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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Introduction

After the original report in China [1], several studies in different countries reported the identification of *mcr-type* genes in *Enterobacteriaceae* isolates from humans, food and companion animals, meat and environment [2].

In Portugal, plasmid-mediated colistin resistance had been detected in *Salmonella enterica* isolates from humans and food [3-4], and more recently in one *Escherichia coli* strain isolated from fresh vegetables [5].

Here we describe the first detection of a novel *mcr* variant, ***mcr-3***, identified in a commensal *E. coli* isolated in 2015, in Portugal, from a swine.

Methods

- MICs were determined by both agar dilution and the microdilution technique and interpreted according to the EUCAST epidemiological cut-off values [6].
- Plasmid DNA was extracted from *E. coli* LV23529, using a NucleoBond Xtra Plus kit (Macherey-Nagel), and quantified using Qubit 1.0 Fluorometer (Invitrogen) [7].
- Plasmid sequencing was performed using 150 bp paired-end reads on a MiSeq (Illumina) [7]. Bioinformatics analysis was performed accordingly with Fig. 1.

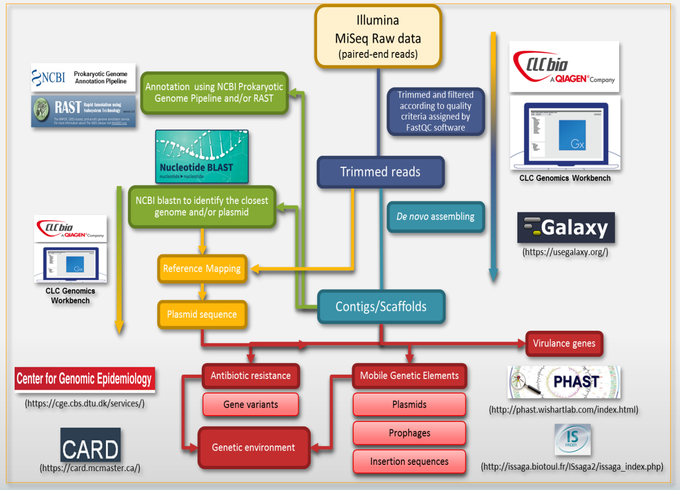
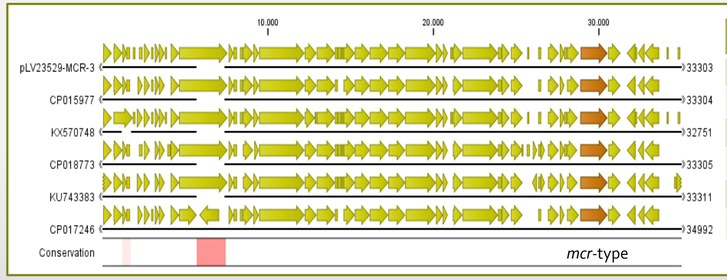


Figure 1. Bioinformatics analysis workflow.

Results

Table 1. Phenotypic and genotypic characterization of Mcr-3-harbouring *E. coli*.

Non-wild-type phenotypes to:	Acquired-antibiotic resistance genes
Colistin (16 mg/L)	*mcr-3* (amino acid substitution Val413Ala)
β-lactams, including ceftazidime (1 mg/L), cefotaxime (32 mg/L) and cefepime (32 mg/L), with synergy with clavulanic acid	bla_{TEM-1} *and* $bla_{CTX-M-8}$
Sulphamethoxazole (>1024 mg/L)	*sul3*
Trimethoprim (>32 mg/L)	*dfrA12*
Chloramphenicol (>128 mg/L)	*cmlA1-type*
Tetracycline (>64 mg/L)	-



Source/Country/Year	E-value	Alignment overlap (%)	Identity (%)
Swine/Portugal/2015	--	--	--
Human patient /Brazil/2016	0.0	100	99.95
Swine feces /China/2016	0.0	100	99.96
Human patient /United States/2016	0.0	100	99.92
Pig slurry /Estonia/2013	0.0	100	99.53
Migratory Penguin /Brazil/2013	0.0	98.3	99.96

Figure 2. Linear comparison of IncX4-pLV23529-MCR-3 with the 5 *mcr-1*-harboring plasmids showing the highest identities (>99,5%), in different *E. coli* isolates.

In all of these plasmids, the genetic environment of *mcr-type* gene is characterized by an *IS26* element upstream of the *mcr-pap2* element and by the absence of *IS*Apl1. The *mcr-3* is the only antibiotic resistance gene in the plasmid.

Conclusion

The presence of colistin resistance gene in food represents a potential public health threat, as it is located in mobile genetic elements that have the potential to spread horizontally. 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.

References

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