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Original

Escherichia coli and *Salmonella* spp. *mcr-1* gene carriers at a pig slaughterhouse in Medellín (Colombia)

Carlos A. Palacio-Arias[1]; Astrid V. Cienfuegos-Gallet[2]; Jorge A Fernández-Silva[1]; Laura Vásquez-Jaramillo[1]*.

[1]Universidad de Antioquia, Facultad de Ciencias Agrarias, Escuela de Medicina Veterinaria, grupo de investigación Centauro, Ciudadela Robledo, Medellín, Colombia.
[2]Universidad de Antioquia, Escuela de Microbiología, Grupo de Microbiología Molecular, Medellín, Colombia.
*Correspondencia: laura.vasquezj@udea.edu.co

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ABSTRACT

Objective. This study aimed to evaluate the acquired *mcr-1* gene-mediated colistin resistance in *Escherichia coli* and *Salmonella* spp. isolates obtained from fecal samples in pigs destined for human consumption at slaughterhouse located in Medellín (Colombia). **Materials and methods.** A descriptive study was carried out, in which 190 fecal samples were collected from pigs at the slaughterhouse in March 2020. Colistin sulfate-supplemented chromogenic and MacConkey agars (2 mg/l) were used for the screening of colistin-resistant enterobacteria. The selected isolates were analyzed by PCR to identify the presence of the *mcr-1* gene. Bacterial identification and antibiotic susceptibility profile were performed on *mcr-1* gene-positive isolates by the automated Microscan® system. The information was collected and analyzed using descriptive statistics. **Results.** The 70.52% (134/190) of the animals were positive for colistin-resistant isolates by the screening test. The 15.78% (30/190) of the isolates were *mcr-1* gene carriers, of which 1.05% (2/190) belong to *Salmonella enterica* species and 4.21% (8/190) were *E. coli*. A multiple antibiotics resistance profile (10/10) and an extended-spectrum beta-lactamase (ESBL) -producing *E. coli* were identified in all the isolates carrying the *mcr-1* gene. Most of the pigs with enterobacteria carrying the *mcr-1* gene came from farms located in the province of Antioquia, and all belonged to the growing-finishing production stage. **Conclusions.** This study evidences the circulation of the *mcr-1* type gene in pigs at the time of slaughter, representing a potentially serious threat to public health due to possible implications in the food chain.

Keywords: Drug resistance; colistin; *Enterobacteriaceae;* Foodborne diseases (*Source: DeSC*).

RESUMEN

Objetivo. Evaluar la resistencia adquirida a colistina mediada por el gen *mcr-1* en aislados de *E. coli* y *Salmonella* spp., de muestras de materia fecal de porcinos con destino a consumo humano en planta

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de beneficio animal de Medellín, Colombia. **Materiales y métodos.** Se realizó un estudio descriptivo, tomando 190 muestras de materia fecal de porcinos durante marzo del 2020. Para la tamización de enterobacterias resistentes a colistina se utilizó agar cromogénico y MacConkey suplementado con sulfato de colistina (2 mg/l). Los aislados fueron analizados mediante PCR para identificar el gen *mcr-1,* identificación bacteriana y perfil de susceptibilidad antibiótica a los aislados positivos al gen *mcr-1* por el sistema automatizado Microscan®. La información fue registrada y analizada mediante estadística descriptiva. **Resultados**. La frecuencia de porcinos con aislados resistentes a colistina en la tamización fue del 70.52% (134/190). El 15.78% (30/190) portadores del gen *mcr-1* de los cuales el 1.05% (2/190) pertenecieron a la especie *Salmonella enterica* y el 4.21% (8/190) *E. coli.* Se identificó en los aislados positivos al gen *mcr-1* la capacidad de resistir la acción de múltiples antibióticos (10/10), y una *E. coli* con la habilidad de producir betalactamasas de espectro extendido (BLEE). La mayoría de los cerdos con enterobacterias portadoras del gen *mcr-1* provenían de granjas del departamento de Antioquia, y todos en etapa de levante y ceba. **Conclusiones**. Este estudio evidencia la circulación del gen *mcr-1* en cerdos al sacrificio, representando un riesgo potencial para la salud pública por su posible entrada a la cadena alimentaria.

Palabras clave: Colistina; enfermedades transmitidas por los alimentos; *Enterobacteriaceae*; Fármacoresistencia microbiana (*Fuente: DeSC*).

INTRODUCTION

Escherichia coli and *Salmonella* spp. are commensal enteric bacteria common to humans and animals, important due to their potential zoonotic risk and in their causal role in foodborne diseases (FBDs), with important effects on the economy of the countries (1). These bacteria are etiological agents that cause diseases in the pig industry as well, leading to important economic losses due to increased mortality, decreased weight gain, and treatment-related costs (2).

Colistin is an antibiotic of the polymyxin family, widely used in veterinary medicine, especially for treating enteric infections in pigs, and even as a growth promoter (3), and for the treatment of enteric infections (4). Likewise, due to the emergence of Gram-negative pathogens resistant to third-generation cephalosporins and carbapenems, colistin was reintroduced for clinical use in humans between the 1980s and the mid-1990s worldwide (5,6).

As a consequence of the colistin's widespread use, an increase in its related resistance has been reported in bacteria isolated from both humans and animals (7). Resistance to colistin is due to the modification of the bacterial lipopolysaccharide through a chromosomal mechanism —mediated by mutations in the regulatory system of PhoPQ-PmrAB, and a plasmid mechanism, mediated by the mobile colistin resistance (*mcr*) gene (4). The latter is considered the most important mechanism because of its rapid spread, even among pathogenic bacteria of different species through horizontal transfer mechanisms (5).

Resistance to colistin by *mcr* was initially reported in China in *E. coli* and *Klebsiella pneumoniae* of pig origin at an animal slaughterhouse, in pork and chicken (4). Currently, 10 *mcr* variants have been reported (8,9,10,11,12,13), being the *mcr-1* the most frequent (14). Specifically, in pigs, the *mcr-1* gene has been identified in *E. coli* and *Salmonella* spp. isolates obtained from healthy and sick pigs at the farm level, in slaughterhouses, and in raw meat in different Asian countries, Europe, and America (15).

In Colombia, the presence of the *mcr-1* gene has been reported in human clinical *E. coli*, *Salmonella enterica* serovar Typhimurium, and *Klebsiella pneumoniae* isolates (16). In addition, the gene was detected in *Salmonella enterica* from food of animal origin, sausages, and raw meat (17), and in 86 isolates of *E. coli* from a pig farm (18). Recently, the detection of this same gene was reported in 43.2% (240/555) of cryopreserved isolates of *Salmonella* spp. from pigs, birds, cattle, horses, dogs, fish, guinea pigs, and morrocoy turtles from different locations in the country (19).

Considering the frequent use of colistin in the pig industry, and the possibility that pigs act as reservoirs of bacteria carrying resistance genes, it is important to monitor the frequency of presentation of resistant pathogenic bacteria at the slaughterhouse level, due to the high probability of reaching humans through the consumption of meat. Therefore, this study aimed to evaluate the acquired *mcr-1* gene-mediated colistin resistance in *Escherichia coli* and *Salmonella* spp. isolates obtained

from fecal samples in pigs destined for human consumption at a slaughterhouse located in Medellín (Colombia).

MATERIALS AND METHODS

Type of study. This is a cross-sectional descriptive study.

Study location. This study was carried out from March 2nd to 11th, 2020, at one slaughterhouses located in Medellín, province of Antioquia (Colombia). The pigs came from the Colombian provinces of Antioquia and Caldas and were all raised on modernized farms, which reached the growing-finishing stage satisfactorily.

Selection of animals. A total of 190 pigs were randomly selected through systematic sampling. The animals were subjected to *antemortem* inspection and identified with the batch mark, which remained visible throughout the process.

Sample collection. One gram (1g) of feces was collected directly from the cecum, then placed in a sterile container, and immediately diluted in 2 ml of saline solution, following the recommendations of the manufacturer of CHROMID Colistin R agar (COLR®) (BioMérieux, Lyon, France). The samples were stored and transported under refrigeration to the Diagnostic Unit of the Faculty of Agricultural Sciences (Universidad de Antioquia, Medellín) for immediate processing. A thermometer was used to ensure that the temperature did not exceed 4°C.

Isolation of colistin-resistant *Escherichia coli* and *Salmonella* spp. For the selective isolation (screening) of *E. coli and Salmonella* spp*,* 50 µl of each sample was diluted in 9ml of brain heart infusion (BHI, Merk, Darmstadt, Germany) broth with a colistin disk (COL 10µg) (Oxoid, Basignstoke, UK). The broths were incubated at 35±2°C for 4 to 5 hours. Subsequently, 50µl of the previously incubated broths were seeded by depletion on CHROMID Colistin R agar (COLR®) chromogenic agar (BioMérieux, France), and incubated again at 35±2°C for 18 to 24 hours. According to the manufacturer's instructions, *E. coli*-presumptive colonies have a burgundy-pink color, and those *Salmonella* spp.-presumptive colonies are white or colorless. The *E. coli* National Type Culture Collection NCTC® 13846® strain was used as the positive control, and the *E. coli* American Type Culture Collection ATCC® 25922® strain was used as the negative one.

A maximum of two colonies of each morphotype (pink-burgundy and white/colorless) were taken from each sample, and later planted on MacConkey agar (Merk, Darmstadt, Germany) supplemented with colistin sulfate at 2 mg/L and then incubated at 35±2°C for 18 to 24 hours.

The colonies with the greatest morphological similarity to the species of interest were selected from the MacConkey agar. Subsequently, the selected colonies were reseeded in 3ml of BHI broth supplemented with colistin sulfate at 2mg/L and incubated again at 35±2°C for 18 to 24 hours, and then frozen at -80°C in a 300µl suspension of 50% glycerol and 700 µl of bacterial growth in BHI.

Detection of the mcr-1 gene. For bacterial DNA extraction, the Wizard® Genomic DNA Purification Kit (Madison, United States) was used. The presence of the mcr-1 gene was evaluated by PCR using the primers described by Liu et al (3). The PCR products were determined by 1% agarose gel electrophoresis. Strain BK47554 (mcr-1 positive isolate) donated by the Kreiswirth Laboratory of the Center for Discovery and Innovation (CDI, New Jersey, USA) was used as the positive control.

Bacterial identification. Bacterial identification of positive isolates for the mcr-1 gene was done using minimum inhibitory concentration (MIC) Gram-negative panels/combined panels and NC72 panels specific for such bacteria (Beckman Coulter, Indianapolis, United States), using the Microscan® system (Beckman Coulter, Indianapolis, United States), and following the manufacturer's recommendations.

Antimicrobial susceptibility. The antimicrobial susceptibility test was performed using the broth microdilution method, via NC72 panels specific for Gram-negative bacteria (Beckman Coulter, Indianapolis, United States).

A total of 21 antibiotics were evaluated and interpreted according to the breakpoints indicated by CLSI (20): ampicillin, ampicillin/sulbactam, aztreonam, cefoxitin, ticarcycline/clavulanic acid, cefotaxime, cefepime, piperacillin/tazobactam, ceftazidime, amikacin, gentamicin, tobramycin, tetracycline, levofloxacin, trimethoprim/sulfamethoxazole, cefuroxime, imipenem, meropenem, ertapenem, and nalidixic acid. The

E. coli ATCC 25922 strain was used for quality control.

Analysis of data. Data were stored, processed, and analyzed using descriptive statistics in Excel 2003 software (Microsoft Office, Redmond, Washington).

Ethical considerations. The study was approved by the Ethics Committee for Experimentation with Animals (CEEA, by its name in Spanish) of the Universidad de Antioquia (Act No. 127, September 2019).

RESULTS

Characteristics of the study population. The animals of study came from 40 different farms, 38 of them from the province of Antioquia, and two from the province of Caldas. Most of the pigs came from the municipalities of Donmatías 41.57% (79/190), Yolombó 8.42% (16/190), and Medellín 6.84% (13/190), all located in Antioquia. All the animals belonged to the growing-finishing production stage, of which 48.42% (92/190) were females and 51.58% (98/190) males (Table 1).

Table 1. Characterization of isolates with phenotypic resistance to colistin and *mcr-1* gene carriers.

Animal features	Isolates with presumptive colistin-resistance (chromogenic agar)			Isolates with plasmid mediated colistin-resistance (mcr-1)		
	Positive (n=134) n (%)	Negative (n=56) n (%)	Total (n=190) n (%)	Positive (n=30)n (%)	Negative (n=19)n (%)	Total (n= 49)n (%)
Municipality of origin						
Barbosa	6 (4.5)	5 (8.9)	**11 (5.7)**	1 (3.3)	3 (15.8)	**4 (8.2)**
Bello	4 (2.9)	1 (1.8)	**5 (2.6)**	1 (3.3)	2 (10.5)	**3 (6.1)**
Belmira	4 (2.9)	0 (0)	**4 (2.1)**	0 (0)	1 (5.2)	**1 (2)**
Betania	7 (5.2)	0 (0)	**7 (3.7)**	2 (6.7)	2 (10.5)	**4 (8.2)**
Donmatías	54 (40.2)	25 (44.6)	**79 (41.8)**	10 (33.3)	4 (21.1)	**14 (28.6)**
El Retiro	1 (0.7)	1 (1.8)	**2 (1.1)**	0 (0)	0 (0)	**0 (0)**
Entrerríos	6 (4.5)	1 (1.8)	**7 (3.7)**	3 (10)	1 (5.2)	**4 (8.2)**
Fredonia	4 (2.9)	3 (5.3)	**7 (3.7)**	1 (3.3)	1 (5.2)	**2 (4.1)**
Maceo	4 (2.9)	1 (1.8)	**5 (2.6)**	0 (0)	1 (5.2)	**1 (2)**
Medellín	10 (7.5)	3 (5.3)	**13 (6.9)**	5 (16.7)	1 (5.2)	**6 (12.2)**
Neira*	3 (2.2)	0 (0)	**3 (1.6)**	1 (3.3)	0 (0)	**1 (2)**
Rionegro	5 (3.7)	7 (12.5)	**12 (6.3)**	3 (10)	0 (0)	**3 (6.1)**
San Andrés de Cuerquia	4 (2.9)	0 (0)	**4 (2.1)**	2 (6.7)	0 (0)	**2 (4.1)**
Santa Rosa de Osos	4 (2.9)	2 (3.6)	**6 (3.2)**	0 (0)	0 (0)	**0 (0)**
Santo Domingo	3 (2.2)	3 (5.3)	**6 (3.2)**	0 (0)	0 (0)	**0 (0)**
Viterbo*	3 (2.2)	0 (0)	**3 (1.6)**	0 (0)	0 (0)	**0 (0)**
Yolombó	12 (8.9)	4 (7.1)	**16 (8.4)**	1 (3.3)	3 (15.8)	**4 (8.2)**
Production stage						
Growing-finishing	134 (100)	56 (100)	**190 (100)**	30 (100%)	19 (100)	**49 (100)**
Sex						
Female	65 (48.5)	27 (48.4)	**92 (48.2)**	14 (46.7)	10 (52.6)	**24 (49)**
Male	69 (51.5)	29 (51.8)	**98 (51.8)**	16 (53.3)	9 (47.4)	**25 (51)**
Bacteria						
Escherichia coli	106 (55.8)	84 (44.2)	**190 (100)**	22 (62.9)	13 (37.1)	**35 (100)**
Salmonella spp.	75 (39.5)	115 ((60.5)	**190 (100)**	8 ((57.1)	6 (42.9)	**14 (100)**

*Municipality of the province of Caldas, Colombia

Colistin-resistant *Escherichia coli* and *Salmonella* spp. isolates. *Escherichia coli* and *Salmonella* spp. growth was obtained in 70.52% (134/190) of the samples cultured in the chromogenic medium for the detection of colistin resistance. A 56% (106/190) were *E. coli*-compatible and 39% (75/190) with *Salmonella* spp. (Table 1). The growth of both bacterial species was found in 20.52% (39/190) of the samples, whereas that no growth was observed in 29.5% (56/190).

Detection of the *mcr-1* gene. 49 colistin-resistant isolates were obtained, all maintaining the phenotypic characteristics related to the species of interest. These isolates were processed for the detection of *mcr-1* by PCR. In total, the *mcr-1* gene was detected in 15.78% (30/190) of the samples (Table 1).

Bacterial identification of isolates carrying the *mcr-1* gene. Of the 30 isolates carrying the mcr-1 gene, 15 isolates could be recovered after freezing. Of these, 4.21% (8/190) corresponded to E. coli, 1.05% (2/190) to S. enterica, and 2.6% (5/190) to *Providencia heimbachae*. The presence of the mcr-1 gene was identified in two different species (*S. enterica* and *P. heimbachae*) in one of the farms in the municipality of Medellín, Antioquia.

Evaluation of antimicrobial susceptibility in Escherichia coli and Salmonella spp. mcr-1 gene carriers. In the 10 isolates of *E. coli* and *S. enterica* carrying the *mcr-1* gene, resistance to tetracycline (10/10) was observed, followed by trimethoprim/sulfamethoxazole, and ampicillin (8/10), ampicillin/sulbactam (5 /10), piperacillin/tazobactam, nalidixic acid and tobramycin (3/10), gentamicin, cefuroxime and cefotaxime (2/10), and ceftazidime (1/10). Sensitivity (10/10) was observed for levofloxacin and carbapenems (Table 2).

For *E. coli*, resistance to ampicillin (7/8), ampicillin/sulbactam (4/8), followed by piperacillin/tazobactam (3/8), cefuroxime (2/ 8), and for aztreonam, ceftazidime and cefotaxime (1/8), and to trimethoprim/sulfamethoxazole (6/8) was observed. In the group of aminoglycosides, resistance was obtained for tobramycin (2/8), gentamicin (1/8), and sensitivity to amikacin (8/8). Quinolones and fluoroquinolones resistance were only observed for nalidixic acid (2/8), and complete sensitivity for levofloxacin (8/8). It was identified that isolate E1092 was positive for ESBL-production, presenting resistance to cefotaxime and cefuroxime. All isolates were sensitive to the group of carbapenems (Table 2).

For *S. enterica*, trimethoprim/sulfamethoxazole and tetracycline resistance (2/2) was observed. In the beta-lactam group, resistance was observed for ampicillin, ampicillin/sulbactam, and aztreonam (1/2). In aminoglycosides, resistance to gentamicin and tobramycin (1/2) and sensitivity to amikacin were observed. Only one isolate resistant to nalidixic acid was found for quinolones and fluoroquinolones. The isolates were sensitive to levofloxacin and to the carbapenem group (Table 2).

In general, it was observed that all the isolates (10/10) presented a multiresistance pattern, characterized by being resistant to an agent in at least three categories of antibiotics (21).

Table 2. Antimicrobial susceptibility profile of *Escherichia coli* and *Salmonella* spp. *mcr-1* gene carriers

Isolate	AM		A/S		AZT		CFX		TIM		CFT		CPE		CL		P/T		CAZ		AK	
	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I
Salmonella spp.																						
4S1	<8	S	<8/4	S	16	R	<8	I	<16	S	2	I	<2	S	>4	R	<16	S	4	S	32	I
153S1	>16	R	>16/8	R	8	I	<8	S	<16	S	2	I	<2	S	>4	R	64	I	<1	S	<16	S
E. coli																						
58E1	>16	R	>16/8	R	<4	S	<8	S	<16	S	<1	S	<2	S	>4	R	<16	S	<1	S	<16	S
181E2	>16	R	8/4	I	<4	S	<8	S	<16	S	<1	S	<2	S	>4	R	<16	S	<1	S	<16	S
183E2	>16	R	>16/8	R	<4	S	<8	S	<16	S	<1	S	<2	S	>4	R	<16	S	<1	S	<16	S
187E1	>16	R	<8/4	S	<4	S	<8	S	<16	S	<1	S	<2	S	>4	R	<16	S	<1	S	<16	S
109E2	>16	R	>16/8	R	8	I	16	I	<16	S	16	R	8	I	>4	R	>64	R	8	I	<16	S
119E1	16	I	<8/4	S	8	I	<8	S	<16	S	2	I	<2	S	>4	R	>64	R	4	S	<16	S
167E1	>16	R	>16/8	R	>16	R	16	I	64	I	8	R	8	I	>4	R	>64	R	>16	R	32	I
57E1	>16	R	<8/4	S	<4	S	<8	S	<16	S	<1	I	<2	S	>4	R	<16	S	<1	S	<16	S

	GM		TO		TE		LVX		T/S		CRM		IMP		MER		ETP		NA	
	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I	MIC	I
Salmonella spp.																				
4S1	8	I	8	I	>8	R	2	S	>2/38	R	<4	S	<1	S	<1	S	<0,5	S	>16	R
153S1	>8	R	>8	R	>8	R	<1	S	>2/38	R	16	S	<1	S	<1	S	<0,5	S	<16	S
E. coli																				
58E1	8	I	<4	S	>8	R	<1	S	>2/38	R	<4	S	<1	S	<1	S	<0,5	S	<16	S
181E2	<4	I	<4	S	>8	R	<2	S	>2/38	R	<4	S	<1	S	<1	S	<0,5	S	<16	S
183E2	<4	S	<4	S	>8	R	<2	S	>2/38	R	<4	S	<1	S	<1	S	<0,5	S	<16	S
187E1	<4	S	<4	S	>8	R	<2	S	<2/38	S	8	I	<1	S	<1	S	<0,5	S	<16	S
109E2	>8	R	>8	R	>8	R	<2	S	>2/38	R	>16	R	<1	S	<1	S	<0,5	S	<16	S
119E1	<4	S	<4	S	>8	R	<2	S	>2/38	R	8	I	<1	S	<1	S	<0,5	S	>16	R
167E1	8	I	>8	R	>8	R	<2	S	>2/38	R	>16	R	<1	S	<1	S	<0,5	S	>16	R
57E1	<4	I	<4	S	>8	R	<2	S	<2/38	S	8	I	<1	S	<1	S	<0,5	S	<16	S

MIC: minimum inhibitory concentration, I: interpretive category. Antibiotics: Ampicillin (AM), ampicillin/sulbactam (A/S), aztreonam (AZT), Cefoxitin (CFX), ticarcycline/clavulanic acid (TIM), cefotaxime (CFT), cefepime (CPE), colistin (CL), piperacillin/tazobactam (P/T), ceftazidime (CAZ), amikacin (AK), gentamicin (GM), tobramycin (TO), tetracycline (TE), levofloxacin (LVX), trimethoprim/sulfamethoxazole (T/S), cefuroxime (CRM), imipenem (IMP) , meropenem (MER), ertapenem (ETP), nalidixic acid (NA), sensitive (S), intermediate (I), resistant (R).

DISCUSSION

In the present study, the presence of *Salmonella* spp. and *E. coli* phenotypically resistant to colistin and carriers of the *mcr-1* gene, was identified in different regions of the province of Antioquia and in a municipality of the province of Caldas in Colombia.

All the pigs belonged to the raising-fattening stage, where the pig is considered ready to be processed in the slaughterhouse. The identification of bacteria with resistance genes at this stage poses a risk due to the possibility of dissemination through the meat chain due to contamination of products (22). The equivalent distribution of isolates harboring the *mcr*-1 gene in relation to the sex of the animals is possibly due to the fact that there is no differential management by sex of the animals destined for fattening.

The identification of the *mcr-1* gene in different animals from the same farm could be explained by different reasons, such as the use of colistin as a prophylactic (23), the direct contact between carrier and non-carrier pigs or exposure to a contaminated environment (24). Therefore, good livestock practices, could help to reduce the permanence of antibiotic resistance genes at the farm level, limiting the therapeutic use of antibiotics such as colistin (4).

The identification of the *mcr-1* gene in pig isolates from different municipalities of Antioquia could suggest that colistin resistance is a widespread problem in the province. The need to produce more and better has led to the implementation of preventive measures based on the use of antibiotics (25). However, the gene has also been reported in other provinces of Colombia, indicating that the presentation of this gene can occur in places with low and high pig production (19).

In particular, a study where clinical isolates of Gram-negative bacteria resistant to colistin from human samples were analyzed between 2002 and 2016, phenotypic resistance was reported in 8.7% (513/5887) and the presence of the *mcr-1* gene by 2.3% (12/513), being Antioquia, Valle del Cauca and Santander, where the largest number of resistant isolates and carriers of the *mcr-1* gene were identified (16).

The frequency of *E. coli* carrying the *mcr-1* gene in the present study (4.21%) is higher than that reported in slaughterhouses in Europe of 0.7% (26), and considerably lower than those reported in pigs at a slaughterhouse in China of 21% (3) and 25.5% at farm-level (24). This could be explained by the strict controls established in the different regulations issued by the European Union in the use of antibiotics in primary production, contrary to the massive use of colistin in agriculture in China (3).

Concerning the frequency of *S. enterica* carrying the *mcr-1* gene (1.05%), a previous study in Colombia reported the presence of the *same* in 23.3% of *Salmonella* spp. isolates from samples collected between 2006 and 2018 in pig farms from 17 provinces of the country (19). Likewise, the differences with the present study, could explain the low frequency found, more so if one considers account, that as of 2018 the use of colistin as a growth promoter was prohibited in Colombia by Resolution 22747 of 2008. Similarly, the frequency found in this study —although slightly higher, is closer to that reported in pig carcasses in European slaughterhouses of 0.1% (26). These results suggest that properly implemented surveillance and control programs to limit the use of colistin in pig production as a growth promoter can have a positive impact on the reduction of resistance, as has been shown by countries such as China (27). This is why the One Health strategy has recognized surveillance of antimicrobial resistance of zoonotic origin throughout the meat chain as beneficial and necessary (28).

Based on the hypothesis by Liu et al. (3), about the possible origin of the *mcr-1* gene at the pig farm level, the detection of the *mcr-1* gene in these enterobacteria isolated from pigs at the time of slaughter, generates great public health concern, and reinforces the need to strengthen quality and safety controls in the different pork transformation processes, guaranteeing product safety and consumer protection.

Similarly, since animal excreta are considered agricultural biofertilizers (29), the presence of enterobacteria carrying the *mcr-1* gene in the feces of farmed pigs could be a source of environmental contamination, generating negative impacts on both water resources and soil, turning them into reservoirs and sources of pollution, when it is used as fertilizer for crops and pastures (30). Particularly for *Salmonella* spp., its capacity to adhere or enter agricultural products such as vegetables and fruits has been identified, defining such products as possible infection or dissemination sources of resistance mechanisms (31).

As a result of the present study, the identification of the *mcr-1* gene in isolates of the genera *Providencia* and *Salmonella* in the same farm could indicate the gene´s horizontal transfer through mobile genetic elements between different species of enterobacteria, accelerating its dissemination (32). *Providencia heimbachae* is a Gram-negative bacterium that has intrinsic resistance to colistin (33), being of public health importance as it has been reported as an etiological agent in diarrhea in piglets (34).

It is possible that the samples with phenotypically resistant isolates in which the *mcr-1* gene was not detected, presented chromosomal mechanisms of antimicrobial resistance through the modification of the antimicrobial target site (33), or harbor another of the 10 variants of the *mcr* gene that have been reported to date (9).

The identification of multiresistant isolates, generates great concern due to their extensive resistance mechanisms, critically reducing the possible therapeutic options (21). In particular, isolates of the *E. coli* 109E2 species are highlighted —which were only susceptible to the carbapenem and fluoroquinolone groups and isolate 167E1. Due to these findings, the problem of the appearance of such bacteria with a wide spectrum of resistance to antimicrobials is emphasized, specifically at the livestock level, where their presence can generate disease outbreaks, high mortality, and economic losses (22).

The findings of our study show that the isolates that harbored the *mcr-1* gene showed high resistance, especially to ampicillin and tetracycline, in agreement with what was reported by Arenas et al. (22). Authors found that the non-therapeutic use of antibiotics constituted the greatest selective pressure related to the use of antibiotics in livestock practices at the national level in Colombia.

The *E. coli* and *Salmonella* spp. ESBL-producers and *mcr*-type gene carriers have been reported in different contexts in both animals and humans (35). Although in the present study only one isolate presented such a feature, the finding of joint resistance to third and fourth generation cephalosporins, as well as to colistin mediated by mobile genetic elements in enterobacteria, continues to represent a great challenge in control strategies in the use of antibiotics in both humans and animals under the One health approach (36).

The present study had limitations. First, due to the health emergency of COVID-19, decreed in March 2020 at the national level, it was not possible to reach the *a priori* (n=321) sample size calculated to estimate the prevalence of both colistin-resistance and the presence of *mcr-1* gene. This study focused on the detection of the *mcr-1* gene, due to its high frequency; however, it is possible that the isolates may present other variants of this gene. Finally, it was not possible to carry out a greater characterization of the productive systems at the farm level due to the limited records available in the slaughterhouse, which would have provided valuable data to study the *mcr-1* gene frequency and dissemination in *E. coli* and *Salmonella* spp. of pig origin.

In conclusions this study found a high frequency of acquired phenotypic resistance to colistin and the circulation of the *mcr-1*-like gene in pigs at slaughter, demonstrating the effect that prior use of colistin may have on the selection of resistant isolates in these animals. Therefore, and in order to limit the spread of these resistance genes in the food chain and the environment, monitoring of this phenomenon from a One Health approach should be considered to determine the impact of widespread use of antibiotics such as colistin in food-producing animals in human and animal health and identify the critical points that must be intervened to ensure the safety of products and the protection of ecosystems.

Conflicts of interest

The authors have no conflicts of interest.

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