First Report of *Salmonella* Serovar Typhimurium and Monophasic Typhimurium Clinical Isolates Harboring *mcr*-9 in Portugal

Primeiros Casos Reportados de Isolados Clínicos de *Salmonella* Serovar Typhimurium e Typhimurium Monofásica com *mcr*-9 em Portugal

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Palavras-chave: Colistina/farmacologia; Farmacorresistência Bacteriana; Salmonella typhimurium/genética; Testes de Sensibilidade Microbiana

Dear Editor,

During the 1990s, the emergence of multidrug resistance (MDR) microorganisms led to the rediscovery of colistin as a last-resort therapeutic solution for MDR Gram-negative infections.[1] Naturally, the rate of colistin resistance began to increase, and the first reports of resistance described chromosomally mediated mechanisms. Since 2016, when plasmid-mediated colistin resistance was firstly described, ten alleles (*mcr*-1 to *mcr*-10) and several variants have been identified.[1]

Even though reports of plasmid-mediated colistin resistance in *Salmonella* are not frequent, *mcr* genes have been identified in several isolates from different sources in recent years.[2–4] We report the first two clinical isolates of *Salmonella* spp. harboring *mcr*-9, identified in Portugal.

Both isolates, recovered from feces of a 4-month-old baby and a 2-year-old child with gastrointestinal disease, were sent to the National Reference Laboratory for Gastrointestinal Infections of the National Institute of Health Doutor Ricardo Jorge (INSA) for serotyping, and were sequenced in 2021. Resistance to antibiotics was determined by disk diffusion and broth microdilution for colistin, according to EUCAST guidelines. DNA was extracted and short reads were obtained by paired-end sequencing on a NextSeq 550 instrument (Illumina, USA). Read quality analysis, improvement, and trimming were performed using FastQC v0.11.5 and Trimmomatic v0.36. Raw reads were submitted on the web server of the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/), for identification of antimicrobial resistance genes, *in silico* sequence type (ST) and presence of plasmids. BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to confirm the presence of *mcr*-9 gene in the IncHI2/ST1 plasmid. Sequencing reads were deposited on the European Nucleotide Archive (ENA) under the bioproject PRJEB32515 (Table 1).

The two isolates revealed a MDR phenotype (Table 1), both presenting resistance to beta-lactams, sulfonamides, and tetracycline. Additionally, isolate Se_248167 presented resistance to fluoroquinolones and aminoglycosides. Although whole genome sequencing (WGS) revealed the presence of a *mcr*-9 gene in the IncHI2/ST1 plasmid, both isolates were susceptible to colistin (2 µg/mL). The wide spread of $bla_{CTX\text{-}M\text{-}9}$/*mcr*-9 in the IncHI2/ST1 plasmid has been previously described in *Escherichia coli* and *Enterobacter cloacae* isolated from wild animals.[5] Here we confirm the presence of IncHI2/ST1 harboring *mcr*-9 and $bla_{CTX\text{-}M\text{-}9}$ genes, in *Salmonella* clinical isolates. The presence of *mcr*-9 has been previously described in *Salmonella,* and seems to confer resistance in some isolates.[2,3] Indeed, the presence of this gene in a highly successful mobile element such as the IncHI2/ST1 plasmid is worrying, since the spread of this resistance marker can occur intra- and inter-species of Enterobacterales.[5] Additionally, as previously described, exposure to sub-inhibitory concentrations of antimicrobials can induce the expression of silent genes, leading to resistant phenotypes.[4] To our knowledge, this is the first report in Portugal of *Salmonella* isolates carrying *mcr*-9 gene recovered from human samples.

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Table 1 – Isolate characterization

Isolate	Year of isolation	Patient age	Serovar	Resistance phenotype	Antibiotic resistance genes	ST	Plasmid incompatibility type	Ena acession #
Se_10169	2019	4 months	Monophasic Typhimurium	AMP, TET, FOX, FEP, SMX	*aac(6')-Iaa*, *ant(2")-Ia*, *aph(6)-Id*, *aph(3")-Ib*, *blaCTX-M-9*, *blaTEM-1B*, *mcr-9*, *sul1*, *sul2*, *tet(B)*	34	IncHI2/ST1, IncHI2A IncQ1	ERS13570778
Se_248167	2021	2 years	Typhimurium	AMP, TET, CAZ, FOX, FEP, CRO, GMN, PEF, SMX	*aac(6')-Iaa*, *ant(2")-Ia*, *blaCTX-M-9*, *mcr-9*, *qnrA1*, *sul1*, *tet(A)*	19	IncHI2/ST1, IncHI2A, IncFIB(S), IncFII(S)	ERR10372088

ST: sequence type; AMP: ampicillin; TET: tetracycline; CAZ: ceftazidime; FOX: cefotaxime; FEP: cefepime; CRO: ceftriaxone; GMN: gentamycin; PEF: pefloxacin; SMX: sulfamethoxazole

Laboratory, under the scope of the national surveillance program.

AUTHOR CONTRIBUTIONS

LS: Study design, data analysis, research, and writing of the manuscript.

AP: Study design, data analysis, research, and critical review of the manuscript.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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