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Whole-genome sequencing of *Escherichia coli* bearing *mcr-1* from sewage water

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Background: *mcr-1*, the first mobile colistin resistance mechanism, has been largely described in multiple Enterobacteriaceae from human and animal sources. However, the dissemination of this gene and the bacteria and mobile elements involved remains unclear in the environmental context. Therefore, the objective of this work was to elucidate this spread of *mcr-1* in Enterobacteriaceae in sewage samples from Barcelona (Spain)¹ using whole genome sequencing.

Material/methods: 16 *mcr-1*-positive *E. coli* from two waste-water treatment plants were selected to be fully sequenced using Illumina technology (MiSeq). *De novo* assembly was carried out with Spades v.3.5.0. The consequent analysis was assessed by srst v0.1.8 software and the Center for Genomic Epidemiology services, in order to determine the sequence type (PubMLST of Pasteur web), serotype (SerotypeFinder database), antibiotic resistance genes (ARGannot and ResFinder databases),

virulence genes (VirulenceFinder database) and plasmid incompatibility groups (PlasmidFinder database). The structure of genetic mobile elements associated to *mcr-1* was obtained with Geneious v8.1.0 software.

Results: The 16 *mcr-1*-positive isolates analysed belonged to the infrequent and recently described sequence type ST632, which has been related to nosocomial infections caused by carbapenemase-producing *E. coli*². Likewise, all isolates were identified as *E. coli* O91:H28, a Shiga-toxin-producing *E. coli* serotype. The antibiotic resistance genes found in all the isolates, together with *mcr-1*, were *bla*_{TEM-1}, *bla*_{CTX-M-55}, *rmtB* and *sul1*. Furthermore, the isolates belonging to pulsotype I shared the genes *dfrA12*, *aadA2*, *strA* and *strB*, and those belonging to pulsotype II bore in addition *dfrA17*, *mphA* and *aadA5*. The virulence genes found in all the isolates were *lpfA* and *astA*, associated with extraintestinal pathogenic and enterotoxigenic *E. coli*. Regarding the plasmid incompatibility groups, all isolates harboured IncI2, IncFII and IncI1 plasmids. Transformation experiments showed that *mcr-1* was borne by IncI2 in all the cases. The analysis of the genetic context of *mcr-1* revealed the association of this gene with the commonly described IS*ApI1* in all the *mcr-1*-positive isolates.

Conclusions: This work reveals the high frequency of *mcr-1* in pathogenic *E. coli* belonging to the sequence type ST632 from two waste-water treatment plants of Barcelona (Spain). Furthermore, the whole genome sequencing analysis confirmed that only one plasmid was responsible of the dissemination of this gene, and showed the association of *mcr-1* with IS*ApI1*. The study of the dissemination of *mcr-1* through whole genome sequencing in environmental samples is essential to identify the flux of *mcr-1* and implement measures to control further spread of this gene.

¹ Ovejero CM, Delgado-Blas JF *et al.* Spread of *mcr-1*-carrying Enterobacteriaceae in sewage water from Spain. Accepted in *J Antimicrob Chemother.*

² Tavares CP, Pereira PS *et al.* Molecular epidemiology of KPC-2-producing Enterobacteriaceae (non-*Klebsiella pneumoniae*) isolated from Brazil. *Diagn Microbiol Infect Dis* 2015; 82: 326-30.