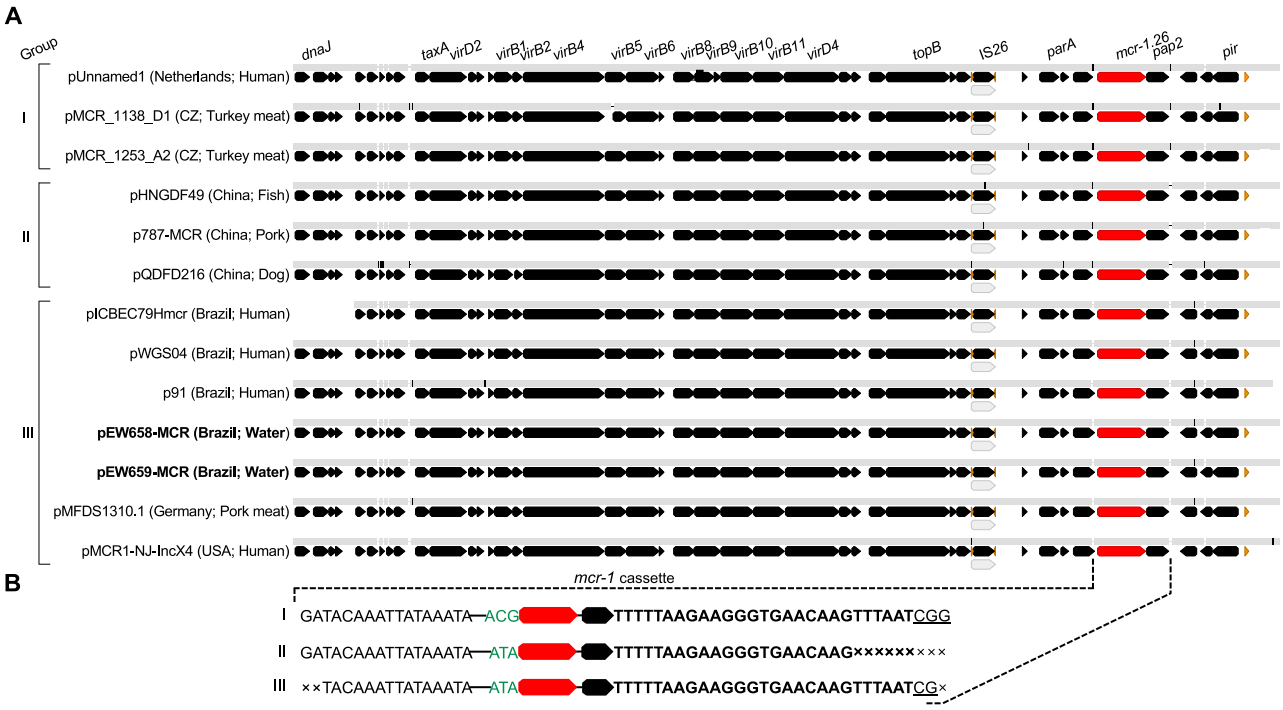

Fig. 1. Comparison of IncX4 plasmids and *mcr-1.26* genetic contexts identified in this study and in sequences from public databases. (A) Full-length alignment of ~33 kb *mcr-1.26*-bearing IncX4 plasmids. *E. coli* strains from humans (pUnnamed1, GenBank accession no. LR882927; pICBEC79Hmcr GenBank accession no. CP020376; pWGS04, GenBank accession no. MH298055; p91, GenBank accession no. MK940858; pMFDS1310.1, GenBank accession no. MK875284), animals (pHNGDF49, GenBank accession no. MF978387; p787-MCR, GenBank accession no. MG825367; p QDFD216, GenBank accession no. CP053212), foods (pMCR_1138_D1, GenBank accession no. MT929278; pMCR_1253_A2, GenBank accession no. MT929276; pMFDS1310.1, GenBank accession no. MK875284), and water samples (pEW658-MCR, GenBank accession no. OM735812; pEW659-MCR, GenBank accession no. OM735813). The plasmids from this study are highlighted in bold. The gray lines represent shared regions of homology. CZ, Czech Republic; USA, United States of America. (B) Adjacent sequences of the *mcr-1.26* gene. The first three nucleotides before the *mcr-1.26* gene, IRR2, and DR are shown in green, bold, and underlined, respectively.

Brazil, Germany, and the United States (Fig. 1B). In addition, the genetic environment of *mcr-1.1*-harbouring IncX4 plasmids identified in this study was identical to that of group iii, and all IncX4 plasmids shared >99.9% identity. Therefore, specific genetic signatures can be used to trace different genetic contexts observed in certain geographic regions.

In this study, we show a high incidence and diversity of colistin-resistant bacteria (identified in all samples) and MCR-1 producers (identified in 75% of the samples) in surface water samples from Brazil, which is probably underestimated and influenced by anthropogenic activities nearby (Fig. S1). We identified a new and previously unrecognized *mcr-1.26* allelic variant, highlighting the need to deepen the recognition of *mcr* variants and their distribution and epidemiological trends.

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Competing interests

None declared

Ethical approval

Not required

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.05.030.

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