# *mcr*-4 carrying *Escherichia coli* isolates of Europe exhibit a high genetic diversity but a highly conserved plasmid type encoding the colistin resistance

Authors: Isidro García-Meniño[1,2,4], Ana Oliveira[3], Annemarie Käsbohrer[4], Azucena Mora[1,2]*, Jens Andre Hammerl[4]*. *(Shared senior researcher authorship)

[1]Laboratorio de Referencia de *Escherichia coli* (LREC), Departamento de Microbioloxía e Parasitoloxía, Facultade de Veterinaria, Universidade de Santiago de Compostela (USC), 27002 Lugo, Spain. [2]Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), 15706 Santiago, Spain. [3]CEB (Centre of Biological Engineering). (Lab. de Investigação em Biofilmes Rosário Oliveira), University of Minho, Braga, Portugal. [4]Department for Biological Safety, German Federal Institute for Risk Assessment, 10589 Berlin, Germany.

**Background**

Colistin represents an important antimicrobial for the veterinary and human sector. Besides its outstanding antimicrobial use in gastrointestinal infections in animals, it is currently a last-line treatment option for human infections caused by multidrug-resistant Gram-negative bacteria. Successively after the description of the first mobile colistin resistance (*mcr*-1) element in *Escherichia coli*, the identification of other genes (*mcr*-2 to *mcr*-10) and variants has forced the understanding of the colistin resistance and dissemination mechanisms in Enterobacterales.

**Material and Methods**

For comparative analysis, a collection of Spanish (n=28), German (n=14) and Portuguese (n=9) *E. coli* isolates of porcine origin exhibiting the mobile colistin resistance determinant *mcr*-4, were subjected to phenotypic and genotypic *in depth* characterization. The isolates were investigated for their antimicrobial susceptibility, macrorestriction profiles (XbaI-PFGE), plasmid patterns (S1-PFGE) and plasmid transmission (*in vitro* filter mating studies). Short-read whole-genome sequencing (WGS) data were used for *in silico*-based typing of the genomes.

**Results**

Overall, the investigated isolates of the three countries differed substantially in their macrorestriction profiles. While Portuguese and Spanish isolates exhibited a closer phylogenetic identity, in relation with their geographic origin, German isolates showed high heterogeneity. Similar results were observed from S1-PFGE analysis. However all isolates showed a low size *mcr*-4 carrying plasmid (range10 to 25 kb). *In vitro* transmission to the sodium azide-resistant *E. coli* J53 strain was confirmed in at least 50% of the Spanish and Portuguese isolates. Interestingly, most of the transconjugants harbored two plasmids of which only one carried the mobilizable *mcr*-4 plasmid, while the second would probably act as a helper for the transmission. While the *mcr*-4 plasmids seem to be based on a highly conserved ColE10 plasmid backbone, the majority of their host are highly heterogeneous.

**Conclusions**

Our results indicate a close relationship of the individual *mcr*-4 carrying plasmids of Portugal, Spain and Germany. Thus, we suppose that dissemination of the conserved plasmid-type is based on a common

ancestor However, the impact of this gene is currently unknown, since no comprehensive information on *mcr* determinants in colistin-resistant isolates from human infections exists.

**Acknowledgement and funding**

L. Lestón acknowledges the Ministry of Education of Spain for her pre-doctoral grant FPU19/01127. I. García-Meniño acknowledges (FEMS-GO-2020-205). This research was funded through: PID2019-104439RB-C21/AEI/10.13039/501100011033 from the Agencia Estatal de Investigación (AEI, Spain), cofounded by the Euro-pean Regional Development Fund of the European Union: a Way to Making Europe (FEDER). The research of J.A. Hammerl was supported by the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 773830 (FULL-FORCE).