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Letter to the Editor

Increased colistin resistance upon acquisition of the plasmid-mediated *mcr-1* gene in *Escherichia coli* isolates with chromosomally encoded reduced susceptibility to polymyxins

Sir,

Colistin is a last-resort antibiotic for treating infections due to multidrug-resistant enterobacterial isolates. The emergence of chromosomal mutations in genes involved in the modification of lipopolysaccharide, e.g. in *pmrAB*, is responsible for colistin resistance [1,2]. However, the major source of concern is related to the recent discovery of plasmid-mediated colistin resistance genes (*mcr-1* to *-3*) owing to the risk of spread of colistin resistance [2,3]. In *Escherichia coli*, chromosomally encoded PmrAB mutations and plasmid-mediated colistin resistance are responsible for low levels of colistin resistance [colistin minimum inhibitory concentrations (MIC) < 16 mg/L] [2,4,5].

The objective of this study was to determine the level of colistin resistance resulting from the combination of chromosomal mutations and plasmid-mediated *mcr-1* gene in *E. coli*.

Four *E. coli* isolates were used in this study, with isolates FRO and MAL being recovered from human urine samples and isolate 41331 from an animal sample. The colistin-susceptible *E. coli* reference strain K12 was used for mating-out assays. MICs of colistin were determined by broth microdilution method (BMD) as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org). Briefly, BMD manual panels were prepared extemporaneously in non-treated 96-well polystyrene microplates (Sarstedt, Nümbrecht, Germany). Dilutions of colistin sulfate (Sigma-Aldrich, St Louis, MO) ranging from 0.12–128 mg/L were made in cation-adjusted Mueller–Hinton broth. The results were interpreted based on EUCAST recommendations, with susceptibility and resistance breakpoints being at ≤2 mg/L and >2 mg/L, respectively.

Isolate FRO showed decreased susceptibility to colistin, with an MIC of 2 mg/L, and sequencing of the *pmrAB* genes identified an amino acid substitution (L110*) in the PmrB protein known to be responsible for an increased colistin MIC [4]. Isolate MAL showed low-level colistin resistance (MIC = 8 mg/L) and sequencing identified an amino acid substitution (T114P) in the HAMP domain of the PmrB protein (Table 1). Neither of the isolates presented mutations in *phoP*, *phoQ* or *mgrB* genes.

Plasmid p41331 recovered from *E. coli* isolate 41331 (colistin MIC = 8 mg/L) carried both the *mcr-1* gene and the $bla_{CTX\text{-}M\text{-}1}$ gene encoding resistance to ticarcillin and broad-spectrum cephalosporins.

To determine the level of colistin resistance resulting from acquisition of the plasmid-mediated *mcr-1* gene in *E. coli* isolates MAL and FRO, plasmid p41331 was first transferred into *E. coli* K12 by mating-out assay and was then transferred into the colistin-resistant *E. coli* FRO and MAL isolates, respectively. To avoid selection with colistin that could be responsible for acquisition of additional mutations, the *E. coli* transconjugants carrying plasmid p41331 were selected using Luria–Bertani agar plates supplemented with ticarcillin (100 mg/L). Presence of the *mcr-1* gene in transconjugants was confirmed by PCR using specific primers as described previously [6].

Table 1
Molecular features and colistin minimum inhibitory concentrations (MICs) of the *Escherichia coli* parental strains and transconjugants.

Isolate [a]	Molecular features				Colistin MIC (μg/mL)	Other antimicrobial resistance
	PmrA	PmrB	MCR-1	CTX-M		
41331	WT	WT	+	+	8	NAL, CIP
K12	WT	WT	–	–	0.25	RIF, NAL
p41331-K12	WT	WT	+	+	4	RIF, NAL
FRO	WT	L110	–	–	2	None
P41331-FRO	WT	L110	+	+	8	None
MAL	WT	T114P	–	–	8	SXT, NAL, CIP
p41331-MAL	WT	T114P	+	+	32	SXT, NAL, CIP

WT, wild type; NAL, nalidixic acid; CIP, ciprofloxacin; RIF, rifampin; SXT, trimethoprim/sulfamethoxazole.
[a] 41331 was the donor strain for mating assay; K12, FRO and MAL were the recipients.

Determination of the MICs of the transconjugants revealed that the *E. coli* K12 transconjugant (p41331-K12) presented a low level of resistance (MIC = 4 mg/L), whereas *E. coli* FRO and MAL transconjugants (harbouring plasmid p41331 in addition to mutations in PmrB) exhibited higher MICs (8 mg/L and 32 mg/L, respectively) (Table 1). Acquisition of the plasmid-mediated *mcr-1* gene in isolates FRO and MAL was responsible for a four-fold increase in the MICs of colistin compared with the parental strains (2 mg/L to 8 mg/L for FRO and 8 mg/L to 32 mg/L for MAL).

This study indicates that an increase in resistance level to colistin may be achieved upon acquisition of the *mcr-1* gene in strains harbouring chromosomally encoded mutations. The two-step process leading to a higher level of resistance to colistin mirrors what is known for quinolone resistance, with a plasmid determinant conferring low-level resistance that may facilitate further selection of chromosomally encoding mechanisms eventually leading to high-level resistance [7]. These results further highlight that acquisition of the *mcr-1* gene may have a very significant clinical impact, contributing to a higher level of colistin resistance.

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