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SHORT REPORT
Colistin-resistant *Escherichia coli* clinical isolate harbouring the *mcr-1* gene in Ecuador

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SUMMARY

Colistin resistance mediated by the *mcr-1* gene has been reported worldwide, but to date not from the Andean region, South America. We report the first clinical isolate of *Escherichia coli* harbouring the *mcr-1* gene in Ecuador. The strain was isolated from peritoneal fluid from a 14-year-old male with acute appendicitis, and subjected to molecular analysis. The minimum inhibitory concentration of colistin for the strain was 8 mg/ml and it was susceptible to carbapenems but resistant to tigecycline. The strain harboured *mcr-1* and $bla_{\text{CTX-M-55}}$ genes and was of sequence type 609. The recognition of an apparently commensal strain of *E. coli* harbouring *mcr-1* serves as an alert to the presence in the region of this recently described resistance mechanism to one of the last line of drugs available for the treatment of multi-resistant Gram-negative infections.

Key words: Ecuador, *Escherichia coli*, *mcr-1*.

The most important antimicrobial drug-resistance genetic determinants are those related to mobile extra-chromosomal elements because of their potential for rapid spread and global dissemination of resistance [1]. Until recently, only chromosomally encoded resistance to polymyxins, including colistin, had been reported in the Enterobacteriaceae. However, in late 2015, horizontal transmission of low to moderate levels of colistin resistance mediated by the presence of the plasmid-borne gene *mcr-1* was described first in China by Liu *et al.* [2] and subsequently in several other countries. This was a novel antimicrobial resistance mechanism with an enhanced capacity for dispersion and the report raised global concern owing to the use of polymyxins as a last-resort antibiotic for the treatment of serious infections caused by carbapenem-resistant Gram-negative bacteria [2]. To date, *mcr-1* has been detected in clinical isolates from four of the five continents and there is evidence from analysis of whole genome databases of its circulation since 2009 or earlier [3]. However, to our knowledge, there are no reports in the indexed literature of the isolation of Enterobacteriaceae harbouring the *mcr-1* gene in the Andean region. We record here the first isolation in Ecuador of a strain of *Escherichia coli* from a clinical infection harbouring the *mcr-1* gene.

Since March 2016, all clinical isolates of Enterobacteriaceae showing resistance to colistin identified in our laboratory were screened for the presence of the *mcr-1* gene. Five such isolates (four *Klebsiella pneumoniae* and one *E. coli*) were identified, but only the *E. coli* isolate proved positive for the *mcr-1*

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Table 1. *Antimicrobial susceptibility profile of the strains analysed using the VITEK 2 system*

Isolate	MIC, mg/l (S/R)														
	FOX	CAZ	CRO	FEP	DOR	ETP	IMI	MER	AMK	CN	CIP	TGC	COL	*mcr-1*	*bla*	ST
KPN 238	⩽4 (S)	⩽1 (S)	⩽1 (S)	⩽1 (S)	⩽0·12 (S)	⩽0·5 (S)	⩽0·25 (S)	⩽0·25 (S)	⩽2 (S)	⩽1 (S)	0·5 (S)	2 (R)	16 (R)	–	–	n.d.
KPN 1204191	⩾6 (R)	⩾64 (R)	⩾64 (R)	⩾64 (R)	⩾8 (R)	⩾8 (R)	⩾16 (R)	⩾16 (R)	⩾64 (R)	⩾16 (R)	⩾4 (R)	4 (R)	⩾16 (R)	–	KPC-2	n.d.
KPN 1220672	⩾6 (R)	⩾64 (R)	⩾64 (R)	⩾64 (R)	⩾8 (R)	⩾8 (R)	⩾16 (R)	⩾16 (R)	⩾64 (R)	⩾16 (R)	⩾4 (R)	4 (R)	⩾16 (R)	–	KPC-2	n.d.
ECO Z&Z1409	⩽4 (S)	16 (R)	⩾64 (R)	2 (R)	⩽0·12 (S)	⩽0·5 (S)	⩽0·25 (S)	⩽0·25 (S)	⩽2 (S)	⩽1 (S)	⩾4 (R)	2 (R)	8 (R)	+	CTX-M-55	609

FOX, Cefoxitin; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; DOR, doripenem; ETP, ertapenem; IMI, imipenem; MER, meropenem; AMK, amikacin; CIP, ciprofloxacin; TGC, tigecycline; COL, colistin; ST, sequence type; n.d., not determined.

gene. The isolate originated from a 14-year-old male patient admitted to the emergency service with a diagnosis of acute appendicitis. The appendix was removed by laparoscopy, and the presence of necrotic areas was confirmed. Samples of peritoneal liquid were taken for culture, resulting in the isolation of the colistin-resistant *E. coli* strain Z&Z1409 (susceptible to carbapenems, amikacin and gentamicin and resistant to tigecycline, ciprofloxacin and cephalosporins, according to CLSI guidelines [4] (Table 1). Species identification and antimicrobial susceptibility profiling were performed using the VITEK 2 system (bioMérieux, France). Total DNA was isolated using a High Pure PCR Template Preparation kit (Roche Diagnostics, Switzerland) and ESBL genes (bla_{SHV}, bla_{TEM}, bla_{CTX}) were amplified and sequenced as previously described [5–7]. The *mcr-1* gene was screened, and the complete coding sequence was obtained with an in-house approach designed for this study (Fig. 1). Multi-locus sequence typing analysis of the strain was performed according to the scheme of Wirth *et al.* [8]. The *E. coli* strain Z&Z1409 was of sequence type (ST) 609. This rare genotype had previously been reported in isolates from rooks (*Corvus frugilegus*) in Poland (harbouring $bla_{CTX\text{-}M\text{-}1}$) [9], Glaucous-winged gulls (*Larus glaucescens*) from Russian islands (harbouring $bla_{CTX\text{-}M\text{-}14}$) [10], dog faeces from public gardens in Denmark (harbouring $bla_{CTX\text{-}M\text{-}15}$) [11], and from a patient in Canada (harbouring $bla_{CTX\text{-}M\text{-}14}$) [12]. These reports highlight the commensal nature of ST609 in wild and companion animals and its capacity to cause human infections. It is noteworthy that the ESBL gene ($bla_{CTX\text{-}M\text{-}55}$) gene identified in our isolate (GenBank accession no. KU896134) has not previously been reported in this sequence type.

Of the four *K. pneumoniae* isolates resistant to colistin, but negative for *mcr-1*, one showed a minimum inhibitory concentration (MIC) of 8 mg/l which was within the 4–8 mg/l range recorded by Liu *et al.* [2] in their *E. coli mcr-1*-positive isolates; the remaining *K. pneumoniae* isolates gave MICs of 16 mg/l. The *mcr-1*-positive *E. coli* strain Z&Z1409 colistin MIC was consistent with the colistin MIC levels in the *E. coli* isolates observed by Liu *et al.* [2]. However, low colistin MICs (2 mg/l) have been described in *K. pneumoniae* harbouring *mcr-1* [13], raising the possibility of differences in phenotypic expression of resistance in clinical isolates and highlighting the necessity of additional research to establish the range of colistin MICs in Gram-negative bacteria other than *E. coli*

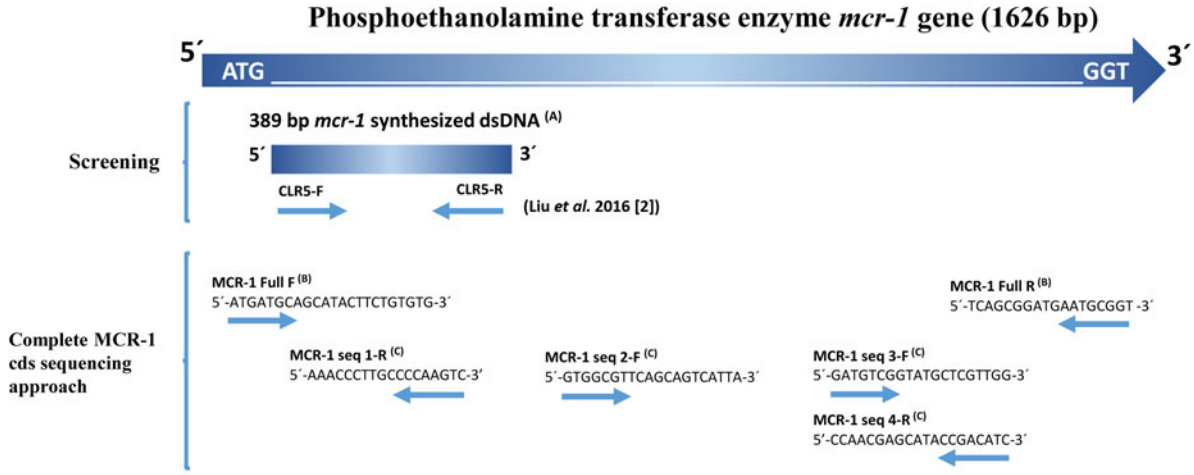

Fig. 1. Screening of *mcr-1* and sequencing approach. (A) 389 bp *mrc-1* DNA-positive control synthesized by Invitrogen (USA) in a pMA-T plasmid. (B) Primers used for amplification and sequencing. (C) Internal sequencing primers.

harbouring this gene. The complete sequence of *mcr-1* (GenBank accession no. KU886144) described here has maximum identity to the sequences deposited in GenBank, suggesting a unique *mcr-1* origin and supporting the reports of global dissemination of the gene [14]. To date, this resistance determinant has been the subject of over 30 reports from 17 countries worldwide and found in isolates from food, food animals and river water as well as infections in hospitalized patients [2, 13, 15]. The fact that our isolate was recovered from a case of acute appendicitis presenting as an emergency from the community strongly suggests that the strain was part of the patient's commensal gut flora. *E. coli* harbouring *mrc-1* has been previously described as commensal flora in Dutch travellers, presumably acquired in the Andean region [16]. On the other hand, our patient recorded his residence in the outer urban area of Quito with frequent visits to an intensive food animal production area 160 km from Quito. The increasing incidence of *mcr-1* strains has been proposed to be the result of the abuse of polymyxins in food animals [2], which can act as reservoirs and spread this resistance in the environment. In Ecuador polymyxins are approved for use in veterinary and animal food production, but the amount of usage is not available. Therefore, the contact of the patient with animal production areas was most likely a contributory risk factor for the acquisition of the strain from the environment and this is supported circumstantially by the reported association of the ST609 genotype with birds and animals [9–11]. Furthermore, the incident highlights the risk of maintaining resistant bacteria as part of the intestinal flora.

Our strain showed substantial differences in susceptibility profile and production of ESBL genes (CTX-M variant) from the clinical strains reported by Rapoport *et al.* in Argentina [17] and by Fernandes *et al.* in Brazil [18], which suggests a diversity of genetic backgrounds of *mcr-1*-positive strains and its dissemination in South America. Finally, although, the Z&Z1409 strain was not pan-resistant to antibiotics, the possible dissemination of the *mcr-1* gene remains a great concern, especially for the risk of its acquisition by carbapenem-resistant Enterobacteriaceae strains present in the microbiota or the acquisition of more resistance determinants in plasmids harbouring *mcr-1* which may result in infections that are virtually untreatable. Conjugation and plasmid analysis are currently in progress.

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DECLARATION OF INTEREST

None.

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