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 blaCTX-M or blacmy-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 has 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 blacTX-M or blacmy-2 genes (Table 1). These isolates were found to be genetically unrelated by pulsed-field gel electrophoresis (PFGE) (17) and were not
<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Date (mo/yr)</th>
<th>Sample meat</th>
<th>Region</th>
<th>Resistance profile</th>
<th>MIC (μg/ml)</th>
<th>Additional resistance genes</th>
<th>Plasmid group</th>
<th>Virulence genotype</th>
<th>Phylogroup</th>
<th>PFGE profile (MLST [ST])</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF1.2</td>
<td>8/2016</td>
<td>Breast</td>
<td>W</td>
<td>CTF, CRO, CTX</td>
<td>8</td>
<td>CTX-M-2</td>
<td>aadA1, aadB, sul1</td>
<td>IncFlB, IncFlC, IncX4</td>
<td>D</td>
<td>A (48)</td>
</tr>
<tr>
<td>CF101</td>
<td>8/2016</td>
<td>Breast</td>
<td>W</td>
<td>CTF, CRO, CTX, FOX, SXT, TET, GEN</td>
<td>8</td>
<td>CTX-M-2</td>
<td>aadA1, aac(3)-VIa, sul1, sul2, tetB</td>
<td>IncHI2A, IncQ1</td>
<td>gad, ireA, iss</td>
<td>A</td>
</tr>
<tr>
<td>CF111</td>
<td>8/2016</td>
<td>Breast</td>
<td>S</td>
<td>AMC, CRO, CTX, FOX</td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>IncX4</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>CF121</td>
<td>9/2016</td>
<td>Breast</td>
<td>S</td>
<td>AMC, CTF, CRO, CTX, FOX</td>
<td>2</td>
<td>CMY-2</td>
<td>aadA2</td>
<td>IncFlI, IncN, IncR</td>
<td>–</td>
<td>A</td>
</tr>
<tr>
<td>CF131</td>
<td>9/2016</td>
<td>Breast</td>
<td>S</td>
<td>AMC, CTF, CRO, CTX, CTX, FOX</td>
<td>4</td>
<td>CTX-M-8</td>
<td>ND</td>
<td>IncX4</td>
<td>B1</td>
<td>E</td>
</tr>
<tr>
<td>CF132</td>
<td>9/2016</td>
<td>Breast</td>
<td>S</td>
<td>AMC, CTF, CRO, CTX, TET, GEN</td>
<td>4</td>
<td>CTX-M-8</td>
<td>aadA1, aac(3)-VIa, sul1, sul2</td>
<td>IncI1, IncX1, IncX4</td>
<td>gad</td>
<td>A</td>
</tr>
<tr>
<td>CF341</td>
<td>10/2016</td>
<td>Breast</td>
<td>N</td>
<td>CTF, CRO, CTX, TET, GEN</td>
<td>4</td>
<td>CTX-M-2</td>
<td>ND</td>
<td>IncX4</td>
<td>B2</td>
<td>G</td>
</tr>
<tr>
<td>CF351</td>
<td>10/2016</td>
<td>Breast</td>
<td>N</td>
<td>CTF, CRO, CTX, TET, GEN</td>
<td>8</td>
<td>CTX-M-2</td>
<td>aadA1, aadAS, aac(3)-VIa, aph(3′)-Ic, sul1, tetA</td>
<td>IncI1</td>
<td>–</td>
<td>B2 (H (132))</td>
</tr>
</tbody>
</table>

аН, west; S, south; N, north.
амС, amoxicillin-clavulanic acid; CTF, ceftriaxone; CTX, cefotaxime; FOX, cefoxitin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; GEN, gentamicin.
амB, polymyxin B.
амC, CTX-M-2 phenotype not confirmed by PCR.
амD, not determined.
амE, the replicon type of plasmid carrying the mcr-1 gene is in boldface.
амF, 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, CF131, and CF132) 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 v9 (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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