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Characterization of CMY-2-type beta-lactamase-producing *Escherichia coli* isolated from chicken carcasses and human infection in a city of South Brazil

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Abstract

Background: Food-producing animals, mainly poultry, have been associated with the maintenance and dissemination of antibiotic-resistant bacteria, such as plasmid-mediated AmpC (pAmpC)-producing Enterobacteriaceae, to humans, thus impacting food safety. Many studies have shown that *Escherichia coli* strains isolated from poultry and humans infections share identical cephalosporin resistance, suggesting that transmission of resistance from poultry meat to humans may occur. The aim of this study was to characterize pAmpC-producing *E. coli* strains isolated from chicken carcasses and human infection in a restrict area and to determine their antimicrobial resistance profiles, and molecular type by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE).

Results: A total of 14 pAmpC-producing *E. coli* strains were isolated, including eight strains from chicken carcasses and six strains from human infections (from urine, tissue and secretion). The *bla*_{CMY-2} gene was identified in all pAmpC-producing *E. coli* strains by polymerase chain reaction (PCR) and DNA sequencing. High percentages of strains resistant to tetracycline, nalidixic acid and sulfamethoxazole-trimethoprim (78–92%) were detected, all of which were considered multidrug-resistant. Among the non-beta-lactam resistance genes, the majority of the strains showed *tetA*, *tetB*, *sulI* and *sulII*. No strain was considered an extended-spectrum beta-lactamases (ESBL) producer, and the *bla*_{TEM-1} gene was found in 2 strains isolated from human infection. Six strains from chicken carcasses and four strains from humans infections were linked to an *ISEcp1*-like element. Through MLST, 11 sequence types were found. Three strains isolated from human infection and one strain isolated from chicken carcasses belonged to the same sequence type (ST354). However, considerable heterogeneity between the strains from chicken carcasses and humans was confirmed by PFGE analysis.

Conclusion: This study showed the prevalence of *E. coli* strains producing *bla*_{CMY-2} linked to *ISEcp1* that were present in both chickens and humans in a restricted area. Our results also suggest the presence of a highly diverse strains that harbor pAmpC, indicating no clonal dissemination. Therefore, continuous monitoring and comparative analyses of resistant bacteria from humans and food-producing animals are needed.

Keywords: *Escherichia coli*, Chicken, Plasmid-mediated AmpC, Zoonotic risk

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Background

Food-producing animals have been associated with the maintenance and dissemination of antimicrobial-resistant bacteria to humans, impacting food safety. Studies have indicated that poultry meat is an important reservoir for resistance problems rapidly emerging worldwide due to bacterial selection caused by antimicrobial agents used as growth promoters or for prophylactic and therapeutic purposes [1–4].

In recent years, the frequency of resistance to third-generation cephalosporins has increased both in strains isolated from human infections and from the colonization of food-producing animals, mainly mediated by extended-spectrum beta-lactamases (ESBL) and the AmpC-beta-lactamase. However, the epidemiology of AmpC-producing bacteria may be underreported due to the lack of a phenotypic test for the detection of this mechanism of resistance. Failure to detect this beta-lactamase has contributed to its uncontrolled spread and occasional therapeutic failure [5–8].

Bacteria overexpressing AmpC beta-lactamases are usually resistant to all beta-lactam antibiotics, except cefepime, ceftazidime, and carbapenems, which is an important clinical concern because the bacteria often express a multidrug-resistant phenotype, leaving limited therapeutic options. The AmpC beta-lactamase can be encoded by genes located on chromosomes or plasmids. In *Escherichia coli*, the expression of the chromosome-encoded AmpC beta-lactamase is very low, due to the absence of the *ampR* regulator gene. On the other hand, the genes that encode plasmid-mediated AmpC beta-lactamases (pAmpC) in *E. coli* are often overexpressed and have been found around the world in nosocomial and non-nosocomial isolates. Plasmid-mediated *ampC* genes originated from chromosomal *ampC* genes carried by several gram-negative species and are classified into at least five phylogenetic groups, namely, the *Enterobacter* group (MIR, ACT), the *Citrobacter freundii* group (CMY-2-like, LAT, CFE), the *Morganella morganii* group (DHA), the *Hafnia alvei* group (ACC), and the *Aeromonas* group (CMY-1-like, FOX, MOX), with the most prevalent and widely disseminated being CMY-2-like enzymes. The presence of AmpC in plasmids has contributed to the rapid spread of this mechanism of resistance [5, 7, 9].

The prevalence of pAmpC-producing *E. coli* varies significantly depending on the geographical region and host, with a high prevalence in both humans and food-producing animals mainly in North America [2–4]. In Brazil, pAmpC beta-lactamases were first reported in strains isolated from humans between 2007 and 2008 [10, 11]. Since then, AmpC-producing bacteria have been reported in food-producing animals, such as poultry carcasses [12–16]. However, there are few studies on

AmpC-mediated resistance in human clinical and veterinary medicine in Brazil. As Brazil is one of the largest chicken meat exporters in the world and our work has pointed out a significant increase in the presence of beta-lactamases in chicken meat from Brazil [12], an investigation of the spread of AmpC genes in food-producing animals is also important to elucidate the origin of resistant strains. The aim of our study was to characterize pAmpC-producing *E. coli* strains from both chicken carcasses and human clinical samples from a city in southern Brazil (Paraná state) within close time periods to determine whether chicken meat might act as a reservoir and dissemination route for pAmpC-producing *E. coli*. These strains were studied regarding their antimicrobial resistance profiles and molecular typing by multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE).

Methods

Bacterial isolates

In 2013, a study performed by our group [12] isolated 121 *E. coli* strains from commercial refrigerated chicken carcasses intended only for local consumption that were sold in a city in southern Brazil. From these strains, 8 were screened and confirmed as pAmpC-producing strains by polymerase chain reaction (PCR) described by Pérez-Pérez and Hanson (2002) [7]. These strains belong to the collection of the Basic and Applied Bacteriology Laboratory from State University of Londrina (UEL), Londrina, PR, Brazil. Between 2013 and 2015, 6 *E. coli* strains isolated from human infection (from urine, tissue and secretion) were confirmed as pAmpC by the Vitek system GNID card (bioMérieux, Marcy l'Etoile, France) and PCR [7] (Table 1). Only one isolate was selected per patient. These strains were provided by the University Hospital of Londrina, Londrina, Paraná, Brazil.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *E. coli* isolates was performed using the standard disk-diffusion method recommended by the Clinical and Laboratory Standards Institute [24, 25], with the following antimicrobials: ciprofloxacin (5 µg), gentamicin (10 µg), norfloxacin (10 µg), enrofloxacin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), tetracycline (30 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), nitrofurantoin (300 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) and amoxicillin-clavulanic acid (20/10 µg) (Oxoid Ltd., Basingstoke, Hants, UK). For the negative control, we used *E. coli* strain ATCC 25922. All strains resistant to 3rd generation cephalosporins were tested for phenotypic confirmation of ESBL production by standard ceftazidime and cefotaxime disks combined with clavulanic acid [25] and by the double-disk diffusion method with disks containing cefepime, cefotaxime, ceftazidime and

Table 1 Oligonucleotide used for amplification in the PCR

Target	Gene	Oligonucleotide sequence (5' to 3')	References
<i>bla</i> _{ESBL}	<i>bla</i> _{CTX-M-1} group	F- AAA AAT CAC TGC GCC AGT TC R- AGC TTA TTC ATC GCC ACG TT	[17]
	<i>bla</i> _{CTX-M2} group	F- CGA CGC TAC CCC TGC TAT T R- CCA GCG TCA GAT TTT TCA GG	
	<i>bla</i> _{CTX-M8} group	F- TCG CGT TAA GCG GAT GAT GC R- AAC CCA CGA TGT GGG TAG C	
	<i>bla</i> _{CTX-M9} group	F- CAA AGA GAG TGC AAC GGA TG R- ATT GGA AAG CGT TCA TCA CC	
	<i>bla</i> _{CTX-M25} group	F- GCA CGA TGA CAT TCG GG R- AAC CCA CGA TGT GGG TAG C	
	<i>bla</i> _{TEM}	F- CAT TTC CGT GTC GCC CTT ATT C R- CGT TCA TCC ATA GTT GCC TGA C	[18]
	<i>bla</i> _{SHV}	F- CAC TCA AGG ATG TAT TGT G R- TTA GCG TTG CCA GTG CTC G	[19]
<i>bla</i> _{pAmpC}	<i>bla</i> _{MOX-1} , <i>bla</i> _{MOX-2} , <i>bla</i> _{CMY-1} , <i>bla</i> _{CMY-8} to <i>bla</i> _{CMY-11}	F- GCT GCT CAA GGA GCA CAG GAT R- CAC ATT GAC ATA GGT GTG GTG C	[7]
	<i>bla</i> _{LAT-1} to <i>bla</i> _{LAT-4} , <i>bla</i> _{CMY-2} to <i>bla</i> _{CMY-7} , <i>bla</i> _{BIL-1}	F- TGG CCA GAA CTG ACA GGC AAA R- TTT CTC CTG AAC GTG GCT GGC	
	<i>bla</i> _{DHA-1} and <i>bla</i> _{DHA-2}	F- AAC TTT CAC AGG TGT GCT GGG T R- CCG TAC GCA TAC TGG CTT TGC	
	<i>bla</i> _{ACC}	F- AAC AGC CTC AGC AGC CGG TTA R- TTC GCC GCA ATC ATC CCT AGC	
	<i>bla</i> _{MIR-1T} , <i>bla</i> _{ACT-1}	F- TCG GTA AAG CCG ATG TTG CGG R- CTT CCA CTG CGG CTG CCA GTT	
	<i>bla</i> _{FOX-1} to <i>bla</i> _{FOX-5b}	F- AAC ATG GGG TAT CAG GGA GAT G R- CAA AGC GCG TAA CCG GAT TGG	
PMQR	<i>qnrA</i>	F- ATG ATG AAA AAA TCG TTA TGC TGC R- GCT TTT CAA GAA TGC GCC AGG	[20]
	<i>qnrB</i>	F- AGA GGA TTT CTC ACG CCA GG R- TGC CAG GCA CAG ATC TTG AC	[21]
	<i>qnrS</i>	F- GGM ATH GAA ATT CGC CAC TG R- TTT GCY GYY CGC CAG TCG AA	
Tetracycline resistance	<i>tetA</i>	F- GCA AGT TCA TTG AAC AGG GT R- TCT AAA CCG TCG AGT TCG GCG	
	<i>tetB</i>	F- GCC TTT CCT TTG GGT TCT CT R- TGT CCG ACA AGT TGC ATG AT	[22]
Sulfonamide resistance	<i>sulI</i>	F- GCT TTC AGG GAT CAC AGG AG R- GAC CAA GAC CCG CTA ATG AA	
	<i>sulII</i>	F- ACG AGA TTG TGC GGT TCT TC R- GGT TTC CGA GAT GGT GAT TG	[22]
Insertion sequence	<i>sulI</i>	F- CCG TCT CGC TCG ACA GTT AT R- GTG TGT GCG GAT GAA GTC AG	
	ISEcp1 - CMY	F- AAA AAT GAT TGA AAG GTG GT R- TTT CTC CTG AAC GTG GCT GGC	[7, 23]

aztreonam placed 25 mm apart (center to center) to a disk containing a beta-lactamase inhibitor (amoxicillin-clavulanic acid) [26].

Screening of antimicrobial resistance genes and insertion sequence

All *E. coli* strains were screened by PCR for *bla*_{CMY} gene as described by Dierikx and collaborators (2010) and sequenced [20] (Table 1). For sequencing, amplicons were purified with a column-based kit (Pure Link Quick PCR Purification Kit, Invitrogen, Germany). The purified product was sequenced based on Sanger methodology using an ABI PRISM 3500xL Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequencing was performed at the Multiuser Laboratory of Genotyping and Sequencing from State University of Campinas (UNICAMP) and in the Soil Biotechnology Laboratory from the Brazilian Agricultural Research Corporation (Embrapa).

After sequencing, homology searches were done based on the BLAST algorithm available at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. The DNA sequences were compared with reference sequences from the LAHEY home page (<http://www.lahey.org/Studies/>).

The strains were also analyzed for the presence of other major beta-lactamase genes (*bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM}), plasmid mediated quinolone resistance (PMQR) determinants (*qnrA*, *qnrB* and *qnrS*), sulfonamide resistance genes (*sulI* and *sulIII*) and tetracycline resistance genes (*tetA* and *tetB*) [7, 17–22]. To define the mechanism of transmission of the pAmpC gene, we screened *bla*_{ampC} for the insertion sequence *ISEcp1* by *ISEcp1*-*bla*_{CMY} linkage PCR [23]. Strains positive for this PCR were sequenced as previously described. Details regarding oligonucleotide primers and references are illustrated in Table 1. The PCR mixture was prepared using Promega PCR Master Mix (Promega, USA). PCR amplicons were visualized on 2.0% agarose gels stained with GelRed (Biotium). After gel electrophoresis, the images were captured using an Image Capture System (LPixImageHE). Strains positive for beta-lactamases (non AmpC-type) were also sequenced as previously described.

Multilocus sequence typing

MLST was performed according to the Achtman scheme (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli>), for sequencing the PCR amplicon *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*. Sequencing was performed as previously described in this manuscript.

Pulsed-field gel electrophoresis

Genomic relationships were analyzed by *XbaI* restriction digestion followed by pulsed-field gel electrophoresis (PFGE) using the CHEF DR III PFGE System (BioRad,

Hercules, CA, USA). Electrophoresis conditions consisted of an initial time of 2.2 s, a final time of 54.2 s at a gradient of 6 V cm⁻¹ and an included angle of 120°. The gels were electrophoresed for 18 h. The results were evaluated with BioNumerics (version 7.6; Applied Maths, Austin, TX, USA) using the cut-off value of 80% similarity to distinguish PFGE types.

Results

A total of 14 strains (8 strains isolated from chicken carcasses and 6 strains isolated from humans infections) were confirmed as pAmpC-producing strains by PCR. The strains from humans infections were isolated from urine (*N* = 4), a fragment of sacral ulcer tissue (*N* = 1) and secretion of an abdominal surgical wound (*N* = 1) (Fig. 1). PCR and sequencing, using specific primers (Table 1) identified the *bla*_{CMY-2} gene in all pAmpC-producing *E. coli* strains.

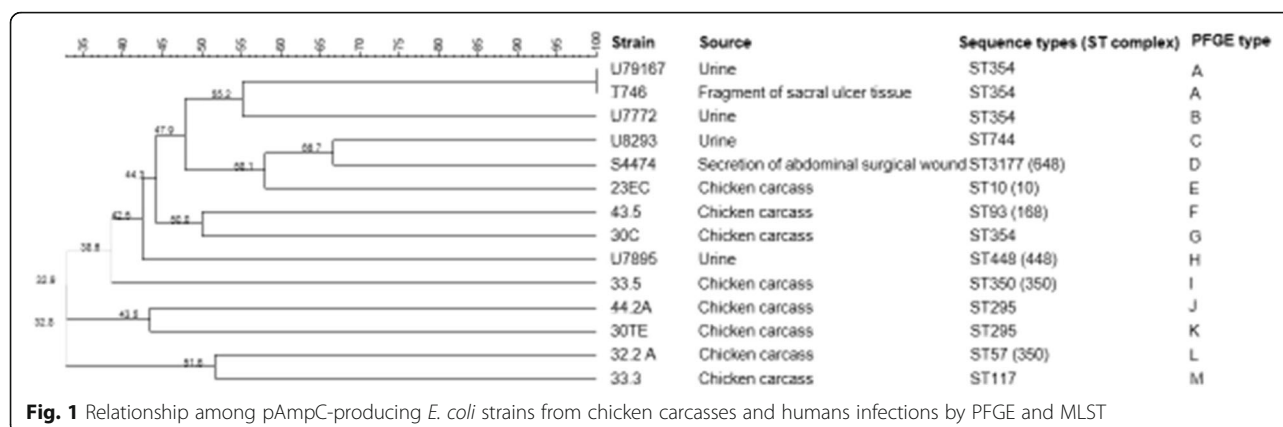
All the strains were resistant to amoxicillin-clavulanic acid, and 92.86% of the strains were resistant to cefoxitin. According to the antimicrobial susceptibility profile for non-beta-lactam antimicrobials, the strains presented a high frequency of resistance for mainly tetracycline (92.86%), nalidixic acid (92.86%) and sulfamethoxazole-trimethoprim (78.57%) (Table 2). Among the non-beta-lactamase genes, the strains showed *tetA* (7 from chickens and 2 from humans), *tetB* (6 from chickens and 3 from humans), *sulI* (8 from chickens) and *sulIII* (7 from chickens and 1 from humans) (Table 2). PMQRs were not found. All strains were considered multidrug-resistant (non-susceptible to at least 1 agent in 3 or more antimicrobial categories) [27].

ESBL production, by phenotypic testing, was not observed for any strain. In addition, the *bla*_{TEM-1} gene was found in 2 strains isolated from human.

To detect whether *ISEcp1* is upstream of *bla*_{CMY-2}, PCR with a forward primer targeting the *ISEcp1* element and a reverse primer targeting the *bla*_{CMY} genes was performed, and the amplicons of positive strains were sequenced. Ten strains (6 from chicken carcasses and 4 from human infection) were positive, and sequencing confirmed that *bla*_{CMY-2} genes are linked to an upstream *ISEcp1*-like element.

By MLST, 11 sequence types (STs) were found. Three strains isolated from human infection (2 from urine and 1 from tissue) and one strain isolated from a chicken carcass belonged to ST354 (Fig. 1).

The AmpC-beta-lactamase-producers were classified within 13 PFGE types, showing high diversity among strains. Only two strains of ST354 from human urine and tissue (U79167 and T746 strains) showed 100% similarity (Fig. 1).



Discussion

The emergence of third-generation cephalosporin-resistant Enterobacteriaceae, such as expressing ESBL and AmpC, in food-producing animals and their products has impacted the health of consumers, leading to the hypothesis that animals might become antimicrobial resistance sources and/or even contribute to the spread of these bacteria. Recent studies have shown that poultry and humans share similar antimicrobial resistance genes, and *E. coli* strain types, suggesting that transmission from poultry to humans may occur [2–4]. The presence of similar pAmpC in strains isolated from chicken meat and human clinical samples, in the same city and similar time period led us to compare the similarity of these strains by PFGE

and MLST methodologies and to determine their antimicrobial resistance profiles to understand the dissemination of this mechanism of resistance.

In 2013, our group identified 8 pAmpC-producing *E. coli* isolates from chicken carcasses [12]. Near this time period, 6 pAmpC-producing *E. coli* were also isolated from patients with infection from a hospital in the same city where our study was conducted with chicken carcasses. All pAmpC of these strains, from chicken carcasses and human infection, were identified as the *bla*_{CMY-2} gene by sequencing. According to the literature, *bla*_{CMY-2} is the most common pAmpC gene identified from widespread human and veterinary medical cases [2–4]. Initially, in Brazil, pAmpC-producing bacteria

Table 2 Antimicrobial resistance profiles, presence of resistance genes and *ISEcp1* in AmpC beta-lactamase positive *E. coli* strains

Strains	Antimicrobial resistance profile to non beta-lactams	Betalactamase genes	Non beta-lactam resistance genes	<i>ISEcp1</i>
Chicken carcass				
23EC	tet, gen, nal, sut	<i>bla</i> _{CMY-2}	<i>tetA</i> , <i>tetB</i> , <i>sull</i> , <i>sullI</i>	+
30C	tet, gen, clo, nal, cip, nor, enr, sut	<i>bla</i> _{CMY-2}	<i>tetB</i> , <i>sull</i> , <i>sullI</i>	+
30TE	tet, nal, sut	<i>bla</i> _{CMY-2}	<i>tetA</i> , <i>tetB</i> , <i>sull</i> , <i>sullI</i>	+
32.2 A	tet, nal, sut	<i>bla</i> _{CMY-2}	<i>tetA</i> , <i>tetB</i> , <i>sull</i> , <i>sullI</i>	+
33.3	tet, nit, nal, cip, enr	<i>bla</i> _{CMY-2}	<i>tetA</i> , <i>tetB</i> , <i>sull</i> , <i>sullI</i>	+
33.5	tet, nal, cip, nor, enr, sut	<i>bla</i> _{CMY-2}	<i>tetA</i> , <i>tetB</i> , <i>sull</i> , <i>sullI</i>	–
43.5	tet, gen, clo, nit, sut	<i>bla</i> _{CMY-2}	<i>tetA</i> , <i>sull</i> , <i>sullI</i>	–
44.2 A	tet, nal, sut	<i>bla</i> _{CMY-2}	<i>tetA</i> , <i>sull</i>	+
Human samples				
U79167	tet, nal, cip, nor, enr, sut	<i>bla</i> _{CMY-2} ; <i>bla</i> _{TEM-1}	<i>tetA</i>	+
U7895	tet, nal, cip, nor, enr, sut	<i>bla</i> _{CMY-2}	<i>tetA</i>	–
T746	tet, nal, cip, nor, enr, sut	<i>bla</i> _{CMY-2} ; <i>bla</i> _{TEM-1}	<i>tetB</i>	–
U7772	tet, gen, nal, cip, nor, enr, sut	<i>bla</i> _{CMY-2}	<i>tetB</i>	+
S8293	tet, nit, nal, nor, enr	<i>bla</i> _{CMY-2}	<i>tetB</i> , <i>sullI</i>	+
S4474	nal, cip, nor, enr	<i>bla</i> _{CMY-2}	–	+

tetracycline (tet), gentamicin (gen), chloramphenicol (clo), nitrofurantoin (nit), nalidixic acid (nal), ciprofloxacin (cip), norfloxacin (nor), enrofloxacin (enr), trimethoprim-sulfamethoxazole (sut)

(+) Presence

(–) Absence

were only observed in human medical settings. FOX-5 like and CMY-2-like were the first pAmpC beta-lactamases reported in Brazilian isolates and were detected in *E. coli* from patients in hospitals [10, 11]. Studies have shown an increase in the frequency of pAmpC in human clinical setting, but few studies have described the frequency of pAmpC in Enterobacteriaceae in Brazil [6, 11, 28, 29].

However, since 2015, studies have found pAmpC-producing bacteria in food-producing animals, mainly chicken meat in Brazil, leading to the hypothesis that this might become an infection source or reservoir that contributes to the spread of these bacteria. The *bla*_{CMY-2} gene is also the pAmpC variant more frequently found in food-producing animals in Brazil [12–16, 30]. Studies have detected a high prevalence of *bla*_{CMY-2} genes harbored on different plasmids in *E. coli* from poultry [13, 14]. In Brazil, da Silva and collaborators (2017) [16] found *bla*_{CMY-2} in avian pathogenic *E. coli* (APEC) from turkey, with airsacculitis, showing that this antimicrobial resistance mechanism can also be found in pathogenic strains.

The true rate of occurrence of pAmpC in strains of *E. coli* remains unknown because only a few surveillance studies have examined this resistance mechanism in Brazil [6]. Moreover, the lack of a standardized phenotypic method for the detection of AmpC-producing isolates contributes to underreporting in human clinical laboratories and veterinary medicine [5]. This higher frequency of pAmpC found in food-producing animals in recent years may be linked to changes in molecular epidemiology of AmpC beta-lactamase and to the indiscriminate use of antimicrobials in the production of these animals, which may be selecting this resistance mechanism.

Infections caused by pAmpC-producing bacteria limit therapeutic options since these organisms are usually resistant to all beta-lactam antibiotics, except cefepime, ceftazidime, and the carbapenems [5]. In our study, all strains were considered multidrug-resistant and were nonsusceptible to at least 1 agent in 3 or more antimicrobial categories [27]. The highest frequencies of resistance to non-beta-lactam antimicrobials were for tetracycline (92.86%), nalidixic acid (92.86%) and sulfamethoxazole-trimethoprim (78.57%). In addition, by PCR, our strains showed that chicken meat is a reservoir of non-beta-lactam resistance genes such as *tetA*, *tetB*, *sulI* and *sulII* (Table 2), which corroborates the high frequency of phenotypic resistance for tetracycline and sulfamethoxazole-trimethoprim. In addition, two strains from humans have the *bla*_{TEM-1} gene, which encodes a beta-lactamase with a lower spectrum of action. The *bla*_{pAmpC} genes are usually present in mobile genetic elements, which carry resistance genes encoding other beta-lactamases and/or genes encoding resistance to

other classes of antimicrobials, as pAmpC-producing bacteria are commonly multiresistant [5]. Co-resistance phenotypes are involved in the maintenance of resistance genes and plasmids in *E. coli* thus, the use of antimicrobials in animal production may also play a role in the selection of multidrug-resistant isolates in the animals' environment [4].

A variety of genetic elements has been implicated in the mobilization of *bla*_{ampC} genes onto plasmids. It has been reported that beta-lactamase genes can be genetically linked to an upstream insertion elements, as *ISEcp1*. Many studies have shown that *bla*_{AmpC}-type genes are associated with mobile genetic elements, including insertion sequences such as *ISEcp1*, most of which are carried on transferable plasmids [5, 31, 32]. In Brazil, only one study reported the presence of the *ISEcp1*-*bla*_{CMY-2} gene present on a plasmid from an *E. coli* strain isolated from chickens [13]. In our study, six strains from chicken carcasses and four strains from humans infection (71.4% of the total strains) showed the *bla*_{CMY-2} gene linked to an upstream *ISEcp1*-like element. This insertion element can be responsible for the transposition of *bla*_{CMY-2} to different plasmids and can also have an important role in the dissemination of CMY-2 beta-lactamases.

MLST is a methodology that can reflect the microevolution of the *E. coli* core genome, providing a true picture of the population structure of this bacterial species [4]. Eleven STs were found in this study (Fig. 1), all of which were reported both in birds and humans, with the exception of ST3177, which has never been reported in birds. All the STs have been reported in Brazil, with the exception of ST448 and ST3177 [4, 13, 33–42]. STs 10, 57, 93 and 117 were reported in avian pathogenic *E. coli* (APEC) and extraintestinal pathogenic *E. coli* (ExPEC) in Brazil, showing that these strains may be related to strains pathogenic, for both poultry and humans [38].

Four strains were grouped as ST354 (3 strains isolated from human infection and 1 strain isolated from a chicken carcass) suggested the possibility that they share the same clonal origin. However, PFGE revealed considerable heterogeneity among these strains. The most closely related strains were the 2 strains isolated from urine and tissue of human infection. PFGE also revealed 13 different PFGE types, with the dendrogram clearly showing a good distinction between the strains isolated from humans and chicken carcasses (Fig. 1). These data suggest a high diversity of strains that carry pAmpC genes and show possible parallel microevolution [4].

According to our study, we found a diverse *E. coli* population from both chicken carcasses and in human infection carrying the *bla*_{CMY-2} gene. Some studies also concluded that dissemination of AmpC-producing *E. coli* does not occur by clonal strains in these hosts [43, 44].

However, in our study, the presence of *ISEcp1* upstream of *bla_{CMY-2}* in some strains suggests that mobile genetic elements are being disseminated between bacteria from humans and animals, mainly poultry.

Antimicrobials are normally used in animal husbandry as veterinary drugs or feed additives [45]. Although a withdrawal time for antimicrobial use is required before the animal is sacrificed for sale, Wang and collaborators (2017) found residues of antimicrobials in meat and even detected some human antimicrobials, that are not used as veterinary drugs. The spread of antimicrobial resistance genes in poultry may be associated with the prophylactic use of cephalosporins injected into eggs to control *E. coli* omphalitis in broiler chickens [46]. In Brazil, third-generation cephalosporins have been associated with *in ovo* vaccination on the 18th day of incubation because the vaccine can also select antimicrobial resistant bacteria in poultry [33].

Further research about the dissemination of resistant bacteria need to be conducted in a given time and geographical area to trace the flow of resistant bacteria because there are few studies about this dissemination [4]. Our study allows us to understand some aspects of the dissemination of this resistance mechanism in a restricted area, which is important step for developing strategies aimed at preventing the propagation of this resistance through food ingestion. These data show the presence of the *bla_{CMY-2}* gene linked with an *ISEcp1*-type insertion element in both chicken carcasses and in human infection in a restricted region. Our results suggest the presence of highly diverse strains that harbor pAmpC, indicating no clonal dissemination. In a “One-Health” context, continuous collaboration among professionals in human and animal healthcare, the food industry and the environmental sector is needed to characterize the occurrence and routes of dissemination of these antimicrobial resistance determinants.

Conclusion

Since Brazil is one of the largest exporters of chicken meat in the world, surveillance studies are essential to identify resistance genes and bacterial clones that may spread from chickens to humans. Our results show the presence of highly diverse strains that harbor pAmpC, indicating no clonal dissemination. However, the presence of *bla_{CMY-2}*, linked to the *ISEcp1* element, was present both in chicken meat and human infection, suggesting that mobile genetic elements can be responsible for the spread of this resistance mechanism in this restricted area. Therefore, continuous monitoring and comparative analyses of resistant bacteria from humans and food-producing animals are needed.

Abbreviations

APEC: Avian pathogenic *E. coli*; CLSI: Clinical and Laboratory Standards Institute; Embrapa: Brazilian Agricultural Research Corporation; ESBL: Extended-spectrum beta-lactamase; ExPEC: Extraintestinal pathogenic *E. coli*; MLST: Multilocus sequence typing; pAmpC: Plasmid-mediated AmpC; PCR: Polymerase chain reaction; PFGE: Pulsed-field gel electrophoresis; PMQR: Plasmid mediated quinolone resistance; ST: Sequence typing; UEL: State University of Londrina; UNICAMP: State University of Campinas

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Authors' contributions

VLK performed the experimental research, data analysis and wrote the manuscript. ECV performed the microbiological analysis of the strains isolated from human infections. RPM and WDS participated in the PFGE and MLST tests and in drafting and analyzing the results of these tests. RAR and MH participated in the sequencing tests. GN participated in drafting the manuscript. RKT coordinated the manuscript and the overall study. All authors have read and approved the manuscript.

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Availability of data and materials

All the data supporting our findings are contained in the manuscript. The raw data and scientific records are saved in our laboratory and can be obtained from the corresponding author per a reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics and Research Committee of the State University Londrina (CAAE 43013315.8.0000.5231).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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