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Chicken Meat as a Reservoir of Colistin-Resistant *Escherichia coli* Strains Carrying *mcr-1* Genes in South America

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ABSTRACT The detection and rapid spread of colistin-resistant *Enterobacteriaceae* carrying the *mcr-1* gene has created an urgent need to strengthen surveillance. In this study, eight clonally unrelated colistin-resistant *Escherichia coli* isolates carrying *mcr-1* and bla_{CTX-M} or bla_{CMY-2} genes were isolated from commercial chicken meat in Brazil. Most *E. coli* strains carried IncX4 plasmids, previously identified in human and animal isolates. These results highlight a new reservoir of *mcr-1*-harboring *E. coli* strains in South America.

KEYWORDS *mcr*-1, ESBL, CTX-M-8, IncX4, Brazil, polymyxins

The detection and rapid spread of colistin-resistant *Escherichia coli* isolates carrying the *mcr-1* gene have created an urgent need to strengthen surveillance. Recently, *mcr-1*-harboring *Enterobacteriaceae* isolates were identified in food-producing animals, foods, aquatic environments, and humans (1–12). Although the *mcr-1* gene has spread rapidly in Asia, Europe, Africa, North America, and South America, few studies reported its presence in *Enterobacteriaceae* isolates from foods. So far, *mcr-1*-positive *E. coli* strains have been described in meats or vegetables in Europe (4–7, 9–11), Asia (8), and North America (12). In this study, we report for the first time, to our knowledge, the identification of colistin-resistant *E. coli* strains carrying the *mcr-1* gene in commercial chicken meat in Latin America.

As part of a local investigation conducted to monitor the presence of colistin-resistant bacteria carrying *mcr-1* in chicken meat sold in markets in São Paulo, southeastern Brazil, 41 samples, including breast ($n = 20$), thigh ($n = 20$), and liver ($n = 1$), were collected from 12 markets between August and October 2016. Samples (25 g) were dispensed in sterile plastic bags (Whirl-Pak; Nasco, WI) containing 225 ml of MacConkey broth and incubated at 37°C for 24 h. After incubation, a 1-ml aliquot of MacConkey broth was serially diluted in buffered peptone water, inoculated onto MacConkey agar plates containing colistin (2 μg/ml) (Sigma-Aldrich, St. Louis, MO), and incubated at 37°C for 24 h (13). Next, antimicrobial susceptibility profiles and MIC values of polymyxin B and colistin were determined by disk diffusion (14) and a microdilution method (15), respectively; *mcr-1*, extended-spectrum β-lactamase (ESBL), and plasmid-mediated AmpC β-lactamase genes were screened by PCR and sequencing (1, 16).

Eight colistin-resistant *E. coli* isolates from chicken meat samples (19.5%), collected from markets located in the north, south, and west regions of São Paulo, tested positive for *mcr-1* and bla_{CTX-M} or bla_{CMY-2} genes (Table 1). These isolates were found to be genetically unrelated by pulsed-field gel electrophoresis (PFGE) (17) and were not

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TABLE 1 Characteristics of colistin-resistant *E. coli* isolates carrying the *mcr-1* gene, isolated from commercial chicken meat samples

E. coli strain	Date (mo/yr)	Sample meat	Region[a]	Resistance profile[b]	MIC (μg/ml)[c]		ESBL/pAmpC	Additional resistance genes	Plasmid group[f]	Virulence genotype	Phylogroup	PFGE profile[g] (MLST [ST])
					Colistin	PMB						
CF1.2	8/2016	Breast	W	CTF, CRO, CTX	8	4	CTX-M-2	*aadA1*, *aadB*, *sul1*	IncFIB, IncFIC, **IncX4**	*iss*, *iroN*, *lpfA*, *mchB*, *mchC*, *mchF*, *ireA*	D	A (48)
CF101	8/2016	Breast	W	CTF, CRO, CTX, FOX, SXT, TET, GEN	8	4	CTX-M-2	*aadA1*, *aac(3)-VIa*, *sul1*, *sul2*, *tetB*	IncHI2A, IncQ1	*gad*, *ireA*, *iss*	A	B (10)
CF111	8/2016	Breast	S	AMC, CRO, CTX, FOX	4	2	–[d]	ND[e]	**IncX4**	ND[e]	A	C
CF121	9/2016	Breast	S	AMC, CTF, CRO, CTX, FOX	2	2	CMY-2	*aadA2*	IncFII, IncN, IncR	–	A	D (522)
CF131	9/2016	Breast	S	AMC, CTF, CRO, CTX, GEN	4	4	CTX-M-8	ND[e]	**IncX4**	ND[e]	B1	E
CF132	9/2016	Breast	S	AMC, CTF, CRO, CTX, TET, GEN	4	4	CTX-M-8	*aadA1*, *aac(3)-VIa*, *sul1*, *sul2*	IncI1, IncX1, **IncX4**	*gad*	A	F (4419)
CF341	10/2016	Breast	N	CTF, CRO, CTX, TET, GEN	4	2	CTX-M-2	ND[e]	**IncX4**	ND[e]	B2	G
CF351	10/2016	Breast	N	CTF, CRO, CTX, TET, GEN	8	4	CTX-M-2	*aadA1*, *aadA5*, *aac(3)-VIa*, *aph(3′)-Ic*, *sul1*, *tetA*	IncI1	–	B2	H (132)

[a]W, west; S, south; N, north.
[b]AMC, amoxicillin-clavulanic acid; CTF, ceftiofur; CRO, ceftriaxone; CTX, cefotaxime; FOX, cefoxitin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; GEN, gentamicin.
[c]PMB, polymyxin B.
[d]–, ESBL phenotype not confirmed by PCR.
[e]ND, not determined.
[f]The replicon type of plasmid carrying the *mcr*-1 gene is in boldface.
[g]PFGE patterns were analyzed using the Dice similarity with coefficient optimization set at 1% and tolerance at 2% (BioNumerics software; Applied Maths, Kortrijk, Belgium).

related to other *mcr-1*-positive *E. coli* isolates previously identified in food-producing animals (2) and humans (3) in Brazil. However, plasmid characterization by PCR-based replicon typing revealed the presence of IncX4-type plasmids in five *mcr-1*-positive *E. coli* isolates (Table 1) (18), which has been reported globally (3).

Genomic DNA from five representative colistin-resistant *E. coli* isolates (CF1.2, CF101, CF121, CF132, CF351) was extracted to construct a Nextera XT DNA library, which was sequenced using the MiSeq v3 platform (Illumina, San Diego, CA) with paired-end reads (300bp). *De novo* assembly was performed using the A5-MiSeq pipeline, and this assembly was optimized using Geneious vR9 (Biomatters, Ltd., New Zealand). Serotypes, MLST, plasmid replicons, antimicrobial resistance genes, and *E. coli* virulence genes were identified using multiple databases, including SerotypeFinder 1.1, MLST 1.8, PlasmidFinder 1.3, ResFinder 2.1, and VirulenceFinder 1.5, respectively, available from the Center for Genomic Epidemiology (http://genomicepidemiology.org/).

Most *E. coli* isolates exhibited an MDR phenotype, and, indeed, clinically important genes conferring resistance to aminoglycosides, quinolones, sulfonamide, and tetracyclines were identified by whole-genome sequencing (Table 1). On the other hand, MLST analysis from sequence reads identified the sequence types ST132, ST48, ST4419, ST522, and ST10 (Table 1). In particular, the ST10 has been widely identified in animal, food, human, and environmental samples and associated with the production of CTX-M-type ESBLs and, more recently, the MCR-1 enzyme, denoting great versatility of this lineage for adaptation to different hosts (3, 19–23). Furthermore, the presence of IncX4 plasmids carrying the *mcr-1* gene in *E. coli* isolates CF1.2, CF131, and CF132 was confirmed, as previously reported in human and animal clinical samples collected in this region (3, 19). In this regard, contigs of 5,545 kbp containing the *mcr-1* gene were found to bear an IncX4 replicon signature, as determined by the PlasmidFinder database (https://cge.cbs.dtu.dk/services/PlasmidFinder/). Moreover, these contigs were found to host a hit showing 100% identity to another IncX4 plasmid harboring *mcr-1* (GenBank accession no. CP015977). CTX-M-type encoding genes were not located in the same plasmid that carried the *mcr-1* gene. In fact, IncX4 plasmids have been associated with only the mobilization of *mcr-1* (24).

Colistin has been widely used in animal feed as a growth promoter in Brazilian livestock, mainly pigs and poultry. In 2008, the Ministry of Agriculture, Livestock, and Supply (MAPA) established appropriate levels for colistin use in broilers (2 to 10 g/ton of feed), poultry (4 to 10 g/ton of feed), pigs (20 to 40 g/ton of feed), and cattle (5 to 40 g/ton of feed). However, after the presence of colistin-resistant *E. coli* carrying the *mcr-1* gene was confirmed in humans and animals (including livestock), the use of colistin in animal feed was banned by MAPA (regulatory instruction no. 45 [http://www.agricultura.gov.br/]) in November 2016, following the international recommendations of the World Health Organization.

In summary, these results highlight that commercial chicken meat can be an important reservoir of *mcr-1*-carrying *E. coli* isolates, which is a cause for public health concern, since this could contribute to acceleration of the spread of the *mcr-1* gene. In fact, in the agribusiness, Brazil is the third-largest chicken meat producer and the largest exporter of this product, with high domestic consumption (25). Finally, the occurrence of *E. coli* isolates carrying the *mcr-1* gene in chicken meat could be favored by the versatility of *E. coli*, i.e., host adaptability, ubiquity, and persistence along the food chain; IncX4 plasmids might be key vectors responsible for the dissemination of this gene. So, surveillance of colistin-resistant *E. coli* carrying the *mcr-1* gene in the food chain needs to be established as a priority, to prevent their spread.

Accession number(s). Partial IncX4 plasmid sequences were deposited in the GenBank database under accession numbers KY550358 (pCF1-2), KY550357 (pCF131), and KY550359 (pCF132).

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We have no conflicts of interest to declare.

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