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Letter to the Editor

Escherichia coli ST167 carrying plasmid mobilisable *mcr-1* and $bla_{CTX\text{-}M\text{-}15}$ resistance determinants isolated from a human respiratory infection

Sir,

The emergence of polymyxin resistance in Enterobacteriaceae is a matter of major concern and has instigated the recommendation for its surveillance since it compromises the use of colistin, a last-resort antibiotic [1].

The *mcr-1* gene, conferring transferable colistin resistance, has spread worldwide and has become highly prevalent in poultry and pigs, suggesting that positive pressure of antimicrobial treatments might have selected this genetic element [2]. Even worse, emerging multiresistant bacteria carrying *mcr-1* together with genes encoding extended-spectrum β-lactamases (ESBLs) and/or carbapenemases could compromise the effectiveness of last-resort antimicrobial therapy and, although the occurrence of the *mcr-1* resistance determinant is not yet frequent in bacteria isolated from humans, its presence requires a thorough evaluation [1].

One year after the first description of Enterobacteriaceae carrying *mcr-1* (from pigs) in the Iberian Peninsula [3], screening of human isolates with possible zoonotic origin is being approached to monitor the transfer of colistin resistance to human microbiota, where its co-expression with ESBL or carbapenemase enzymes might present the highest clinical relevance. During the first 6 months of 2016, 48 *Escherichia coli* isolates were obtained from non-urinary samples in 'S. Pedro de Alcántara' Hospital (Extremadura, Central-West Region of Spain) and were identified as ESBL- or AmpC-producers using a MicroScan® WalkAway® 96 Plus System (Beckman Coulter, Barcelona, Spain). Among them, the *mcr-1* gene was detected by PCR [2] in a unique *E. coli* strain, named HSP38, which was isolated from a 67-year old man. The patient died 11 days after hospital admission, following aggravation of his chronic obstructive pulmonary disease by a respiratory infection and failure of standard treatments with ceftriaxone administered since first day of hospitalisation and changed 1 week later to cefepime plus linezolid. Culture of sputum obtained close to the fatal outcome (Day 9) revealed the presence of strain HSP38 with an antimicrobial resistance phenotype including third-generation cephalosporins, quinolones, aminoglycosides, trimethoprim/sulfamethoxazole (SXT) and colistin, whereas the bacterium was found to be sensitive to amoxicillin/clavulanic acid (AMC), piperacillin/tazobactam (TZP), carbapenems and tigecycline. The minimum inhibitory concentration (MIC) for colistin was determined as 4 mg/L by Etest (bioMérieux, Madrid, Spain).

The genome of HSP38 was sequenced using an Ion Torrent™ Personal Genome Machine™ (Thermo Fisher Scientific, Madrid, Spain) at STAB facilities (University of Extremadura, Badajoz, Spain) according to the manufacturer's instructions. A total of 5.5 M reads (217 depth) were obtained and were assembled with Assembler SPAdes v.3.9.0 plugin (Thermo Fisher Scientific) to yield a draft spanning 313 contigs (>201 bp). This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. **MWUH00000000** (**MWUH01000000**).

ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/) showed the presence in the HSP38 genome of linked gene cassettes with the antimicrobial resistance determinants *aadA1–cmlA1–sul3*, *aadA2–dfrA12*, *aadA5–sul1–dfrA17* and *strA–strB–sul2*, in addition to the non-clustered genes *aac(3)-IIa*, *aph(3′)-Ia*, $bla_{CTX\text{-}M\text{-}15}$, *lnu*(F), *mcr-1*, *tet*(B) and *tet*(34). Moreover, the quinolone resistance-determining region (QRDR) of GyrA presented mutations S83L and D87N, a genotype that confers quinolone resistance [4].

The clinically relevant determinants conferring antimicrobial resistance found in HSP38 were those encoding resistance to most β-lactam antibiotics and quinolones, first-choice agents used in the treatment of enterobacterial infections, and to colistin, the last-resort agent for treatment of infections with multiresistant strains. Mobilisation of plasmids carrying *mcr-1* and $bla_{CTX\text{-}M\text{-}15}$ was evidenced by conjugation of HSP38 with *E. coli* J53 and selection in medium with sodium azide plus colistin or plus ampicillin. The genes *mcr-1* and $bla_{CTX\text{-}M\text{-}15}$ were transferred with efficiencies of 5×10^{-2} and 2×10^{-5}, respectively, indicating their location in different plasmids and that colistin resistance is highly mobile.

Genome analysis of HSP38 using PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/) allowed the detection of plasmid replicons IncX4 and IncN. The IncX4 replicon from isolate HSP38 spans the *mcr-1* gene and, assembled by plasmidSPAdes (http://spades.bioinf.spbau.ru/plasmidSPAdes/), it covers most of the 30-kb sequence from IncX4 plasmids carrying *mcr-1* that was recently described in enterobacteria isolated worldwide [1].

In silico typing of isolate HSP38 using MLST 1.8 (multilocus sequence typing) (https://cge.cbs.dtu.dk/services/MLST/) identified the bacterium as ST167, a genetic background that has been associated with clinically relevant outbreaks and ESBL expression [5]. Involvement of this bacterium in a human respiratory infection with fatal outcome, carrying a multiresistant phenotype for colistin, most β-lactam antibiotics and quinolones, with an extremely high mobilisation potential (mainly) for colistin resistance, is a matter of serious concern that must be taken into consideration before use of antimicrobial therapies and should be further surveyed in clinical units.

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