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Plasmidic resistance to colistin mediated by *mcr-1* gene in *Escherichia coli* clinical isolates in Argentina: A retrospective study, 2012–2018

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ABSTRACT

Objective. To describe the resistance profile and the genetic characteristics of *Escherichia coli* isolates that harbor the mobilizable colistin resistance gene *mcr-1* in Argentina.

Methods. This was a retrospective study of 192 *E. coli* isolates positive for *mcr-1* obtained from 69 hospitals of Buenos Aires City and 14 Argentinean provinces in 2012 – 2018. The antimicrobial susceptibility was performed by agar diffusion, broth macrodilution, and/or agar dilution. Standard polymerase chain reaction (PCR) was performed to detect resistance genes and incompatibility groups; specific PCR was applied to discriminate between $bla_{CTX\text{-}M}$ allelic groups and *mcr-1.5* variant. The genetic relatedness among isolates was evaluated by XbaI-pulsed field gel electrophoresis and multilocus sequence typing in a subset of isolates.

Results. All *E. coli* isolates showed minimal inhibitory concentrations to colistin ≥ 4μg/mL; nearly 50% were resistant to third-generation cephalosporins, with CTX-M-2 being the main extended-spectrum β-lactamase detected. Five *E. coli* were carbapenemase-producers (3 NDM, 2 KPC). The *mcr-1.5* variant was detected in 13.5% of the isolates. No genetic relationship was observed among the *mcr-1*-positive *E. coli* clinical isolates, but a high proportion (164/192; 85.4%) of IncI2 plasmids was detected.

Conclusions. The presence of IncI2 plasmids among highly diverse *E. coli* clones suggests that the *mcr-1* gene's wide distribution in Argentina may be driven by the horizontal transmission of IncI2 plasmids.

Keywords: Drug resistance, multiple; colistin; *Enterobacteriaceae*; *Escherichia coli;* Argentina.

Polymyxins, including polymyxin B and colistin, are "last-line" treatment options against multidrug-resistant (MDR) gram-negative bacteria, such as carbapenem-resistant *Enterobacterales*. Until November 2015, the main colistin resistance mechanisms reported were chromosome-mediated mutations involving alterations in the PmrAB or PhoPQ, a two-component regulatory system (1). The situation changed with the report of mobile colistin resistance mediated by *mcr-1* gene, revealing for the first time the horizontal spread of a colistin resistance determinant (2). This gene encodes a plasmid-borne phosphoethanolamine transferase that has been reported in *Escherichia coli* isolates from animal, food, environment, and human samples worldwide (3, 4). At this time, nine *mcr* genes have been described; but, *mcr-1* is, by far, the most prevalent (3 – 5). *Mcr-1* has been described in almost all countries in the Region of the Americas, while *mcr-3* and *mcr-5* genes have been sporadically

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described in only Brazil and Colombia, respectively (3, 5 – 7). Although detected in other *Enterobacterales*, the *mcr-1* gene has been associated mainly with *E. coli* isolates, including *Klebsiella pneumoniae* and *Salmonella* spp. (5). Regardless of species, *mcr-1* has been associated with a ~2609 bp DNA fragment containing the *mcr-1* and *pap2* genes, and has mobilized into an *ISApI1*-based composite transposon and different plasmid replicons, of which IncI2, IncX4, and IncHI2 are the most common incompatibility groups described so far (8 – 10).

Mcr-1-positive *E. coli* isolates causing bloodstream infections are still rare (1.0%), and generally, they remain susceptible to many antimicrobial agents (11). However, it is especially worrisome that *mcr-1* gene acquisition by extended-spectrum β-lactamase- (ESBL) or carbapenemase-producing *Enterobacterales*, render extensively- or pan-drug resistant strains (3, 10 – 15). NDM has been the main carbapenemase reported in *mcr-1*-positive isolates, not only in hospitalized patients where it colonizes or causes infections, but also in healthy individuals within the community; sporadic cases of *mcr-1*-producers harboring KPC or OXA-48 carbapenemases have also been described (10 – 15).

After the description of *mcr-1* in November 2015, the National Reference Laboratory on Antimicrobial Resistance in Argentina (NRL) set national and regional alerts for detection of *mcr-1* gene in *E. coli* clinical isolates (16). Through December 2018, a total of 192 *E. coli* clinical isolates were confirmed as positive for *mcr-1* at the NRL. The present study aims to describe the resistance profiles and the genetic characteristics of *mcr-1*-producing *E. coli* clinical isolates in Argentina.

FIGURE 1. Geographical distribution of 192 *mcr-1* positive *Escherichia coli* clinical isolates, by province and capital city, Argentina, 2012 – 2018

Source: Prepared by the authors from the study results.

MATERIALS AND METHODS

This retrospective study comprised the entire NRL collection: 192 *E. coli* clinical isolates (one per patient) previously confirmed as *mcr-1* positive, submitted by 69 hospitals from 14 of Argentina's provinces and Buenos Aires City (Figure 1). Isolates were recovered mainly from urine (n = 117; 60.9%), blood (n = 26; 13.6%), and other samples (n = 49; 25.5%). The first 10 isolates were recovered in July 2012 – January 2016, as previously described by Rapoport and colleagues (17) and Martino and colleagues (18). The remaining 182 isolates were submitted from February 2016 – December 2018, with minimal inhibitory concentration (MIC) to colistin > 2µg/ml and/or a positive growth on Mueller-Hinton screening agar plates containing 3 µg/mL colistin (19).

Data on sample type and patient age and sex were obtained from the clinical laboratory documentation that accompanied each isolate. Patient data were anonymized to preserve the patient´s identity.

Susceptibility profiles were determined by the agar diffusion method, with the exception of colistin which was tested by broth macrodilution and/or agar dilution according to the Clinical and Laboratory Standards Institute guidelines (CLSI; 20). CLSI criteria were used to interpret all results, except for colistin and tigecycline, for which the 2018 European Committee on Antimicrobial Susceptibility Testing guidelines (21) were used. ESBL- or plasmidic-AmpC-phenotype were defined as resistant to third-generation cephalosporins with clavulanic acid or phenyl boronic acid inhibition, respectively.

PCR was performed to detect *mcr-1*, plasmidic-AmpC, broad-spectrum and ESBL, and carbapenemases using the primers and conditions described in Table 1. Briefly, DNA templates were prepared by boiling for 10 min a suspension of one or two colonies of each isolate in 100 µL of Milli-Q water; 2.5µL were used for the PCR reactions. A final volume of 25µL containing 10 pmol of each primer, 25 µM of each dNTP, 1.5 mM MgCl2, 1X Taq buffer, and 2.5 U of Taq polymerase (Invitrogen,™ ThermoFisher Scientific Inc., Waltham, MA, United States) was used. Amplifications were performed using a 2720 Thermal Cycler™ (Applied Biosystems, ThermoFisher Scientific Inc., Waltham, MA) following a standard program: pre-denaturation for 5 min at 94°C; 35 cycles of 94°C for 30 sec,

TABLE 1. Primers for PCR analysis of antimicrobial resistance mechanisms.

Target	Primer	Oligonucleotide sequence	Amplicon size (bp)	T °C annealing
mcr-1	CLR5-F	5´ CGGTCAGTCCGTTTGTTC 3´	309	45
	CLR5-R	5´ CTTGGTCGGTCTGTAGGG 3´		
mcr-1.5	MCR-1.5-F	5´ TCCAGTGGCTGCAGAAGT 3´	288	62
	MCR-1 Full-R	5´ TCAGCGGATGAATGCGGT 3´		
bla_{NDM}	NDM-F	5´ AGCACACTTCCTATCTCGAC 3´	512	50
	NDM-R	5´ GGCGTAGTGCTCAGTGTC 3´		
bla_{IMP}	IMP-UF1	5´ GGYGTTTWTGTTCATACWTCKTTYGA 3´	404	50
	IMP-UR1	5´ GGYARCCAAACCACTASGTTATCT 3´		
bla_{VIM}	VIM-F	5´ AGTGGTGAGTATCCGACAG 3´	261	50
	VIM-R	5´ ATGAAAGTGCGTGGAGAC 3´		
bla_{CTX-M}	CTX-MU1	5´ ATGTGCAGYACCAGTAARGT 3´	593	52
	CTX-MU2	5´ TGGGTRAARTARGTSACCAGA 3´		
CTX-M-G2	CTXM2G-F	5´ GCCGCTCAATGTTAACGGTGA 3´	851	55
	CTXM2G-R	5´ ACCGTGGGTTACGATTTTCGC 3´		
CTX-M-G9	CTXM9G-F	5´ ATGGTGACAAAGAGAGTGCAACG 3´	808	56
	CTXM9G-R	5´ GCGGCTGGGTAAAATAGGTCACC 3´		
CTX-M-G1/15	CTXM1/15G-F	5´ CAGTTCACGCTGATGGCGACG 3´	756	60
	CTXM1/15G-R	5´ CGGCGCACGATCTTTTGGCCA 3´		
CTX-M-G8/25	CTXM8/25G-F	5´ CTGGAGAAAAGCAGCGGGGG 3´	604	58
	CTXM8/25G-R	5´ CGCTGCCGGTTTTATCCCCGAC 3´		
bla_{PER}	PER-U-Fw	5´ GTGTGGGAGCCTGACGATCT 3´	524	59
	PER-U-Rv	5´ CTSTGGTCCTGTGGTGGTTTC 3´		
bla_{TEM}	OT-1	5´ TTGGGTGCACGAGTGGGTTA 3´	504	55
	OT-2	5´ TAATTGTTGCCGGGAAGCTA 3´		
bla_{SHV}	OS1	5´ TCGGGCCGCGTAGGCATGAT 3´	626	59
	OS2	5´ AGCAGGGCGACAATCCCGCG 3´		
bla_{CMY}	CITMF	5´ TGGCCAGAACTGACAGGCAAA 3´	462	64
	CITMR	5´ TTTCTCCTGAACGTGGCTGGC 3´		

Source: Prepared by the authors from the study results.

T°C annealing (Table 1) for 30 sec, 72°C for 30 sec, and final extension at 72°C for 5 min. If the expected amplicon was ≥ 700 bp, the elongation step of cycling was increased to 1 min and the final elongation to 10 min. PCR products were run on 1% agarose gel for 60 min and stained with ethidium bromide. PCR was also used to discriminate between $bla_{CTX-M-2}$, $bla_{CTX-M-1/15}$, $bla_{CTX-M-8/25}$, and $bla_{CTX-M-9/14}$ groups (Table 1). Identification of incompatibility groups IncI2, IncX4, and IncHI2 was analyzed by PCR using previously described conditions (22, 23). To identify the *mcr-1.5* variant, we developed an allele specific PCR that detected the C1354T modification (H452Y). A degenerated primer, MCR-1.5-F, with a mismatch at the third base from the 3´extreme was designed to increase the PCR specificity (Table 1).

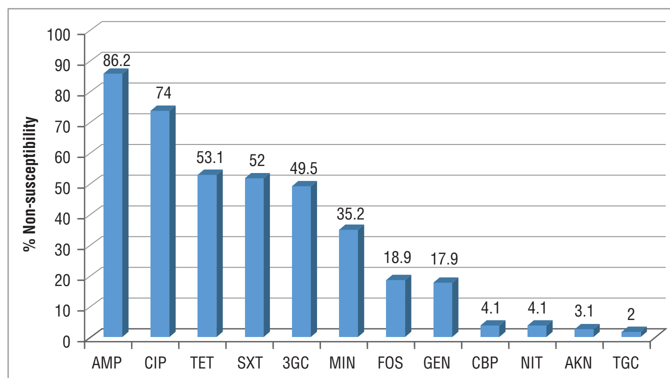
The genetic relatedness between the isolates was evaluated by XbaI-digested pulsed-field gel electrophoresis (PFGE) using a Chef-DR® III System (Bio-Rad™, Hercules, CA, United States) as previously reported (24). DNA fragments were resolved in 1% agarose gel applying a switch time of 2.4 to 54.2 seconds during 20 hr at 14°C. Those PFGE patterns showing > 6 bands of difference were considered to be non-genetically related. Selected isolates were also genotyped by multilocus sequence typing (MLST) and the sequence types (STs) were analyzed according to the *E. coli* MLST (available from http://enterobase.warwick.ac.uk/species/index/ecoli).

RESULTS

All 192 strains from the *E. coli* clinical isolates harboring *mcr-1* strains grew on Mueller-Hinton screening agar plates containing 3 µg/mL colistin and showed MIC to colistin ≥ 4µg/mL. According to Figure 2, the percentage of resistance to other antimicrobials was as follows: ampicillin (85.9%), ciprofloxacin (74.9%), tetracycline (65.2%), trimethoprim-sulfamethoxazole (52.7%), third-generation cephalosporins (cefotaxime and/or ceftazidime; 48.9%), minocycline (39.7%), fosfomycin (23.9%), gentamicin (17.2%), carbapenems (ertapenem and/or imipenem; 3.6%), nitrofurantoin (5.0%), amikacin (2.6%), and tigecycline (2.3%). Nine isolates were resistant only to colistin, while 80.2% showed an MDR phenotype (resistance to > 2 families of antimicrobials).

Nearly one-half of the isolates (*n* = 94; 48.9%) showed resistance to third-generation cephalosporins, with ESBLs being the main mechanism (*n* = 77; 81.9%), followed by AmpC (*n* = 12; 12.8%) and carbapenemases (*n* = 5; 5.3%). The ESBLs detected were CTX-M (*n* = 73; 94.8%), SHV (*n* = 3; 3.9%), and PER-2 (*n* = 1; 1.3%). Bla_{CTX-M} genes were grouped by sequence-similarity-based PCR as follow: CTX-M-2 (*n* = 37; 50.7%); CTX-M-9/14 (*n* = 21; 28.8%); CTX-M-8/25 (*n* = 11; 15.0%), and CTX-M-1/15 (*n* = 4; 5.5%). Nine of 12 isolates showing AmpC-phenotype were positive for bla_{CMY-2} plasmidic-AmpC gene. Carbapenemases

FIGURE 2. Resistance profile of 192 *mcr-1*-positive *Escherichia coli* clinical isolates in Argentina, 2012 – 2018



Note: AMP = ampicillin; CIP = ciprofloxacin; TET = tetracycline; SXT = trimethoprim-sulfamethoxazole; 3GC = third-generation cephalosporins (cefotaxime and/or ceftazidime); MIN = minocycline; FOS = fosfomycin; GEN = gentamicin; CBP = carbapenem (ertapenem and/or imipenem); NIT = nitrofurantoin; AKN = amikacin; TGC = tigecycline.
Source: Prepared by the authors from the study results.

were detected in 5 isolates from five hospitals in four cities that had been recovered from screening (n = 2), blood (n = 2), and urine samples (n = 1). As shown in Table 2, these carbapenemases were characterized as bla_{NDM-1} (n = 3) and bla_{KPC-2} (n = 2). Those isolates harboring bla_{NDM-1} were also positive for bla_{CMY-6} variant and *rmtC* genes. The presence of the *mcr-1.5* variant was confirmed in 26 isolates (13.5%). Other *mcr-1* variants or mcr-genes (*mcr-2* to *mcr-9*) were not evaluated.

Among 110 *E. coli* analyzed by PFGE (57.3%), a high genetic diversity was observed defining 103 pulsotypes, while 7 isolates were repeatedly non-typeable. MLST was analyzed in the 5 carbapenemase-producing *E. coli* isolates identifying 5 unrelated STs: ST10, ST156, ST354, ST8492, and a SLV-ST5208 (adk: 10; fum: 7; gyr: 265; icd: 8; mdh: 12; purA: 8; recA: 194).

Mcr-1-bearing plasmids previously characterized from Argentina belonged to the IncI2 incompatibility group with ca. 60 Kb in size (26 – 28). Considering these previous results, a PCR to detect the IncI2 group was performed on all 192 isolates. A high prevalence (164/192; 85.4%) of IncI2 group was observed among the strains. In the 28 IncI2-negative isolates, the presence of IncX4 and IncHI2 incompatibility groups was evaluated, with IncX4 being detected in 18 isolates (9.4%). The remaining 10 isolates (5.2%) were negative for IncI2, IncX4, and IncHI2 incompatibility groups.

DISCUSSION

The global distribution of *mcr-1* gene shows that most belong to *E. coli* and only a few belong to other bacterial species (7, 25). This low frequency of *mcr-1* in non-*E. coli* species is an epidemiological characteristic of the *mcr-1* gene dissemination (5, 7, 25). The prevalence of *mcr-1* gene in *E. coli* and *K. pneumoniae* recovered from bloodstream infections is still low, ~1.0% and ~0.2%, respectively; nevertheless, the clinical impact of this mechanism is not fully understood (10, 26). However, in two recent global surveillance studies, a high proportion of *mcr-1* gene was detected among colistin-resistant *E. coli* clinical isolates, ranging from 32.2% – 42.2% (3, 6). Analyzing data collected in 2012 – 2018 through the National Antimicrobial Resistance Surveillance Network WHONET-Argentina (91 hospitals), among *Enterobacterales* clinical isolates (excluding community onset infections), an incremental 4.3-fold in colistin resistance was observed in *E. coli* (0.3% to 1.3%; $P < 0.0001$) and 2.7-fold in *K. pneumoniae* (3.3% to 8.9%; $P < 0.0001$); but no significant difference was observed for *E. cloacae* (2.4% to 2.1%; $P = 1$). The rise of colistin resistance in hospital settings may be associated with its increased use in treating human infections caused by MDR or extensively-drug resistant *Enterobacterales*, particularly KPC-producing *K.* pneumoniae, and carbapenem-resistant *Pseudomonas aeruginosa* or *Acinetobacter baumannii*.

The present study observed a wide distribution of *mcr-1 E. coli* clinical isolates, most recovered from urine samples, in several provinces and Buenos Aires City. A high proportion of the isolates showed an MDR profile, and nearly one-half were resistant to third-generation cephalosporins, which reduces therapeutic options for systemic infections mainly to carbapenems, aminoglycosides, or combined therapies. The main mechanism of resistance to third-generation cephalosporins was mediated by ESBLs, with CTX-M being the more relevant, as observed by Wise and colleagues in a global analysis (3).

Infections caused by carbapenemase-producing bacteria have high morbidity and mortality rates, so colistin has become a last-resort option for treatment. In Argentina, bla_{KPC-2} is the main carbapenemase among *Enterobacterales*; however, during recent years, an increase in detection of bla_{NDM-1} has been observed, especially in *Providencia* spp. (14, 18, 24, 27). In the present collection, 5 carbapenemase-producing *mcr-1*-positive *E. coli* isolates were detected, yielding an extensively drug-resistance phenotype for which the unique therapeutic option was tigecycline. These 5 carbapenemase-producing *E. coli* isolates were assigned to unrelated STs; nevertheless, one of them was ST10, the predicted founder of clonal complex 10 (CC10). The CC10 lineage has been defined as an epidemic clone, presents intrinsic ability to acquire antimicrobial resistance genes (including *mcr-1)*, and has been detected in human and animal samples (8). In a previous study, we reported the case of a pediatric patient infected or colonized with 5 NDM-1-producing *Enterobacterales*, including *E. coli* M17386 *mcr-1* isolate (Table 2). This study provides evidence of intra-patient dissemination of a bla_{NDM-1} harboring plasmid among 5 *Enterobacterales* species (18). Additionally, a *C. amalonaticus* clinical isolate—a species rarely reported to cause human infections, harboring 16 resistance genes including bla_{NDM-1} and *mcr-1* determinants borne on different plasmids—was recently described in our country (14). Worldwide, detection of carbapenem- and *mcr-1* colistin-resistant *Enterobacterales* clinical isolates has also been reported, with NDM, KPC, and OXA-48 being the main enzymes described (10 – 15).

The *mcr-1.5* variant was previously described only in three countries: Japan, Bolivia, and Argentina (13, 28, 29). In the present collection, 13.5% of the isolates were positive for this variant. Moreover, the *mcr-1.5* variant was detected in 8 of 10 plasmids from *E. coli* isolates recovered from healthy chickens on commercial farms, and in 4 of 12 *E. coli* from diarrheic piglets and healthy fattening pigs in Argentina (30). Therefore, tracking the distribution of *mcr-1.5* among *E. coli* isolates from food-producing animals and from humans may be a clue to understanding the dissemination of this gene among different sources.

High clonal diversity among *mcr-1 E. coli* isolates was observed, as it has been by other authors (2, 3), suggesting that clonal expansion is not involved in the spread of this mechanism. To date, all *mcr-1* plasmids from human isolates in

TABLE 2. Epidemiological data of five carbapenemase-producing *mcr-1* positive *Enterobacterales* clinical isolates

INEI ID	Species	Date	Sample	Hosp.	Province	Genes	CIM COL	Resistance profile	ST
M17386	*E. coli*	May 2014	Blood	A	CABA	bla_{NDM-1}; bla_{CMY-6}; *mcr-1*	≥ 4µg/mL	3GC, CBP, GEN, AMK, COL	10
M19637	*E. coli*	September 2015	Blood	B	Córdoba	bla_{KPC-2}; *mcr-1*	≥ 4µg/mL	3GC, CBP, CIP, MIN, SXT, COL	156
M21069	*E. coli*	April 2016	Screening	C	Santa Cruz	bla_{KPC-2}; *mcr-1*	≥ 4µg/mL	3GC, CBP, TET, MIN, COL	5208-like
M23101	*E. coli*	March 2018	Screening	D	CABA	bla_{NDM-1}; bla_{CMY-6}; *mcr-1*	≥ 4µg/mL	3GC, CBP, CIP, TET, MIN, GEN, AMK, SXT, NIT, COL	354
M23335	*E. coli*	May 2018	Urine	E	Entre Ríos	bla_{NDM-1}; bla_{CMY-6}; *mcr-1*	≥ 4µg/mL	3GC, CBP, CIP, GEN, AMK, COL	8492

Note: Hosp = hospital; CABA = Ciudad Autonoma de Buenos Aires; 3GC = third generation cephalosporins (cefotaxime and/or ceftazidime); CBP = carbapenem (ertapenem and/or imipenem); GEN = gentamicin; AKN = amikacin; COL = colistin; CIP = ciprofloxacin; MIN, minocycline; SXT = trimethoprim-sulfamethoxazole; TET = tetracycline; NIT = nitrofurantoin; NA = not applicable; ST = sequence type. ST5208-like is a SLV of ST5208 (adk: 10; fum: 7; gyr: 265; icd: 8; mdh: 12; purA: 8; recA: 194).

Source: Prepared by the authors from the study results.

Argentina were characterized as a ca. 60kb IncI2 plasmid (14, 28, 31). Similarly, in the present study, a high proportion (85.4%) of IncI2 plasmids was detected. Additionally, the same IncI2 plasmids have been reported in *mcr-1 E. coli* isolates recovered from gulls, chicken, dogs, and pigs in Argentina (30, 32, 33). The high proportion of IncI2 plasmids among genetically diverse *E. coli* isolates suggests that in Argentina, these plasmids might be the main vehicle for horizontal dissemination of *mcr-1* among human and animal isolates. However, finding IncX4 plasmids in 9.4% of the isolates may indicate recent changes in the genetic platforms involved in *mcr-1* dissemination in our country. A different epidemiological scenario seems to occur in Brazil, where IncX4 has been the major incompatibility group reported among *mcr-1* harboring plasmids, while IncA/C2 and IncHI2 are rarely reported (7, 8). Moreover, IncX4 and IncI2 were the main incompatibility groups detected in other Latin American countries as well (5, 8). Interestingly, both IncX4 and IncI2 plasmids carry *mcr-1* gene as the unique determinant of resistance, while IncHI2 generally harbor multiple resistance determinants (7, 8).

The One Health approach is directed to design and implement programs, policies, legislation, and research to combat antibiotic resistance among multiple sectors, including human, animal, and environmental health. Colistin is known to be used widely to prevent infection and promote growth in food-producing animals (1, 2, 34). The use of colistin in food animals is believed to be responsible for the emergence and transmission of *mcr*-genes (1, 2). It has been suggested that *mcr*-carrying plasmids move from animals to humans, since *mcr*-genes are prevalent in animal food production, which is where the most colistin is consumed (1, 4, 34). According to the National Antimicrobial Resistance Surveillance (35), within the National Service for Safety and Quality of Food and Agriculture of Argentina, high levels of colistin resistance were observed in poultry (31.5%), cattle (16.5%), and pigs (15.0%). In Argentina, *mcr-1* gene has been reported not only in *E. coli* isolates recovered from chicken and swine, but also from pets and wild birds (32, 33). This suggests that this gene is successfully circulating among different environments. Given the situation and to reserve this drug for treating human infections, in January 2019 the Ministry of Agriculture of Argentina banned the use of colistin for veterinary purposes (available from http://servicios.infoleg.gob.ar/infolegInternet/anexos/315000-319999/318811/norma.htm). This initiative aims to intensify stewardship efforts for this last-resort antibiotic.

Limitations

This study's main limitation was that the presence of other *mcr*-genes (*mcr-2* to *mcr-9*) was not evaluated, nor were *mcr*-genes among species other than *E. coli*. Even though this collection of *mcr-1 E. coli* isolates represents the largest and most diverse in Argentina, local and/or regional epidemiological differences are probable and expected.

Conclusions

The study findings show that *mcr-1 E. coli* is circulating among several provinces in Argentina. *Mcr-1 E. coli* is associated with MDR, with CTX-M being the main ESBL. Although infrequently, *mcr-1 E. coli* co-producing NDM or KPC carbapenemases have emerged. The presence of IncI2 plasmids among highly diverse *E. coli* clones indicates that they have driven wide distribution of the *mcr-1* gene among clinical isolates through horizontal transmission.

This study provides a basic framework for understanding the molecular epidemiology of *mcr-1*-positive *E. coli* in Argentina. For comprehensive picture from the perspective of One Health, further studies are essential to understanding the dissemination of *mcr-1* gene through the environment.

Author contributions. DF and AC conceived the original idea. DF, MR, EA, FP, and AC planned the experiments. DF, MR, EA, FC, JDM, DDB, CL, SG, DD, and MG collected, contributed, and analyzed the data. DF, MR, EA, CL, SG, FP, and AC interpreted the results. DF and AC wrote the paper. All authors reviewed and approved the final version.

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Resistencia plasmídica a colistina mediada por el gen *mcr-1* en aislamientos clínicos de *Escherichia coli* de Argentina: Un estudio retrospectivo, 2012–2018

RESUMEN

Objetivo. Describir el perfil de resistencia y las características genéticas de aislamientos clínicos de *Escherichia coli* que portan el gen movilizable de resistencia a colistina *mcr-1* en Argentina.

Métodos. Se realizó un estudio retrospectivo para analizar 192 aislamientos de *E. coli mcr-1* positivo, obtenidos en 69 hospitales de la Ciudad de Buenos Aires y 14 provincias de Argentina entre 2012 y 2018. La sensibilidad a los antimicrobianos se analizó mediante los métodos de difusión en agar, macrodilución en caldo y/o dilución en agar. Se aplicó la técnica estándar de reacción en cadena de la polimerasa (PCR) para detectar genes de resistencia y grupos de incompatibilidad; se aplicó PCR específica para distinguir entre variantes alélicas del gen $bla_{CTX\text{-}M}$ y la variante *mcr-1.5*. La relación genética entre los aislamientos fue evaluada mediante la técnica de electroforesis en gel de campo pulsado usando la enzima XbaI y la tipificación por secuencias de múltiples locus en un subconjunto de aislamientos.

Resultados. Todos los aislamientos de *E. coli* mostraron concentraciones inhibitorias mínimas de colistina ≥ 4µg/mL. Casi el 50% mostró resistencia a las cefalosporinas de tercera generación y CTX-M-2 fue la β-lactamasa de espectro extendido que más se detectó. Cinco aislamientos de *E. coli* mostraron ser productoras de carbapenemasas (3 NDM, 2 KPC). La variante *mcr-1.5* se detectó en 13,5% de las cepas aisladas. No se observó relación genética entre los aislamientos clínicos estudiados de *E. coli* positivas para *mcr-1*, aunque sí se detectó una proporción elevada (164/192; 85,4%) de plásmidos IncI2.

Conclusiones. La elevada ocurrencia de plásmidos IncI2 en un grupo altamente diverso de clones de *E. coli* podría indicar que la amplia difusión del gen *mcr-1* en Argentina estaría asociada a la transmisión horizontal de plásmidos IncI2.

Palabras clave Resistencia a múltiples medicamentos; colistina; *Enterobacteriaceae*; *Escherichia coli;* Argentina.

Resistência à colistina em plasmídeos mediada pelo gene *mcr-1* em isolados clínicos de *Escherichia coli* da Argentina: Um estudo retrospectivo, 2012–2018

RESUMO

Objetivo. Descrever o perfil de resistência e as características genéticas de isolados clínicos de *Escherichia coli* que carregam o gene mobilizábel de resistência à colistina *mcr-1* na Argentina.

Métodos. Neste estudo retrospectivo, foram analizados 192 isolados de *E. coli* positivos para *mcr-1* obtidos em 69 hospitais da Cidade de Buenos Aires e 14 províncias da Argentina, entre 2012 e 2018. A sensibilidade aos antimicrobianos foi examinada usando métodos de difusão em ágar, macrodiluição em caldo e/ou diluição em ágar. A técnica padrão de reação em cadeia da polimerase (PCR) foi aplicada para detectar genes de resistência e grupos de incompatibilidade; a PCR específica foi aplicada para discriminar entre variantes alélicas do gene $bla_{CTX\text{-}M}$ e a variante *mcr-1.5*. A relação genética entre os isolados foi avaliada por eletroforese em gel de campo pulsado usando a enzima XbaI e a tipagem por sequências de múltiplos lócus, em um subconjunto de isolados.

Resultados. Todos os isolados de *E. coli* apresentaram concentrações inibitórias mínimas de colistina ≥4µg/mL. Quase 50% foram resistentes às cefalosporinas de terceira geração, e CTX-M-2 foi a β-lactamase de espectro estendido mais detectada. Cinco isolados de *E. coli* foram produtores de carbapenemase (3 NDM, 2 KPC). A variante *mcr-1.5* foi detectada em 13,5% dos isolados. Não foi observada relação genética entre os isolados clínicos de *E. coli* positivos para *mcr-1*, mas foi detectada uma alta proporção (164/192; 85,4%) de plasmídeos IncI2.

Conclusões. A alta ocorrência de plasmídeos IncI2 em um grupo altamente diverso de clones de *E. coli* sugere que a ampla distribuição do gene *mcr-1* na Argentina estaria associada a transmissão horizontal de plasmídeos IncI2.

Palavras-chave Resistência a múltiplos medicamentos; colistina; *Enterobacteriaceae*; *Escherichia coli;* Argentina.