Extended-spectrum cephalosporin-resistant *Escherichia coli* isolated from chickens and chicken meat in Brazil is associated with rare and complex resistance plasmids and pandemic ST lineages

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**Objectives:** Brazil is the greatest exporter of chicken meat (CM) in the world. It is of utmost importance to monitor resistance to extended-spectrum cephalosporins (ESCs) in this sector because resistance to ESCs in *Escherichia coli* isolated from food-producing animals may contaminate humans through the food chain. Thus, the aim of this study was to characterize and compare ESC-resistant *E. coli* isolated from chickens and retail CM produced in south-eastern Brazil.

**Methods:** Five CM samples and 117 chicken cloacal swabs (CCSs) were inoculated on MacConkey agar supplemented with cefotaxime. Presumptive *E. coli* colonies were identified and antimicrobial susceptibility was tested. Virulence and acquired *bla*ESBL and *bla*AmpC genes were sought and genetic environments characterized. Isolates were typed by phylogenetic grouping, XbaI-PFGE and MLST.

**Results:** All five CM samples and 36 CCSs (30.8%) were positive for the presence of ESC-resistant *E. coli*, leading to the selection of 58 resistant isolates. ESC resistance was mostly due to the presence of the chromosome-encoded *bla*CTX-M-2 gene, but plasmid-mediated *bla*CTX-M-2, *bla*CTX-M-8, *bla*CTX-M-15, *bla*TX-M-55, and *bla*CMY-2 were also detected. Multireplicon plasmids were sporadically identified, such as IncH12-P-blaCTX-M-2 and IncFII/N-blaCTX-M-55. Phylogroup D predominated, while PFGE and MLST revealed a high genetic diversity.

**Conclusions:** Live Brazilian chickens and CM act as reservoirs of ESC-resistant *E. coli* and resistance genes are located on highly diverse genetic determinants. Potentially pathogenic strains, which may represent a threat to human health and a source of environmental contamination, were also identified. Active surveillance is therefore essential in Brazil’s chicken production line.

**Introduction**

The prevalence of MDR *Escherichia coli* is increasing worldwide.1 In a ‘One-Health’ context, characterizing the occurrence of *E. coli* resistant to extended-spectrum cephalosporins (ESCs) and producing ESBLs or plasmid-mediated AmpC β-lactamas in samples from human, animal and food reservoirs is of utmost importance to understand the routes of dissemination of these resistance determinants.2–4

Chicken meat (CM) is considered a potential source of human contamination with antimicrobial-resistant extraintestinal pathogenic *E. coli* (ExPEC).4–6 As Brazil is the greatest exporter of CM worldwide, surveillance studies are essential to identify resistance genes and bacterial clones that may spread in chickens and ultimately contaminate humans. Thus, the aim of this study was to characterize and compare ESC-resistant *E. coli* strains isolated from chickens and retail CM produced in south-eastern Brazil.

**Materials and methods**

**Samples and isolates**

Five CM pieces were bought between June and September 2014 in different marketplaces in the State of São Paulo, Brazil. Twenty-five grams was dispensed in 225 mL of peptone water with 4 mg/L cefotaxime, then incubated at 37°C for 18–20 h. Additionally, 117 chicken cloacal swabs (CCSs) were obtained between August and November 2014 on three different farms in the State of São Paulo, Brazil. All the samples were plated on MacConkey agar supplemented with 4 mg/L cefotaxime. Presumptive
The bla<sub>CTX-M-2</sub> gene was detected in 15/17 isolates from CM and 26/41 isolates from CCSs (farms 1 and 2). Only three other bla<sub>CTX-M</sub> genes were detected: bla<sub>CTX-M-8</sub> and bla<sub>CTX-M-15</sub> in two different CM samples, and bla<sub>CTX-M-55</sub> in seven CCS isolates from farm 2. Finally, the eight plasmid-mediated AmpC β-lactamase producers from farm 3 carried the bla<sub>CMY-2</sub> gene (Table 1).

Twenty-four virulence genotypes were detected (Table S3). Six isolates presented no virulence gene, while six carried up to six genes. The most frequently identified gene was fimH (48/58 isolates).

### Table 1. Proportion (%) of ESC resistance genes found in CM samples and CCSs from three farms

<table>
<thead>
<tr>
<th>Source</th>
<th>ESC resistance gene</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM</td>
<td>CTX-M-2</td>
<td>15 (88.2)</td>
</tr>
<tr>
<td></td>
<td>CTX-M-8</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td></td>
<td>CTX-M-15</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>CCSs F1</td>
<td>CTX-M-2</td>
<td>20 (100)</td>
</tr>
<tr>
<td>CCSs F2</td>
<td>CTX-M-2</td>
<td>6 (46.2)</td>
</tr>
<tr>
<td></td>
<td>CTX-M-55</td>
<td>7 (53.8)</td>
</tr>
<tr>
<td>CCSs F3</td>
<td>CMY-2</td>
<td>8 (100)</td>
</tr>
</tbody>
</table>

F1, farm 1; F2, farm 2; F3, farm 3.

E. coli colonies were identified with the Vitek2 compact system (bioMérieux, Marcy-l’Étoile, France) and susceptibility to 32 antimicrobials was tested by disc diffusion according to the French Society for Microbiology (www.sfm-microbiologie.org). ESBL production was screened using the double-disc synergy test. E. coli ATCC 25922 was used for quality control purposes.

### Detection of resistance and virulence genes
ESC resistance genes were screened by PCR and sequencing, and virulence genes were detected by PCR (for primers see Table S1, available as Supplementary data at JAC Online).

### Characterization and transferability of plasmids
Conjugation was performed by broth mating using E. coli K-12 J53 as a recipient strain. Plasmids were typed applying the replicon typing scheme using the commercial PBRT kit (Diatheva, Fano, Italy) and subtyped according to adequate schemes (http://pubmlst.org/plasmid/). Southern blot analysis on PFGE-S1 gels or PFGE-I-CeuI was performed using adequate probes.  

### Typing of bacteria
Phylogroups were determined by PCR (Table S1). XbaI-PFGE was performed and profiles were compared using BioNumerics<sup>TM</sup> (Applied Maths). A cluster was defined when isolates presented ≥85% similarity. MLST was carried out using the Achtman scheme (https://pubmlst.org/bigsdb? db=pubmlst_mlst_seqdef).

### Results

#### Antimicrobial susceptibility
All five CM pieces and 36/117 (30.8%) CCSs presented cephalosporin-resistant E. coli, leading to the detection of 17 and 41 (n = 58) isolates, respectively. Fifty isolates (86.2%) collected on farms 1 and 2 and from CM were ESBL producers, whereas eight (13.8%) from farm 3 were plasmid-mediated AmpC β-lactamase producers. All the isolates were MDR (non-susceptible to at least one agent in three or more antimicrobial categories)<sup>7</sup> (Table S2).

#### ESC resistance and virulence genes

The bla<sub>CTX-M-2</sub> gene was detected in 15/17 isolates from CM and 26/41 isolates from CCSs (farms 1 and 2). Only three other bla<sub>CTX-M</sub> genes were detected: bla<sub>CTX-M-8</sub> and bla<sub>CTX-M-15</sub> in two different CM samples, and bla<sub>CTX-M-55</sub> in seven CCS isolates from farm 2. Finally, the bla<sub>CMY-2</sub> gene was non-conjugative and its chromosomal location was confirmed by Southern blot analyses on I-CeuI-PFGE. The last bla<sub>CTX-M-2</sub> gene was located on an IncH12-ST2/P conjugative plasmid of ~240 kb. The IS10-bla<sub>CTX-M-8</sub> gene was located on an ~100 kb IncI1/ST113 conjugative plasmid. The IS<sup>Ecp1</sup>-bla<sub>CTX-M-15</sub> gene was located on an ~55 kb IncX1 conjugative plasmid. Six isolates carried IS<sup>Ecp1</sup>-bla<sub>CTX-M-55</sub> on an IncFII/N-ST1 conjugative plasmid of ~100 kb and one on an ~90 kb IncFII conjugative plasmid. While IncF subtyping was constant, IncF belonged to IncF29A::-B-, IncF33A::-B- or IncF38A::-B-. The IS<sup>Ecp1</sup>-bla<sub>CMY-2</sub> genes were carried by non-typeable plasmids of ~90 kb (Figure 1 and Table S4).

None of the transconjugants presented additional resistance, except the one carrying the IncH12/P-bla<sub>CTX-M-2</sub> plasmid, which was resistant to kanamycin and tetracycline.

### Typing of ESC-resistant E. coli isolates

XbaI-PFGE typing revealed high genetic diversity, with 22/58 isolates distributed in eight clusters. No isolate from CM grouped with any from CCSs (Figure 1). All four phylogroups were detected, phylogroup B being the most prevalent (Table S3). ST10/CC10, ST117, ST155/CC155, ST350/CC350 and ST1775 were present, but never shared the same PFGE profile. Numerous other STs were also detected, including ST131 (in CM) and ST69 (in CCSs) (Figure 1).

### Discussion

ESC-resistant E. coli presenting an MDR phenotype were found in all CM pieces and in 30.8% of CCSs. Proportions of resistance to chloramphenicol, aminoglycosides, quinolones/fluoroquinolones and sulphonamides were significantly higher in isolates from CM (Table S2). This high level of resistance in CM, which has also been reported in other countries,<sup>5,8,9</sup> most probably highlights contamination during the transformation process.

bla<sub>CTX-M-2</sub> was the most frequently reported bla<sub>ESBL</sub> gene in this study and has also been frequently detected in E. coli isolated from food, food-producing animals and clinical samples in Brazil.<sup>10-12</sup> This gene was most probably highlights contamination during the transformation process.

Here, ESC resistance genes other than bla<sub>CTX-M-2</sub> were only sporadic, but the presence of bla<sub>CTX-M-15</sub>, bla<sub>CTX-M-8</sub>, bla<sub>CMY-2</sub> and

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Figure 1. Dendrogram of ESC-resistant E. coli isolated from chickens and retail CM generated after genomic restriction with Xbal followed by PFGE. Dendrograms were generated using Dice’s similarity and unweighted pair-group method using arithmetic averages, with 0% optimization and 1% tolerance. PhG, phylogenetic group; AMX, amoxicillin; AMC, amoxicillin/clavulanate; CEF, cefalotin; CXM, cefuroxime; CTX, cefotaxime; XNL, cefotiofur; PIP, piperacillin; TIC, ticarcillin; TAZ, piperacillin/tazobactam; TIM, ticarcillin/clavulanate acid; ETP, ertapenem; CAZ, ceftazidime; FOX, cefoxitin; FEP, ceftazime; ATM, aztreonam; CEF, cefquinome; STR, streptomycin; KAN, kanamycin; AMK, amikacin; APR, apramycin; GEN, gentamicin; TOB, tobramycin; NET, netilmicin; CHL, chloramphenicol; FFC, florfenicol; TET, tetracycline; CST, colistin; SSS, sulphonamides; TMP, trimethoprim; NAL, nalidixic acid; ENR, enrofloxacin; OFX, ofloxacin. In the ‘Antimicrobial susceptibility profile’ section, white squares represent susceptibility, grey squares intermediate resistance and black squares resistance. In the ‘Virulence genes’ section, white squares represent absence and black squares presence.
bla<sub>CTX-M-8</sub> dissemination in Brazil and possibly worldwide. The bla<sub>B2</sub> gene is widespread in Brazil and has previously been detected in E. coli isolates from chickens and CM, also located on IncI1/ST113 plasmids, which seem to be a major vector of bla<sub>CTX-M-8</sub> dissemination in Brazil and possibly worldwide. The bla<sub>CTX-M-55</sub> gene is endemic in Asian countries but appears to be emerging worldwide. Here, we report the occurrence of this gene on farm 2, which may further disseminate it. The IncF<sub>33:A-B</sub>-/IncI<sub>1/ST113</sub> plasmid may be particularly successful since it was described in E. coli isolated from chickens in China. The detection of bla<sub>CTX-M-55</sub> in slightly different multiplication plasmids may indicate microevolution events. Finally, bla<sub>CMY-2</sub>, frequently detected in E. coli isolated from food and food-producing animals in North America and Northern Europe, was only recently identified in E. coli from chickens in Brazil, mostly carried by IncK and IncI1 plasmids. Here, one farm was exclusively contaminated by this gene, confirming its sporadic success.

PFGE and MLST showed a high genetic variability. As expected, phylogroup D was predominant in CM and CCSs, while B2 is scarce. Identical or highly similar isolates according to XbaI-PFGE typing mainly originated from the same animal or meat, but very similar isolates carrying bla<sub>CTX-M-2</sub> or bla<sub>CTX-M-8</sub> were also obtained from different pieces of meat. Interestingly, the bla<sub>CTX-M-55</sub> gene was carried by different plasmids in two identical isolates obtained from two animals on farm 2. Conversely, different isolates were also recovered from the same meat or animal (Figure 1). As already suggested by other studies, MLST confirms the hypothesis that poultry meat production acts as a reservoir of ExPEC lineages such as ST10, ST117, ST69 and ST131. Other clones identified in CCSs, such as ST48 and ST1158, have previously been detected in CM in Brazil and seem to be more adapted to the animal host. Even though a few lineages were present in both CM and CCSs, there is overall little overlap. Most of the isolates presented at least one virulence factor and five isolates presented up to six of the virulence genes tested. Of note, E. coli with a similar diversity of virulence genes was isolated from CM exported to Japan from Brazil.

In conclusion, this study shows the high occurrence of ESC-resistant E. coli in the meat and gastrointestinal tract of chickens in Brazil. This resistant E. coli can be transferred to humans through meat handling or consumption. ESC resistance was mainly associated with chromosomally encoded bla<sub>CTX-M-2</sub>, but also with plasmid-borne bla<sub>CTX-M-2</sub>, bla<sub>CTX-M-8</sub> and bla<sub>CAR-M-15</sub>. bla<sub>CTX-M-55</sub> and bla<sub>CMY-2</sub>. Despite the small number of samples, these findings reveal that retail CM acts as a vehicle for the dissemination of potential pathogenic ESC-resistant E. coli and that chickens are reservoirs, thus highlighting the importance of active surveillance.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 to S4 are available as Supplementary data at JAC Online.

References


