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# Characterization of extended-spectrum and CMY-2 ß-lactamases, and associated virulence genes in *Escherichia coli* from food of animal origin in México

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## Abstract

**Purpose** – Broad-spectrum cephalosporin resistance is rapidly increasing in *Escherichia coli*, representing a food safety problem. The purpose of this paper is to characterize eight extended-spectrum-β-lactamase (ESBL) and acquired AmpC β-lactamase-producing *E. coli* isolates and virotypes associated, obtained from chicken and pork food samples in Puebla, Mexico.

**Design/methodology/approach** – Samples (36 from chicken and 10 from pork) were cultured on Levine agar plates supplemented with cefotaxime (2 mg/L) for isolation of cefotaxime-resistant (CTXR) *E. coli*. CTXR-*E. coli* isolates were detected in 33 of 46 samples (72 percent), and one isolate/sample was characterized (28 from chicken and 5 from pork), for ESBL production, phylogenetic group, sequence typing, resistance and virulence genes by PCR and sequencing.

**Findings** – Results showed 16 ESBL-*E. coli* (35 percent) (12/16 belonging to phylogroup B1) and 8 CMY-2-*E. coli* (17 percent). ESBL detected were as follows (number of isolates): CTX-M-2 (8); CTX-M-1 (2); CTX-M-15 (1); SHV-2a (4) and TEM-52c (1). In total, 20 different sequence types (STs) were identified among the ESBL- or CMY-2-producing *E. coli* strains, which included four new ones. The CTX-M-15 β-lactamase was detected in one *E. coli* ST617-ST10 Cplx-B1 strain that also carried *ibeA* gene. One CMY-2-positive strain of lineage ST224-B2 was detected and it carried the *qnrA*1 gene.

Conflict of interest: competing interests – none declared.

Ethical approval: not required

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**Originality/value** – In this study, a ST131-based virotyping scheme for strains from food of animal origin was established since this kind of strains constitutes an important vehicle of virulent ESBL- and CMY-2-producing *E. coli* isolates, which could be transmitted to humans by direct contact or through the food chain.

**Keywords** *Escherichia coli*, Chicken meat, CMY-2, ESBL, Pork meat, Virotype

**Paper type** Research paper

## 1. Introduction

*Escherichia coli* is a common inhabitant of the intestinal tract of most animals and humans (Torres, 2016), and can easily contaminate food products of animal origin destined to human consumption (Apata, 2009). These strains are reservoir of extended-spectrum $\beta$-lactamases and integrons that could be transmitted to humans through the food chain (Liebana *et al.*, 2013). Extended-spectrum-$\beta$-lactamase (ESBL) of the CTX-M type represent a major problem and a therapeutic challenge in human health (Livermore *et al.,* 2007; Rocha-Gracia *et al.*, 2015).

The detection of ESBL-positive *E. coli* isolates from food-producing animals and food products has been increasingly reported in the last years worldwide. Nevertheless, this type of studies is scarce in Latin America (Bevan *et al.*, 2017). To our knowledge, there are no reports about ESBL-producing *E. coli* isolated from food-producing animals in Mexico. Likewise, acquired AmpC $\beta$-lactamases (acAmpC) represent another important mechanism of resistance, because of the broad spectrum of resistance they confer (Jacoby, 2009), as well as their co-existence with other encoding resistance genes (Alonso *et al.*, 2016). The enzymes AmpC inactivate broad-spectrum cephalosporins as well as cephamycins (cefoxitin) and are not inhibited by clavulanic acid. Among them, those of the CMY class cause problems for therapy of *E. coli* infections and are the most commonly found acAmpC worldwide (Guo *et al.*, 2014; Shin *et al.*, 2017). It is known that all *E. coli* isolates harbor a chromosomal *ampC* $\beta$-lactamase encoding gene not associated to $\beta$-lactam resistance. Nevertheless, specific nucleotidic point mutations in the promoter/attenuator region of this gene (particularly at the −42 or −32 positions) respect to the one of *E. coli* K12 are associated with the hyperproduction of this chromosomal AmpC $\beta$-lactamase (Caroff *et al.*, 2000). This hyperproduction is associated with resistance to some $\beta$-lactamic antimicrobials as ampicillin, amoxicillin–clavulanic acid and cefoxitin, and also confers low-level resistance to broad-spectrum cephalosporins. Several studies show the dissemination of this resistance mechanism among *E. coli* isolates from food samples of animal origin (Ben Sallem *et al.*, 2014; Dhanji *et al.*, 2010; Jones-Dias *et al.*, 2016; Kawamura *et al.*, 2015; Martin *et al.*, 2012; Sheikh *et al.*, 2012). To our knowledge, in Mexico there are only two reports about the dissemination of CMY enzyme in *Salmonella* Typhimurium and *E. coli* of human and animal origin, respectively (Zaidi *et al.*, 2007; Aguilar-Montes de Oca *et al.*, 2015). In the clinical practice, *E. coli* that produce ESBLs and acAmpC usually also carry resistance genes for other antibiotics, which limit the therapeutic options (Paterson and Bonomo 2005). Additionally, the co-occurrence of resistance and virulence determinants implies a clinical challenge (Da Silva and Mendonça, 2012). These resistant-virulent bacteria could enter the food chain, representing an important food safety problem because they can transfer resistance genes to other pathogenic or non-pathogenic bacteria. The objective of our study was to detect ESBL- and acAmpC-producing *E. coli* isolates in food samples of animal origin in Puebla, Mexico and to characterize the $\beta$-lactamases, genetic lineages, resistance and virulence genes of recovered isolates.

## 2. Material and methods

### *2.1 Samples and E. coli isolates*

Food samples of animal origin (36 of chicken and 10 of pork) were obtained from five local open-markets and five supermarkets in Puebla City, Mexico, between February and June of 2009. Samples were kept on ice during transport to the laboratory and all of them were tested within 24 h of collection. One gram of each sample was vigorously homogenized with

3 mL of brain heart infusion (BD Bioxon®) broth supplemented with bacitracin at a concentration of 20 mg/L and incubated for 18 h at 37°C. Later on, 40 μl of the suspension was streaked onto Levine agar plates (Oxoid, LTD, England) supplemented with cefotaxime (2 mg/L) (Sigma-Aldrich®), and incubated for 24 h at 37°C (Ruiz *et al.*, 2012). Isolates with typical *E. coli* morphology were selected and identified by classical biochemical methods, and by the API 20E system (BioMérieux, La Balme Les Grottes, France).

### *2.2 Antimicrobial susceptibility testing*

Antimicrobial susceptibility was performed by the agar disk diffusion method following the Clinical and Laboratory Standards Institute recommendations (Clinical and Laboratory Standards Institute (CLSI), 2017), and a total of 21 antimicrobial agents were tested: ampicillin, amoxicillin–clavulanic, ampicillin-sulbactam, cephalotin, cefoxitin, cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam, imipenem, meropenem, nalidixic acid, ciprofloxacin, streptomycin, gentamicin, amikacin, tobramycin, tetracycline, trimethoprim–sulfamethoxazole and chloramphenicol (Beckton, Dickinson, Heidelberg, Germany). *E. coli* ATCC 25922 was used as a quality control strain. Strains exhibiting resistance to three or more classes of antibiotics were defined as multidrug resistant (MDR). Cefotaxime-resistant ($CTX^R$) *E. coli* isolates (up to four colonies) were initially selected per sample and the antimicrobial resistance phenotype was determined. One $CTX^R$ *E. coli* isolate per sample was kept for further studies, except if they presented different antimicrobial resistance phenotype.

The double disk synergy test with cefotaxime (CTX), ceftazidime (CAZ), aztreonam (ATM) and cefepime (FEP) in proximity to AMC was used for the screening of ESBL (CLSI, 2017). The AmpC-phenotype was considered when the strains showed resistance to amoxicillin-clavulanate (AMC) and cefoxitin (FOX).

### *2.3 Partial characterization of β-lactamase genes and genetic environment of $bla_{CTX\text{-}M}$ genes*

Genes encoding TEM, SHV, OXA and CTX-M enzymes, and the genetic environment of blaCTX-M genes were analyzed by gene-specific PCR and DNA sequencing on both strands (Rocha-Gracia *et al.*, 2015). Nucleotide segments and their deduced amino acid sequences were compared with those included in the GenBank database and on the Lahey Clinic website (www.lahey.org/Studies) in order to confirm the specific β-lactamase gene. The presence of acAmpC was tested by multiplex PCR in AMC and FOX-resistant strains (Maamar *et al.*, 2016). The genetic environment of blaCMY-2 gene was also determined by PCR and sequencing (Guo *et al.*, 2014). The mutation in the promoter/attenuator region of the chromosomal *ampC* gene was also analyzed in all AMC and FOX-resistant *E. coli* isolates by PCR amplification, sequencing and comparison to the same region of the *E. coli* K12 (Briñas *et al.*, 2005).

### *2.4 Detection of genes conferring resistance to non-β-lactam antimicrobial agents and characterization of class 1 integrons*

The presence of genes associated with resistance to tetracycline (*tetA* and *tetB*), chloramphenicol (*cmlA*), sulphonamide (*sul1* and *sul3*) and quinolones (*qnr*, *qepA* and *aac*(6´)Ib-cr) were determined by PCR and sequencing (Ruiz *et al.*, 2012; Sáenz *et al.*, 2004; Vinué *et al.*, 2009). *gyrA* and *parC* genes were amplified by PCR and sequencing to determine the amino acid changes. The obtained sequences were compared with those previously reported for GyrA (GenBank accession number X06373) and ParC (M58408 with the modification included in L22025) proteins (Sáenz *et al.*, 2004). The presence of *intI*1 (encoding class 1 integrase) and the 3´-conserved region (*qacE*Δ1- *sul1* genes) of class 1 integrons were examined by PCR. The variable region of class 1 integrons were characterized by PCR (Ruiz *et al.*, 2012), sequencing and compared with those included in GenBank to identify the gene cassettes. Likewise, the presence of *intI*2 (encoding class 2 integrase) was examined by PCR. Positive and negative controls from the bacterial collection of the University of La Rioja were included in all PCR assays.

*2.5 Genetic typing of E. coli strains*

Phylogenetic groups were determined using an established multiplex PCR (Clermont *et al.*, 2013). This method classified *E. coli* strains into eight phylogenetic groups (A, B1, B2, C, D, E, F and clade I), based on detection of *arpA, chuA, yjaA* or TspE4.C2 genes. ESBL- or CMY-2-producing *E. coli* isolates were typed by multilocus sequence typing (MLST) as described by Wirth *et al.* (2006). MLST was performed by PCR amplification of the standard seven housekeeping loci: *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*. All the amplicons were sequenced and compared with the MLST database (http://mlst.warwick.ac.uk/mlst/mlst/dbs/Ecoli) in order to ascertain the corresponding sequence type (ST) and clonal complex (CC).

*2.6 Virotyping of E. coli strains*

Eleven virulence factor (VF) genes were determined for the CTX-M- or CMY-2-producing *E. coli* isolates using an established PCR protocol. This scheme identifies *pap* (adhesin encoding P fimbriae), *cnfI* (cytotoxic necrotizing factor), *sat* (secreted autotransporter toxin), *kpsM*II (group 2 capsule synthesis), *iroN* (catecholate siderophore receptor), *afa/draBC* (Afa/Dr adhesins), *ibeA* (invasion of brain endothelium), *hlyA* ($\alpha$ hemolysin) and *cdtB* (cytolethal distending toxin) genes distributed into five established virotypes of *E. coli* ST131 (A-E) (Nicolas-Chanoine *et al.*, 2014).

## 3. Results

*3.1 Recovery of CTX$^R$ E. coli isolates*

*E. coli* isolates with CTX$^R$ phenotype were collected in 28 of 36 chicken samples (78 percent) and in five of ten pork samples (50 percent) (Table I); 28 of the 33 positive samples were obtained in markets (24 chicken; 4 pork) and five in supermarkets (four chicken; one pork). Four CTX$^R$ isolates were initially selected from each positive sample, however, after bacterial identification, susceptibility testing and PCRs for identification of $\beta$-lactamase genes; as isolates of the same sample looked similar, only one isolate/sample was selected for further characterization, rendering a collection of 33 CTX$^R$ *E. coli* isolates (Table I). Of this collection, 16 of them were ESBL-producers (14 of 187 chicken and 2 of pork origin) and 17 were ESBL-negative but AmpC-phenotype positive; eight isolates of this last group corresponded to the genotype acAmpC and carried the gene encoding CMY-2 (six of chicken and two of pork origin); taking together, 24 of the 46 food samples tested (52 percent) carried ESBL or CMY-2-producing *E. coli* isolates (Table I).

*3.2 Characterization of ESBL-positive E. coli strains*

Table II shows the characteristics of the 16 ESBL-producing *E. coli* isolates. All isolates showed a multiresistant phenotype, exhibiting resistance to tetracycline and nalidixic acid; and most of them also to ciprofloxacin and trimethoprim-sulphamethoxazole ($n = 14$), streptomycin ($n = 13$), chloramphenicol ($n = 12$) or gentamicin ($n = 10$). The $\beta$-lactamases detected among our isolates were (number of 200 isolates): CTX-M-2 (8); CTX-M-1 (2); CTX-M-15 (1); SHV-2a (4, one of them co-producing CTX-M-2) and TEM-52c (1). Besides, no tested

**Table I.** Origin of food samples analyzed, and number of *E. coli* isolates with ESBL or acquired AmpC

| Origin (no. samples) | Number (%) of samples containing *E. coli* with | | | |
|---|---|---|---|---|
| | CTX$^R$ | ESBL | acAmpC (CMY-2) | No acAmpC |
| Chicken (36) | 28 (78) | 14 (39) | 6 (17) | 8 (22) |
| Pork (10) | 5 (50) | 2 (20) | 2 (20) | 1 (10) |
| Total (46) | 33 (72) | 16 (35) | 8 (17) | 9 (19) |

**Notes:** CTXR, resistance to cefotaxime; ESBL, extended-spectrum $\beta$-lactamase; acAmpC, acquired AmpC enzyme (resistance to amoxicillin-clavulanic and cefoxitin)

**Table II.**
Characteristics of the 16 ESBL-producing *E. coli* strains recovered from food samples of animal origin in Puebla, Mexico

| Strain (origin[a])[b] | Phylogenetic group | MLST ST/CC | Resistance phenotype to non-β-lactam antibiotics | β-lactamase detected | Others resistance genes detected | Mutations in chromosomal *ampC* gene[c] | Amino acid changes in GyrA/ParC | Class 1 integron | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *intI1*/ *qacEΔ1-sul1* | Gene cassettes |
| C3315 (C) | A | ST3498[e] | STR,GM,TOB,NA, CIP, TE,C,SXT | CTX-M-2 +SHV-2a | – | ND | S83L-D87N/ S80I | +/+ | *aadA*(1/2) |
| C3320 (C) | B1 | ST602/ST446 Cplx | STR,NA,TE,C,SXT | CTX-M-2 | *tet*(A), *sul3* | ND | S83L-D87N/ S80I | +/+ | *dfrA1-aadA1* |
| C3298 (C) | B1 | ST3377[d] | STR,NA,CIP,TE,C, SXT | CTX-M-2 | – | ND | S83L-D87N/ S80I | –/+ | – |
| C3314 (C) | B1 | ST1196 | STR,NA,CIP,TE,C, SXT | CTX-M-2 +TEM-1b | *tet*(A), *cml*A, *sul3* | ND | S83L-D87N/ S80I | +/+ | *dfrA12-orfF-aadA2* |
| C3329 (C) | B1 | ST501 | STR,GM,TOB,NA, CIP, TE,C,SXT | CTX-M-2 | *tet*(A), *cml*A, *sul3* | ND | S83L-D87N/ S80I-E84G | +/+ | 2 integrons 1:*dfrA17-aadA5*; 2: *dfrA12-orfF-aadA2* |
| C3319[g] (C) | B1 | ST1771 | STR,GM,NA,CIP,TE, C, SXT | CTX-M-2 | *tet*(A) | −42,−18,+1,+58 | S83L-D87N/ S80I | +/+ | – |
| C3318 (C) | D | ST501 | STR,GM,AMK,TOB, NA, CIP,TE,C,SXT | CTX-M-2 | *tet*(A), *cml*A | Wild | S83L-D87N/ S80I-E84G | +/+ | *aadA1* |
| C3302 (P) | E | ST1266 | STR,NA,CIP,TE,C, SXT | CTX-M-2 +OXA-1 | *tet*(A), *tet*(B), *cml*A, *sul3* | ND | S83L-D87N/ S80I-E84G | +/+ | 3 integrons 1: 1100 bp (*aadB-aadA*); 2: 1,400 bp; 3: 1,800 pb |
| C3300 (C) | B1 | ST1249 | STR,GM,TOB,NA, CIP, TE,C,SXT | CTX-M-1 | – | ND | S83L-D87N/ S80I | –/+ | – |
| C3313 (C) | B1 | ST155/ST155 Cplx | AMK,TOB,NA,CIP, TE,SXT | CTX-M-1 | *tet*(A), *cml*A, *sul3* | ND | S83L-D87N/ S80I | +/+ | 2 integrons 1: *dfrA1-aadA1*; 2 *dfrA12-aadA2* |

(*continued*)

Table II.

| Strain (origin[a])[b] | Phylogenetic group | MLST ST/CC | Resistance phenotype to non-$\beta$-lactam antibiotics | $\beta$-lactamase detected | Others resistance genes detected | Mutations in chromosomal *ampC* gene[c] | Amino acid changes in GyrA/ParC | Class 1 integron *intI1*/*qacE*Δ*1-sul1* | Class 1 integron Gene cassettes |
|---|---|---|---|---|---|---|---|---|---|
| C3327[g] (C) | B1 | ST617/ST10 Cplx | STR,GM,TOB,NA, CIP, TE,C,SXT | CTX-M-15 +TEM-1b | *tet*(A), *tet*(B), *cml*A, *sul3* | ND | S83L-D87N/ S80I | +/+ | *estx-psp-aadA2-cmlA-aadA1-qacH-IS330-sul3* |
| C3317 (C) | B1 | ND | NA,CIP,TE,SXT | SHV-2a | *sul3* | −18,+1,+58 | S83L-D87N/ S80I | −/+ | *aadA*(1/2) |
| C2337 (C) | B1 | ST162/ST469 Cplx | STR,GM,TOB,NA, CIP,TE | SHV-2a | – | −18,+1,+58 | S83L-D87N/ S80I | −/+ | *aadA1* |
| C2339[g] (P) | B1 | ST162/ST469 Cplx | STR,GM,TOB,NA, CIP,TE | SHV-2a +OXA-2 | *tet*(A), *sul3* | −18,+1,+58 | S83L-D87N/ S80I | −/+ | – |
| C3297[g] (C) | B1 | ST155/ST155 Cplx | STR,GM,TOB,NA, TE,C, SXT | TEM-52c | *sul3* | ND | S83L/Wild | +/− | *aadA1* |
| C4424 (C) | F | ST3379[d] | GM,NA,CIP,TE,C, SXT | NG[f] | *sul3* | −28,+58 | S83L-D87N/ S80I | +/− | *dfrA1-aadA1* |

**Notes:** [a]C, chicken, P, pork; [b]Strains in bold letters showed AmpC phenotype in addition to the ESBL phenotype; [c]ND: Non determined; [d]New ST introduced in the database; [e]In the strain C3315 new allele *mdh* number 290 was encounter; [f]NG, no ESBL genes were detected; [g]Strains with Class 2 integron; Abbreviations: STR: streptomycin; GM: gentamicin; AMK: amikacin; TOB: tobramycin; NA: nalidixic acid; CIP: ciprofloxacin; TE: tetracycline; C: chloramphenicol; SXT: trimethoprim-sulphamethoxazole

ESBL genes were detected in one isolate with ESBL phenotype (C4424 strain). Five isolates contained, in addition of ESBL, other types of β-lactamases (TEM-1b, OXA-1 and OXA-2).

A high variety of genetic lineages were detected among the ESBL-producing strains with the identification of 12 different ST, where three of them were new (ST3377 and ST3379, with new allelic combinations, and ascribed to B1 and F phylogroups, respectively; ST3498, with a new *mdh* allele (290), and associated with phylogroup A). Most of the ESBL-producing isolates were ascribed to phylogroup B1 (12 strains), although phylogroups A, D, E and F were also found (one strain each). In total, 12 of 16 ESBL-producing isolates (75 percent) carried class 1 integrons and seven different gene cassette arrays were identified in their variable region (Table II); six of them corresponded to classic class 1 integrons and one to the *sul3*-atypical class 1 integron. Moreover, other genes associated with non-β-lactam resistance were found (number of strains): *sul3* (10), *tetA* (9, in two of them associated with *tetB*), and *cmlA* (6). Four ESBL-producing strains were positive for type 2 integrase (Table II). Three ESBL-producing strains contained more than one class 1 integron (Table II).

All 16 isolates but 1 (C3297) presented two amino acid changes in GyrA (S83L+D87N) and the S80I change in ParC (in three strains associated with E84G) (Table II). On the other hand, C3297 only presented one amino acid change in GyrA (S83L) and a wild ParC sequence. Six of the ESBL-positive isolates also presented the phenotype AmpC, although none of them carried the blaCMY-2 gene or other acAmpC encoding genes. Mutations in *ampC* promoter/attenuator region were studied in these six isolates with AmpC-phenotype: one isolate contained mutations at positions −42, −18, +1 and +58; another isolate had a wild sequence; c) three isolates presented the mutations −18, +1 and +58; and one strain the mutations −28 and +58 (Table II).

### *3.3 Characterization of AmpC-positive and ESBL-negative E. coli strains*

Table III shows the characteristics of the 17 $CTX^R$ *E. coli* isolates with AmpC-phenotype and ESBL-negative. Eight of them were CMY-2-producers, and the remaining nine isolates were negative for all acAmpC β-lactamase genes tested. The strains with the CMY-2 encoding gene, presented the classical environment (*ampR-bla*$_{CMY-2}$*-blc*) described by Jacoby (2009), but one strain of chicken origin, which showed an unusual upstream region with IS*1* truncating IS*Ecp1*. Two of the eight CMY-2-producing strains presented mutations in the promoter/attenuator region of *ampC* gene (−18, +1 and +58), and the other six showed a wild sequence. All CMY-2-positive isolates were ascribed to different ST, one of them new (ST3378), and corresponded to phylogroups: A (3 isolates), B1 (1), B2 (1), D (1), E (1) and F (1). All these strains showed ciprofloxacin, streptomycin and tetracycline resistance. One of the strains presented the *qnrB*10 gene and another one the *qnrA*1 gene. Five CMY-2-positive strains harbored a classic class 1 integron (intI1/ qacEΔ1-*sul*1) showing all of them different gene cassette arrays. Five CMY-2-producing strains were positive for type 2 integrase (Table III). Besides, all eight CMY-2-positive strains presented one or two amino acid changes in GyrA and ParC proteins. The nine strains negative for acAmpC encoding genes, six of them presented mutations in the promoter/attenuator region of ampC gene (−18, +1, +58 (5 strains); +58 (1 strain)), and the remaining three strains showed a wild sequence. On the other hand, these strains were ascribed to phylogroups: B1 ($n = 4$); D ($n = 3$); A ($n = 1$) and 251 C ($n = 1$). Only three of these strains carried class 1 integrons and the *aadA*(1/2) gene cassette was detected in two of them. Most of the strains presented amino acid changes 253 in GyrA ($n = 8$) and ParC proteins ($n = 6$) (Table III).

### *3.4 Virotyping of E. coli strains*

In total, 6 of the 19 tested strains were not carriers of VF, so they were classified as Virotype 0; nevertheless, we identified eight virotypes (A–H) where only virotype B may correspond to the same virotype in *E. coli* ST131 (detected in three strains with CMY-2), ten strains were grouped in seven defined virotypes (A, C–H). It is remarkable that 1 strain (C3315) harbored 4 of the 11 VF (Table IV).

**Table III.**
Characteristics of the 17 CTX$^{R}$ *E. coli* strains ESBL-negative and with AmpC phenotype, isolated from food samples of animal origin in Puebla, Mexico

| Strain (origin[a]) | Phylogenetic group | MLST[b] ST/CC | Resistance phenotype to non-$\beta$-lactam antibiotics | Plasmidic class C $\beta$-lactamase detected | Other resistance genes detected | Mutations in chromosomal *ampC* gene | Amino acid changes in GyrA/ParC | Class 1 integron *intI1*/ *qacEΔ1-sul1* | Gene cassettes |
|---|---|---|---|---|---|---|---|---|---|
| C3306 (C) | A | ST93/ST168 Cplx | STR,NA,CIP,TE,C, SXT | CMY-2[d] | – | Wild | S83L-D87N/ S80I | +/+ | *dfrA12-orfF-aadA2* |
| C3305 (C) | A | ST2207 | STR,NA,CIP,TE, SXT | CMY-2 | *qnrB10* | Wild | S83L/S80I | +/– | – |
| C3316[e] (P) | A | ST10/ST10 Cplx | STR,NA,CIP,TE,C, SXT | CMY-2 | *tet(A), tet(B), cmlA, sul3* | Wild | S83L-D87N/ S80I | +/+ | 2 integrons 1: *dfrA1-aadA1* 2: *dfr12-aadA2* |
| C3321[e] (C) | B1 | ST359 | STR,NA,CIP,TE | CMY-2 | *tet(A), cmlA, sul3* | −18,+1,+58 | S83L-D87N/ S80I | +/+ | *estx-psp-aadA2-cmlA-aadA1-qacH-IS330-sul3* |
| C3325[e] (C) | B2 | ST224 | STR,GM,NA,CIP, TE,C, SXT | CMY-2 | *qnrA1, cmlA, sul3* | −18,+1,+58 | S83L-D87N/ S80I | +/+ | *aad*A1 |
| C3311[e] (C) | D | ST2309 | STR,GM,TOB,NA, CIP,TE,C,SXT | CMY-2 | – | Wild | S83L-D87N/ S80I | +/– | *aadA*(1/2) |
| C3309[e] (P) | E | ST57/ST350 Cplx | STR,NA,CIP,TE, SXT | CMY-2 | – | Wild | S83L/S80I | +/– | – |
| C3304 (C) | F | ST3378[c] | STR,GM,AMK, TOB,NA, CIP,TE,C,SXT | CMY-2 | *tet(B)* | Wild | S83L-D87N/ S80I-E84G | +/+ | *estx-smr2-aadA* |
| C3307 (C) | B1 | ND | GM,NA,CIP,TE,C, SXT | – | *tet(A)* | −18,+1,+58 | S83L-D87N/ S80I | +/+ | *aadA*(1/2) |
| C3308 (P) | B1 | ND | STR,GM,TOB,NA, CIP,TE,C,SXT | – | – | −18,+1,+58 | S83L-D87N/ S80I | +/+ | – |

(*continued*)

**Table III.**

| Strain (origin[a]) | Phylogenetic group | MLST[b] ST/CC | Resistance phenotype to non-β-lactam antibiotics | Plasmidic class C β-lactamase detected | Other resistance genes detected | Mutations in chromosomal *ampC* gene | Amino acid changes in GyrA/ParC | Class 1 integron *intI1*/*qacEΔ1-sul1* | Gene cassettes |
|---|---|---|---|---|---|---|---|---|---|
| C3322 (C) | B1 | ND | STR,GM,TOB,NA, CIP,TE,C,SXT | – | *tet(A), cmlA, sul3* | −18,+1,+58 | S83L-D87N/ S80I | +/+ | *aadA*(1/2) |
| C3326 (C) | B1 | ND | STR,NA,TE,C | – | *tet(A), sul3* | −18,+1,+58 | S83L/Wild | –/+ | – |
| C3328 (C) | C | ND | STR,NA,CIP,TE, SXT | – | – | −18,+1,+58 | S83L-D87N/ S80I | –/– | – |
| C3330 (C) | D | ST12/ST12 Cplx | STR,GM,TOB,NA, TE,C | – | – | +58 | D87G/Wild | –/– | – |
| C3299 (C) | D | ND | STR,NA,CIP,TE,C, SXT | – | – | Wild | S83L-D87N/ S80I | +/– | – |
| C3323 (C) | D | ND | STR,GM,NA,TE,C, SXT | – | – | Wild | S83L/S80I | –/– | – |
| C3324 (C) | A | ND | STR,TE | – | *tet(B)* | Wild | Wild/Wild | –/– | – |

**Notes:** [a]C, chicken, P, pork; [b]ND, not determined; [c]new ST introduced in the database; abbreviations: STR, streptomycin; GM, gentamicin; AMK, amikacin; TOB, tobramycin; NA, nalidixic acid; CIP, ciprofloxacin; TE, tetracycline; C, chloramphenicol; SXT, trimethoprim-sulphamethoxazole; [d]this strain presented IS1-ΔIS*Ecp1*-CMY-2 as upstream region (Figure 1); [e]strains with class 2 integron

## 4. Discussion

ESBL-producing bacteria could spread through different sources; there are studies that report that ESBL-carrying *E. coli* of animal origin can disseminate via the food chain or water worldwide (Kluytmans *et al.*, 2013; Leverstein-van Hall *et al.*, 2011; Meunier *et al.*, 2006); nevertheless, few data do exist so far about the prevalence of ESBL or acAmpC producing *E. coli* isolates in food samples of animal origin for human consumption in Latin America (Bevan *et al.*, 2017), and these data are almost lacking in Mexico. Our study shows that more than half of the food samples analyzed, carried *E. coli* isolates producers of ESBL (35 percent) or acAmpC, concretely CMY-2 (17 percent). Moreover, the prevalence of ESBL-producing *E. coli* isolates was higher among chicken samples (39 percent) than among pork samples (20 percent).

Half of the ESBL-positive isolates contained the $bla_{\text{CTX-M2}}$ gene. This gene is unusual among *E. coli* isolates in Europe but it is more frequent in Latin America (Bevan *et al.*, 2017). Several Brazilian reports detected CTX-M-2 as predominant ESBL in *E. coli* obtained from chicken meat (Bonelli *et al.*, 2014; Botelho *et al.*, 2015; Casella *et al.*, 2015; Ferreira *et al.*, 2014). Moreover, in a study carried out in Sweden, CTX-M-2-producing *E. coli* strains were detected in broiler meat samples from South America (Egervärn *et al.*, 2014), suggesting the specific endemic situation of this ESBL. The presence among food samples of two CTX-M-1-positive *E. coli* strains (frequent in Europe and Magreb (Alonso *et al.*, 2017; Jouini *et al.*, 2013)), and of a CTX-M-15-producer strain of lineage ST617-B1 and that also carried *ibeA* gene, is relevant. The epidemic clone *E. coli* ST131-B2/CTX-M15 has been disseminated in clinical settings worldwide (Ranjan *et al.*, 2015), but it seems that other clones harboring CTX-M-15 could be also spreading in other ecosystems; such as the clone ST617-B1, detected in a chicken sample in this study, and also in a healthy dog in a previous report in Mexico (Rocha-Gracia *et al.*, 2015).

The detection of four strains producers of SHV-2a (one of them together with CTX-M-2) is of relevance; SHV-2a and SHV-2 are persisting ESBL in *E. coli* from food samples in Canada, particular in those of chicken origin (Pouget *et al.*, 2013). Most of our SHV-2a-positive strains of chicken origin carried a class 1 integron with *aadA*1 as gene cassette (streptomycin resistance), similar to previous data (Pouget *et al.*, 2013). On the other hand, a chicken sample was contaminated with a TEM-52c-producer *E. coli* strain (1/16, 6.2 percent). This type of ESBL has also been detected in low frequency in broiler chickens and turkey's meat in Great Britain (0.5–1.3 percent) (Randall *et al.*, 2011), or in piglets with post-weaning diarrhea in China province (2.6 percent) (Xu *et al.*, 2015), and more frequently in diverse *E. coli* clones from different piggeries in Portugal (59 percent) 296 (Rodrígues *et al.,* 2013). To our knowledge, this is first report of the blaTEM-52c gene in chicken meat *E. coli* isolates in Mexico.

In this study, the high rate of samples contaminated with CMY-2-producing *E. coli* strains (17 percent) is surprising, higher than in studies performed in food or food-producing animals in Africa or Europe (Ben Sallem *et al.*, 2014; Liebana *et al.*, 2013). Additionally, six of these CMY-2-producing strains carry some virulence genes (Table IV). Curiously, in other study realized in UK, CMY-type enzymes are a very frequent mechanism of resistance (42 percent) among oxymino-cephalosporin-resistant *E. coli* isolates obtained of raw chicken samples of South American origin (Dhanji *et al.*, 2010). In Mexico, there is only one previous study in

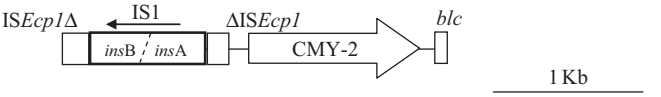


**Figure 1.**
Genetic environment of $bla_{\text{CMY-2}}$ in strain C3306

**Table IV.**
Virulence coding genes presents in CTX-M(−1, −2, 15)- or CMY-producing *E. coli* strains isolated from food samples of animal origin in Puebla, Mexico

| Virotype | Virulence factors coding genes | | | | | | | | | | Strain |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *afa/draBC* | *afa* operon | *iroN* | *sat* | *ibeA* | *papG*II | *cnf1* | *hlyA* | *papG*III | *cdtB* | *neuC*K1 | |
| A[a] | − | − | + | − | − | − | − | − | − | − | − | C3298[c], C3300[d], C3302[c] |
| B[a] | − | − | + | − | − | − | + | − | − | − | − | C3304[f], C3305[f], C3306[f], |
| C[a] | + | − | − | − | − | − | − | − | − | − | − | C3311[f] |
| D[a] | − | − | + | − | + | − | + | − | − | − | − | C3315[c] |
| E[a] | − | − | + | | − | − | − | − | − | − | − | C3314[c], C3316[f] |
| F[b] | − | − | − | − | + | − | − | − | − | − | − | C3320[c] |
| G[b] | − | − | − | − | − | − | − | − | − | + | − | C3321[f] |
| H[b] | − | − | − | − | + | − | − | − | − | − | − | C3327[e] |
| 0 | − | − | − | − | − | − | − | − | − | − | − | C3309[f], C3313[d], C3318[c], C3319[c], C3325[f], C3329[c] |

**Notes:** [a]Major virotypes in *E. coli* ST131; [b]new vyrotipes; [c]CTX-M-2 producer; [d]CTX-M-1 producer; [e]CTX-M-15 producer; [f]CMY-2 producer. +, positive PCR result; −, negative PCR result. *afa/draBC*, Afa/Dr adhesin; *afa* operon, FM955459; *iroN*, catecholate iron receptor; *sat*, autotransporter secreted toxin; *ibeA*, epithelial brain invasion protein; *papG*II, *papG* allele II; *cnf1*, citotoxic necrotizing factor type 1; *hlyA*, α-hemolysine; *papG*III, *papG* allele III; *cdtB*, cytolethal distention toxin; *neuC*K1, group II capsule K1 variant (based on *E. coli* ST131 scheme; Nicolas-Chanoine *et al.*, 2014)

which a CMY-producing *E. coli* strain is detected in one bovine meat sample (Aguilar-Montes de Oca *et al.*, 2015), without reporting the CMY variant. On the other hand, Zaidi *et al.* (2007) reported the detection of CMY-2 in Salmonella Typhimurium isolated from samples of retail meat and food animal intestines in Yucatan, Mexico, with a frequency of 3.9 percent in retail chicken and 7.4 percent in swine intestine. Moreover, prevalence CMY-2-producing *E. coli* strains of 11 and 9.8 percent were reported in healthy dogs and in cloacal samples of healthy turtles, in Mexico, respectively (Rocha-Gracia *et al.*, 2015; Cortés-Cortés *et al.*, 2016). Interestingly, since 2010 in Mexico the free sale of antibiotics in drugstores was regularized; however, the use of antibiotics as promoters of animal growth has not been legislated. The high variety of ST (with four new ones) and high carriage of virulence genes detected among the ESBL- and CMY-2-producing *E. coli* strains indicate that the dissemination of this resistance do not correspond to a specific clone but to different strains that could share similar plasmids. However, most of the ESBL- or CMY-2-producing isolates were typed as phylogroup B1 or A, respectively. Phylogroup B2, frequent among clinical isolates, was only detected in one CMY-2-positive *E. coli* isolate of chicken origin that corresponded to the lineage ST224, and carried the *qnrA*1 gene. This ST has been reported in *E. coli* strains isolated from human (non-pathogenic/pathogenic strain), chicken and horse (both pathogenic) (Alonso *et al.*, 2016; Silva *et al.*, 2016; Aizawa *et al.*, 2014), which indicates its wide distribution and easy spread; in our case carrying classes 1 and 2 integrons (Table III).

The high content of resistance genes for non-$\beta$-lactam antibiotics (Tables II and III) and the presence of amino acid changes in GyrA and ParC proteins in our ESBL or CMY-2-positive strains is of concern due to the potential difficulties of therapeutic options in case of being implicated in human infections.

Interestingly, since 2010 in Mexico the free sale of antibiotics in drugstores was regularized; however, the use of antibiotics as animal growth promoters has not been legislated (Dreser *et al.*, 2012). Multidrug resistant *E. coli* isolates may act as reservoirs for intra- and inter-species exchange and thereby a source for spread of multidrug-resistant determinants. Food samples could get contaminated by these strains during animal slaughter. Furthermore, other points of the food chain (markets, supermarkets and others) could also be a source for contamination (Szmolka and Nagy, 2013). Moreover, during handling of meat in markets and supermarkets, susceptible human might acquire multidrug-resistant *E. coli* strains, either by direct contact or through the environment which could be relevant to human medicine. In addition, sanitation problems, inadequate and unsanitary handling of food of animal origin, pet defecation outdoors, socioeconomic factors and environmental (air and water), could contribute to increase the presence of ESBL or acAmpC-enzyme-*E. coli* strains (Ljungquist *et al.*, 2016; Baede *et al.*, 2015). Also, contaminated food could be a source of resistant bacteria, at the kitchen level, for other food or for humans. All of the above leads us to recommend activities to improve surveillance and monitoring of food control in open-markets and supermarkets since the human could acquire MDR *E. coli* strains by cross-contamination, establishing in the intestinal microbiota with implications in the public health. It have been suggested that ExPEC (Extra intestinal Pathogenic *E. coli*) is defined by the presence of $\geqslant 2$ VF genes among *pap*, *sfa*, *afa/draBC*, *iutA* and *kpsM*TII (Johnson and Stell, 2000) and the virotype the strains belong to is important to infer the distribution of VF and the potential of damage that the pathogenic strain could deploy to the host cell; this scheme was originally proposed for *E. coli* ST131 since this clone is widespread and harbor genetic determinants of resistance and virulence (Nicolas-Chanoine *et al.,* 2014). Here, we detected 13 ESBL or acAmpC-enzyme-*E. coli* strains harboring VF genes previously reported in pathogenic ST131 *E. coli* strains; interestingly, virotype B-*E. coli* ST131 was detected in one strain and seven new virotypes were obtained. It highlights the importance of VF genes distribution worldwide that can be placed into Mobilizable Genetic Elements which also can harbor resistance determinants; interestingly, three CMY-2 and one CTX-M-2 producer

strains, were positive for *cnfI*; similarly, the CTX-M-15 producer strain C3327 carries the *ibeA* gene, which has been implied in brain endothelium invasion; otherwise, C3321 harbors *cdtB* gene, a cytolethal distention toxin; these genetic determinants may fit up the strains to be more aggressive, which could compromise intestinal stability leading to states of dysbiosis with the complications involved.

In conclusion, the high rate of ESBL and CMY-2-producing *E. coli* and some with virulence genes, in the food chain, represent an important problem of public health, acting as source for the spread of MDR determinants and/or VFs from contaminated food of animal origin to humans. In countries, like ours, it is necessary to monitor the food-producing animals for the presence of MDR bacteria, enhance the hygiene in food practices to limit the spread of these microorganisms and to promote the prudent use of antibiotics in animals and to limit its use as animal growth promoters.



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