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Genome Note

Draft genome of a ST443 *mcr-1*- and $bla_{CTX\text{-}M\text{-}2}$-carrying *Escherichia coli* from cattle in Brazil

Josman Dantas Palmeira[a,*], Helena Ferreira[a], Jean-Yves Madec[b], Marisa Haenni[b]

[a] Microbiology, Biological Sciences Department, Faculty of Pharmacy, University of Porto, Porto, Portugal
[b] Unité Antibiorésistance et Virulence Bactériennes, Université Claude Bernard Lyon 1, Anses Laboratoire de Lyon, Lyon, France

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ABSTRACT

Objectives: Colistin is used in Brazil for the treatment of food-producing animals. The colistin resistance gene *mcr-1* has already been reported from chicken and swine in this country. Here we report the draft genome of an *Escherichia coli* isolate presenting both an extended-spectrum β-lactamase (ESBL) gene and the *mcr-1* gene in a healthy cow in Brazil.
Methods: Whole genomic DNA from *E. coli* E12 was extracted and 2× 150-bp paired-end reads were generated using Illumina sequencing technology. De novo genome assembly was performed using SPAdes v.3.11 and the draft genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Further analyses were performed using Center for Genomic Epidemiology databases. Southern blots were performed to characterise plasmid location.
Results: The 5 024 393-bp genome displayed several resistance genes, including the *mcr-1* and $bla_{CTX\text{-}M\text{-}2}$ genes. These two genes were located on different plasmids (*mcr-1* on an IncX4 plasmid and $bla_{CTX\text{-}M\text{-}2}$ on an IncF plasmid).
Conclusion: The genome sequence reported here can be compared with previously published genomes for *mcr*-1-producing isolates. This will ultimately help to understand the routes of dissemination of the resistance genes.

Colistin has been used in veterinary medicine since the 1960s in food-producing animals on all continents [1]. Following the discovery of the first plasmid-borne colistin resistance gene *mcr-1*, reports of *mcr-1*-positive *Escherichia coli* in food-producing animals have emerged worldwide, and dissemination of the *mcr-1* gene has been mainly attributed to IncI2, IncHI2 and IncX4 plasmids, with both global and local spread [2]. In Brazil, colistin is authorised not only for the treatment of diseased animals but also as a growth promoter in feed for broilers, swine and cattle [3]. In line with colistin use, the *mcr-1* gene has been reported in Brazil in food-producing animals (chicken and swine), food (chicken meat), water and, ultimately, humans, but not yet in cattle. Here we report the detection and draft genome of an *mcr*-1-positive *E. coli* collected from a healthy cow in Brazil.

Strain *E. coli* E12 was collected in 2014 from a 5-year old cow living on an intensive production farm in the Northeast Region of Brazil. This strain was detected in the framework of a study evaluating faecal carriage of extended-spectrum cephalosporin resistant *E. coli* in healthy cattle. Strain *E. coli* E12 was isolated from a faecal sample by selection on MacConkey agar supplemented with ceftazidime (2 μg/mL) following an enrichment step in non-selective tryptic soy broth. Identification was confirmed using API® 20E strip tests (bioMérieux, Marcy-l'Étoile, France). Antimicrobial susceptibility testing performed by the disk diffusion method showed an extended-spectrum β-lactamase (ESBL) phenotype and additional resistance to tetracycline and gentamicin. Moreover, *E. coli* E12 was resistant to colistin [minimum inhibitory concentration (MIC) of 2 mg/L] as determined by broth microdilution assay. The *mcr*-1 and $bla_{CTX\text{-}M\text{-}2}$ genes were detected by PCR. The transferability of *mcr*-1 and $bla_{CTX\text{-}M\text{-}2}$ was confirmed by conjugation assays using non-lactose-fermenting *E. coli* HB101 as recipient strain.

Whole-genome sequencing was performed on genomic DNA extracted using a Microbial DNA Extraction Kit (Macherey Nagel, Hoerdt, France). A genomic library was prepared using a Nextera XT DNA Library Preparation Kit (Illumina Inc., Cambridge, UK) according to the manufacturer's protocol, and 2× 150-bp

* Corresponding author.
E-mail address: josmandantasp@gmail.com (J.D. Palmeira).

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paired-end reads were generated using Illumina sequencing technology (Illumina Inc., San Diego, CA). De novo genome assembly was performed using SPAdes v.3.11 and the draft genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). In total, 1789672 paired-end reads were generated with a minimum 53-fold coverage, which were assembled into 152 contigs. The genome size was calculated as 5024393 bp, which comprised a total of 4858 protein-coding sequences. The G + C content of this strain was 50.6%.

Escherichia coli E12 belonged to sequence type 443 (ST443) as determined by in silico typing according to Achtman multilocus sequence typing (MLST) scheme using the MLST 1.8 online tool available at the Center for Genomic Epidemiology (CGE) (https://cge.cbs.dtu.dk/services/MLST/). This rare ST has previously been reported in Shiga toxin-producing *E. coli* (STEC) isolates from food-producing animals in Germany and in water effluents, suggesting a non-human origin. Further characterisation of *E. coli* E12 was performed through online tools available at the CGE (http://www.genomicepidemiology.org). The $bla_{CTX\text{-}M\text{-}2}$, *mcr*-1, *aadA1*, *aac(3)-VIa*, *sul1* and *tet*(A) antibiotic resistance genes were detected using ResFinder 2.1 (https://cge.cbs.dtu.dk/services/ResFinder/), which confer resistance to extended-spectrum cephalosporins, colistin, streptomycin, gentamicin, sulphonamides and tetracycline, respectively. The IncX4, IncI1, IncFII and IncFIB incompatibility groups were found using PlasmidFinder 1.3 (https://cge.cbs.dtu.dk/services/PlasmidFinder/), and plasmid subtyping using an online database (http://pubmlst.org/plasmid/) showed that the IncI1 plasmid belonged to ST12 and the IncF plasmid presented the F4:A-:B1 formula. Southern blot experiments on *S1* nuclease pulsed-field gel electrophoresis (S1-PFGE) gels proved that *mcr*-1 was carried by the IncX4 plasmid and $bla_{CTX\text{-}M\text{-}2}$ by the IncF plasmid. Furthermore, the *aadA1*, *aac(3)-VIa*, *sul1* and $bla_{CTX\text{-}M\text{-}2}$ genes were found on the same contig, proving their occurrence on the same IncF4:A-:B1 plasmid. Detection of *mcr-1* on an IncX4 plasmid has already been reported in Brazil in chicken meat, recreational water, penguins and humans, thus strongly suggesting that IncX4 is a major driver of the *mcr*-1 gene in all sectors [4,5]. This is different from the situation in Europe (France, Portugal, Spain and the Netherlands) where the *mcr-1* gene reported from cattle was more often carried by IncHI2 plasmids.

The ST443 *E. coli* E12 strain belonged to phylogroup B1 and presented an O153/O178:H19-*fim31* profile (https://cge.cbs.dtu.dk/services/SerotypeFinder/and https://cge.cbs.dtu.dk/services/FimTyper/). The virulence factors (https://cge.cbs.dtu.dk/services/VirulenceFinder/) *iss*, *mchF*, *gad*, *cma* and *iroN* were also detected, along with 684 proteins of pathogenic families (https://cge.cbs.dtu.dk/services/PathogenFinder/).

In conclusion, here we report the draft genome of a bovine *E. coli* harbouring the *mcr-1* gene on an IncX4 plasmid, an association that has already been described several times in Brazil, but in *E. coli* from different STs. Accumulation and comparison of data from the whole country and from different sectors will help to decipher the routes and determinants of transmission of the *mcr-1* gene. Finally, considering that Brazil is a major exporter of beef, this report should prompt further studies in this food-producing sector.

This Whole Genome Sequencing project has been deposited at DDBJ/EMBL/GenBank under the accession no. **PUFY00000000**.

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Competing interests

None declared.

Ethical approval

Not required.

References

[1] Kempf I., Jouy E, Chauvin C. Colistin use and colistin resistance in bacteria from animals. Int J Antimicrob Agents 2016;48:598–606.
[2] Matamoros S, van Hattem JM, Arcilla MS, Willemse N, Melles DC, Penders J, et al. Global phylogenetic analysis of *Escherichia coli* and plasmids carrying the *mcr-1* gene indicates bacterial diversity but plasmid restriction. Sci Rep 2017;7:15364.
[3] de Silva KC, Knobl T, Moreno AM. Antimicrobial resistance in veterinary medicine: mechanisms and bacterial agents with the greatest impact on human health. Braz J Vet Res Anim Sci 2013;50:171–83.
[4] Monte DF, Fernandes MR, Cerdeira L, de Souza TA, Mem A, Franco B, et al. Draft genome sequences of colistin-resistant MCR-1-producing *Escherichia coli* ST1850 and ST74 strains isolated from commercial chicken meat. Genome Announc 2017;5: pii: e00329-17.
[5] Fernandes MR, McCulloch JA, Vianello MA, Moura Q, Pérez-Chaparro PJ, Esposito F, et al. 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. Antimicrob Agents Chemother 2016;60:6415–7.