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Short Communication

First three *Escherichia coli* isolates harbouring *mcr-1* in Uruguay

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ABSTRACT

Objective: This report described the first *Escherichia coli* (*E. coli*) isolates harbouring *mcr-1* in Uruguay.
Methods: Three *E. coli* isolates were obtained from blood, urine and rectal swabs from different patients in two hospitals. Extended-spectrum β-lactamases (ESBL), plasmid-encoded (pAmpC) β-lactamases, plasmid-mediated quinolone resistance (PMQR) genes, class 1 integrons, and *mcr-1*, *mcr-2* and *mcr-3* were sought and characterised in three *E. coli* isolates. Transfer of resistance determinants was assessed by conjugation. Clonality was analysed by multilocus sequence typing.
Results: All isolates were categorised as being colistin-resistant and the *mcr-1* gene was detected. Two isolates were also resistant to oxyimino cephalosporins: one on account of bla_{CMY-2} and the other due to $bla_{CTX-M-15}$, the latter also harbouring transferable quinolone-resistance genes (*aac(6′)Ib-cr* and *qnrB*). All *mcr-1* genes were transferred by conjugation to recipient strains. The *mcr-1*-bearing isolates belonged to sequence types ST10, ST93 and ST5442.
Conclusions: ST10 is considered as a high-risk clone worldwide. This type of *mcr-1*-harbouring clone is a major concern for human and animal health and must be under close surveillance. This study detected the presence of *mcr-1* for the first time in Uruguay, albeit in an allodemic manner, associated with different antibiotic-resistance genes and from diverse clinical contexts. Considering that colistin is often the last therapeutic option available for multidrug-resistant Gram-negative bacilli infections, it is important to maximise precautions to avoid dissemination of isolates carrying *mcr-1*.

1. Introduction

Gram-negative bacilli (GNB) infections are a growing problem in clinical practice mainly due to the emergence of antimicrobial resistance mechanisms to all or almost all antibiotics used for treating patients [1]. The increasing resistance to β-lactams, including carbapenems, has led to the inclusion of older antibiotics, such as fosfomycin or polymyxins, for treating multidrug-resistant (MDR) GNB infections [2]. Polymyxins have been clinically used for the treatment of GNB since 1950. The mechanism of action of polymyxins is explained by the electrostatic interaction between positively charged residues of these antibiotics and the negatively charged lipid A moieties of lipopolysaccharides (LPS) anchored to the Gram-negative's outer membrane [3]. The emergence of transferable colistin resistance mediated by *mcr* genes and their dissemination is currently a global concern [3].

This report describes the first three isolates of *Escherichia coli* (*E. coli*) harbouring *mcr-1* in Uruguay. Interestingly, these cases occurred in different clinical contexts and featured different genetic environments.

2. Methods

2.1. Patients

Patients' clinical data were retrospectively collected by reviewing the medical records.

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2.2. Bacterial identification and susceptibility assays

Bacterial identification and antibiotic susceptibility were determined using the VITEK 2 Compact System (bioMérieux, Durham, NC, USA) and/or Etest (bioMérieux, Marcy l'Étoile, France) in accordance with the manufacturers' recommendations. Susceptibility to fosfomycin was performed by the disk diffusion method, and resistance to colistin was confirmed by the Sensititre microdilution method (Thermo Fisher Scientific Waltham, MA, USA). Susceptibility results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (http://www.eucast.org). Colistin resistant isolates were sent to the Department of Bacteriology and Virology of the Faculty of Medicine for further characterization.

2.3. Detection of antibiotic resistance genes

The presence of $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{TEM}, $bla_{\text{PER-2}}$, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-2}}$ subgroups, carbapenemases and plasmid-encoded (pAmpC) β-lactamases, and plasmid-mediated quinolone resistance (PMQR) genes were determined using PCR and sequencing, as previously described [4–7]. Detection of plasmid-mediated colistin resistance genes (*mcr-1*, *mcr-2* and *mcr-3*) was performed by Real-Time PCR according to Li et al. [8].

2.4. Characterisation of class 1 integrons

To improve the characterisation of the isolates carrying *mcr*, the presence of class 1 integrons was determined by PCR with primers I5/I3 [9]. Variable regions and 3′- and 5′-conserved regions were determined in *intI1*-positive isolates by PCR, with primers 5CS/3CS and qacE1F/sul1b [9].

2.5. Conjugation and transformation experiments

Conjugation assays were carried out using *E. coli* J53-2 (rifampicin-resistant) as the recipient strain. Transconjugants (Tc) were selected on Luria-Bertani (LB) agar plates supplemented with 150 mg/L rifampicin and 1 mg/L colistin [7].

For Tc with more than one plasmid, chemical transformation assays were performed using the *E. coli* TOP10 strain as the recipient and plasmid DNA extracted with the Plasmid Miniprep kit (ZymoPURE®) [10]. Transformants were selected on LB agar plates supplemented with colistin (2 mg/L).

2.6. Plasmid characterisation

Plasmid size was estimated for both transformants and transconjugants by treatment with S1 nuclease followed by pulsed-field gel electrophoresis. Incompatibility groups (Inc.) were determined by PCR-based replicon-typing using genomic DNA obtained from both transconjugants and transformants as templates [11–14].

2.7. Multilocus sequence typing

The three isolates underwent multilocus sequence typing (MLST) by gene amplification and sequencing of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) according to the MLST database https://enterobase.warwick.ac.uk/species/index/ecoli.

2.8. Ethics

This study was approved by the institutional ethics committees, and signing informed consent was waived because no additional tests or clinical intervention were performed. Additionally, the identity of the patients was not disclosed.

3. Results

3.1. Patients

Case 1. In January 2017, an 84-year-old man was admitted to hospital 'A' from Montevideo exhibiting a cold jaundice syndrome and severe cachexia. After imaging studies, biliary tract cancer was diagnosed. Because of his unstable basal clinical state, a percutaneous external biliary bypass was performed. The patient developed a fever and empirical treatment with sulbactam-ampicillin plus amikacin was added. Blood culture results were received after 48 h: *E. coli* was isolated in both samples (designated as HAEc-1 in the laboratory). The patient had a Charlson Comorbidity Index of 6 (mortality of 85% at 3 years) and died 24 h after obtaining the blood cultures. No colonisation studies were conducted.

Case 2. In January 2017, a 26-year-old woman consulted a primary care clinic at institution 'B' with community acquired lower urinary tract syndrome. The patient reported an antecedent of urinary tract infection 4 months before and no previous hospital admissions. A urine culture was collected, leading to the isolation of $\geq 10^5$ cfu/mL of an *E. coli* strain (designated as HBEc-1 in the laboratory).

Case 3. In September 2017, an 80-year-old man with a history of chronic obstructive pulmonary disease and arterial hypertension was admitted to the intensive care unit of hospital 'A' with severe respiratory insufficiency derived form a pulmonary infection. The patient was treated with ampicillin–sulbactam plus clarithromycin and no clinical samples were obtained from the respiratory tract. A rectal swab was performed 48 h later, in the context of microbiological surveillance from the hospital's infection control program, isolating an *E. coli* strain (designated as HAEc-2 in the laboratory).

3.2. Susceptibility testing

All three isolates displayed resistance to colistin by Vitek-2 and were confirmed as colistin-resistant by the sensititre microdilution method; additionally, HAEc-1 and HAEc-2 were also resistant to oxyimino cephalosporins (Table 1). Conversely, the three isolates were susceptible to carbapenems, gentamicin, amikacin and fosfomycin.

3.3. Antibiotic resistance genes and class 1 integrons

The three isolates carried the *mcr-1* gene. Furthermore, strain HAEc-1 carried $bla_{\text{CMY-2}}$, a pAmpC β-lactamase, and strain HAEc-2 carried $bla_{\text{CTX-M-15}}$ and PMQR genes *aac(6)Ib-cr* and *qnrB*.

Class 1 integrons were detected in HAEc-2 and HBEc-1; however, only the integron in HBEc-1 featured a variable region. Said variable region harboured the *dfr1A* and *aadA1* gene cassettes (Table 1).

Table 1
Susceptibility test results, resistance genes detected and plasmids characterised in all three strains, and either their transconjugants and/or transformants.

	HAEc-1	Tc HAEc-1	HAEc-2	Tc HAEc-2	Tf HAEc-2	HBEc-1	Tc HBEc-1	Tf HBEc-1	*E. coli* J53-2	*E. coli* Top10
Sample	Blood	–	Rectal swab	–	–	Urine	–	–	–	–
Sequence type	93 (168 cplx)	–	10	–	–	5442	–	–	–	–
Susceptibility test — MIC (mg/L)										
Ampicillin	≥32	4	≥32	≥32	4	≥32	4	4	4	4
Sulbactam Ampicillin	≥32	≤2	≥32	≥32	≤2	≥32	≤2	≤2	≤2	≤2
Cefuroxime	≥64	4	≥64	≥64	4	8	4	4	4	4
Cefotaxime	≥64	≤1	≥64	≥64	≤1	≤1	≤1	≤1	≤1	≤1
Ceftazidime	≥64	≤1	≥64	≥64	≤1	≤1	≤1	≤1	≤1	≤1
Cefepime	≤1	≤1	≥64	≥64	≤1	≤1	≤1	≤1	≤1	≤1
Tazobactam Piperacillin	8	≤4	≥128	≥128	≤4	≤4	≤4	≤4	≤4	≤4
Ertapenem	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Imipenem	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Meropenem	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Gentamicin	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1
Amikacin	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2
Ciprofloxacin	1	≤0.25	2	0.12	0.032	≤0.25	≤0.25	0.032	0.032	0.032
Trimethoprim–Sulfamethoxazole	≤20	≤20	≥320	≥320	≤20	≥320	≥320	≤20	≤20	≤20
Fosfomycin[a]	26	28	27	28	28	25	28	28	28	28
Colistin	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≤0.5	≤0.5
Resistance genes										
	mcr-1, $bla_{\text{CMY-2 like}}$	*mcr-1*	*mcr-1*, $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, *aac(6′)Ib-cr*, *qnrB*, *sul-2*, *dfrA1*	*mcr-1*, $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, *aac(6′)Ib-cr*, *sul-2*	*mcr-1*	*mcr-1*, $bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$, *dfrA1*, *aadA1*, *sul-1*	*mcr-1*, *dfrA1*, *aadA1*, *sul-1*	*mcr-1*	–	–
Plasmids										
Size		60 kb		33 kb, 120 kb	33 kb		35 kb, 104 kb	35 kb	–	–
Incompatibility group		IncI2		IncX4, IncFII-K	IncX4		IncX4, IncI1	IncX4	–	–

[a] Diameter (mm).

3.4. Plasmid transference experiments

Colistin-resistant Tc harbouring *mcr-1* were obtained for all the three strains. A single conjugative 60 kb IncI2 plasmid was transferred in TcHAEc-1 harbouring only *mcr-1*. Two plasmids were transferred in each TcHAEc-2 (33 kb and 120 kb) and TcHBEc-1 (35 kb and 104 kb) (see Table 1).

Besides *mcr-1* the different Tc harboured different resistance genes. TcHAEc-2 carried $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, *aac(6')Ib-cr* and *sul-2*, and TcHBEc-1 carried *dfrA1*, *aadA1* and *sul-1* (as shown in Table 1). Transformants obtained from both TcHAEc-2 and TcHBEc-1 displayed a single plasmid in S1 digestion sized 33 kb and 35 kb, respectively; both belonged to the IncX4 group and carried only *mcr-1* (Table 1).

3.5. Multilocus sequence typing analysis

HAEc-1 belonged to ST93, HAEc-2 to ST10 and HBEc-1 to ST5442. ST5442 is a single locus variant of ST10, the former featuring the allele *adk* 468 instead of *adk* 10. Conversely, ST93 belongs to clonal complex 168 and has four different alleles compared with ST10.

4. Discussion

This study reported the first three identified *E. coli* isolates carrying *mcr-1* in Uruguay. Interestingly, the three cases corresponded to unrelated *E. coli* strains isolated in dissimilar clinical contexts and accompanied by diverse resistance determinants. *Mcr-1* was found in plasmids belonging to either IncI2 or IncX4 incompatibility groups, which have already been described to spread *mcr* variants in Latin America, namely Argentina and Brazil [15].

Apart from displaying resistance to colistin, strain HAEc-1 (MLST ST93) also showed resistance to third-generation cephalosporins, on account of a plasmid-borne CMY-2-like enzyme, similar to what was recently reported in faecal samples from healthy humans in Finland [16].

The current study also detected *E. coli* ST10; this clone has been recovered from birds, swine, sheep and humans, and is usually associated with several resistance genes such as $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, *qnrB*, *aac(6′)Ib-cr* and *mcr-1*, *mcr-4* and *mcr-5* [7,17–19]. In South America, *E. coli* ST10 carrying *mcr-1* has recently been reported in Colombia, but the information on the clones carrying these genes in human isolates is still scarce in the continent [20]. Worldwide dissemination of high-risk *E. coli* clones (such as ST10) harbouring *mcr-1* is a major concern for human and animal health and should be kept under close surveillance within the concept of One Health [21].

HBEc-1 belonged to ST5442. Although ST5442 is a single locus variant of ST10, it does not belong to Clonal Complex 10, and there are no reports of such ST in the literature. However, detecting *mcr* genes in this kind of patient (i.e. an outpatient) should raise an alarm, since susceptibility to colistin is not usually tested in community-acquired urinary tract infections.

In this study, *E. coli* isolates obtained from both clinical samples (i.e. blood - HAEc-1 and urine - HBEc-1) were susceptible to various antimicrobials such as carbapenems, gentamicin, amikacin and fosfomycin. In this scenario, colistin susceptibility testing would not have been necessary or colistin could not have been considered for treatment. Nonetheless, routine susceptibility testing allowed the later detection of *mcr-1*, which could have already been circulating undetected in Uruguay.

In summary: *mcr-1* has emerged in Uruguay in an allodemic manner, is associated with different antibiotic resistance genes and

is from diverse clinical contexts. It is important to maximise the surveillance measures to avoid dissemination of colistin resistance, given that said antibiotic often constitutes the last therapeutic option available for MDR-GNB infections.

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Competing interests

Nothing to declare.

Ethical approval

The study did not intervene in the treatment (clinical or paraclinical) of the patients. Data were used and results presented without disclosing the identity of the patients.

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