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CASE REPORT

Plasmid-mediated colistin resistance *mcr-1* in a clinical isolate of *Escherichia coli* ST19 in Algeria

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ABSTRACT

We describe the emergence of plasmid-mediated colistin resistance *mcr-1* in a clinical isolate of *Escherichia coli* ST19. The strain was isolated from a urine sample of a 75-year-old male patient. The identification was carried out by an automated system. The antibiogram was performed according to CLSI recommendations. The colistin MIC was determined by broth micro-dilution according to CA-SFM/EUCAST criteria. The detection of *mcr-1* was carried out by immunochromatographic test and PCR *mcr-1*. Genetic transfers and determination of the plasmid incompatibility group were made. The ST was carried out by MLST using the Pasteur scheme.

The strain has a high-level penicillinase resistance phenotype. It was susceptible to amikacin and resistant to gentamicin, tobramycin, fluoroquinolones, tetracycline, chloramphenicol, and cotrimoxazole. The strain was resistant to colistin (MIC = 4 µg/ml).

The genetic transfer is given a colistin-resistant transconjugant. The plasmid carrying *mcr-1* gene belongs to the incompatibility group IncHI2.The strain belongs to ST19. *J Microbiol Infect Dis 2019; 10(4):222-224.*

Keywords: *Colistin resistance, mcr-1, Escherichia coli ST19*

INTRODUCTION

Colistin, a polypeptide antibiotic, is showing renewed interest in the treatment of multidrug-resistant Gram-negative bacilli. However, resistance appeared either by chromosomal mutations or by plasmid acquisition. The plasmid-mediated colistin resistance (*mcr* gene) was first described in 2015 [1]. Since then, several authors have reported the emergence of this mechanism in both veterinary medicine and human medicine [2]. The risk of transfer of *mcr* gene makes fearful its spread, especially if selection pressure by the polypeptides is exerted.

We describe the emergence of plasmid-mediated colistin resistance *mcr-1* in a clinical isolate of *Escherichia coli* sequence type (ST) 19.

CASE

In 2014, an *E. coli* strain was isolated from a urine sample of a 75-year-old male patient, diabetic and hypertensive, presenting an adenoma of the prostate. CBEU (a bacteriological urine test) was motivated by the presence of pollakiuria, urinary incontinence, and urinary burns.

The identification of the strain was carried out by the "Vitek 2 Systems". The antibiogram was performed according to CLSI recommendations [3].

Colistin minimal inhibitory concentration (MIC) was determined by broth micro dilution, reference technique recommended by CA-SFM / EUCAST (4), using the colistin sulfate powder and the cations-adjusted MH broth (CAMHB), the dilution range of colistin were from 0.016 to 128 µg/ml.

The detection of *mcr-1* was performed by immunochromatographic rapid test "NG-Test

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MCR-1" ® and confirmed by polymerase chain reaction (PCR) *mcr-1* using primers; F: 5' GCAGCATACTTCTGTGTGGTAC 3', R: 5' TATGCACGCGAAAGAAACTGGC 3' [5].

In order to demonstrate that the resistance is carried by a plasmid, genetic transfers by conjugation in *E. coli* J5 Azide were made. Genetic transfer was followed by typing the plasmid to determine the incompatibility group.

The Sequence Type of the isolated strain was carried out by Multi Locus Sequence Typing (MLST) according to Pasteur scheme (bigsdb.pasteur.fr/ecoli/ecoli.html) by sequencing the genes*: dinB, icdA, pabB, polB, putP, trpA, trpB* and *uidA* [6].

In addition, we conducted the search for genes for β-lactams resistance: penicillinases (*bla*TEM, *bla*SHV) and extended spectrum β-lactamase (*bla*CTXM1, *bla*CTXM2, *bla*CTXM8, *bla*CTXM9), for plasmid fluoroquinolones resistance (qnrA, qnrB, qnrS and aac 6 '-Ib-cr) and we also search for class 1, 2 and 3 integrons.

Antibiotic susceptibility testing reveals a high-level penicillinase resistance phenotype with resistance to ampicillin, ticarcillin and piperacillin, intermediate resistance to cefazolin, susceptibility to amoxicillin / clavulanic acid, 3rd and 4th generation cephalosporins and carbapenems.

The strain is susceptible to amikacin and resistant to gentamicin, tobramycin, fluoroquinolones, tetracycline, chloramphenicol, sulfonamides, trimethoprim, cotrimoxazole and colistin with a MIC of 4 µg / ml (MICs breakpoint values CA-SFM / EUCAST: susceptible: ≤2 µg/ml, Resistant: >2 µg/ml).

The results of rapid test "NG-Test *MCR-1*" ® and *mcr-1* PCR were positives. The transconjugant obtained by conjugation was colistin resistant, with detection of *mcr-1*gene by PCR demonstrating the transferability of this resistance. The plasmid carrying this gene belongs to Incompatibility Group IncHI2.

PCR revealed, also, the presence of the penicillinase gene *bla*TEM as well as the class 1 integron. The result of the MLST shows an *E. coli* ST19 genotype (dinB: 7, icdA: 37, pabB: 4, polB: 10, putP: 26, trpA: 7, trpB: 4, uidA: 2).

Table1. Comparison between the isolated strain in our case and strains described in the literature.

Cases	Origin	ST	MLST scheme	colistin MIC (µg/ml)	ESBL	NDM	Plasmid carrying *mcr-1*	Reference
Our Case	Human (Urine)	*E. coli* ST19	Pasteur	4	-	-	IncHI2	
Venezuela	Human (Feces)	*E. coli* ST19	Pasteur	4	+	+ NDM1	IncI2	(10)
Brazil	Human (blood)	*E. coli* ST156*	Warwick	4	-	-	IncX4	(11)
Algeria	Human (sperm)	*E. coli* ST405	Warwick	8	+	-	IncFIB	(8)
Algeria	Human (urine)	*E. coli* ST405	Warwick	4	+	-	/	(9)
China	Animal (Duck)	*E. coli* ST156*	Warwick	/	+	+ NDM5	IncI2	(12)
Algeria	Animal (Barbary macaques)	*E. coli* ST405	Warwick	4	+	-	/	(7)

*Corresponding to ST19 in Pasteur scheme: ST= Sequence type, MLST= Multi locus sequence typing, MIC= Minimal inhibitory concentration, ESBL= Extended-spectrum beta-lactamase, NDM= New Delhi Metallo-beta-lactamase.

DISCUSSION

Plasmid-mediated colistin resistance in E coli has already been described in Algeria in wild animals [7] and clinical isolates [8,9]. These strains belonged to the ST 405 clone according to the Warwick scheme

(https://enterobase.warwick.ac.uk/species/ecoli/allele_st_search), while our isolate belongs to the ST 19 clone (bigsdb.pasteur.fr/ ecoli / ecoli.html) which corresponds to ST 156 of the Warwick data base.

The bibliography reports human cases of plasmid-mediated colistin resistance in E coli ST19: In Venezuela, the strain was also producing a metallo-carbapenemase type NDM1 [10] and in Brazil, in this case the strain was susceptible to carbapenems [11]. Animal case have also been described in China in ducks [12].

In our case, we report the notion of eating frozen meat that may be the source of the contamination.

The plasmid carrying the *mcr-1* gene belonged to incompatibility group IncHI2, already described in the literature [13,14] . Indeed, the plasmids most frequently encountered belong to incompatibility group IncI2, IncX4 and more rarely IncP, IncFII, IncHI1 and IncHI2 [2].

In table 1, we compare the mains results obtains with those of the literature

Conclusion:

The emergence of plasmid mediated colistin resistance raises fears of horizontal spread with a risk of therapeutic impasse if faced with a multidrug resistant bacterium. It is therefore imperative to monitor the emergence of this type of resistance in the laboratory, by the determination of colistin MIC, the performance of rapid diagnostic tests and PCR.

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