1 **Short-Form Paper**

2 Chicken Meat as Reservoir of Colistin-Resistant *Escherichia coli* Carrying 3 *mcr-1* Genes in South America

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5 **Daniel F. M. do Monte,[a,b] Andressa Mem,[c] Miriam R. Fernandes,[d] Louise Cerdeira,[d]**
6 **Fernanda Esposito,[d] Julia A. Galvão,[c] Bernadette D. G. M. Franco,[a,b] Nilton Lincopan,[d,e]**
7 **Mariza Landgraf[a,b]**
8 Department of Food and Experimental Nutrition, School of Pharmacy, University of São Paulo,
9 Brazil[a]; Food Research Center, University of São Paulo, Brazil[b]; Department of Veterinary
10 Medicine, Federal University of Paraná, Brazil[c]; Department of Clinical Analysis, School of
11 Pharmacy, University of São Paulo, Brazil[d]; Department of Microbiology, Institute of Biomedical
12 Sciences, University of São Paulo, Brazil[e]

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22 Address correspondence to Nilton Lincopan, lincopan@usp.br

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 $bla_{\text{CTX-M}}$ or $bla_{\text{CMY-2}}$ genes, were isolated from commercial chicken meat in Brazil. Most *E. coli* carried IncX4 plasmids, previously identified in human and animal isolates. These results highlight a new reservoir of *mcr-1*-harboring *E. coli* in South America.

46 **Text**

47 The detection and rapid spread of colistin-resistant *Escherichia coli* isolates carrying the *mcr-1*
48 gene has created an urgent need to strengthen surveillance. Nowadays, *mcr-1*-harboring
49 Enterobacteriaceae isolates have been identified in food-producing animals, foods, aquatic
50 environments, and humans (1-12). Although, the *mcr-1* gene has spread rapidly in Asia, Europe,
51 Africa, North America and South America, studies reporting its presence in Enterobacteriaceae
52 from foods have been unfrequent. In this regard, *mcr-1*-positive *E. coli* have been described in
53 meats or vegetables in Europe (4-7, 9-11), Asia (8) and North America (12), so far. In this study,
54 we report for the first time the identification of colistin-resistant *E. coli* carrying the *mcr-1* gene
55 in commercial chicken meat, in Latin America.

56 As part of a local investigation conducted for monitoring the presence of colistin-resistant
57 bacteria, carrying *mcr-1*, in chicken meat sold in markets in São Paulo, southeastern Brazil, forty-
58 one samples including breasts (*n*= 20), thighs (*n*= 20), and liver (*n*= 1) were collected from 12
59 markets, between August and October of 2016. Samples (25 g) were dispensed in sterile plastic
60 bags (Whirl-Pak, Nasco, WI, USA) containing 225 ml of MacConkey broth and incubated at
61 37°C for 24 h. After incubation, an aliquot of 1 ml of MacConkey broth was serially diluted on
62 buffered peptone water and inoculated onto MacConkey agar plates containing colistin (2 µg/ml)
63 (Sigma-Aldrich, St. Louis, MO), being incubated at 37°C for 24 h (13). Next, antimicrobial
64 susceptibility profiles and MIC values of polymyxin B and colistin were determined by disc
65 diffusion (14) and microdilution method (15), respectively, and *mcr-1*, ESBL and pAmpC genes
66 were screened by PCR and sequencing (1, 16).

67 Eight colistin-resistant *E. coli* isolated from chicken meat samples (19.5%) collected from
68 markets located in the North, South, and West region of São Paulo city, were 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), as well as to other *mcr-1*-positive *E. coli* isolates, previously identified in food-producing animals (2) and human (3), in Brazil However, plasmid characterization by PCR-based replicon typing (PBRT) (18) revealed the presence of IncX4-type plasmids in five *mcr-1*-positive *E. coli* isolates (Table 1), which has been globally reported (3).

Genomic DNA from five representative colistin-resistant *E. coli* isolates (CF1.2, CF 101, CF 121, CF132, CF351) was extracted to construct a Nextera XT DNA library, which was sequenced using the MiSeq v3 platform (Illumina, San Diego, CA), using paired-end reads (300bp). *De novo* assembly was performed using A5-Miseq pipeline and this assembly was optimized using Geneious v.R9 (Biomatters Ltd, New Zealand). Serotypes, MLST, plasmid replicons, antimicrobial resistance genes, and *E. coli* virulence genes were identified using multiple databases as 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 a MDR phenotype and, indeed, clinically important genes conferring resistance to aminoglycosides, quinolones, sulphonamide and tetracyclines were identified by whole genome sequencing (WGS) (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 identify from animal, food, human and environmental samples, and associated to the production of CTX-M-type ESBLs and, more recently, the MCR-1 enzyme, denoting a great versatility of this lineage for adaptation to different hosts (3, 19-23). Furthermore, presence of IncX4 plasmids carrying the *mcr-1* gene in *E. coli* isolates CF1.2, CF131 and CF132 was confirmed, as previoulsy reported in human and

92 animal clinical samples collected in this region (3, 19). In this regard, contigs of 5545-kbp,
93 containing the *mcr-1* gene were found to bear an IncX4 replicon signature, as determined by the
94 PlasmidFinder database (https://cge.cbs.dtu.dk/services/PlasmidFinder/). Moreover these contigs
95 were found to host a hit showing 100% identity to another IncX4 plasmid harbouring *mcr-1*
96 (GenBank accession no. CP015977). Partial IncX4 plasmid sequences were deposited in the
97 GenBank database under the accession numbers: KY550358 (pCF1-2), KY550357 (pCF131) and
98 KY550359 (pCF132). CTX-M-type-encoding genes were not located in the same plasmid that
99 carried the *mcr-1* gene. In fact, IncX4 plasmids have only been associated with the mobilization
100 of *mcr-1* (24).

101 Colistin has been widely used in animal feed as a growth promoter in Brazilian livestock,
102 mainly in pigs and poultry. From 2008, the Ministry of Agriculture, Livestock, and Supply
103 (MAPA) has established appropriate levels for colistin use in broilers (2-10 g/tonne of feed),
104 poultry (4-10 g/tonne of feed), pigs (20-40 g/tonne of feed) and cattle (5-40 g/tonne of feed).
105 However, after the presence of colistin-resistant *E. coli* carrying the *mcr-1* gene was confirmed in
106 human and animals (includying livestock), following international recommendations of the World
107 Health Organization (WHO), the use of colistin in animal feed was banned by the MAPA
108 (Regulatory Instruction no. 45) in November 2016, available at http://www.agricultura.gov.br/.

109 In summary, these results highlight that commercial chicken meat can be an important
110 reservoir of *mcr-1*-carrying *E. coli*, which is a cause for public health concern, since this could
111 contribute to the acceleration of the spread of the *mcr-1* gene. In fact, in the agribusiness, Brazil
112 is the third largest chicken meat producer country and the largest exporter of this product with a
113 high domestic consumption (25). Finally, the occurrence of *E. coli* carrying the *mcr-1* gene in
114 chicken meat could be favoured by the versatility of *E. coli*, regarding host adaptability, ubiquity,

and persistence along the food chain; whereas IncX4 plasmids might be key vectors responsible for the dissemination of this gene. So, surveillance of colistin-resistant *E. coli* carrying *mcr-1* gene in the food chain needs to be established as a priority, to prevent their spread.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

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238 **TABLE 1** Characteristics of colistin-resistant *E. coli* isolates, carrying the *mcr-1* gene, isolated from commercial chicken meat
239 samples.
240

E. coli strain	Date (m/y)	Sample meat	Region[a]	Resistance profile[b]	MIC (µg/ml)[c] Colistin	PMB	ESBL/pAmpC	Additional resistance genes	Plasmid Inc group[f]	Virulence genotype	Phylogroup	PFGE profile[g] (MLST, ST)
CF 1.2	08/2016	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)
CF 101	08/2016	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)
CF 111	08/2016	Breast	S	AMC, CRO, CTX, FOX	4	2	-[d]	ND[e]	IncX4	ND[e]	A	C
CF 121	09/2016	Breast	S	AMC, CTF, CRO, CTX, FOX	2	2	CMY-2	*aadA2*	IncFII, IncN, IncR	-	A	D (522)
CF 131	09/2016	Breast	S	AMC, CTF, CRO, CTX, GEN	4	4	CTX-M-8	ND[e]	IncX4	ND[e]	B1	E
CF 132	09/2016	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)
CF 341	10/2016	Breast	N	CTF, CRO, CTX, TET, GEN	4	2	CTX-M-2	ND[e]	IncX4	ND[e]	B2	G
CF 351	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)

241 [a] W, West; S, South; N, North.
242 [b] AMC, amoxicillin-clavulanic acid; CTF, ceftiofur; CRO, ceftriaxone; CTX, cefotaxime; FOX, cefoxitin; SXT, trimethoprim-sulfamethoxazole; TET,
243 tetracycline; GEN, gentamicin.
244 [c] PMB, polymyxin B.
245 [d] ESBL phenotype was not confirmed by PCR.
246 [e] ND, not determined.
247 [f] The replicon type of plasmids carrying the *mcr*-1 gene is underlined.
248 [g] PFGE patterns were analysed using the Dice similarity with coefficient optimisation set at 1% and tolerance at 2% (BioNumerics software; Applied Maths,
249 Kortrijk, Belgium).