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

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. 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. 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. 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 blaCTX-M-9 in the IncHI2/ST1 plasmid has been previously described in Escherichia coli and Enterobacter cloacae isolated from wild animals. Here we confirm the presence of IncHI2/ST1 harboring mcr-9 and blaCTX-M-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. 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 Enterobacteriales. Additionally, as previously described, exposure to sub-inhibitory concentrations of antimicrobials can induce the expression of silent genes, leading to resistant phenotypes. 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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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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REFERENCES

Table 1 – Isolate characterization

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

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