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IncX4 plasmid carrying the new mcr-3 gene variant in a CTX-M-8-producing Escherichia coli isolate recovered from swine

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Background: After the original report in China, several studies in different countries reported the identification of *mcr-1* in *Enterobacteriaceae* isolates from humans, food and companion animals, meat and environment. Here we describe the first detection of a novel *mcr* variant identified in a commensal *Escherichia coli* isolated in 2015 from a swine.

Material/methods: MICs were determined by both agar dilution and microdilution technique. Interpretation of results was done according to the EUCAST epidemiological cut-off values. Plasmid DNA was extracted from *E. coli* LV23529, using a NucleoBond Xtra Plus kit (Macherey-Nagel), and quantified using Qubit 1.0 Fluorometer (Invitrogen). The Nextera XT DNA Sample Preparation Kit (Illumina) was used to prepare sequencing libraries from 1ng of genomic DNA according to the manufacturer's instructions. Plasmid sequencing was performed using 150 bp paired-end reads on a MiSeq (Illumina). Sequence reads were trimmed and filtered according to quality criteria, and de novo assembled into contigs by means of CLC Genomics Workbench 9.0 (Qiagen). The NCBI prokaryotic genome automatic annotation pipeline (PGAAP) was used for annotation. ResFinder 2.1, VirulenceFinder 1.4, PlasmidFinder 1.3, pMLST 1.4 and PHAST were used to estimate the acquired antibiotic resistance genes, virulence factors, plasmid MLST and phage regions, respectively.

Results: Determination of the MICs showed non-wild-type phenotypes to third- and fourth-generation cephalosporins (ceftazidime 1mg/L, cefotaxime 32mg/L, cefepime 32 mg/L) with synergy with

clavulanic acid; to sulphamethoxazole (>1024mg/L), trimethoprim (>32mg/L), tetracycline (>64mg/L), and colistin (16mg/L). LV23529 remained wild-type to carbapenems, fluoroquinolones, aminoglycosides and tigecycline. *In silico* antimicrobial resistance analyses, with a threshold of 90% identity and a minimum length of 60%, revealed genes conferring resistance to β-lactams ($bla_{CTX\text{-}M\text{-}8}$) and to colistin (a new *mcr* gene variant*).* The hereafter named *mcr-3* gene differed from *mcr-1* by one point mutation (T1238C) that leads to the amino acid substitution Val413Ala. Bioinformatics analysis revealed the presence of three plasmids: IncF [F2:A-:B-], IncX4-harbouring *mcr-3* (named pLV23529-MCR), and IncI1-ST113 carrying the $bla_{CTX\text{-}M\text{-}8}$ (pLV23529-CTX); at our knowledge, this represents the first description of this CTX-M variant in Portugal. The closest matches (99% of identity) of the MCR-3-containing contigs as identified by BLASTn analysis were the *E. coli* pICBEC7Pmcr (CP017246), *E. coli* pMCR1-NJ-IncX4 (KX447768) and the *Klebsiella pneumoniae* pMCR1.2-IT (KX236309) plasmids. In all of these plasmids, the genetic environment of *mcr* gene is characterized by an *IS26* element upstream of the *mcr-pap2* element and by the absence of *IS*Apl1.

Conclusions: The information presented herein will enable further studies aiming to assess which will be the potential impact of the acquisition of plasmid-mediated *mcr-3* variant for the treatment of pan-drug-resistant Gram-negative bacteria.