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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Aurélie Jayol
Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland
INSERM European Unit (LEA-IAME Paris, France), University of Fribourg, Switzerland
National Reference Center for Emerging Antibiotic Resistance, University of Fribourg, Switzerland
CNRS UMR5234, University of Bordeaux, France
Laboratory of Bacteriology, University Hospital of Bordeaux, France

Patrice Nordmann
Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland
INSERM European Unit (LEA-IAME Paris, France), University of Fribourg, Switzerland
National Reference Center for Emerging Antibiotic Resistance, University of Fribourg, Switzerland
University of Lausanne and University Hospital Center, Lausanne, Switzerland

Catherine André
CNRS UMR5234, University of Bordeaux, France

Véronique Dubois
CNRS UMR5234, University of Bordeaux, France
Laboratory of Bacteriology, University Hospital of Bordeaux, France

Laurent Poirel *
Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland
INSERM European Unit (LEA-IAME Paris, France), University of Fribourg, Switzerland
National Reference Center for Emerging Antibiotic Resistance, University of Fribourg, Switzerland

* Corresponding author. Medical and Molecular Microbiology, Department of Medicine, Faculty of Science, University of Fribourg, rue Albert Gockel 3, CH-1700 Fribourg, Switzerland.
E-mail address: laurent.poirel@unifr.ch (L. Poirel)

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