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Short Communication

Multidrug-resistant *Escherichia coli* harbouring *mcr-1* and bla_{CTX-M} genes isolated from swine in Argentina

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ABSTRACT

Objectives: Multidrug-resistant *Escherichia coli* isolates recovered from food-producing animals are a global public-health concern, especially those with transferable mechanisms of antimicrobial resistance such as extended-spectrum β-lactamase (ESBL) and *mcr-1* genes. Here we report for the first time *E. coli* recovered from diarrhoeic and healthy pigs harbouring bla_{CTX-M} and/or *mcr-1* from Argentinean farms.
Methods: During 2017, a total of 34 *E. coli* were recovered from 31 faecal samples from diarrhoeic piglets and healthy fattening pigs from five pig farms in three Argentinean provinces. Antimicrobial susceptibility was evaluated by agar diffusion and resistance genes were identified by PCR. Multiplex PCR was applied to screen for ST69, ST73, ST95 and ST131 clones. Genetic relationships were evaluated by *Xba*I-PFGE.
Results: A high diversity of resistance profiles was observed (20 profiles among 34 isolates), and 71% of isolates were multidrug-resistant. Resistance to third-generation cephalosporins (3GCs) was observed in 28 isolates and was associated with bla_{CTX-M} (24), bla_{CMY} (3) and bla_{PER-2} (1) genes. bla_{CTX-M} alleles were grouped by specific PCR as follow: 17 $bla_{CTX-M-8/25}$; 4 $bla_{CTX-M-1/15}$; 2 $bla_{CTX-M-2}$; and 1 $bla_{CTX-M-9/14}$. Twelve isolates were positive for *mcr-1*, of which six were also resistant to 3GCs and were positive for $bla_{CTX-M-8/25}$ (4), $bla_{CTX-M-1/15}$ (1) or bla_{CMY} (1). High genetic diversity was observed, discriminating 29 profiles. One ST131 and two ST95 human-associated clones were detected.
Conclusion: Here we describe *E. coli* isolates recovered from diarrhoeic piglets and healthy fattening pigs harbouring ESBL and/or *mcr-1* genes. 3GC resistance was mainly associated with CTX-M, in particular with $bla_{CTX-M-8/25}$ alleles.

1. Introduction

Antimicrobial use in food-producing animals can lead to the selection and dissemination of antimicrobial-resistant bacteria, which can then be transmitted to humans via food and other transmission routes [1]. Reports of multidrug-resistant (MDR) *Escherichia coli* isolates recovered from food-producing animals are a global public-health concern. In veterinary medicine, colistin and third-generation cephalosporins (3GCs) have been used both as prophylaxis and metaphylaxis [2,3]. Colistin has been widely used to prevent infection and as a growth promoter in food-producing animals. In January 2019, use of colistin for veterinary purposes was banned in Argentina by the former Ministry of Agriculture. This measure included the preparation, distribution, import, use and possession of any formulation containing colistin and its salts (http://servicios.infoleg.gob.ar/infolegInternet/anexos/315000-319999/318811/norma.htm). This innovative regulation sought to preserve the use of colistin only for the treatment of human infections. Recently, a mobile colistin resistance gene (*mcr-1*) located on transferable plasmids has been reported in several bacterial species from animals, animal food products, humans and environmental samples [4]. Production of extended-spectrum β-lactamase (ESBL) enzymes, including the TEM-, SHV- and CTX-M-types, confers resistance to frequently used β-lactam antimicrobials, including 3GCs such as ceftriaxone, ceftazidime and ceftiofur [5]. The bla_{CTX-M} family are the most widely reported ESBLs in

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Gram-negative bacilli [6]. Notably, CTX-M -type ESBL-producing isolates have acquired co-resistance to additional classes of antimicrobial agents such as fluoroquinolones and aminoglycosides [7]. The *mcr-1* gene has also been identified in several MDR bacteria such as ESBL-producing and carbapenemase-producing *E. coli* of chicken and swine origin [3]. Since 2005, the World Health Organization (WHO) has produced a regularly updated list of all antimicrobials currently used for human medicine (most also used in veterinary medicine) grouped into three categories based on their importance to human medicine. The list is intended to assist in managing antimicrobial resistance, ensuring that all antimicrobials, especially critically important antimicrobials (including 3GCs and colistin), are used prudently both in human and veterinary medicine [8].

The aim of this study was to determine the presence of *mcr-1* and $bla_{\text{CTX-M}}$ genes in *E. coli* isolated from diarrhoeic and healthy pigs in Argentina.

2. Materials and methods

During 2017, a total of 31 faecal samples from diarrhoeic piglets (2–3 days old) and healthy fattening pigs from five pig farms in three Argentinean provinces (Entre Ríos, Santa Fe and San Luis) were recovered. Samples were incubated overnight at 37 °C in buffered peptone water. Enriched cultures (30 μL) were inoculated on Mac Conkey agar plates containing 4 μg/mL cefotaxime and/or 2 μg/mL colistin. Antimicrobial susceptibility was evaluated by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [9], except for colistin for which resistance was evaluated as growth or not on Mueller–Hinton screening agar plates containing 3 μg/mL colistin.

PCR was performed to detect *mcr-1* and common ESBL and plasmidic AmpC β-lactamase genes, and specific PCR was also used to discriminate between $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-1/15}}$, $bla_{\text{CTX-M-8/25}}$ and $bla_{\text{CTX-M-9/14}}$ groups [10]. The genetic relationship between the isolates was evaluated by *Xba*I-digested pulsed-field gel electrophoresis (*Xba*I-PFGE) [11].

3. Results

A total of 34 *E. coli* were recovered from 31 faecal samples, including 22 from cefotaxime-containing plates, 6 from colistin-containing plates and 6 from cefotaxime + colistin-containing plates. The rates of non-susceptibility of the *E. coli* isolates were as follows: ampicillin, 100%; tetracycline, 82%; cefotaxime, 80%; chloramphenicol, 53%; ciprofloxacin, 56%; minocycline, 51%; trimethoprim/sulfamethoxazole, 41%; colistin, 35%; gentamicin, 15%; and fosfomycin, 6%. All of the isolates (100%) were susceptible to amikacin, imipenem and tigecycline. A high diversity of resistance profiles was observed, with 20 profiles among the 34 isolates (Fig. 1). Moreover, 24 isolates (71%) were categorised as MDR.

A total of 24 *E. coli* were positive for a $bla_{\text{CTX-M}}$ gene, comprising 17 $bla_{\text{CTX-M-8/25}}$, 4 $bla_{\text{CTX-M-1/15}}$ (1 strain was also positive for bla_{CMY}), 2 $bla_{\text{CTX-M-2}}$ and 1 $bla_{\text{CTX-M-9/14}}$. Three isolates were positive only for bla_{CMY} and one isolate was positive for the $bla_{\text{PER-2}}$ gene. Previous studies have shown that $bla_{\text{CTX-M-1/15}}$ genes are the main CTX-M variants associated with ESBL-producing Enterobacteriaceae isolates recovered from pigs [5,6,12]. Interestingly, in the current study $bla_{\text{CTX-M-8/25}}$ genes were the dominant CTX-M-variant.

All 12 isolates that grew on Muller–Hinton agar containing 3 μg/mL colistin were positive for the *mcr-1* gene. Six of them were also resistant to 3GCs and harboured the following ESBL genes in addition to *mcr-1*: $bla_{\text{CTX-M-8/25}}$ (4); $bla_{\text{CTX-M-1/15}}$ (1); and bla_{CMY} (1).

Profile of resistance								No. Isolates	MDR
C3G	TET	CMP	CIP	SXT	COL	GEN	FOS		
■	■							5	NO
■			■					3	NO
■								2	NO
■	■	■	■		■			3	YES
■	■	■	■	■				2	YES
■	■	■	■	■		■		2	YES
■	■		■	■		■		2	YES
■	■	■	■	■	■			2	YES
■	■	■						2	YES
■	■		■	■				1	YES
■	■	■	■					1	YES
■	■	■		■	■			1	YES
■		■		■				1	YES
■	■		■					1	YES
	■	■		■	■			1	YES
	■	■	■	■	■		■	1	YES
	■		■		■	■		1	YES
	■	■			■		■	1	YES
	■	■			■			1	YES
	■			■	■			1	YES

Fig. 1. Resistance profile of *Escherichia coli* ($n = 34$) isolated from pigs. C3G, third-generation cephalosporin; TET, tetracycline; CMP, chloramphenicol; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; COL, colistin; GEN, gentamicin; FOS, fosfomycin; MDR, multidrug-resistant.

The 34 *E. coli* isolates were grouped into 29 PFGE patterns, with 5 patterns including 2 isolates each. These five pairs of isolates grouped by PFGE were recovered from different pig samples from the same farm. The presence of clinically relevant *E. coli* clones from humans was evaluated by Multiplex PCR to detect sequence type ST69, ST73, ST95 and ST131 clones [13]. One CTX-M-8/25-producing isolate was identified as ST131 clone, the hyperepidemic clone associated mainly with extraintestinal infection in humans. In addition, two genetically related isolates harbouring bla_{CMY} were associated with ST95. Therefore, high genetic diversity between the isolates was observed, although some common human-associated clones were detected.

4. Discussion

In Argentina, the *mcr-1* gene, with or without CTX-M ESBLs, was previously reported in *E. coli* clinical isolates from inpatients and from healthy poultry [11,14]. MDR *E. coli* isolates recovered from pig farms from Argentina were already described, however no ESBL or *mcr-1* genes were reported [15]. Here we describe the finding of ESBL and/or *mcr-1* genes in *E. coli* isolates recovered from diarrhoeic piglets and healthy fattening pigs. Resistance to 3GCs was mainly associated with CTX-M, in particular with those grouped as CTX-M-8/25, and multidrug resistance was observed in 71% of the isolates.

Prudent use of antimicrobial agents is mandatory, as well as the implementation of international measures to control zoonotic pathogens and to limit the global emergence of antimicrobial resistance traits. In November 2017, the WHO published guidelines on the use of medically important antimicrobials in food-producing animals, recommending complete restriction on the use of all classes of medically important antimicrobials in food-producing animals for growth promotion as well as for the prevention, control and treatment of infectious diseases. Considering the WHO recommendation and the local evidence of transferable mechanisms of resistance in a common human pathogen, restriction of 3GCs and colistin to only the treatment of human infections should be highly recommended.

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Competing interests

None declared.

Ethical approval

Not required.

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