UNIVERSIDADE
ESTADUAL DE LONDRINA

VANESSA LUMI KOGA

CARACTERIZAÇÃO DE *ESCHERICHIA COLI* PRODUTORA DE ESBL E AMPC DE AMOSTRAS CLÍNICAS HUMANAS E DE CARCAÇAS DE FRANGO

Londrina
2019

VANESSA LUMI KOGA

CARACTERIZAÇÃO DE *ESCHERICHIA COLI* PRODUTORA DE ESBL E AMPC DE AMOSTRAS CLÍNICAS HUMANAS E DE CARCAÇAS DE FRANGO

Tese apresentada ao Programa de Pós-graduação em Microbiologia da Universidade Estadual de Londrina, como requisito parcial à obtenção do Título de Doutor em Microbiologia.

Orientadora: Profa. Dra. Renata Katsuko Takayama Kobayashi

Londrina
2019

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

K78 Koga, Vanessa Lumi.

Caracterização de *Escherichia coli* produtora de ESBL e AmpC de amostras clínicas humanas e de carcaças de frango / Vanessa Lumi Koga. - Londrina, 2019.

88 f. : il.

Orientador: Renata Katsuko Takayama Kobayashi.

Tese (Doutorado em Microbiologia) - Universidade Estadual de Londrina, Centro de

1. *Escherichia coli* - Tese. 2. ESBL - Tese. 3. AmpC - Tese. 4. Infecção urinária - Tese. I. Kobayashi, Renata Katsuko Takayama. II. Universidade Estadual de Londrina. Centro de Ciências Biológicas. Programa de Pós-Graduação em Microbiologia. IV. Título.

CDU 579

VANESSA LUMI KOGA

CARACTERIZAÇÃO DE *ESCHERICHIA COLI* PRODUTORA DE ESBL E AMPC DE AMOSTRAS CLÍNICAS HUMANAS E DE CARCAÇAS DE FRANGO

Tese apresentada ao Programa de Pós-graduação em Microbiologia da Universidade Estadual de Londrina, como requisito parcial à obtenção do Título de Doutor em Microbiologia.

BANCA EXAMINADORA

Renata Kobayashi

Orientadora: Profa. Dra Renata K. T. Kobayashi
Universidade Estadual de Londrina – UEL

Prof. Dr. Gerson Nakazato
Universidade Estadual de Londrina – UEL

Prof. Dra. Eliana Carolina Vespero
Universidade Estadual de Londrina – UEL

Profa. Dra. Marcia Regina Eches Perugini
Universidade Estadual de Londrina – UEL

Prof. Dr. Benito Guimarães de Brito
Instituto de Pesquisas Veterinárias Desidério
Finamor – IPVDF

Londrina, 23 de agosto de 2019.

Dedico este trabalho aos meus pais e aos meus irmãos que representam tudo para mim.

"Desistir... eu já pensei seriamente nisso, mas nunca me levei realmente a sério; é que tem mais chão nos meus olhos do que o cansaço nas minhas pernas, mais esperança nos meus passos, do que tristeza nos meus ombros, mais estrada no meu coração do que medo na minha cabeça."

(Cora Coralina)

AGRADECIMENTOS

Agradeço imensamente a Deus por todas as oportunidades dadas, por todas as pessoas incríveis que colocou em minha vida e por me ajudar a construir meu caminho.

Aos meus pais Nelson M. Koga e Emiko M. Koga pelo amor incondicional, pelas ajudas e apoios, e por tudo que me ensinaram até aqui. Agradeço aos meus irmãos, Tatiane K. M. Koga, Daniele S. Koga e Fabrício M. Koga, por todos esses anos de convivência, companheirismo e apoio. Eu não seria nada sem a ajuda de cada membro da minha família.

Agradeço imensamente à minha orientadora Drª Renata K. T. Kobayashi, por todo ensinamento, por abrir as portas da microbiologia para mim, por me acompanhar durante a graduação, mestrado e agora no doutorado, e por ser a maior responsável pelo caminho que segui. Obrigada por fazer a diferença em minha vida! A você toda a minha admiração, e respeito.

Ao professor Dr. Gerson Nakazato, por todo ensinamento, ajuda, conselhos e motivação. O professor é um grande exemplo de profissional, esteve sempre à disposição para me ajudar, e seu acompanhamento em toda a minha trajetória foi de imenso aprendizado. Obrigada por tudo!

A professora Drª Eliana Carolina Vespero, por todo ensinamento, ajuda com projetos, por me acompanhar durante toda a minha caminhada na graduação, mestrado, especialização e doutorado. Obrigada também pela oportunidade de novos projetos e por estar sempre à disposição para me ajudar. A você toda a minha admiração e respeito da excelente profissional.

A professora Drª Márcia R. E. Perugini, por aceitar fazer parte da minha banca de doutorado. Também é uma grande profissional, quem me ensinou muito sobre microbiologia, e tenho grande admiração. Muito obrigada por todo ensinamento e ajuda.

Ao Dr. Benito G. de Brito, por aceitar fazer parte da banca. Agradeço também pela oportunidade dada a novos projetos e por me acompanhar no mestrado. Obrigada pelas correções e parcerias.

Ao Dr. Renato P. Maluta e Dr. Wanderley Dias da Silveira, por terem nos acolhidos em seu laboratório na UNICAMP, por todo ensinamento quanto as técnicas de PFGE e sequenciamentos. O aprendizado que tive com vocês foi de grande crescimento para o projeto que desenvolvi em meu doutorado.

Ao Dr. Renan A. Ribeiro e a Drª Mariangela Hungria pelo serviços prestados aos sequenciamentos, uso do PFGE e do programa Bionumerics. Muito obrigada pela disposição e toda a ajuda.

A Drª Sara Mataroli de Godoy e profª Claudete de Fátima Ruas, pelos sequenciamentos realizado no Laboratório de Marcadores Moleculares e Citogenética de Plantas.

Agradeço a todos os integrantes do Laboratório de Bacteriologia Básica e Aplicada, vulgo "NIP3", tanto aos atuais quanto aos que passaram por lá. Tenho uma admiração muito grande por cada um de vocês, já foram muitos anos de convivência, que sentirei muita falta. Essa família que está sempre unida, mesmo com os diversos problemas que enfrentamos em nosso dia a dia de laboratório. Obrigada por cada momento, cada ensinamento, cada diversão. Me farão muita falta.

À todos os professores do Programa de Pós-Graduação em Microbiologia pelas parcerias com os laboratórios, por todo apoio e ensinamentos.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

Agradeço à todos que de alguma forma esteve envolvido na minha formação tanto profissional quanto pessoal.

Muito obrigada!

KOGA, Vanessa Lumi. **Caracterização de *Escherichia coli* produtora de ESBL e AmpC de amostras clínicas humanas e de carcaças de frango.** 2019. 88 f. Tese (Doutorado em Microbiologia) – Universidade Estadual de Londrina, Londrina, 2019.

RESUMO

A emergência de bactérias produtoras de betalactamases de espectro estendido (ESBL) e/ ou AmpC em humanos e nas carnes de origem animal tem criado uma grande preocupação quanto à possibilidade de transmissão da resistência aos antimicrobianos por meio da cadeia alimentar. O objetivo do nosso trabalho foi estudar amostras de *Escherichia coli* produtoras de ESBL e AmpC mediadas por plasmídios (pAmpC), isoladas da carcaça de frango e da clínica humana na cidade de Londrina, e estes foram analisados quanto ao perfil de resistência antimicrobiana, virulência e relação clonal. O primeiro estudo refere-se às amostras de *E. coli* produtoras de ESBL isoladas de infecção do trato urinário (ITU) e de carcaças de frango. Entre as amostras isoladas de ITU, identificamos genes de ESBL, como $\textit{bla}_{\text{CTX-M-2}}$, $\textit{bla}_{\text{CTX-M-3}}$, $\textit{bla}_{\text{CTX-M-8}}$, $\textit{bla}_{\text{CTX-M-14}}$, $\textit{bla}_{\text{CTX-M-15}}$ e $\textit{bla}_{\text{CTX-M-27}}$, enquanto que nas amostras de carcaças de frango identificamos $\textit{bla}_{\text{CTX-M-1}}$, $\textit{bla}_{\text{CTX-M-2}}$ e $\textit{bla}_{\text{CTX-M-8}}$. As amostras apresentaram fatores de virulência associados a *E. coli* patogênica aviária (APEC) (93.1% em ITUs e 71.43% em carcaças de frango) e ilhas de patogenicidade (52.4% em ITUs e 31% em carcaças de frango). Por meio do “Pulsed Field Gel Electrophoresis” (PFGE) e do “Multilocus Sequence Typing” (MLST), nossos resultados demonstraram uma alta diversidade de cepas entre ambos os grupos de amostras. O segundo estudo refere-se às amostras produtoras de pAmpC isoladas de carcaças de frango e amostras clínicas humanas. Nesse trabalho, todas as amostras apresentaram o gene $\textit{bla}_{\text{CMY-2}}$, e foram consideradas multirresistentes. A maioria das amostras (71.43%) estavam associadas a sequência de inserção IS*Ecp1*. Por meio do MLST, 11 “Sequence types” foram identificados, sendo apenas o ST354 encontrado em ambos os grupos de amostras. PFGE demonstrou uma alta diversidade entre as amostras clínicas com as amostras de frango. Nossos trabalhos demonstraram uma alta diversidade bacteriana entre as amostras clínicas com as isoladas de carcaças de frango. No entanto, a presença de fatores de virulência e genes de resistência, associados a infecções humanas, nas amostras isoladas de carne de frango representam um potencial zoonótico ao homem.

Palavras-chave: ESBL. AmpC. *Escherichia coli*. Infecção do trato urinário. Fatores de virulência.

KOGA, Vanessa Lumi. **Characterization of ESBL- and AmpC-producing *Escherichia coli* from human clinical samples and chicken carcasses.** 2019. 88 p. Thesis (Microbiology Doctorate) – Universidade Estadual de Londrina, Londrina, 2019.

ABSTRACT

The emergence of extended-spectrum beta-lactamase (ESBL) and/ or AmpC-producing bacteria from humans and meat of animal origin has raised great concern about the possibility of transmission of antimicrobial resistance through the food chain. The aim of our study was research ESBL- and plasmid-mediated (pAmpC)-producing *Escherichia coli* strains isolated from chicken carcasses and human clinic samples from Londrina, and these strains were analyzed for antimicrobial resistance and virulence profile, and relationship clonal. The first study refers to ESBL-producing *E. coli* strains isolated from urinary tract infection (UTI) and chicken carcasses. Among the strains isolated from UTI, we identified ESBL genes, as $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$, while in chicken carcass we identified $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-8}}$. The strains presented virulence factors associated with avian pathogenic *E. coli* (APEC) (93.1% in UTIs and 71.43% in chicken carcasses) and pathogenicity islands (52.4% in UTIs and 31% in chicken carcasses). Through Pulsed Field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST), our results demonstrated a high diversity of strains between both strains groups. The second study refers to pAmpC-producing strains isolated from chicken carcasses and human clinical samples. In this work, all strains presented the $bla_{\text{CMY-2}}$ gene and they were considered multidrug resistant. The most of the strains (71.43%) was associated with the IS*Ecp*1 insertion sequence. Though MLST, 11 Sequence types were identified, with only ST354 found in both strains groups. PFGE demonstrated a high diversity between strains from clinical samples with chicken. Our study demonstrated a high bacterial diversity between clinical samples with chicken carcass. However, the presence of virulence factors and resistance genes associated with human infections in strains isolated from chicken meat represents a zoonotic potential to human.

Keywords: ESBL. AmpC. *Escherichia coli.* Urinary tract infection. Virulence factors.

LISTA DE ILUSTRAÇÕES

ARTIGO I

ARTIGO II

LISTA DE TABELAS

ARTIGO I

ARTIGO II

LISTA DE SIGLAS E ABREVIATURAS

Amc	amoxicillin-clavulanic acid (amoxicilina- ácido clavulânico)
APEC	avian pathogenic *E. coli* (*E. coli* patogênica aviária)
ATCC	American Type Culture Collection (Coleção de microrganismos norte Americana)
Caz	ceftazidime (ceftazidima)
CC	Chicken carcass (carcaça de frango)
CDC	Centers for Disease Control and Prevention (Centro de Controle e Prevenção de Doenças)
Cfo	cefoxitin (cefoxitina)
CIM	concentração inibitória mínima
Cip	ciprofloxacin (ciprofloxacino)
Clo	chloramphenicol (cloranfenicol)
CLSI	Clinical and Laboratory Standards Institute (Instituto de Normas Clínicas e Laboratoriais)
Cn	gentamicin (gentamicina)
Ctx	cefotaxime (cefotaxima)
CTX-M	cefotaximase
CVP	conserved virulence plasmidic
DAEC	*E. coli* aderente difusa
DEC	Diarrheagenic *E. coli* (*E. coli* diarreiogênica)
DNA	ácido desoxirribonucleico
EAEC	*E. coli* enteroagregativa
EHEC	*E. coli* enterohemorrágica
EIEC	*E. coli* enteroinvasiva
Enr	enrofloxacin (enrofloxacino)
EPEC	*E. coli* enteropatogênica
ESBL	extended-spectrum beta-lactamase (beta-lactamase de espectro estendido)
EUCAST	European Committee on Antimicrobial Susceptibility Testing (Comitê europeu de testes de susceptibilidade antimicrobiana)
ExPEC	Extraintestinal Pathogenic *E. coli* (*E. coli* patogênica extraintestinal)
Fos	fosfomycin (fosfomicina)
IRAS	infecções relacionadas à assistência à saúde

ITU	infecção do trato urinário
MLST	multilocus sequence typing
Nal	nalidixic acid (ácido nalidixico)
ND	not defined (não definido)
Nit	nitrofurantoin (nitrofurantoína)
NMEC	Neonatal Meningitis *E. coli* (*E. coli* associada à meningite neonatal)
Nor	norfloxacin (norfloxacino)
PAI	pathogenic island (ilha de patogenicidade)
pAmpC	plasmid-mediated AmpC (AmpC mediada por plasmídio)
PBP	penicillin binding proteins (proteína de ligação à penicilina)
PCR	polymerase chain reaction (Reação em cadeia da polymerase)
PFGE	pulsed-field gel electrophoresis (Eletroforese em gel de campo pulsado)
PG	phylogenetic group (grupo filogenético)
PMQR	plasmid mediated quinolone resistance (resistência a quinolonas mediada por plasmídios)
RNA	ácido ribonucleico
SEPEC	Sepsis-associated *E. coli* (*E. coli* associada a sepse)
ST Cplx	sequence type complex
ST	sequence typing
STEC	*E. coli* produtora de toxina Shiga
Sut	trimethoprim-sulfamethoxazole (sulfametoxazol-trimetoprim)
Tet	tetracycline (tetraciclina)
UPEC	Uropathogenic *E. coli* (*E. coli* uropatogênica)
USA	United States of America (Estados Unidos da América)
UTI	urinary tract infection (infecção do trato urinário)

SUMÁRIO

1. INTRODUÇÃO

Os antimicrobianos têm sido utilizados na produção animal. Apesar dos vários benefícios proporcionados pelo uso de antimicrobianos na produção animal, o uso indiscriminado dessas drogas tem levado a seleção de bactérias resistentes aos antimicrobianos em seus produtos (BRUMANO; GATTÁS, 2009; MANGES; JONHSON, 2012; MELLATA, 2013).

Na avicultura os antimicrobianos são utilizados tanto como promotores de crescimento para melhorar o desempenho das aves, como também para a prevenção e profilaxia das doenças infecciosas (BRUMANO; GATTÁS, 2009). No entanto, a carne de frango tem sido considerada uma das carnes com os maiores níveis de contaminação por *Escherichia coli* resistentes aos antimicrobianos. Assim, *E. coli* é uma bactéria que tem sido utilizada em muitos estudos como um bioindicador do uso de antimicrobianos na avicultura. Além disso, *E. coli* presente em aves pode ser patogênica para os humanos, podendo a carne de frango e seus derivados serem veículos de transmissão dos mesmos (EWERS et al., 2012; MANGES; JONHSON, 2012; MELLATA, 2013).

Amostras de *E. coli* produtoras de betalactamases de espectro estendido (ESBL) e AmpC estão sendo encontradas nos animais de produção, principalmente em carnes de frango (CASELLA et al., 2018; EWERS et al., 2012; KOGA et al., 2015; SMET et al., 2010). A produção de betalactamases constitui o principal mecanismo relacionado à resistência aos beta-lactâmicos em bactérias Gram-negativas. As betalactamases são enzimas capazes de hidrolisar os antibióticos beta-lactâmicos, através da hidroxilação irreversível da ligação amida do anel beta-lactâmico, inativando a ação dessas drogas (DHILLON; CLARK, 2011; GUTKIND et al., 2013). É comum que bactérias produtoras de ESBL e/ou AmpC sejam multirresistentes, devido ao fato dos genes codificadores de betalactamases estarem normalmente localizados em plasmídios onde estão presentes diversos outros genes que determinam a resistência para outras classes de antimicrobianos como quinolonas, sulfas, aminoglicosídeos, cloranfenicol entre outros (JACOBY, 2009; DHILLON; CLARK, 2011).

No entanto, a presença, tanto de bactérias produtoras de ESBL quanto de AmpC, em carnes de frango tem preocupado a sociedade, visto que a produção destas enzimas são uma das principais causas de falhas terapêuticas em tratamentos de infecções humanas por enterobactérias. Assim, a emergência das bactérias produtoras de betalactamases, inclusive em animais, se tornou um risco a saúde humana devido à possibilidade de patógenos resistentes aos antimicrobianos poderem colonizar o homem, ou à possibilidade de ocorrer uma transferência desses genes de resistência entre as bactérias, levando a um possível risco zoonótico (MANGES, JONHSON, 2012; MELLATA, 2013).

Muitos estudos tem demonstrado uma alta similaridade entre *E. coli* de origem humana com as de origem aviária, principalmente em relação às características de virulência, grupos filogenéticos e no perfil de resistência. Em 2008, o clone *E. coli* sequence type 131 (ST131), produtor de uma betalactamase do tipo CTX-M-15, emergiu em diversos países, sendo este caracterizado como uma cepa altamente virulenta, responsável por diversos casos de infecções como as do trato urinário, bacteremias e meningites em humanos. No entanto, estudos recentes tem demonstrado também a presença da ST131 em animais de produção, representando um risco aos consumidores (EWERS et al., 2012; PLATELL, et al., 2011; MÜLLER et al., 2016; LEVERSTEIN-VAN HALL et al., 2011).

A elevada taxa de resistência aos antimicrobianos em bactérias isoladas de produtos de origem animal tem se tornado um importante desafio para a produção de alimentos, sendo necessário que haja uma regulamentação do uso de antimicrobianos no agronegócio em âmbito nacional, além de medidas que possam diminuir a disseminação de enteropatógenos resistentes aos antimicrobianos em alimentos de origem animal (EWERS et al., 2012; MELLATA, 2013). Este trabalho tem a finalidade de estudar amostras de *E. coli* produtoras de betalactamases do tipo ESBL e AmpC mediadas por plasmídios, isoladas de infecções humanas e de carcaças de frango, visando esclarecer a disseminação de bactérias produtoras de betalactamases entre o homem e alimentos de origem animal, e desta forma, minimizar o risco zoonótico.

2. OBJETIVOS

2.1 Objetivo Geral

Caracterizar as amostras de *E. coli* produtoras de ESBL e de pAmpC isoladas de amostras clínicas humanas e da carcaça de frango, da cidade de Londrina- PR, quanto ao perfil de resistência antimicrobiana e virulência e avaliar, por clonalidade, quanto ao potencial zoonótico.

2.2 Objetivos Específicos

-Verificar quais os genes codificadores de ESBL e de pAmpC estão presentes nas amostras selecionadas;

-Caracterizar as amostras quanto ao perfil fenotípico e genotípico de genes de resistência a quinolonas, tetraciclina, sulfanamida, fosfomicina e colistina;

-Caracterizar as amostras quanto ao perfil de virulência por meio da pesquisa de fatores de virulência e ilhas de patogenicidade;

-Classificar as amostras quanto aos grupos filogenéticos;

-Estudar a proximidade genética entre as amostras isoladas de amostras clínicas com as isoladas da carcaça de frango, por meio do PFGE e MLST.

3. REVISÃO DE LITERATURA

3.1. *Escherichia coli*

E. coli foi descrita pelo médico alemão Theodor Von Escherich, em 1885, sendo inicialmente denominado como *Bacterium* (*Bacillus*) *coli commune*. *E. coli* é uma bactéria Gram-negativa, pertencente à família Enterobacteriaceae (domínio Bacteria, filo Proteobacteria, classe Gammaproteobacteria, ordem "Enterobacteriales"). Está amplamente distribuída na natureza, tendo como habitat natural o trato gastrointestinal humano e animal, geralmente são móveis, possuindo flagelos peritríquios, e não são formadores de endósporos. Apresentam metabolismo anaeróbio facultativo, utilizam glicose como única fonte de carbono e energia, são oxidase-negativos, catalase-positivos, podem fermentar tanto a glicose quanto a lactose, produzem indol e reduzem nitratos a nitritos (KAPER; NATARO; MOBLEY, 2004; WINN et al., 2014).

E. coli é a espécie predominante entre as enterobactérias encontradas no trato gastrointestinal em humanos e animais, sendo a maioria comumente comensais. Por comporem a microbiota intestinal, trazem benefícios ao hospedeiro por sintetizarem ácidos graxos, vitaminas K e do complexo B, além de competirem com bactérias patogênicas por sítios de colonização do trato gastrointestinal (TENAILLON et al., 2010).

Apesar dos vários benefícios promovidos aos seres humanos, *E. coli* é uma espécie bacteriana comumente isolada em laboratórios clínicos, sendo agente de infecção em praticamente todos os tecidos e sistemas orgânicos dos seres humanos (TENAILLON et al., 2010; WINN et al., 2014).

3.2. *Escherichia coli* patogênica

Entre os patotipos de *E. coli*, há aqueles que apresentam uma combinação de fatores de virulência que lhes permitem causar infecções do trato gastrointestinal, denominados como *E. coli* diarreiogênica (DEC - "*Diarrheagenic E. coli*") e há aqueles que causam infecções extraintestinais, denominados como *E. coli* patogênica extraintestinal (ExPEC – "*Extraintestinal Pathogenic E. coli*") (KAPER; NATARO; MOBLEY, 2004; KARIYAWASAM; HAN, 2019).

Entre DECs, existem seis categorias patogênicas de *E. coli* que causam infecção intestinal em homens e animais, sendo essas diferenciadas pela presença de fatores de virulência (adesinas fimbriais e afimbriais, toxinas e invasinas), manifestações clínicas e sua interação com células epiteliais cultivadas *in vitro*, e classificadas em: *E. coli* enteropatogênica (EPEC), *E. coli* enterotoxigênica (ETEC), *E. coli* enteroinvasiva (EIEC), *E. coli* enterohemorrágica (EHEC), *E. coli* produtora de toxina de Shiga (STEC), *E. coli* enteroagregativa (EAEC) e *E. coli* aderente difusa (DAEC) (KAPER; NATARO; MOBLEY, 2004; SOUZA et al., 2016).

ExPECs apresentam diversos fatores de virulência responsáveis pela colonização em diferentes superfícies anatômicas, pela evasão das defesas do hospedeiro, aquisição de nutrientes essenciais como ferro, além por permitir a invasão no hospedeiro e a estimulação de processo inflamatório. Esses patógenos são classificados quanto ao sítio anatômico acometido, fazendo parte desse grupo: *E. coli* uropatogênica (UPEC – “Uropathogenic *E. coli*”), *E. coli* associada a meningite neonatal (NMEC – “Neonatal Meningitis *E. coli*”), *E. coli* associada a sepse (SEPEC – “Sepsis-associated *E. coli*”), e aqueles que podem acometer as aves, pela *E. coli* patogênica aviária (APEC – “Avian Pathogenic *E. coli*”) (DALE; WOODFORD, 2015).

UPEC é o principal agente etiológico das infecções do trato urinário (ITU), responsáveis por 70% a 95% das ITUs adquiridas na comunidade e 50% em ambientes hospitalares. A patogênese das UPECs está associada à expressão de diversos fatores de virulência, como adesinas (fímbria P e tipo 1), formação de biofilmes, produção de toxinas tais como hemolisinas, entre outros. A presença desses fatores de virulência permite que a bactéria colonize e provoque danos às células do hospedeiro, resistindo aos mecanismos de defesa nestes sítios, estimulando uma resposta inflamatória, e causando doença (WILES; KULESUS; MULVEY, 2008; CROXEN; FINLAY, 2009; PITOUT, 2012).

Outro patotipo extraintestinal, o NMEC, é a causa mais comum de meningite por bactérias Gram-negativas em neonatos, com uma taxa de letalidade de 15 a 40%, além de deixar graves sequelas neurológicas nos sobreviventes. Nesta patologia, a bactéria chega ao sistema nervoso central, mais comumente pela via hematogênica, resultando em inflamação das meninges, razão pela qual a meningite está fortemente associada à sepse neonatal. Estas bactérias apresentam diversos fatores de virulência, sendo o antígeno capsular K1 o mais comum, presente em aproximadamente 80% das NMECs (KAPER; NATARO; MOBLEY, 2004).

E. coli é o principal agente etiológico de infecções sanguíneas, entre as bactérias Gram-negativas. SEPECs apresentam vários fatores de virulência em comum com UPEC e APEC. Apesar de ter tido um grande aumento de casos de sepse e bacteremia causados por *E. coli* nos últimos anos, ainda há poucos estudos relacionados à patogênese de SEPEC. ExPEC causadoras de infecções na corrente sanguínea pertencem às linhagens que apresentam uma grande variedade de fatores de virulência como adesinas, sistema de captação de ferro, resistência sérica e toxinas (DAGA et al., 2019; KOGA et al., 2014).

Uma pequena porcentagem de *E. coli* é patogênica para aves, conhecida como APEC, sendo um dos principais causadores de colibacilose, doença responsável por elevados prejuízos para a indústria aviária. Dentre os principais fatores de virulência encontrados em APEC estão os relacionados ao sistema de captação de ferro (*iut*A e *iro*N), hemolisina (*hly*F), resistência lítica ao soro (*iss*) e proteases (*omp*T). Muitos trabalhos demonstraram que cepas de APEC pertencem normalmente aos sorogrupos O1, O2, O18 e O78, sendo sorogrupos comuns a ExPECs que causam infecções em humanos (KARIYAWASAM; HAN, 2019; JOHNSON et al., 2008; MOULIN-SCHOULEUR et al., 2007).

3.3. Patogenicidade bacteriana em ExPEC

A patogenicidade de *E. coli* se deve à presença de diversos genes que codificam fatores de virulência, sendo os principais fatores encontrados em ExPEC, os sideróforos, adesinas, resistência sérica, toxinas e a capacidade de invasão (PITOUT, 2012; DALE; WOODFORD, 2015).

O ferro é um importante elemento para as bactérias, pois este é um co-fator essencial para a multiplicação bacteriana, e também é importante em alguns processos biológicos como na produção de energia e transporte do oxigênio. Pelo fato da concentração de ferro na forma solúvel no homem ser muito baixa, a expressão de fatores envolvidos na captação de ferro se mostra importante para a sobrevivência da bactéria, tendo um importante papel na aquisição de ferro. Devido à baixa concentração de ferro nos locais de infecção extraintestinal, ExPECs apresentam uma alta prevalência de sideróforos (como por exemplo a enterobactina e aerobactina), que promovem o sequestro e a aquisição do ferro através da remoção por proteínas carreadoras (DZIVA; STEVENS, 2008; DALE; WOODFORD, 2011).

A habilidade da bactéria em aderir e colonizar as células epiteliais do hospedeiro é um passo essencial para o início de uma infecção. A adesão da bactéria é promovida por estruturas proteicas presentes na superfície bacteriana, que permite que esta se ligue a receptores específicos, presentes nas células do hospedeiro. Estas estruturas, conhecidas como adesinas, podem se localizar em organelas filamentosas denominadas pili ou fímbrias, ou se caracterizarem como moléculas monoméricas ou multiméricas ancoradas na parede celular bacteriana, chamadas de adesinas afimbriais. Entre os genes de virulência relacionados a adesão encontram-se os genes *pap*C e *pap*G, sendo estes constituintes do operon da fímbria P (DALE; WOODFORD, 2015).

As bactérias podem apresentar também resistência ao soro, que é uma habilidade de se evitar a atividade bactericida do soro. A resistência ao soro é devida à presença de vários fatores de virulência: lipopolissacarídeos, cápsula e proteínas de membrana externa, que tem sido associados a infecções intestinais e extraintestinais causadas por *E. coli* (DALE; WOODFORD, 2015; JOHNSON; STEEL, 2000). Essas características permitem que a bactéria possa sobreviver nos fluidos e órgãos internos do hospedeiro (DZIVA; STEVENS, 2008; JOHNSON et al., 2008).

As toxinas são capazes de causar citotoxicidade direta no tecido dos hospedeiros. Os principais genes relacionados à produção de toxinas, presentes em ExPECs são o *cnf*1, *hly*A, *hly*F e *cva*C. O gene *cnf*1 codifica uma proteína que contribui para a invasão do sistema nervoso central, e tem sido relatado em infecções extraintestinais, incluindo a sepse. As hemolisinas são endotoxinas capazes de formar poros na membrana celular em diferentes tipos de células, levando à morte celular, e dessa forma disponibilizando o íon ferro ao microrganismo. O gene *hly*A está envolvida na síntese da α-hemolisina e é frequentemente encontrada em isolados de bacteremias. O gene *hly*F, está relacionada com *E. coli* aviária, e codifica uma hemolisina aviária. O gene *cva*C é responsável pela codificação da colicina V, normalmente associado a *E. coli* uropatogênica e em casos de meningites (DALE; WOODFORD, 2015; JOHNSON et al., 2008).

Já a protease OmpT está localizada na membrana externa da parede bacteriana e tem sido caracterizado como um ativador do plasminogênio, e também capaz de degradar peptídeos antimicrobianos catiônicos (JOHNSON et al., 2008).

A presença de elementos genéticos móveis como as ilhas de patogenicidade (PAIs) e os plasmídios, permitem a transferência horizontal de genes entre as bactérias, sendo responsável pela intensa variabilidade genética entre os microrganismos, permitindo a aquisição ou perda de genes codificadores de fatores de virulência, além de conferir uma melhor adaptação à novos sítios anatômicos, ampliando o espectro da doenças (PITOUT, 2012; DALE; WOODFORD, 2015).

As PAIs são segmentos de DNA inseridos no cromossomo bacteriano, variando de 10.000 a 200.000 pares de bases, são elementos genéticos móveis, no qual, são flanqueados por pequenas sequências diretas repetidas localizadas adjacentes aos genes que codificam para o RNA transportador. Apresentam também genes que codificam integrases, transposases e sequências de origem de replicação, podendo ser transferidos por conjugação. As PAIs contêm genes associados à patogenicidade, que atribuem uma variedade de características de virulência aos microrganismos que a possuem (SABATÉ et al., 2006). Muitos dos fatores de virulência de UPECs estão situados nas PAI, havendo 9 ilhas bem estudadas nesse agente: PAI I e II em *E. coli* J96, PAI I e II em *E. coli* CTF073 e PAI I ao V em *E. coli* 536 (KAPER; NATARO; MOBLEY, 2004; SABATÉ et al., 2006).

Assim como as PAIs, os plasmídeos também são elementos genéticos móveis, com genes capazes de atribuir uma variedade genética bacteriana, porém são extracromossomais, constituídos por duas cadeias de DNA circulares. Em ExPECs, os plasmídios ColV e ColBM foram bastante encontrados. Estes plasmídios têm sido associados a fatores de virulência relacionados com a resistência ao soro, aquisição de ferro, produção de colicinas entre outros, permitindo o estabelecimento de patógenos que a contém em infecções sanguíneas e do trato urinário. Também já foi relatado a presença de uma região plasmidial denominada “Conserved Virulence Plasmidic” (CVP), em ExPECs. Essa região apresenta oito operons ou genes, que codificam sistemas sideróforos (genes *iro*N, *iuc*, *sit*), proteína de membrana externa (gene *omp*T), colicina V (gene *cva*), α-hemolisina (gene *hly*F), sistema de secreção tipo I (gene *ets*) e gene relacionado a resistência sérica (gene *iss*) (CYOIA et al., 2015; DZIVA; STEVENS, 2008).

De acordo com a classificação filogenética, as cepas de *E. coli* podem ser classificadas em até 8 grupo filogenéticos: A, B1, B2, C, D, E e F. De acordo com esse esquema a maioria das ExPEC humanas são classificadas dentro dos grupos B2 e D, enquanto as cepas de APEC normalmente são classificadas no grupo C, seguidos do grupo F, B1 e B2 (CLERMONT et al., 2013; LOGUE et al., 2017).

A patogenicidade da ExPEC se deve a um resultado multifatorial, na qual, o produto de genes cromossomais e plasmidiais, juntamente com as características relacionadas ao hospedeiro e ao ambiente, levam ao potencial patogênico destes isolados (DALE; WOODFORD, 2015).

3.4. Betalactamases

Os betalactâmicos estão entre os principais antimicrobianos utilizados no tratamento de infecções por enterobactérias. Os antimicrobianos betalactâmicos caracterizam-se por possuírem um anel betalactâmico, cuja estrutura química mimetiza a terminação D-alanina-D-alanina da cadeia lateral pentapeptídica dos blocos de peptideoglicanos, que formam a parede celular das bactérias. Dessa forma, o anel beta-lactâmico liga-se às transpeptidases (ou PBPs- do inglês, *Penicillin Binding Proteins*), impedindo-as de se ligarem ao pentapetídeo e realizarem a ligação cruzada das cadeias de glicano e, assim, estabilizar a estrutura da parede celular (KATZUNG; MASTERS; TREVOR, 2017).

Um dos principais mecanismos de resistência relacionado aos betalactâmicos, está associado com a produção de betalactamases. Essas enzimas hidrolisam o anel betalactâmico, inativando o antimicrobiano, e estão amplamente distribuídas entre bactérias Gram-negativas (PEIRANO; PITOUT, 2019; da SILVA; LINCOPAN, 2012).

As betalactamases são classificadas, com base em sua estrutura molecular ou por suas características enzimáticas, em dois esquemas. O esquema proposto por Bush-Jacoby-Medeiros baseia-se na preferência do substrato da enzima e na inativação diante de inibidores específicos, agrupando as betalactamases em grupos de 1 a 4, com subdivisões (BUSH; JACOBY, 2010). Já o esquema proposto por Ambler considera a similaridade entre as cadeias de aminoácidos das enzimas, e elas foram agrupadas nas classes de A a D, na qual, as enzimas pertencentes às classes A, C e D são serina-beta-lactamases, pois possuem o aminoácido serina no centro ativo da enzima, enquanto as enzimas da classe B são classificadas como metalo-beta-lactamases (AMBLER. 1980).

A primeira betalactamase codificada por elemento genético móvel em Gram-negativos foi a enzima $bla_{\text{TEM-1}}$, identificada em *E. coli* isolada de um paciente na Grécia, chamado Temorieira, nome que designou a enzima TEM-1. Pelo fato dessa enzima estar associada a plasmídios e transposons, sua disseminação ocorreu de forma muito rápida em vários países (DATTA; KONTOMICHALOU, 1965).

Devido à rápida disseminação das betalactamases, as cefalosporinas de terceira geração foram introduzidas na prática médica, no Brasil, na década de 1980. No entanto, devido à pressão seletiva exercida pelo uso desses novos antimicrobianos, ocasionou a emergência de cefalosporinases com espectro de atividade estendido (da SILVA; LINCOPAN, 2012).

A rápida disseminação dessas enzimas está ligado ao fato desses genes estarem presentes em elementos genéticos altamente móveis como plasmídeos conjugativos, transposons, sequências de inserção, integrons e, mais raramente, em elementos relacionados a bacteriófagos (PITOUT, 2012).

As sequências de inserção apresentam três funções básicas: codificam a transposase, responsável pela mobilidade de um elemento genético; apresentam promotores que ativam genes silenciados ou aumentam a expressão de genes *downstream* a elas; e movem os genes ligados a elas entre integrons, transposons, plasmídeos e cromossomos, fazendo com que estes genes sejam altamente transferíveis. Esses elementos podem também carrear genes relacionados à resistência a outras classes de antimicrobianos, o que pode favorecer a manutenção das betalactamases por processos de co-seleção. As principais sequências de inserção ligadas aos genes codificadores de ESBL tem sido ISE*cp*1 e IS*CR*1, mas também tem sido relatado a presença de IS10 e IS26 (CANTÓN; GONZÁLEZ-ALBA; GALÁN, 2012).

A presença da sequência de inserção potencializa a transferência de genes entre cromossomos e plasmídios. Interessantemente, testes *in vitro* tem demonstrado que quando IS*Ecp1 é* inserido *upstream* ao gene $bla_{\text{klu/CTX-M}}$, em situações de estresse como altas temperaturas (40°C) ou na presença de antimicrobianos, como ceftazidima ou cefotaxima, ocorre um aumento da mobilização da IS*Ecp1*, o qual, carrega consigo suas sequências *downstream*. Assim, tem sido especulado que o uso de antimicrobianos, bem como o aquecimento global ou temperaturas maiores que a temperatura corpórea humana, presentes em certos animais, tem contribuído para o aumento e disseminação dos genes da betalactamase (CANTÓN; GONZÁLEZ-ALBA; GALÁN, 2012; LARTIGUE et al., 2006).

A rápida disseminação dessas betalactamases tem direcionado ao maior uso de antimicrobianos carbapenêmicos, levando a seleção de isolados produtores de carbapenemases no país. A alta frequência de resistência em bactérias patogênicas de importância clínica fizeram a Agência Nacional de Vigilância Sanitária (Anvisa), no ano de 2010, proibir a venda de antimicrobianos sem prescrição médica, com o intuito de diminuir o uso inadequado desses medicamento no país. No entanto, a emergência de betalactamases, não só nas infecções relacionadas à assistência à saúde (IRAS), como também em infecções comunitárias, e também em produtos de origem animal, tem demonstrado a complexidade envolvendo a disseminação de betalactamases em Enterobacteriaceae (da SILVA; LINCOPAN, 2012).

3.5. ESBL

A emergência e a disseminação de ESBL entre os membros da família Enterobacteriaceae têm sido descritas mundialmente como ponto de urgência clínica devido à grande incidência desses isolados nas IRAS (PEIRANO; PITOUT, 2019; CANTÓN; GONZÁLEZ-ALBA; GALÁN, 2012).

As ESBLs são enzimas capazes de hidrolisar cefalosporinas de terceira e quarta gerações e o aztreonam, além de serem inibidas por inibidores de betalactamases, como clavulanato, sulbactam e tazobactam. Essas enzimas fazem parte da classe A de Ambler e ao grupo 2be pelo esquema de Bush-Jacoby-Medeiros (DHILLON; CLARK, 2011).

Frequentemente, as ESBLs são codificadas por genes situados em elementos genéticos móveis, como plasmídeos, transposons e integrons, na qual, muitas vezes também estão presentes genes de resistência a outras classes de antimicrobianos. Esse fato faz com que, normalmente, cepas produtoras de ESBL sejam multirresistentes, sendo uma das principais causas de falha terapêutica, levando ao aumento considerável de morbidade por infecções bacterianas (CANTÓN; GONZÁLEZ-ALBA; GALÁN, 2012; DHILLON; CLARK, 2011).

As ESBLs tem sido encontradas em diversos patógenos da família Enterobacteriaceae, como *Klebsiella* spp., *Escherichia coli*, *Salmonella enterica*, *Citrobacter* spp., *Enterobacter* spp., *Proteus mirabilis*, *Serratia marcescens*, entre outros. Contudo, o ponto de urgência clínica tem sido a alta prevalência dessa enzima em *Klebsiella* spp. e *E. coli* (da SILVA; LINCOPAN, 2012; CANTÓN; GONZÁLEZ-ALBA; GALÁN, 2012).

A primeira descrição de ESBL foi da SHV-2, derivada de enzimas de espectro restrito do tipo SHV-1, na qual, foi encontrada em uma amostra de *Klebsiella ozaenae*, na Alemanha, em 1983 (KNOTHE et al., 1983). Já as ESBLs do tipo TEM foram derivadas de TEM-1 e TEM-2. A primeira ESBL do tipo TEM foi isolada de uma amostra de *Klebsiella oxytoca*, na Inglaterra (DU-BOIS; MARRIOTT; AMYES, 1995).

Em 1990, foi reportado na França a presença de uma cefalosporinase diferente das do tipo TEM ou SHV, a CTX-M, denominada assim devido à sua atividade de “cefotaximase”. Diferentemente das enzimas do tipo TEM e SHV, todas as variantes do tipo CTX-M apresentam perfil de ESBL. Desde a sua identificação, as enzimas da família CTX-M disseminaram-se de forma acelerada no mundo todo (CANTÓN; GONZÁLEZ-ALBA; GALÁN, 2012; GUTKIND et al., 2013). Essa família de enzima originou-se de genes de cefotaximases intrínsecos, codificados por genes cromossômicos em uma bactéria ambiental denominada *Kluyvera* spp. A primeira ESBL do tipo CTX-M foi identificada em uma *E. coli* na Alemanha em 1989 e foi designada como CTX-M-1 por conta da sua atividade hidrolítica contra a cefotaxima (CANTÓN; GONZÁLEZ-ALBA; GALÁN, 2012).

Baseado na árvore filogenética das sequências de aminoácidos, as enzimas da família CTX-M são divididas em cinco grupos, cuja diferença entre os grupos é maior ou igual a 10% de resíduos de aminoácidos e, dentro de cada grupo, a diferença dos resíduos é menor ou igual a 5% entre as enzimas. Assim as variantes de CTX-M foram agrupadas em cinco grupos: grupos CTX-M-1. CTX-M-2, CTX-M-8, CTX-M-9 e CTX-M-25 (GUTKIND et al., 2013).

Diversas outras famílias de ESBL também têm sido identificadas, embora em menor número, como: PER, GES, VEB, BES, TLA, SFO e IBC (da SILVA; LINCOPAN, 2012).

Um dos métodos para identificação fenotípica de ESBL é por meio da utilização de fitas comerciais de E-test ESBL® (Biomérieux, Marcy I'Etoile, France), em que há uma queda de pelo menos três vezes na concentração inibitória mínima (CIM) do antimicrobiano, quando este é associado a um inibidor que indica a produção de ESBL. Há também o teste de aproximação de discos, na qual, discos de cefalosporinas, como cefotaxima, ceftazidima, cefepima e aztreonam, são colocados a 30mm de distância do disco com inibidor (clavulanato, tazobactam e sulbactam), e a presença de sinergismo entre os substratos e o inibidor; a produção de ESBL é confirmada. Outro teste fenotípico utilizado na detecção de ESBLs é o teste do disco combinado, na qual, utiliza-se discos de cefalosporinas, como cefotaxima e ceftazidima, junto com os mesmos antimicrobianos combinados com um inibidor. Nesse teste, a presença de diferença maior ou igual a 5mm do diâmetro do halo de inibição entre o disco de cefalosporina e seu respectivo disco combinado com inibidor, infere-se a produção da ESBL (da SILVA; LINCOPAN, 2012, JALIER et al., 1988).

3.6. AmpC

As enzimas do tipo AmpC, apresentam serina no sítio ativo, e são resistentes aos inibidores clavulanato, sulbactam e tazobactam, além de conferirem resistência às cefalosporinas de primeira, segunda, terceira geração e às cefamicinas. Essas enzimas podem ser cromossomais ou plasmidiais e pertencem ao grupo 1 na classificação de Bush-Jacoby-Medeiros e ao grupo C na classificação de Ambler (JACOBY, 2009).

As AmpCs cromossomais são codificadas por diversas espécies de Enterobacteriaceae, como *Citrobacter freundii*, *Enterobacter* spp., *E. coli, Hafnia alvei, Morganella morganii, Proteus* spp., *Providencia* spp., *Serratia* spp., e *Shigella* spp. Nesses hospedeiros, há uma hiperprodução induzível pela exposição aos betalactâmicos. O gene que codifica a AmpC pode sofrer uma desrepressão quando a bactéria é submetida a tratamento com antimicrobianos betalactâmicos, resultando em resistência aos mesmos (JACOBY, 2009; PÉREZ-PÉREZ; HANSON, 2002; GUTKIND et al., 2013).

As AmpCs plasmidiais (pAmpCs) tem sido conhecidas desde 1989, e desde então, tem sido muito encontrado principalmente em *Klebsiella pneumoniae* e *E. coli*. Os genes codificadores de pAmpCs são derivados dos genes cromossômicos de várias espécies da família Enterobacteriaceae. Nas pAmpC a expressão não é induzível, como nas cromossomais, mas é regulada por promotores e mecanismos de atenuação. Normalmente cepas produtoras de pAmpC apresentam caráter de multirresistência, pois os plasmídios que carreiam esses genes normalmente carreiam genes de resistência a outros antimicrobianos como quinolonas, sulfas, aminoglicosídeos, cloranfenicol, além de genes codificadores de ESBL (PÉREZ-PÉREZ; HANSON, 2002; JACOBY, 2009).

As principais pAmpCs são CMY, MIR, MOX, LAT, FOX, DHA, ACT, ACC e CFE. Entre essas variantes, CMY é a pAmpC mais amplamente disseminada no mundo todo (PÉREZ-PÉREZ, HANSON, 2002; JACOBY, 2009).

No entanto, ainda não há nenhum método padrão para detecção de pAmpC na prática clínica. A dificuldade na detecção fenotípica de pAmpC leva a falhas terapêuticas, e impede a estimativa da prevalência desta enzima. A investigação molecular tem se mostrado um importante instrumento na detecção desse mecanismos de resistência (PÉREZ-PÉREZ; HANSON; JACOBY, 2009).

3.7. Tipagem molecular

A principal função da tipagem molecular é a determinação da relação entre clones de microrganismos de uma mesma espécie, por meio da análise da variação genética observada no DNA cromossomal. Este método permite a realização de estudos da similaridade genômica para identificação e monitoramento da disseminação de clones patogênicos, ou ainda para disseminação de cepas resistentes. Atualmente, várias técnicas baseadas na análise do DNA estão padronizadas. Entre elas, o PFGE (do inglês, *Pulsed Field Gel Electrophoresis*) e o MLST (do inglês, *Mulilocus Sequence Typing*) têm sido amplamente utilizadas, permitindo a identificação de fontes e rotas de infecção e o reconhecimento de linhagens virulentas (DALE; WOODFORD, 2015; RANJBAR et al., 2014).

O PFGE é um método de DNA “fingerprinting” com alta capacidade discriminatória, utilizada para analisar a relação genética de uma variedade de bactérias. Essa técnica se baseia na macrorrestrição do DNA bacteriano após a digestão com enzimas de restrição e posterior separação desses fragmentos de DNA gerados em um gel de agarose, após eletroforese em campo elétrico pulsado. Devido à alta capacidade discriminatória, o PFGE é considerado padrão ouro para tipagem molecular. Uma desvantagem dessa técnica é a baixa capacidade de comparação interlaboratorial, a demanda de vários dias para sua execução e o alto custo (DALE; WOODFORD, 2015; RANJBAR et al., 2014).

O MLST é baseada no sequenciamento de genes housekeeping, que são genes conservados que variam entre as espécies. Essas sequências são depositadas em um banco de dados e por meio de análises de mutações pontuais nos genes sequenciados, se obtém um perfil de alelos, gerando o sequence type (ST). Através do MLST, é possível a comparação entre amostras bacterianas isoladas de regiões geográficas distintas, e em períodos de tempo diferentes. A desvantagem desse método é que essa metodologia não está disponível para todas as espécies bacterianas, além do alto custo. Atualmente, existem dois esquemas mais utilizados para a realização do MLST para *E. coli*, na qual analisam genes conservados diferentes (DALE; WOODFORD, 2015; RANJBAR et al., 2014). O esquema do Instituto Pasteur, se baseia no sequenciamento de 9 genes housekeeping: *din*B (DNA polimerase), *icd*A (isocitrato desidrogenase), *put*P (prolina permease), *pol*B (polimerase Pol II), *trp*B (triptofano sintase subunidade B), *trp*A (triptofano sintase subunidade A), *pab*B (p-aminobenzoato sintase) e *uid*A (beta-glicuronidase) (https://pubmlst.org/databases.shtml). Já o MLST pelo esquema da Universidade de Warwick, se baseia no sequenciamento de 7 genes housekeeping: *adk* (adenilato quinase), *fum*C (fumarato hidratase), *gyr*B (DNA girase), *icd* (isocitrato/ isopropilmalato desidrogenase), *mdh* (malato desidrogenase), *pur*A (adenilsuccinato desidrogenase) e *rec*A (motivo ligador de ATP/GTP) (http://enterobase.warwick.ac.uk/).

Enquanto o PFGE tem sido utilizado em estudos epidemiológicos localizados, para a comparação de linhagens isoladas em programas de vigilância locais e em períodos de tempo mais curtos, o MLST é, geralmente, aplicado em estudos de epidemiologia amplos, permitindo comparar linhagens virulentas e resistentes aos antimicrobianos isoladas no mundo todo (RANJBAR et al., 2014).

3.8. Epidemiologia de betalactamases do tipo ESBL e AmpC no Brasil

As cefalosporinas de terceira geração começaram a serem utilizadas na prática clínica no Brasil, no início dos anos 80 (SAMPAIO; GALES, 2016). Em 1997, foi relatado o primeiro caso de *bla*$_{\text{CTX-M-2}}$ em uma cepa de *Proteus mirabilis* de uma paciente em ambiente hospitalar no Brasil (BONNET et al., 2000). Já em 2007, foi reportada o primeiro caso de uma *E. coli* produtora de CTX-M-2 em ambiente comunitário (MINARINI et al., 2007). Desde então, muitos trabalhos tem relatado a rápida disseminação de cepas produtoras de ESBL em ambientes hospitalares e comunitários no Brasil (da SILVA; LINCOPAN, 2012; ROCHA et al., 2015; NOGUEIRA et al., 2015).

Atualmente no Brasil, as ESBL tem sido mais frequentemente encontrados em cepas de *Klebsiella pneumoniae* e *E. coli* em amostras clínicas humanas (ROCHA et al.,2015; SAMPAIO; GALES, 2016), sendo as enzimas do tipo CTX-M-2 e CTX-M-15 as predominantes em nosso país, seguido de enzimas do tipo CTX-M-8, CTX-M-9 e CTX-M-59 (ROCHA et al., 2015; da SILVA; LINCOPAN, 2012; GONÇALVES et al., 2016; NOGUEIRA et al., 2015).

Posteriormente ao primeiro relato de ESBL de origem comunitária, foi reportado a presença de ESBLs em carnes de frango em diversas regiões do país, como Sul, Sudeste e Centro-Oeste (SILVA et al., 2013; KOGA et al., 2015; FICH et al., 2015). Interessantemente, um trabalho realizado por Fernandes e colaboradores (2009), pesquisou cepas isoladas de amostras clínicas de pacientes hospitalizados e de carne de frango e seu ambiente, entre os anos de 2003 a 2004, e encontraram a presença de ESBL do tipo CTX-M-2 nessas amostras, demonstrando que a muito tempo antes da detecção de ESBL na comunidade essas betalactamases já circulavam entre a produção aviária. CTX-M-2 tem sido a variante de ESBL mais frequentemente descrita no Brasil em enterobactérias tanto em amostras clínicas quanto em animais de produção (SAMPAIO; GALES, 2016; ROCHA et al., 2015; FICH et al., 2015; da SILVA; LINCOPAN, 2012).

Entre os animais de produção, cepas produtoras de ESBL tem sido descritas principalmente em produtos de origem aviária (frango e ovos) e fontes relacionadas (granjas produtoras de frango e peru) (da SILVA; LINCOPAN, 2012; KOGA et al., 2015; FICH et al., 2015; BOTELHO et al., 2015; CASELLA et al., 2018). Entre os estudos relacionados a pesquisa de bactérias produtoras de ESBL em carnes de frango, tem sido relatado principalmente em amostras de *E. coli*. Entre as ESBLs encontradas em carnes de frango, as variantes do tipo CTX-M-2 e CTX-M-8 tem sido as mais frequentemente encontradas, embora também tenha sido relatado, em menor frequência, a presença de CTX-M-1, CTX-M-14, CTX-M-15, CTX-M-44, CTX-M-55 (KOGA et al., 2015; FICH et al., 2015; IARK et al., 2018; BOTELHO et al., 2015; FERREIRA et al., 2018; CUNHA et al., 2017). Alguns estudos também tem reportado a disseminação de *Salmonella* spp. produtoras de ESBL do tipo CTX-M1, CTX-M-2, CTX-M-8 e CTX-M-14, em carne de frango (SILVA et al., 2012; FERNANDES et al., 2009; FICH et al., 2015). Um estudo europeu também identificou o gene $bla_{\text{CTX-M-2}}$ em *E. coli* isolada de frangos importados do Brasil (WARREN et al., 2008).

A presença de cepas produtoras de ESBL tem sido descritos em outros animais de produção. Em suínos foram relatados a presença de cepas produtoras de CTX-M-15 (SILVA et al., 2016). Palmeiras e colaboradores (2018) relataram a presença de CTX-M-2 concomitante ao gene *mcr*-1 em uma amostra de *E. coli* isoladas de um bovino na região Nordeste do Brasil. Um estudo também detectou a presença de ESBL do tipo CTX-M-8 em amostras de *E. coli*, isoladas de búfalos no estado do Paraná (AIZAWA et al., 2014).

Em animais silvestres também tem sido detectada a presença de betalactamases. Em peixes foi descrito a presença de CTX-M-2 e CTX-M-55 (SELLERA et al., 2018). Da mesma forma, genes de betalactamases foram identificados em pinguins migratórios, que apresentaram CTX-M em concomitância ao gene *mcr*-1 (SELLERA et al., 2017). A presença de genes de betalactamase em animais silvestres demonstra que as rotas de disseminação desses genes vão além do uso de antimicrobianos na clínica humana e na produção animal.

As primeiras cepas produtoras de pAmpC foram reportadas no Brasil entre os anos de 2007 e 2008, sendo identificadas as variantes do tipo FOX-5-like e CMY-2-like em pacientes de hospitais (CASTANHEIRA et al., 2007; PAVEZ et al., 2008). No Brasil, as variantes do tipo CMY-2 tem sido a mais prevalentes em *E. coli* e *K. pneumoniae* produtoras de pAmpC entre amostras clínicas humanas e em animais de produção (CAMPANHA et al., 2013; PAVEZ et al., 2008; ROCHA et al., 2016).

Quanto a estudos com animais de produção no país, além da presença em frango, tem sido detectado a presença de CMY-2 em amostras de APEC isoladas de infecções em perus (SILVA et al., 2017), e colonizando um rebanho de búfalos confinados para a produção de leite no Paraná (AIZAWA et al., 2014). No entanto, ainda há poucos relatos da frequência de pAmpC em enterobactérias no Brasil (SAMPAIO; GALES, 2016).

No Brasil, não existem programas de vigilância de abrangência nacional referentes à epidemiologia da resistência antimicrobiana e seus mecanismos, tornando difícil estimar a proporção das variantes de betalactamases no país (da SILVA; LINCOPAN, 2012). Muito dos dados publicados em relação à epidemiologia de betalactamases, do tipo ESBL e AmpC, estão concentrados nas regiões Sul, Sudeste e Nordeste, e há muito poucos dados nas regiões Norte e Centro-Oeste do Brasil (ROCHA et al., 2015; GONÇALVES et al., 2019).

3.9. Potencial Zoonótico

Antimicrobianos tem sido utilizados na produção animal, principalmente na produção de suínos, bovinos, caprinos, ovinos e aves com objetivos terapêuticos, profiláticos e como promotores de crescimento (ARIAS; CARRILHO, 2012). Os antimicrobianos utilizados como promotores de crescimento são administrados em doses subterapêuticas como suplemento alimentar, e atuam através da alteração da microbiota e destruição de bactérias patogênicas do trato gastrointestinal dos animais de produção, resultado em uma melhor digestão dos alimentos e metabolismo dos nutrientes (ARIAS; CARRILHO, 2012; SANTOS et al., 2013; MARSHALL; LEVY, 2011).

O uso indiscriminado de antimicrobianos na produção e medicina animal, tem levado à disseminação de bactérias multirresistentes. Isso tem sido preocupante, visto que muitas das resistências encontradas estão relacionadas a importantes antimicrobianos utilizados na clínica humana. Além disso, diversos aditivos antimicrobianos utilizados na produção animal são análogos ou apresentam resistência cruzada com antimicrobianos utilizados na medicina humana, como por exemplo o glicopeptídeo avoparcina e a vancomicina, o que pode interferir no aumento de bactérias resistentes (ARIAS; CARRILHO, 2012).

Diante da emergência de isolados resistentes aos antimicrobianos de importância clínica humana em animais de produção, desde o ano de 2006, está proibido o uso de antimicrobianos como aditivos na Europa (MANGES; JOHNSON, 2012). No Brasil, o Ministério da Agricultura Pecuária e Abastecimento (MAPA) a cada ano vêm restringindo o uso de antimicrobianos como promotores de crescimento, sendo proibido o uso de antimicrobianos como tetraciclinas, cloranfenicol, sulfonamidas, penicilinas, entre outros (BRASIL, 2003; BRASIL, 2009). Muitas empresas de agroindústrias nacionais acataram adicionalmente as proibições que vigoram em outros países, como os países da União Européia, como forma de continuar atendendo os mercados internacionais (BRUMANO; GATTÁS, 2009). No final do ano de 2016, com a disseminação da isolados resistentes a colistina em diversos países, e a presença destes no Brasil entre infecções clínicas humanas e em animais destinados a produção de alimentos ao homem, fez com que o seu uso de colistina como aditivo fosse proibido em todo o território nacional (BRASIL, 2016).

A contaminação por bactérias resistentes advindas de animais destinados a alimentos para o homem, pode ocorrer por meio do consumo da própria carne, contaminação em utensílios utilizados no preparo desses alimentos, pelo consumo de vegetais fertilizados com adubo orgânico vindo de aves que tiveram contato com antimicrobianos em sua produção, contaminação do ambiente, entre outras formas (BÉLANGER et al., 2011). A contaminação na carne animal por bactérias resistentes ocorre durante o abate do animal e o processamento para a produção de carne, na qual, bactérias resistentes do trato gastrointestinal dos animais podem contaminar a carcaça e, posteriormente, via cadeia alimentar, chegar ao trato gastrointestinal humano, onde atuam como reservatórios de genes de resistência transmissíveis horizontalmente para a microbiota humana, ou podendo se disseminar para outros sítios corporais, causando infecções de difícil tratamento (MANGES; JOHNSON, 2012).

Entre os animais destinados à produção de alimentos ao homem, a carne de frango tem sido considerada uma das principais fontes de bactérias resistentes aos antimicrobianos, e também fontes de patógenos, como ExPEC, ao homem (KOGA et al., 2015; CYOIA et al., 2019). Diversos trabalhos têm relatado similaridades genotípicas e fenotípicas quanto ao perfil de susceptibilidade aos antimicrobianos e ao perfil de virulência em cepas de *E. coli* produtoras de ESBL isoladas de carnes de frango com cepas isoladas de amostras clínicas humanas. Vicent e colaboradores (2010) demonstraram, por meio de técnicas de tipagem molecular (PFGE e MLST) e sorotipagem, alta relação entre cepas de *E. coli* isoladas de carnes de frango com *E. coli* uropatogênicas, ambos isolados na mesma região e em períodos próximos. Van Hoek e colaboradores (2016) compararam amostras de *E. coli* resistentes a cefalosporinas isoladas de carne de frango, de pessoas em contato com essas carnes (moradores ou trabalhadores), da população em geral e de pessoas com infecção urinária. Nesse estudo, amostras de *E. coli* isoladas de carne de frango e de pessoas que apresentavam contato com essas carnes, apresentaram similaridades em relação a genes de ESBL e pAmpC, fatores de virulência e classificação filogenética. Enquanto que amostras isoladas de infecção urinária e a população em geral apresentaram uma relação mais distante. Voets e colaboradores (2013) estudaram cepas de *E. coli* isolados de humanos e de carne de frango, isolados no mesmo período e na mesma região, e encontraram uma alta similaridade em relação a pAmpC, plasmídios e STs, indicando uma possível transmissão de genes de resistência e/ ou cepas entre humanos e alimento. No entanto, em contraste com esses dados, há também estudos que encontraram diferenças genotípicas e fenotípicas entre amostras isoladas de animais de produção quando comparadas com amostras isoladas de humanos (VALENTIN et al.,2014; BEEN et al., 2014).

Muitos estudos também têm demonstrado uma alta similaridade entre *E. coli* isolada de aves com ExPECs humana, em relação à presença de genes de virulência, STs, grupos filogenéticos e testes *in vivo* (TIVENDALE et al., 2010; MANGES; JOHNSON, 2012; PEIRANO et al., 2012; COHEN STUART et al., 2012). Isso sugere que pode haver pouca ou nenhuma dependência de especificidade ao hospedeiro entre ExPECs humanas e aviárias, demonstrando um potencial risco zoonótico. Um estudo demonstrou que cepas de APEC são capazes de causar meningites em ratos, indicando que esse patógeno pode causar doenças em mamíferos, suportando a hipótese de que APEC seja também um potencial zoonótico (TIVENDALE et al., 2010).

Em 2008, foi identificado um clone pandêmico de *E. coli*, a ST131, que apresenta grande virulência e capacidade de causar diversas infecções, como as do trato urinário, bacteremias e meningites em humanos. Este clone é o mais bem estudado, e tem sido encontrado com grande frequência, tanto em humanos quanto em animais domésticos e de produção, e vários estudos sugerem a transmissão deste patógeno via cadeia alimentar pelo consumo de carne de frango. Diversos trabalhos tem demonstrado uma alta similaridade genética entre ST131 isolada de humanos com aquelas isoladas de carnes de frango (MANGES; JOHNSON, 2012; MORA et al., 2010).

Outas linhagens de ExPECs em humanos também têm sido relacionadas com um possível risco zoonótico. *E. coli* ST69 tem sido identificada com uma alta frequência de ITUs e resistência antimicrobiana nos Estados Unidos, e tem sido encontrada em carne de frango e suína (MANGES; JOHNSON, 2012). O complexo clonal ST10 tem sido associado com a disseminação de ESBL entre infecções hospitalares e comunitárias, como também em animais de produção e seus derivados (PEIRANO et al., 2012; COHEN STUART et al., 2012). O ST117 tem sido relacionado com linhagens de APECs, mas também tem sido encontrada em infecções humanas e em carne de frango (VICENT et al., 2010). Em outro estudo, verificou-se que ST95, reconhecido como um grupo clonal de linhagens de APEC, foi encontrado em 6% de infecções por ExPEC em humanos (GIBREEL et al., 2012).

O uso de antimicrobianos na produção animal tem favorecido a seleção de bactérias resistentes, como as enterobactérias produtoras de betalactamases com potencial para disseminação na comunidade por meio de contato direto e consumo de alimentos contaminados, podendo ainda se estabelecer nos ecossistemas (da SILVA; LINCOPAN, 2012). A emergência de bactérias produtoras de ESBL e/ou AmpC em humanos e nas carnes de origem animal tem causado uma grande preocupação quanto à possibilidade de transmissão da resistência aos antimicrobianos por meio da cadeia alimentar. No contexto “One-Health”, há a necessidade da colaboração conjunta de profissionais da área da saúde humana, saúde animal, indústria alimentícia e da área ambiental para o controle da disseminação da resistência antimicrobiana no mundo (EWERS et al., 2012; VAN PUYVELD, DEBORGGAEVE; JACOBS, 2018).

4. REFERÊNCIAS

1. AIZAWA, J.; NEUWIRT, N.; BARBATO, L.; NEVES, P.R.; LEIGUE, L.; PADILHA, J.; PESTANA DE CASTRO, A.F.; GREGORY, L.; LINCOPAN, N. Identification of fluoroquinolone-resistant extended-spectrum β-lactamase (CTX-M-8)-producing *Escherichia coli* ST224, ST2179 and ST2308 in buffalo (*Bubalus bubalis*). **The Journal of Antimicrobial Chemotherapy**, v. 69, n.10, p. 2866-2869, 2014.

2. AMBLER, R.P. The structure of beta-lactamases. **Philosophical transactions of the Royal Society of London**, v. 289, n. 1036, p. 321- 331, 1980.

3. ARIAS, M.V.B.; CARRILHO, C.M.D.M. Resistência antimicrobiana nos animais e no ser humano. Há motivo para preocupação? **Semina: Ciências Agrárias**, v. 33, n. 2, p. 775 – 790, 2012.

4. BÉLANGER, L.; GARENAUX, A.; HAREL, J.; BOULIANNE, M.; NADEAU, E.; DOZOIS, C.M. *Escherichia coli* from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. **FEMS Immunology and Medical Microbiology**, v.62, n. 1, p. 1 – 10, 2011.

5. BONNET, R.; SAMPAIO, J.L.; LABIA, R.; DE CHAMPS, C.; SIROT, D.; CHANAL, C.; SIROT, J. A novel CTX-M beta-lactamase (CTX-M-8) in cefotaxime-resistant Enterobacteriaceae isolated in Brazil. **Antimicrobial Agents Chemotherapy**, v. 44, n. 7, p. 1936 – 1942, 2000.

6. BOTELHO, L.A.; KRAYCHETE, G.B.; COSTA E SILVA, J.L.; REGIS, D.V.; PICÃO, R.C.; MOREIRA, B.M.; BONELLI, R.R. Widespread distribution of CTX-M and plasmid-mediated AmpC β-lactamases in *Escherichia coli* from Brazilian chicken meat. **Memórias do Instituto Oswaldo Cruz**, v. 110, n. 2, p. 249 – 254, 2015.

7. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa Nº9, de 27 de junho de 2003. Proíbe a fabricação, a manipulação, o fracionamento, a comercialização, a importação e o uso dos princípios ativos cloranfenicol e nitrofuranos e os produtos que contenham estes princípios ativos, para uso veterinário e susceptível de emprego na alimentação de todos os animais e insetos. 2003. Disponível em: <http://www.agricultura.gov.br/arq_editor/file/CRC/IN%2009-2003%20-%20Proibe%20uso%20de%20cloranfenicol%20e%20nitrofuranos>. Acesso em: 30 jan. 2014.

8. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa Nº26, de 9 de julho de 2009. Regulamento técnico para a fabricação, o controle de qualidade, a comercialização e o emprego de produtos antimicrobianos de uso veterinário. 2009. Disponível em : <http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=visualizarAtoPortalMapa&chave=1984822284>. Acesso em: 30 jan. 2014.

9. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa Nº45, de 22 de novembro de 2016. Proíbe, em todo o territórioo nacional, a importação e a fabricação da substância antimicrobiana sulfato de colistina, com a finalidade de aditivo zootécnico melhorador de desempenho na alimentação animal. 2016. Disponível em: < http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-pecuarios/alimentacao-animal/arquivos-alimentacao-animal/legislacao/instrucao-normativa-no-45-de-22-de-novembro-de-2016.pdf/view>. Acesso em: 30 jan. 2018.

10. BRUMANO, G.; GATTÁS, G. Implicações sobre o uso de antimicrobianos em rações de monogástricos. **Revista Eletrônica Nutritime**, v. 6, n. 3, p. 953 – 959, 2009.

11. BUSH, K.; JACOBY, G.A. Updated functional classification of beta-lactamases. **Antimicrobial Agents and Chemotherapy**, v. 54, n. 3, p. 969 – 976, 2010.

12. CANTÓN, R.; GONZÁLEZ-ALBA, J.M; GALÁN, J.C. CTX-M enzymes: origin and diffusion. **Frontiers in Microbiology**, v.3, 2012.

13. CASELLA, T.; HAENNI, M.; MADELA, N.K.; ANDRADE, L.K.; PRADELA, L.K.; ANDRADE, L.N.; DARINI, A.L.D.C.; MADEC, J.Y.; NOGUEIRA, M.C.L. Extended-spectrum cephaloposporin-resistant *Escherichia coli* isolated from chicken and chicken meat in Brazil is associated with rare and complex resistance plasmids and pandemic ST lineages. **Journal of Antimicrobial Chemotherapy,** v. 73, n. 12, p. 3293 – 3297, 2018.

14. CASTANHEIRA, M.; PEREIRA, A.S.; NICOLETTI, A.G.; PIGNATARI, A.C.; BARTH, A.L.; GALES, A.C. First report of plasmid-mediated *qnr*A1 in a ciprofloxacin-resistant *Escherichia coli* strain in Latin America. **Antimicrobial Agents and Chemotherapy**, v. 51, n. 4, p. 1527-1529, 2007.

15. CLERMONT, O.; CHRISTENSON, J.K.; DENAMUR, E.; GORDON, D.M. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. **Environmental Microbiology Reports**, v.5, n.1, p. 58-65, 2013.

16. COHEN STUART, J.; VAN DEN MUNCKHOF, T.; VOETS, G.; SCHARRINGA, J.; FLUIT, A.; HALL, M.L. Comparison of ESBL contamination in organic and conventional retail chicken meat. **International Journal of Food Microbiology**, v. 154, n. 3, p. 212–214, 2012.

17. CROXEN, M.A.; FINLAY, B.B. Molecular mechanisms of *Escherichia coli* pathogenicity. **Nature Reviews Microbiology**, v. 8, n. 1, p. 26 – 38, 2009.

18. CUNHA, M.P.; LINCOPAN, N.; CERDEIRA, L.; ESPOSITO, F.; DROPA, M.; FRANCO, L.S.; MORENO, A.M.; KNÖBL, T. Coexistence of CTX-M-2, CTX-M-55, CMY-2, FosA3, and QnrB19 in Extraintestinal pathogenic *Escherichia coli* from poultry in Brazil. **Antimicrobial Agents and Chemotherapy**, v. 61, n. 4, p. 1- 3, 2017.

19. CYOIA, P.S.; RODRIGUES, G.R.; NISHIO, E.K.; MEDEIROS, L.P.; KOGA, V.L.; PEREIRA, A.P.D.; VESPERO, E.C.; HOULE, S.; DOZOIS, C.M.; NAKAZATO, G.; KOBAYASHI, R.K.T. Presence of virulence genes and pathogenicity islands in extraintestinal pathogenic *Escherichia coli* isolates from Brazil. **The Journal of Infection in Developing Countries**, v. 9, n. 10, p. 1068 – 1075, 2015.

20. CYOIA, P.S.; KOGA, V.L.; NISHIO, E.K.; HOULE, S.; DOZOIS, C.M.; DE BRITO, K.C.T.; DE BRITO, B.G.; NAKAZATO, G.; KOBAYASHI, R.K.T. Distribution of ExPEC virulence factors, *bla* $_{\text{CTX-M}}$, *fos*A3, and *mcr*-1 in *Escherichia coli* isolated from commercialized chicken carcasses. **Frontiers in Microbiology**, v. 10, p. 1–9, 2019.

21. DAGA, A.P.; KOGA, V.L.; SONCINI, J.G.M.; DE MATOS, C.M.; PERUGINI, M.R.E.; PELISSON, M.; KOBAYASHI, R.K.T.; VESPERO, E.C. *Escherichia coli* bloodstream infections in patients at a university hospital: virulence factors and clinical characteristics. **Frontiers in cellular and infection microbiology,** v. 9, 2019.

22. DALE, A.P.; WOODFORD, N. Extra-intestinal pathogenic *Escherichia coli* (ExPEC): disease, carriage and clones. **Journal of Infection**, v. 71, p. 615-626, 2015.

23. DA SILVA, K.C.; LINCOPAN, N. Epidemiologia das betalactamases de espectro estendido no Brasil: impacto clínico e implicações para o agronegócio. **Jornal Brasileiro de Patologia e Medicina Laboratorial**, v. 48, n. 2, p. 91 – 99, 2012.

24. DATTA, N.; KONTOMICHALOU, P. Penicillinase synthesis controlled by infectious R factors in Enterobacteriaceae. **Nature**, v. 208, n. 5007, p. 239 – 241, 1965.

25. DE BEEN, M.; LANZA, V.F.; DE TORO, M.; SCHARRINGA, J.; DOHMEN, W.; DU, Y.; HU, J.; LEI, Y.; TOOMING-KLUNDERUD, A.; HEEDERIK, D.J.; FLUIT, A.C. BONTEN, M.J.; WILLEMS, R.J.; DE LA CRUZ, F.; VAN SCHAIK, W. Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. **PLOS Genetics**, v. 10, n.12, 2014.

26. DHILLON, R.H.-P.; CLARK, J. ESBLs: a clear and present danger? **Critical Care Research and Practice**, v. 2012, p. 1 - 11, 2011.

27. DU-BOIS, S.K.; MARRIOTT, M.S.; AMYES, S.G. TEM- and SHV- derived extended-spectrum beta-lactamases: relationship between selection, structure and function. **The Journal of Antimicrobial Chemotherapy**, v.35, n. 1, p. 7 – 22, 1995.

28. DZIVA, F.; STEVENS, M.P. Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural hosts. **Avian Pathology**, v. 37, n. 4, p. 355 – 366, ago. 2008.

29. EWERS, C.; BETHE, A.; SEMMLER, T.; GUENTHER, S.; WIELER, L.H. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. **Clinical Microbiology and Infection,** v. 18, n. 7, p. 646 – 655, mar. 2012.

30. FERNANDES, S.A.; PATERSON, D.L.; GHILARDI-RODRIGUES, A.C.; ADAMS-HADUCH, J.M.; TAVECHIO, A.T.; DOI, Y. CTX-M-2-producing *Salmonella typhimurium* isolated from pediatric patients and poultry in Brazil. **Microbial Drug Resistance**, v. 15, n. 4, p. 317 - 321, 2009.

31. FERREIRA, J.C.; PENHA FILHO, R.A.C.; KUAYE, A.P.Y.; ANDRADE, L.N.; CHANG, Y.F.; DARINI, A.L.C. Virulence potential of commensal multidrug resistant Escherichia coli isolated from poultry in Brazil. **Infection, Genetics and Evolution**, v. 65, p. 251–256, 2018.

32. FITCH, F.M.; CARMO-RODRIGUES, M.S.; OLIVEIRA, V.G.; GASPARI, M.V.; DOS SANTOS, A.; DE FREITAS, J.B.; PIGNATARI, A.C. β-Lactam Resistance Genes: Characterization, Epidemiology, and First Detection of *bla* $_{\text{CTX-M-1}}$ and *bla* $_{\text{CTX-M-14}}$ in *Salmonella* spp. Isolated from Poultry in Brazil—Brazil Ministry of Agriculture's Pathogen Reduction Program . **Microbial Drug Resistance**, v. 22, n. 2, p. 164–171, 2015.

33. GIBREEL, T.M.; DODGSON, A.R.; CHEESBROUGH, J.; FOX, A.J.; BOLTON, F.J.; UPTON, M. Population structure, virulence potential and antibiotic susceptibility of uropathogenic *Escherichia coli* from Northwest England. **Journal of Antimicrobial Chemotherapy**, v. 67, n. 2, p. 346–356, 2012.

34. GONÇALVES, L.F.; DE OLIVEIRA MARTINS-JÚNIOR, P.; DE MELO, A.B.F.; DA SILVA, R.C.R.M.; DE PAULO MARTINS, V.; PINTODO-SILVA, A.; DE CAMPOS, T.A. Multidrug resistance dissemination by extended-spectrum β-lactamase-producing *Escherichia coli* causing community-acquired urinary tract infection in the Central-Western Region, Brazil. **Journal of Global Antimicrobial Resistance**, v. 6, n. 2010, p. 1–4, 2016.

35. GUTKIND, G.O.; DI CONZA, J.; POWER, P.; RADICE, M. β-lactamase-mediated resistance: a biochemical, epidemiological and genetic overview. **Current Pharmaceutical Design**, v. 19, n. 2, p. 164-208, 2013.

36. IARK, A.S.; KOGA, V.L.; VESPERO, E.C.; KOBAYASHI, R.K.T.; DE OLIVEIRA, T.C.R.M. First report of CTX-M-44 in *Escherichia coli* isolated from chicken meat produced in Brazil. **Journal of Infection in Developing Countries**, v. 12, n. 4, p. 284–285, 2018.

37. JACOBY, G.A. AmpC β-lactamases. **Clinical Microbiology Reviews**, v. 22, n. 1, p. 161 – 182, 2009.

38. JALIER, V. NICOLAS, M.H.; FOURNIER, G.; PHILIPPON, A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. **Reviews of infectious diseases**, v. 10, n. 4, p. 867-878, 1988.

39. JOHNSON, T.J.; WANNEMUEHLER, Y.; DOETKOTT, C.; JOHNSON, S.J.; ROSENBERGER, S.C.; NOLAN, L.K. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. **Journal of Clinical Microbiology,** v. 46, n. 12, p. 3987 - 3996, 2008.

40. JOHNSON, J.R.; STELL, A.L. Extended Virulence Genotypes of *Escherichia coli* Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise. **The Journal of Infectious Diseases**, v. 181, n. 1, p. 261–272, 2002.

41. KAPER, J.B.; NATARO, J.P.; MOBLEY, L.T. Pathogenic *Escherichia coli*. **Nature Reviews Microbiology**, v. 2, p. 123 – 140, 2004.

42. KARIYAWASAM, S.; HAN, J. Avian Pathogenic *Escherichia coli*: Link to Foodborne Urinary Tract Infections in Humans. **Food Safety in Poultry Meat Production**, 2019.

43. KATZUNG, B.G.; MASTERS, S.B.; TREVOR, A.J. **Farmacologia básica e clínica**. 13.ed. Porto Alegre: AMGH, 2017.

44. KNOTHE, H.; SHAH, P.; KRCMERY, V.; ANTAL, M.; MITSUHASHI, S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumonia* and *Serratia marcescens*. **Infection**, v. 11, n. 6, p. 315 – 317, 1983.

45. KOGA, V.L.; TOMAZETTO, G.; CYOIA, P.S.; NEVES, M.S.; VIDOTTO, M.C.; NAKAZATO, G.; KOBAYASHI, R.K.T. Molecular screening of virulence genes in extraintestinal pathogenic *Escherichia coli* isolated from human blood culture in Brazil. **Biomed Research Internacional**, v. 2014, p. 1 - 9, 2014.

46. KOGA, V.L.; RODRIGUES, G.R.; SCANDORIEIRO, S.; VESPERO, E.C.; OBA, A.; DE BRITO, B.G.; DE BRITO, K.C.T.; NAKAZATO, G.; KOBAYASHI, R.K.T. Evaluation of the Antibiotic Resistance and Virulence of *Escherichia coli* Strains Isolated from Chicken Carcasses in 2007 and 2013 from Paraná, Brazil. **Foodborne Pathogens and Disease**, v. 12, n. 6, p. 479–485, 2015.

47. LARTIGUE, M.F.; POIREL, L.; AUBERT, D.; NORDMANN, P. In vitro analysis of IS*Ecp*1B-mediated mobilization of naturally occurring β-lactamase gene *bla*$_{\text{CTX-M}}$ of *Kluyvem ascorbata*. **Antimicrobial Agents and Chemotherapy**, v. 50, n. 4, p. 1282–1286, 2006.

48. LEVERSTEIN-VAN HALL, M.A.; DIERIKX, C.M.; COHEN STUART, J.; VOETS, G.M.; VAN DEN MUNCKHOF, M.P.; VAN ESSEN-ZANDBERGEN, A.; PLATTEEL, FLUIT, A.C.; VAN DE SANDE-BRUINSMA, N., SCHARINGA, J.; BONTEN, M.J.; MEVIUS, D.J. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. **Clinical Microbiology and Infection**, v. 17, n. 6, p. 873 – 880, 2011.

49. LOGUE, C.M.; WANNEMUEHLER, Y.; NICHOLSON, B.A.; DOETKOTT, C.; BARBIERI, N.L.; NOLAN, L.K. Comparative analysis of phylogenetic assignment of human and avian ExPEC and fecal commensal *Escherichia coli* using the (previous and revised) clermont phylogenetic typing methods and its impact on avian pathogenic Escherichia coli (APEC) classification. **Frontiers in Microbiology**, v. 8, 2017.

50. MANGES, A.R.; JOHNSON, J.R. Food-borne origins of *Escherichia coli* causing extraintestinal infections. **Clinical Infectious Diseases**, v. 55, n. 5, p. 712 – 719, 2012.

51. MARSHALL, B.M.; LEVY, S.B. Food animals and antimicrobials: Impacts on human health. **Clinical Microbiology Reviews**, v. 24, n. 4, p. 718–733, 2011.

52. MELLATA, M. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. **Foodborne Pathogens and Disease**, v. 10, n. 11, p. 916 - 932, 2013.

53. MINARINI, L.A.R.; GALES, A.C.; PALAZZO, I.C.; DARINI, A.L. Prevalence of community-occurring extended spectrum beta-lactamase-producing Enterobacteriaceae in Brazil. **Current Microbiology**, v. 54, n. 5, p. 335 – 341, 2007.

54. MORA, A.; HERRERA, A.; MAMANI, R.; LÓPEZ, C.; ALONSO, M.P.; BLANCO, J.E.; BLANCO, M.; DAHBI, G.; GARCÍA-GARROTE, F.; PITA, J.M.; COIRA, A.; BERNÁRDEZ, M.I.; BLANCO, J. Recent emergence of clonal group O25b:K1:H4-B2-ST131 *ibe*A strains among *Escherichia coli* poultry isolates, including CTX-M-9-producing strains, and comparison with clinical human isolates. **Applied and Environmental Microbiology**, v. 76, n. 21, p. 6991–6997, 2010.

55. MOULIN-SCHOULEUR, M.; RÉPÉRANT, M.; LAURENT, S.; BRÉE, A.; MIGNON-GRASTEAU, S.; GERMON, P.; RASSCHAERT, D.; SCHOULER, C. Extraintestinal pathogenic *Escherichia coli* strains of avian and human origin: link between phylogenetic relationships and common virulence patterns. **Journal of Clinical Microbiology**, v. 45, n. 10, p. 3366-3376, 2007.

56. MÜLLER, A.; STEPHAN, R.; NÜESCH-INDERBINEN, M. Distribution of virulence factors in ESBL-producing *Escherichia coli* isolated from the environment, livestock, food and humans. **Science of the Total Environment**, v. 541, n. 2016, p. 667 – 672, 2016.

57. NOGUEIRA, K.S.; CONTE, D.; MAIA, F.V.; DALLA-COSTA, L.M. Distribution of extended-spectrum β-lactamase types in a Brazilian tertiary hospital. **Revista da Sociedade Brasileira de Medicina Tropical,** v. 48, n. 2, p. 162-169, 2015.

58. PALMEIRA, J.D.; FERREIRA, H.; MADEC, J.Y.; HAENNI, M. Draft genome of a ST443 *mcr*-1- and *bla*$_{CTX-M-2}$-carrying *Escherichia coli* from cattle in Brazil. **Journal of Global Antimicrobial Resistance,** v. 13, p. 269–270, 2018.

59. PAVEZ, M.; NEVES, P.; DROPA, M.; MATTÉ, M.H.; GRINBAUM, R.S.; ELMOR DE ARAÚJO, M.R.; MAMIZUKA, E.M.; LINCOPAN, N. Emergence of carbapenem-resistant *Escherichia coli* producing CMY-2-type AmpC β-lactamase in Brazil. **Journal of Medical Microbiology**, v. 57, p. 1590-1592, 2008.

60. PEIRANO, G.; PITOUT, J.D.D. Extended-spectrum β-lactamase-producing Enterobacteriaceae: update on molecular epidemiology and treatment options. **Drugs**, 2019.

61. PEIRANO, G.; VAN DER BIJ, A.K.; GREGSON, D.B.; PITOUT, J.D. Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum β-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. **Journal of Clinical Microbiology**, v. 50, n. 2, p. 294–299, 2012.

62. PÉREZ-PÉREZ, F.J.; HANSON, N.D. Detection of plasmid-mediated ampC β-lactamase genes in clinical isolates by using multiplex PCR. **Journal of Clinical Microbiology**, v. 40, n. 6, p. 2153 – 2162, jun. 2002.

63. PITOUT, J.D.D. Extraintestinal pathogenic *Escherichia coli*: a combination of virulence with antibiotic resistance. **Frontiers in Microbiology**, v. 3, n. 9, p. 1 – 7, jan. 2012.

64. PLATELL, J.L.; JOHNSON, J.R.; COBBOLD, R.N.; TROTT, D.J. Multidrug-resistant extraintestinal pathogenic *Escherichia coli* of sequence type ST131 in animals and foods. **Veterinary Microbiology**, v. 153, p. 99 – 108, 2011.

65. RANJBAR, R.; KARAMI, A.; FARSHAD, S.; GIAMMANCO, G.M.; MAMMINA, C. Typing methods used in the molecular epidemiology of microbial pathogens: a how-to guide. **New Microbiologica**, v. 37, p. 1-15, 2014.

66. ROCHA, F.R.; PINTO, V.P.T.; BARBOSA, F.C.B. The Spread of CTX-M-Type Extended-Spectrum β-Lactamases in Brazil: A Systematic Review. **Microbial Drug Resistance**, v. 22, n. 4, p. 301–311, 2015.

67. SABATÉ, M.; MORENO, E.; PÉREZ T.; ANDREU A.; PRATS, G. Pathogenicity island markers in commensal and uropathogenic *Escherichia coli* isolates. **European Society of Clinical Microbiology and Infectious Disease**, v. 12, n. 9, p. 880 – 886, 2006.

68. SAMPAIO, J.L.M.; GALES, A.C. Antimicrobial resistance in Enterobacteriaceae in Brazil: focus on β-lactams and polymyxins. **Brazilian Journal of Microbiology**, v. 47, p. 31–37, 2016.

69. SANTOS, L.L.; MOURA, R.A.; AGUILAR-RAMIRES, P.; DE CASTRO, A.P.; LINCOPAN, N. Current status of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in animals. **Formatex**, p. 1600-1607, 2013.

70. SELLERA, F.P.; FERNANDES, M.R.; SARTORI, L.; CARVALHO, M.P.; ESPOSITO, F.; NASCIMENTO, C.L.; DUTRA, G.H.; MAMIZUKA, E.M.; PÉREZ-CHAPARRO, P.J.; MCCULLOCH, J.A.; LINCOPAN, N. *Escherichia coli* carrying IncX4 plasmid-mediated *mcr*-1 and *bla* $_{\text{CTX-M}}$ genes in infected migratory Magellanic penguins (*Spheniscus magellanicus*). **Journal of Antimicrobial Chemotherapy**, v. 72, n. 4, p. 1255–1256, 2017.

71. SELLERA, F.P.; FERNANDES, M.R.; MOURA, Q.; CARVALHO, M.P.N.; LINCOPAN, N. Extended-spectrum-B-lactamase (CTX-M)-producing *Escherichia coli* in wild fishes from a polluted area in the Atlantic Coast of South America. **Marine Pollution Bulletin**, v. 135, p. 183–186, 2018.

72. SILVA, K.C.; FONTES, L.C.; MORENO, A.M.; ASTOLFI-FERREIRA, C.S.; FERREIRA, A.J.; LINCOPAN, N. Emergence of extended-spectrum-β-lactamase CTX-M-2-producing *Salmonella enterica* serovars Schwarzengrund and agona in poultry farms. Antimicrobial **Agents and Chemotherapy**, v. 57, n. 7, p. 3458–3459, 2013.

73. SILVA, K.C.; MORENO, M.; CABRERA, C.; SPIRA, B.; CERDEIRA, L.; LINCOPAN, N.; MORENO, A.M. First characterization of CTX-M-15-producing *Escherichia coli* strains belonging to sequence type (ST) 410, ST224, and ST1284 from commercial swine in South America. **Antimicrobial Agents and Chemotherapy**, v. 60, n. 4, p. 2505 – 2508, 2015.

74. SILVA, K.C.; CUNHA, M.P.; CERDEIRA, L.; DE OLIVEIRA, M.G.; DE OLIVEIRA, M.C.; GOMES, C.R.; LINCOPAN, N.; KNÖBL, T.; MORENO, A.M. High-virulence CMY-2- and CTX-M-2-producing avian pathogenic *Escherichia coli* strains isolated from commercial turkeys. **Diagnostic Microbiology and Infectious Disease,** v. 87, n. 1, p. 64-67, 2017.

75. SOUZA, C.O.; MENEZES, Ê.M.; MELO, T.R.B.; CARVALHO, A.C.; MELO, C.S.B.; MONTEIRO, L.C.R. *Escherichia coli* enteropatogênica: uma categoria diarreiogênica versátil. **Revista Pan-Amazônica de Saúde**, v. 7, n. 2, p. 79 – 91, 2016.

76. SMET, A.; MARTEL, A.; PERSOONS, D.; DEWULF, J.; HEYNDRICKX, M.; HERMAN, L.; HAESEBROUCK, F.; BUTAYE, P. Broad-spectrum β-lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. **FEMS Microbiology Review**, v. 34, n. 3, p. 295-316, 2010.

77. TENAILLON, O.; SKURNIK, D.; PICARD, B.; DENAMUR, E. The population genetics of commensal *Escherichia coli*. **Nature Reviews Microbiology**, v. 8, n. 3, p. 207 – 217.

78. TIVENDALE, K.A.; LOGUE, C.M.; KARIYAWASAM, S.; JORDAN, D.; HUSSEIN, A.; LI, G.; WANNEMUEHLER, Y.; NOLAN, L.K. Avian-pathogenic *Escherichia coli* strains are similar to neonatal meningitis *E. coli* strains and are able to cause meningitis in the rat model of human disease. **Infection and Immunity**, v. 78, n. 8, p. 3412 – 3419, 2010.

79. VALENTIN, L.; SHARP, H.; HILLE, K.; SEIBT, U.; FISCHER, J.; PFEIFER, Y.; MICHAEL, G.B.; NICKEL, S.; SCHMIEDEL, J.; FALGENHAUER, L.; FRIESE, A.; BAUERFEIND, R.; ROESLER, U.; IMIRZALIOGLU, C.; CHAKRABORTY, T.; HELMUTH, R.; VALENZA, G.; WERNER, G.; SCHWARZ, S.; GUERRA, B.; APPEL, B.; KREIENBROCK, L.; KÄSBOHRER, A. Subgrouping of ESBL-producing *Escherichia coli* from animal and human sources: an approach to quantify the distribution of ESBL types between different reservoirs. **International journal of medical microbiology : IJMM**, v. 304, n. 7, p. 805–816, 2014.

80. VAN HOEK, A.H.A.M.; STALENHOEF, J.E.; VAN DUIJKEREN, E.; FRANZ, E. Comparative virulotyping of extended-spectrum cephalosporin-resistant *E. coli* isolated from broilers, humans on broiler farms and in the general population and UTI patients. **Veterinary Microbiology**, v. 194, n. 2015, p. 55–61, 2016.

81. VAN PUYVELDE, S.; DEBORGGRAEVE, S.; JACOBS, J. Why the antibiotic resistance crisis requires a One Health approach. **The Lancet Infectious Diseases**, v. 18, p. 132 – 134, 2018.

82. VICENT, C.; BOERLIN, P.; DAIGNAULT, D.; DOZOIS, C.M.; DUTIL, L.; GALANAKIS, C.; REID-SMITH, R.J.; TELLIER, P.P.; TELLIS, P.A.; ZIEBELL, K.; MANGES, A.R. Food reservoir for *Escherichia coli* causing urinary tract infections. **Emerging Infectious Disease**, v. 16, n. 1, p. 88 - 95, 2010.

83. VOETS, G.M.; FLUIT, A.C.; SCHARRINGA, J.; SCHAPENDONK, C.; VAN DEN MUNCKHOF, T.; LEVERSTEIN-VAN HALL, M.A.; STUART, J.C. Identical plasmid AmpC beta-lactamase genes and plasmid types in *E. coli* isolates from patients and poultry meat in the Netherlands. **International Journal of Food Microbiology**, v. 167, n. 3, p. 359–362, 2013.

84. WARREN, R.E.; ENSOR, V.M.; O'NEILL, P.; BUTLER, V.; TAYLOR, J.; NYE, K.; HARVEY, M.; LIVERMORE, D.M.; WOODFORD, N.; HAWKEY, P.M. Imported chicken meat as a potential source of quinolone-resistant *Escherichia coli* producing extended-spectrum β-lactamases in the UK. **Journal of Antimicrobial Chemotherapy**, v. 61, n. 3, p. 504 - 508, 2008.

85. WILES, T.J.; KULESUS, R.R.; MULVEY, M.A. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. **Experimental and Molecular Pathology**, v. 85, p. 11 – 19, 2008.

86. WINN, W.J.; ALLEN, S.; JANDA, W.; KONEMAN, E.; PROCOP, G.; SCHRECKENBERGER, P.; WOODS, G. **Koneman, Diagnóstico Microbiológico: texto e atlas colorido**. 6. ed. Rio de Janeiro: Guanabara Koogan, 2014.

5. ARTIGO CIENTÍFICO

5.1. ARTIGO CIENTÍFICO I

Os resultados e discussão foram apresentados na forma de artigo. O artigo I será submetido e segue as normas da revista “International Journal of Food Microbiology”, e tem como título: **Characterization of virulence factors and antimicrobial resistance profile in genetic diversity of ESBL-producing *Escherichia coli* isolated from human urinary tract infection and chicken carcasses in a restrict area and period.**

Highlights

- CTX-M-2 was the most frequent ESBL gene found in the strains studied
- Similar virulence factors and resistance profile were found in both group
- *mcr*-1 can be present in UTIs in Brazil since 2013
- ST131, ST354, ST359 were found both in UPEC and *E. coli* strain from chicken
- UPEC strains and *E. coli* strains from chicken showed high heterogeneity

Title

Characterization of virulence factors and antimicrobial resistance profile in genetic diversity of ESBL-producing *Escherichia coli* isolated from human urinary tract infection and chicken carcasses in a restrict area and period

Vanessa L. Koga[a], Paula S. Cyoia[a], Larissa C. de Camargo[a], Renato P. Maluta[b], Wanderley D. da Silveira[b], Sébastien Houle[c], Charles M. Dozois[c], Eliana C. Vespero[d], Gerson Nakazato[a], Renata K. T. Kobayashi[a]

[a]Basic and Applied Bacteriology Laboratory, Department of Microbiology, State University of Londrina (UEL), Londrina, PR, Brazil

[b]Bacterial Molecular Biology Laboratory, Department of Genetics, Evolution and Bioagents, Institute of Biology, State University of Campinas (UNICAMP), Campinas, SP, Brazil

[c]Institut Armand-Frappier, Institut National de la Recherche Scientifique, Laval, QC, Canada

[d]Department of Pathology and Clinical and Toxicological Analysis, State University of Londrina (UEL), Londrina, PR, Brazil

Corresponding author: Renata K. T. Kobayashi; kobayashirkt@uel.br

Abstract

Chicken meat is considered a potential source of pathogenic *Escherichia coli* and antimicrobial-resistant genes, as to extended-spectrum beta-lactamase (ESBL) genes, to human. In the present study, we characterized and evaluated the similarities between ESBL-producing *E. coli* from urinary tract infections (UTIs) and from chicken carcasses (CC) about antimicrobial resistance, virulence factors profile, and genetic similarities by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). A total of 50 ESBL-producing *E. coli* strains were selected (21 from UTIs and 29 from CC) in the same period and city (Londrina, Brazil). Among the UPEC strains, we found *bla*$_{\text{CTX-M-2}}$, *bla*$_{\text{CTX-M-3}}$, *bla*$_{\text{CTX-M-8}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{CTX-M-27}}$, while in strains from chicken we found *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-2}}$ and *bla*$_{\text{CTX-M-8}}$. The majority of the strains (96%) were considered multidrug-resistant, with high resistance frequency mainly for tetracycline (96.55%) and nalidixic acid (86,2%) in strains isolated from CC and high frequency of resistance for quinolones and fluoroquinolones (95.23 – 85.7%) in strains isolated from UTIs. This study also showed that some resistance genes as *tet*A, *tet*B, *sul*I and *sul*II are widespread associated with ESBL-producing strains. Interestingly, we also evidenced that *mcr*-1, associated with ESBL-producing *E. coli*, can be disseminated in UTI from Brazil since 2013. The strains were screened for markers of pathogenicity islands (PAI) and virulence factors (*hly*F, *iss*, *omp*T, *iro*N and *iut*A), which 52.4% of UPEC strains and 31% of strains from chicken showed at least one of the PAIs surveyed, while 93.1% of UPEC strains and 71.43% of strains from chicken showed at least one of the virulence genes studied. A total of 23 different sequence types were found, being ST131 the most found. The ST131, ST354 and ST359 were found in both groups of strains. Clonal relatedness was analyzed by PFGE, which revealed a high genetic diversity between the strains. Although we found no evidence of clonal dissemination of ESBL-producing *E. coli* in our region, our results showed that chicken meat has ESBL genes and virulence factors and PAIs similar to UPEC, suggesting that chicken meat can be a reservoir and dissemination route to humans.

Keywords: *Escherichia coli*, chicken, ESBL, UPEC, urinary tract infection

1- Introduction

Escherichia coli is a dynamic bacterial species that inhabits the gut microbiota of humans and warm-blooded animals. However, a small percentage of *E. coli* is pathogenic, which carries specific virulence genes that allow ability to colonize and survive in many environment. It is divided into two groups, the group of diarrheagenic *E. coli* (DEC) that cause intestinal infections, and the group of extra-intestinal *E. coli* (ExPEC), able to cause different types of infections, including septicemia, meningitis, urinary tract infections (UTI), among others (Kaper et al., 2004; Pitout, 2012; Dale and Woodford, 2015; Kariyawasam; Han, 2019). Already in birds, avian pathogenic *E. coli* (APEC) (part of ExPEC group) are among the main cause of avian diseases, called colibacillosis. (Johnson et al., 2008; Manges, 2016).

UTI are among the most common bacterial infections worldwide, being caused mainly by *E. coli*. The UTIs are a serious societal and public health concern owing to the resultant morbidity, productivity loss, and healthcare costs (Manges, 2016; Kariyawasam; Han, 2019). However, the emergence of antimicrobial resistance among strains of uropathogenic *E. coli* (UPEC) have led to limitations in effective antimicrobial treatment options (Kariyawasam; Han, 2019; Nordstrom et al., 2013).

Extended-spectrum beta-lactamases (ESBL)-producing bacteria have emerged in both community and healthcare settings, representing a public health concern. These enzymes hydrolyze the β-lactam ring, and inactivate oxyimino-cephalosporins, and monobactams but not cephamycins or carbapenems. Normally these enzymes are associated with resistance to other antimicrobials agents, as fluoroquinolones, sulphonamides, tetracycline and others, which limits therapeutic options. Although the epidemiology predominant of ESBL families have been for TEM, SHV and CTX-M, in the last years, the incidence of the CTX-M-type has risen dramatically, with particular subtypes associated with different geographic regions (Pitout, 2012; Cantón et al., 2012; Bush and Jacoby, 2010).

The use of antimicrobials in animal production, as prophylactic and therapeutic way, or even as growth promoters (feed additive), has been described as one of the main causes of acquiring resistance bacteria in food-producing animals. The emergence of antimicrobial resistance *E. coli* isolates in food-producing animals, mainly in chicken meat, leading to the hypothesis that chicken meat and its products can be transmission vehicles of antimicrobial resistance *E. coli* to humans infections. Studies also have found similarities between avian and human pathogenic *E. coli* suggesting poultries foods derivatives as sources of ExPEC, as UPECs (Manges, 2016; Mellata, 2013; Ewers et al., 2012; Marshall and Levy, 2011).

In a preview study, we showed a high frequency of ESBL-producing *E. coli* isolated from chicken carcasses (CC) in Brazil, mainly strains producers of CTX-M-2 and CTX-M-8 groups (Koga et al., 2015a, Koga et al., 2015b). The objective of this study was to analyze ESBL-producing *E. coli* strains between CC and UTIs, both isolated in the same city and period, and compare they about antimicrobial resistance and virulence factors profile and the genetic similarities by pulsed-field gel electrophoresis (PFGE) types and multilocus sequence typing (MLST).

2- Material and methods

2.1- Bacterial isolates

Fifty ESBL-producing *E. coli* strains were selected to this study. Of these, 21 strains were isolated from UTIs, being they provided by University Hospital of Londrina (Londrina, city of South of Brazil), and 29 strains were isolated from commercial refrigerated CCs, sold in Londrina too. The ESBL-producing *E. coli* strains isolated from CCs were selected from a previously characterized bacterial collection in 2013 (Koga et al., 2015a; Koga et al., 2015b) and they belong to the collection of the Basic and Applied Bacteriology Laboratory from State University of Londrina (UEL), Londrina, PR, Brazil. All the strains were isolated in 2013 and in the same city (Londrina, Brazil).

The selected strains used in this study were characterized as ESBL producer by the combination disk test with disks supplemented with cefotaxime and ceftazidime alone and coupled with clavulanic acid (CLSI, 2015), and by double-disk sinergy testing with the disc with clavulanic acid along with for cefotaxime, ceftazidime, cefepime and aztreonam (Jarlier et al., 1988).

2.2- Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was determined by the Clinical Laboratory Standard Institute (CLSI) disk-diffusion method. Interpretation was performed according to criteria of the Clinical and Laboratory Standard Institute (CLSI, 2008, 2015). The antimicrobials used included the following: 5µg of ciprofloxacin; 10 µg each of gentamicin, norfloxacin and enrofloxacin; 30 µg each of cefotaxime, cefoxitin, ceftazidime, tetracycline, nalidixic acid, and chloramphenicol; 300 µg of nitrofurantoin; 1.25/23.75 µg of trimethoprim-sulfamethoxazole; and 20/10 µg of amoxicillin-clavulanic acid; and 200 µg of fosfomycin (Oxoid Ltd., Basingstoke, Hants, UK). For susceptibility to colistin, strains were tested by broth microdilution method by VITEK® 2 system (bioMérieux, USA), following the criteria of European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2016). *E. coli* strain ATCC 25922 was used as a negative control. Strains were classified as multidrug-resistant when it to be nonsusceptible to at least 1 agent in 3 or more antimicrobial categories (Magiorakos et al., 2012).

2.3- Detection of antimicrobial resistance genes

All 50 strains were screened for ESBL genes, including TEM-type, SHV-type and CTX-M-type (Dallenne et al., 2010; Jouini et al., 2007; Woodford et al., 2006). DNA from *E. coli* strains was extracted by a standard boiling procedure and they are used for polymerase chain reaction (PCR) assay. Primers specific to $bla_{\text{CTX-M}}$ group were used (Saladin et al., 2002; Woodford et al., 2006; Jouini et al., 2007; Lião et al., 2010; Dallene et al., 2010), and the PCR products were purified and sequenced by Sanger methodology using an ABI PRISM 3500xL Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequencing was performed at Multiuser Laboratory of Genotyping and Sequencing from State University of Campinas (UNICAMP) and in Soil Biotechnology Laboratory from Brazilian Agricultural Research Corporation (Embrapa). β-lactamases genes were identified using the β-lactamase database (http://www.lahey.org/studies/webt.asp) after all the sequences were analyzed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The strains were also analyzed for the presence of others antimicrobial resistance genes, as AmpC beta-lactamase (bla_{CMY}, bla_{MOX}, bla_{FOX}, bla_{LAT}, bla_{ACT}, bla_{MIR}, bla_{DHA}, bla_{BIL}, bla_{ACC}), plasmid mediated quinolone resistance (PMQR) determinants (*qnr*A, *qnr*B and *qnr*S), sulfonamide resistance (*sul*I and *sul*II), tetracycline resistance (*tet*A and *tet*B), plasmid-mediated colistin resistance (*mcr*-1) and plasmid-mediated fosfomycin resistance (*fos*A3) (Pérez-Pérez and Hanson, 2002; Cattoir et al., 2007; Li et al., 2007; Liu et al., 2016; Sato et al., 2013) The PCR reaction mixture was prepared using Promega PCR Master Mix (Promega, USA), and PCR amplicons were visualized on 2.0% agarose gels stained with GelRed (Biotium). Image Capture System (LPixImageHE) were used to captured the images. Strains positive for AmpC genes

(Dierikx et al., 2010) and *mcr*-1 were also sequenced as previously described. All the primers used are in **Table 1**.

2.4- Phylogenetic typing

The isolates were screened by PCR to phylogenetic groups according to the protocol previously described (Clermont et al., 2013). Based on this method, the isolates were classified into one of seven groups (A, B1, B2, C, D, E or F). The primers used are in **Table 1**.

2.5- Screening of virulence factor genes

The strains were screened by PCR for markers of virulence associated with APEC (*hly*F, *iut*A, *iro*N, *omp*T and *iss*) (Johnson et al., 2008) and for pathogenicity islands (PAIs) markers associated with UPEC (PAI I_{536}, PAI II_{536}, PAI IV_{536}, PAI I_{CFT073}, PAI II_{CFT073} and PAI I_{J96}) (Sabaté et al., 2006). The primers used are in **Table 1**.

2.6- Typing of bacteria

Clonal relatedness of the strains were further determined by *XbaI* restriction (New England Biolabs, Ipswich, MA), followed by PFGE according to the Centers for Disease Control and Prevention (CDC) (https://www.cdc.gov/pulsenet/pathogens/pfge.html), using CHEF DR III PFGE System (Biorad, Hercules, CA, USA). Electrophoresis conditions consisted on an initial time of 2.2 seconds, a final time of 54.2 seconds at a gradient of 6V cm^{-1} and an included angle of 120°, and the gels were electrophoresed for 18h. Results were evaluated with Bionumerics (version 7.6; Applied Maths, Austin, TX, USA), and clusters were defined when isolates presented ≥90% of similarity.

2.7- MLST

MLST was carried out using the Achtman scheme (*fum*C, *gyr*B, *icd*, *mdh*, *pur*A, *rec*A, and *adk* genes) as previously described (Wirth et al., 2006). Alleles and sequence types (STs) were determined at EnteroBase, *Escherichia coli* MLST Database. (http://enterobase.warwick.ac.uk/species/ecoli/allele_st_search). For identical or highly strains by PFGE, one strains of this cluster were chosen to be typed by MLST.

3- Results

Fifty ESBL-producing *E. coli* were studied, which they were derived from CC (N= 29) and human UTIs (N= 21). All the strains were confirmed as ESBL producers by phenotypic tests and by PCR.

All the strains are cefotaxime resistant. The antimicrobial susceptibility test revealed also high resistance frequency mainly for tetracycline (96.55%) and nalidixic acid (86.21%) in strains isolated from CC and high frequency of resistance for quinolone and fluoroquinolones (95.24 – 85.71%) in strains isolated from UTIs. UPEC strains showed higher frequencies of resistance for the majority of the antimicrobials tested than *E. coli* strains from chicken, except for amoxicillin-clavulanic acid, tetracycline, gentamicin, nitrofurantoin and fosfomycin (**Figure 1**). The majority of the total of the strains (96%) were considered multidrug-resistant, according to previously described criteria.

Among the ESBL genes identified, UPEC strains showed six variants of *bla*$_{\text{CTX-M}}$, including *bla*$_{\text{CTX-M-2}}$ (N= 6), *bla*$_{\text{CTX-M-3}}$ (N= 2), *bla*$_{\text{CTX-M-8}}$ (N= 4), *bla*$_{\text{CTX-M-14}}$ (N= 5), *bla*$_{\text{CTX-M-15}}$ (N= 3) and *bla*$_{\text{CTX-M-27}}$ (N= 1). Among the strains isolated from CC, we found three variants of *bla*$_{\text{CTX-M}}$, including *bla*$_{\text{CTX-M-1}}$ (N= 2), *bla*$_{\text{CTX-M-2}}$ (N= 18) and *bla*$_{\text{CTX-M-8}}$ (N= 7). Two strains from chicken showed SHV-type. Four CTX-M groups were not identified by sequencing (**Figure 2**).

By investigating of other antimicrobial resistance genes than ESBL genes, we observed a high prevalence of genes related to resistance to tetracycline and sulfonamide between the UPEC strains and strains from CC, as *tet*A (28.57% vs. 37.93%), *tet*B (42.86% vs. 79.31%), *sul*I (57.14% vs. 72.41%), and *sul*II (57.14% vs. 65.52%), respectively. Regarding PMQR, 5 strains isolated from CC showed the *qnr*S. About fosfomycin, only one strain was found as positive to *fos*A3 also isolated from CC. Only one UPEC strain was positive to AmpC genes, following the sequencing, it was identified as *bla*$_{\text{CMY-2}}$ (**Figure 2**).

Interestingly, two strains showed the *mcr*-1 gene, one strain isolated from CC and one UPEC strain (**Figure 2**). By the broth microdilution test, both strains were considered resistant to colistin.

Regarding the presence of UPEC pathogenicity islands, 52.4% of the UPEC strains showed at least 1 of the islands surveyed, while 31.03% of strains isolated from CC presented at least 1 of the islands surveyed. The distribution of PAIs showed the same pattern in both groups of strains, being the PAI IV$_{536}$ the most prevalent (52.4%

UPEC vs. 24.14% from CC), followed by PAI II_{CFT073} (19.05% UPEC vs. 13.79% from CC) and PAI I_{CFT073} (14.29% UPEC vs 3.45% from CC) (**Figure 2**).

According to the presence of APEC virulence factors, 27 strains (93.1%) isolated from CC and 15 UPEC strains (71.43%) showed at least one of the virulence genes studied, and 51.72% from CC and 33.33% from UPEC presented the five virulence markers of APEC (**Figure 2**).

Phylogenetic classification of the strains isolated from chicken carcasses revealed that the group B1 (37.93%) was the most frequent, while among the UPEC strains, group B2 (33.33%) was the most frequent (**Figure 2**). The phylogenetic group B2 was also the group that was most related to the strains that presented the greatest amount of virulence factors in both sampling, in which, 6 strains from group B2 presented from 7 to 8 virulence genes. Interestingly, all strains that presented more than one PAI belonged to group B2 (**Table 2**).

Molecular typing by PFGE showed a high genetic diversity, with no clonal relationship between the UPEC strains with the strains isolated from CC. We observed clonal relationship into of UPEC strains groups, the strain 144 with 145, and the strains 159 with 152, each strains being isolated from a different patient. We also observed a clonal relationship between strains isolated from CC, with the strains 22.2 A with 22TE, and 2.1, 2.3 with 2.6T, being each cluster isolated from the same CC, and the strains 29.3 with 30.2, both isolated from different CC (**Figure 2**).

A total of 23 different STs were found among the strains (15 different STs in *E. coli* isolated from CC and 11 different STs in UPEC strains), being the ST131 the most prevalent. The ST131, ST354 and ST359 were found in both UPEC strains and in strains isolated from CC (**Figure 2**).

Figure 1 – Frequencies of antimicrobial resistance exhibited by UPEC strains and *E. coli* isolated from CC. Amc, amoxicillin-clavulanic acid; cfo, cefoxitin; ctx, cefotaxime; caz, ceftazidime; tet, tetracycline; cn, gentamicin; clo, chloramphenicol; nit, nitrofurantoin; nal, nalidixic acid; cip, ciprofloxacin; nor, norfloxacin; enr, enrofloxacin; sut, trimethoprim-sulfamethoxazole; fos, fosfomycin

Figure 2 – Relationship between ESBL-producing *E. coli* from CC and UTIs by PFGE, MLST, phylogenetic group, antimicrobial resistance genes profile and virulence factors genes profile. In antimicrobial resistance and virulence factors genes, black squares

represent presence of the gene and white squares represent absence of the gene. ST Cplx, sequence type complex; PG, phylogenetic group; ND, not defined

4- Discussion

The prevalence of ESBL-producing bacteria is increasing in different environments, as human medicine, veterinary clinics and livestock industries, particularly in many developing countries, as in Brazil, where there are low investments in healthcare sector (Liu et al., 2018; Koga et al., 2015a; Botelho et al., 2015; Casella et al., 2018; Daga et al., 2019). The characterization of the occurrence of ESBL-producing *E. coli* in samples from humans and animals is of utmost importance to understand the routes of dissemination of these resistance determinants (Casella et al., 2018). As Brazil is one of the most important producer and exporter of chicken meat worldwide, surveillance studies are essential to identify resistance genes and bacterial clones that may spread in chickens and ultimately contaminate humans.

In 2015, a study carried out by our group showed a high frequency of *E. coli* producers of ESBL, mainly by CTX-M -type enzymes, isolated from CC, especially ESBL from the CTX-M2 and CTX-M8 groups (Koga et al., 2015a; Koga et al., 2015b). Close to period of this study, ESBL-producing UPEC were isolated, in the same city. The similarities of antimicrobial resistance patterns found in strains of UTIs and from CC, in the same period and city, drew attention to a possible transmission of antimicrobial resistance genes and/ or pathogenic strains between humans and food-producing animals. Thus, the aim of this study was to characterize and compare ESBL-producing *E. coli* strains isolated from chicken carcasses and from human UTI about antimicrobial resistance and virulence profile, and about the genetic similarity by molecular techniques.

ESBL-producing bacteria are a major cause of morbidity, mortality, multidrug-resistant outbreaks, and increased hospital costs (Rocha et al., 2015). In our study we found ESBL-producing UPEC strains, positive to $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$, being the $bla_{\text{CTX-M-2}}$ the most prevalent. These data corroborate with the literature, in which other studies carried out in Brazil have already demonstrated the presence of these genes in strains isolated from clinical samples (Sampaio and Gales, 2016; Rocha et al., 2015; Peirano et al., 2011), with the exception of $bla_{\text{CTX-M-27}}$ that has never been reported in Brazil. Among the ESBL-producing strains from CC, we found the presence of $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-}}$

$_{\text{M-8}}$, being *bla*$_{\text{CTX-M-2}}$ the most prevalent ESBL gene too. These data corroborate with the literature too, in which other studies carried out in Brazil have already demonstrated the presence of these enzymes in *E. coli* and *Salmonella* spp. from chicken meats, being *bla*$_{\text{CTX-M-2}}$ and *bla*$_{\text{CTX-M-8}}$ the ESBL genes most frequents in food-producing animals in our country (Fernandes et al., 2009; Ferreira et al., 2014; Botelho et al., 2015; Fitch et al., 2015; Casella et al., 2018). CTX-M-2- and CTX-M-8-producing *E. coli* in raw chicken and broiler meat imported into European countries from South America countries, including Brazil, has been previously reported too (Warren et al., 2008; Egervärn et al., 2014). CTX-M2 has commonly been identified the most frequent ESBL variant in bacteria isolated both in animals, foods and human clinical in South America, corroborating with our results (Rocha et al., 2015). The fact that CTX-M-2 is the ESBL-type most prevalent in both groups call us attention to a possible transmission of antimicrobial resistance via food route. In addition, there are still few studies related to the molecular epidemiology of ESBL in different environments in our country, with the majority of studies concentrated in the South, Southeast and Northeast regions of Brazil, with few studies in the North and Central-West region, hindering the real prevalence of ESBLs (Rocha et al., 2015).

Data obtained in this study demonstrated a low genetic diversity of beta-lactamases in *E. coli* strains from chicken, which we found only three variety of ESBL, while in UPEC strains we found six variety. This shows that ESBLs circulating among poultries appear to be well defined in the country, unlike strains isolated from human clinical samples, in which, the variation of ESBL is much larger. This may be related to the fact that our UPEC strains are isolated from hospital environment, in which, there is the use of a greater variety of antimicrobials (Mellata, 2013), besides the use of antimicrobials of greater action spectrum, than in poultry environment.

The ESBL-producing strains normally show multidrug resistance phenotype, since ESBL are often encoded by genes located on plasmids that also carry genes for resistance to other antimicrobials agents (Cantón et al., 2012). The presence of multidrug resistance *E. coli*-carrying *bla*$_{\text{CTX-M}}$ in Brazil has been reported (Dhanji et al., 2010, Egervärn et al., 2014, Ferreira et al., 2014). In our study, 96% of the total of the strains were classified as multidrug-resistant, with high frequency of resistance for tetracycline and nalidixic acid for strains from CC and high frequency of resistance for quinolone and fluoroquinolones in UPEC strains. UPEC strains showed higher frequencies of antimicrobial resistance than *E. coli* isolated from chicken. This is expected, since the UPEC strains came from a hospitable environment, being an environment of greater antimicrobial selection. However, in contrast to our result, Johnson and collaborates (2012) observed that the prevalence of multidrug resistance were more in avian *E. coli* than in human ExPEC, although this study not said whether the origins of strains isolated from humans were from hospital or community.

The presence of other resistance genes can allow the co-selection of ESBL-producing bacteria via the use of other antimicrobials, both in human medicine and in animal production (Marshall and Levy, 2011; Cantón et al., 2012). Among the research of other antimicrobial resistance genes than ESBL, a high prevalence of tetracycline and sulfonamide resistance genes (*tet*A, *tet*B, *sul*I and *sul*II) were found in both strains groups. The other antimicrobial resistance genes studied were found in less number, such as *qnr*S (5 strains from chicken), *fos*A3 (1 strain from chicken), *bla*$_{\text{CMY-2}}$ (1 UPEC strain) and *mcr*-1 (1 UPEC strain and 1 strain from chicken). Although in less frequency, the presence of these genes in these strains is a concern, as they lead to resistance to important antimicrobials used in human medical clinic.

Although we found a low frequency of resistance to colistin, the presence of the *mcr*-1 gene in one UPEC strains and in one strain from chicken is worrying, because colistin has been the last option of choice for the human treatment of infections caused by multiresistant bacteria. Since 2015, studies have shown that the current dissemination of resistance to colistin in humans, food, animals and the environment has occurred mainly through the *mcr*-1 (mobile colistin resistance) gene, being this gene normally mediated by plasmids, presenting high capacity of dissemination (Fernandes et al., 2016a; Liu et al., 2016). A Brazilian study evidences that *mcr*-1 gene has emerging in Brazil since at least 2012 in food-producing animals, as pigs and healthy chickens (Fernandes et al., 2016a). Our study demonstrates that close to this period, the *mcr*-1 gene was already circulating among human clinical samples in our region, since our UPEC strain was isolated in the year of 2013. Others Brazilian studies also found *mcr*-1 in *E. coli* isolated in migratory birds (Sellera et al., 2017) and in human clinical samples (Fernandes et al., 2016b; Castanheira et al., 2016).

The association of ESBL and virulence genes, such as ESBL-producing ExPEC, increases the impact that these bacteria may cause to animal and human health (Ferreira et al., 2018). ExPEC strains harbor specialized virulence factors that confer pathogenic potential, which are infrequent among commensal isolates (Sabaté et al., 2006). Thus, in our study, we investigated virulence factors related to ExPEC, mainly associated with UPEC and APEC in all of our strains.

UPEC strains show blocks of DNA, called as pathogenicity islands, which there are genes of virulence factors that contribute to their virulence (Sabaté et al., 2006). According to our results, PAIs were detected in 52.4% of the our UPEC strains, and in 31% *E. coli* strains from CC. Although PAIs have been more frequently reported in the genomes of human ExPEC than poultry, the presence of PAIs in strains isolated from chicken carcasses demonstrating that animal-producing food can also be a reservoir of UPEC virulence factors. Despite of it, the distribution of PAIs showed the same pattern in both strains groups, being PAI IV_{536} the most frequent pathogenicity island found in both groups of strains, followed by PAI II_{CFT073} and PAI I_{CFT073}. PAI IV_{536} is reported as being the most ubiquitous PAI found in Enterobacteriaceae (Sabaté et al., 2006). This similar pattern of virulence factors is also a fact that demonstrate a possible zoonotic risk. Others studies realized in the same region of our study, but in different periods (Daga et al., 2019; Koga et al., 2014), with *E. coli* strains from human blood culture, showed that over 70% of the isolates displayed PAI, being PAI IV_{536} the most prevalent, followed of the PAI I_{CFT73} and PAI II_{CFT073}, corroborating with our results. This demonstrating that the PAI profile in different *E. coli* pathogenic strains have showed the same in our region.

APEC is one of the main bacterial pathogens diagnosed in avian colibacillosis, being responsible for significant financial losses in avian production, due to mortality, morbidity, reduced productivity, and carcass condemnation at slaughter (Kariyawasam and Han, 2019). According to Johnson and collaborates (2008), the five genes, *iut*A, *hly*F, *iss*, *iro*N and *omp*T, normally carried by a ColV plasmid, are more prevalent in APEC strains than in others *E. coli*, indicating that this genes panel can be used to predict the APEC pathotype. In our study, about the presence of APEC virulence factors, 93.1% of strains isolated from chicken showed at least one of these genes. Interestingly, the majority of UPEC strains (71.43%) showed at least one of these virulence factors too. Although many studies have shown a high frequency of these genes in APEC strains, studies found also in UPEC strains and sepsis-associated *E. coli* (SEPEC) (Daga et al., 2019; Cyoia et al., 2015; Koga et al., 2014). Thus, this study demonstrates that UPEC strains and chicken meat are reservoir for ExPEC genes with possible public health concern both for veterinary medicine as both for human medicine. The detection of virulence factors and PAIs in strains from UTIs and chicken strongly imply relationship between them.

Beyond to virulence attributes, *E. coli* strains also differ with respect to phylogenetic group. According to the phylogenetic typing, *E. coli* strains can be classified into the groups A, B1, B2, C, D, E and F (Clermont et al., 2013). In this scheme, a majority of human ExPEC were categorized into groups B2 and D, whereas majority of APEC strains were reclassified under the phylogenetic group C, followed by F, B1 and B2 (Logue et al., 2017). In our study, classification of the strains isolated from chicken carcasses revealed that the group B1 was the most prevalent, while in UPEC strains, group B2 was the most frequent. The group B2 was the group most related with the high numbers of virulence factors. Interestingly, all strains that presented more than one PAI belonged to group B2, while virulence factors associated to APEC have not a defined phylogroug.

PFGE and MLST showed a high genetic variability between the UPEC strains and *E. coli* from chicken. According to PFGE results, the relationship was found only into the same group. It shows that ESBL-producing *E. coli* disseminate by different routes among human infection and poultry production in our country. These results corroborate with the literature, which studies (Been et al., 2014; Valentin et al., 2014) found considerable heterogeneity between human and poultry associated isolates, suggesting that ESBL genes are mainly disseminated in food-producing animals and humans via mobile elements, as plasmids. However, there are studies that showed many genetic similarities between *E. coli* from poultry and from humans, on the basis of phenotypic and genotypic properties (Müller et al., 2016; Leverstein-van Hall et al., 2011; Vicent et al., 2010).

MLST is commonly used for *E. coli* typing, providing the delineate clonal relationships among ExPEC strains and antimicrobial resistance strains (Kariyawasam; Han, 2019; Ewers et al., 2012). A total of 23 STs were found, and the ST131, ST354 and ST359 were found both in UPEC and *E. coli* from chicken. The presence of same STs in both in human infection and both in chicken suggest the hypothesis that poultry meat production acts as a reservoir of ESBL-producing ExPEC strains. Among the STs identified in this study, all were previously reported in the database (http://mlst.ucc.ie/mlst/dbs/Ecoli), and the majority was associated with animal sources and ExPEC infections, excepting ST1246, ST1551 and ST3258 that were found only in animal source, and ST6761 and ST7973 that were not related to the source.

Some UPEC lineages have been associated with multiple outbreaks in certain geographical regions, as *E. coli* ST131. *E. coli* ST131 is a classic virulent ExPEC clone, belonging to group B2 and carrying *bla*$_{\text{CTX-M-15}}$ gene, being responsible for many *E. coli* infections worldwide, mainly UTI and urosepsis (Kariyawasam and Han, 2019). In this study, ST131 was the ST most found, being four from UPEC strains and one *E. coli* from chicken, all B2 phylogenetic group. Interesting, these strains were clustered together in the PFGE dendrogram. Of these, only one strain carry the *bla*$_{\text{CTX-M-15}}$ (strain 144), while the others carry different ESBL genes, as *bla*$_{\text{CTX-M2,}}$ *bla*$_{\text{CTX-M-14}}$ and *bla*$_{\text{CTX-M-27}}$. These show that, since 2013, the ST131 has disseminated among foods and human infection in our region, carrying others ESBL genes than CTX-M-15, as disseminated in the worldwide (Pitout, 2012).

The small number of *E. coli* strains analyzed is a limitation of this study. Therefore, our study shows a great diversity of *E. coli* isolates carrying *bla*$_{\text{CTX-M}}$ types between UTIs and from chicken, indicating that mobile elements, as plasmids, can be contributed to dissemination of ESBL genes between human infection and food. Interestingly, the strains of both the groups showed similar profile about virulence factors and PAI, and antimicrobial resistance genes. The presence of *E. coli* isolates from poultry carrying many virulence genes, characterized these commensal bacteria as potential ExPEC. These factors associated with multidrug resistance showed the necessity to rational use of antibiotics in poultry industry and human medicine. In Brazil, there are no nationwide surveillance programs that are related to bacterial resistance, as spread of ESBL enzymes, being relatively little is known about the epidemiology of ESBL as in humans as in animals (Rocha et al., 2015). Therefore continuous monitoring and comparative analyses of ESBL from farm animals, foods, and humans, as well as limiting spread within and between different sectors are needed, besides continued collaboration between professionals and agencies working in human healthcare, animal healthcare, and the food industry is needed. The emergence of new variants and the prevalence of ESBL in isolates of human infections, environmental and animal origin has demonstrates the complexity in establishing the origin of ESBL dissemination.

5- Conclusion

The emergence of ESBL-producing *E. coli* between human infection and food-producing animals is a public health concern, especially if pathogenic strains are also involved. We aimed to improve overall knowledge regarding the influence of foods on the frequency of ESBLs in humans and food-producing animals. CTX-M-2 was the most prevalent ESBL gene in both strains groups, and the majority of the strains was classified as multidrug resistant. We also evidenced that *mcr*-1 in *E. coli* can be disseminated in UTIs from Brazil since 2013. There is no a clonal ESBL-producing *E. coli* between ITUs and chickens, however similar virulence factors and antimicrobial resistance profile were found, and the same STs were found in both groups, as ST131, ST354 and ST359, suggesting that chicken carcasses as a potential pathogen, associated to multidrug resistance to important antibiotics to humans.

Conflict of interest statement

None to declare.

Acknowledgements

The authors would like to thank all colleagues of department of microbiology for their laboratory cooperation.

Funding

This work was partially supported by “Programa Institucional de Pesquisa Básica e Aplicada, by Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná (Chamada Pública CP09/2016 – Protocol 10748)” and “Coordenação de Aperfeiçoamento do Pessoal de Ensino Superior” (CAPES) – Finance Code 001”.

References

de Been, M., Lanza, V.F., de Toro, M., Scharringa, J., Dohmen, W., Du, Y., Hu, J., Lei, Y., Li, N., Tooming-Klunderud, A., Heederik, D.J.J., Fluit, A.C., Bonten, M.J.M., Willems, R.J.L., de la Cruz, F., van Schaik, W., 2014. Dissemination of Cephalosporin Resistance Genes between *Escherichia coli* Strains from Farm Animals and Humans by Specific Plasmid Lineages. PLoS Genet. 10. https://doi.org/10.1371/journal.pgen.1004776

Botelho, L.A.B., Kraychete, G.B., e Silva, J.L.C., Regis, D.V.V., Picão, R.C., Moreira, B.M., Bonelli, R.R., 2015. Widespread distribution of CTX-M and plasmid-mediated AmpC β-lactamases in *Escherichia coli* from Brazilian chicken meat. Mem. Inst. Oswaldo Cruz 110, 249–254. https://doi.org/10.1590/0074-02760140389

Bush, K., Jacoby, G.A., 2010. Updated functional classification of β-lactamases. Antimicrob. Agents Chemother. 54, 969–976. https://doi.org/10.1128/AAC.01009-09

Cantón, R., González-Alba, J.M., Galán, J.C., 2012. CTX-M enzymes: Origin and diffusion. Front. Microbiol. 3. https://doi.org/10.3389/fmicb.2012.00110

Casella, T., Haenni, M., Madela, N.K., Andrade, L.K. De, Pradela, L.K., Andrade, L.N. De, Darini, A.L.D.C., Madec, J.Y., Nogueira, M.C.L., 2018. 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. J. Antimicrob. Chemother. 73, 3293–3297. https://doi.org/10.1093/jac/dky335

Castanheira, M., Griffin, M.A., Deshpande, L.M., Mendes, R.E., Jones, R.N., Flamm, R.K., 2016. Detection of *mcr*-1 among *Escherichia coli* Clinical Isolates Collected Worldwide as Part of the SENTRY Antimicrobial Surveillance Program in 2014 and 2015. Antimicrob. Agents Chemother. 60, 5623–5624. https://doi.org/10.1128/aac.01267-16

Cattoir, V., Poirel, L., Rotimi, V., Soussy, C.J., Nordmann, P., 2007. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. J. Antimicrob. Chemother. 60, 394–397. https://doi.org/10.1093/jac/dkm204

Clermont, O., Christenson, J.K., Denamur, E., Gordon, D.M., 2013. The Clermont *Escherichia coli* phylo-typing method revisited: Improvement of specificity and detection of new phylo-groups. Environ. Microbiol. Rep. 5, 58–65. https://doi.org/10.1111/1758-2229.12019

CLSI, 2008. Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals; 3rd edition, CLSI Document M31-A3, Wayne, Pennsylvania (PA).

CLSI, 2015. Performance standards for antimicrobial susceptibility testing; Twenty-Fifth Informational Supplement. vol. 31, CLSI Document M02-A12, M07-A10, and M11-A8), Wayne, Pennsylvania (PA).

Cyoia, P.S., Rodrigues, G.R., Nishio, E.K., Medeiros, L.P., Koga, V.L., Pereira, A.P.D., Vespero, E.C., Houle, S., Dozois, C.M., Nakazato, G., Kobayashi, R.K.T., 2015. Presence of virulence genes and pathogenicity islands in extraintestinal pathogenic *Escherichia coli* isolates from Brazil. J. Infect. Dev. Ctries. 9, 1068–1075. https://doi.org/10.3855/jidc.6683

Daga, A.P., Koga, V.L., Soncini, J.G.M., de Matos, C.M., Perugini, M.R.E., Pelisson, M., Kobayashi, R.K.T., Vespero. E.C., 2019. *Escherichia coli* bloodstream infections in patients at a university hospital: virulence factors and clinical characteristics. Front. Cell. Infect. Microbiol. 9. https://doi.org/10.3389/fcimb.2019.00191

Dale, A.P., Woodford, N., 2015. Extra-intestinal pathogenic *Escherichia coli* (ExPEC): Disease, carriage and clones. J. Infect. 71, 615–626. https://doi.org/10.1016/j.jinf.2015.09.009

Dallenne, C., da Costa, A., Decré, D., Favier, C., Arlet, G., 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J. Antimicrob. Chemother. 65, 490–495. https://doi.org/10.1093/jac/dkp498

Dhanji, H., Murphy, N.M., Doumith, M., Durmus, S., Lee, S.S., Hope, R., Woodford, N., Livermore, D.M., 2010. Cephalosporin resistance mechanisms in *Escherichia coli* isolated from raw chicken imported into the UK. J. Antimicrob. Chemother. 65, 2534–2537. https://doi.org/10.1093/jac/dkq376

Dierikx, C., van Essen-Zandbergen, A., Veldman, K., Smith, H., Mevius, D., 2010. Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. Vet. Microbiol. 145, 273–278. https://doi.org/10.1016/j.vetmic.2010.03.019

Egervärn, M., Börjesson, S., Byfors, S., Finn, M., Kaipe, C., Englund, S., Lindblad, M., 2014. Escherichia coli with extended-spectrum beta-lactamases or transferable AmpC beta-lactamases and *Salmonella* on meat imported into Sweden. Int. J. Food Microbiol. 171, 8–14. https://doi.org/10.1016/j.ijfoodmicro.2013.11.005

European Committee on Antimicrobial Susceptibility Testing. 2016. Breakpoint tables for interpretation of MICs and zone diameters, v.6. http:// www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint _tables/v_6.0_Breakpoint_table.pdf.

Ewers, C., Bethe, A., Semmler, T., Guenther, S., Wieler, L.H., 2012. Extended-spectrum b-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin. Microbiol. Infect. 18, 646-655. https://doi.org/10.1111/j.1469-0691.2012.03850.x

Fernandes, S.A., Paterson, D.L., Ghilardi-Rodrigues, Â.C., Adams-Haduch, J.M., Tavechio, A.T., Doi, Y., 2009. CTX-M-2–Producing *Salmonella* Typhimurium Isolated from Pediatric Patients and Poultry in Brazil . Microb. Drug Resist. 15, 317–321. https://doi.org/10.1089/mdr.2009.0050

Fernandes, M.R.,Moura, Q., Sartori, L., Silva, K.C., Cunha, M.P. Esposito, F., Lopes, R., Otutumi, L.K., Gonçalves, D.D., Dropa, M., Matté, M.H., Monte, D.F., Landgraf, M., Francisco, G.R., Bueno, M.F., de Oliveira, G.D., Knöbl, T., Moreno, A.M., Lincopan, N., 2016a. Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr*-1 gene. Euro Surveill. 21, 1–6. https://doi.org/10.2807/1560-7917.ES.2016.21.17.30214

Fernandes, M.R., McCulloch, J.A., Vianello, M.A., Moura, Q., Pérez-Chaparro, P.J., Esposito, F., Sartori, L., Dropa, M., Matté, M.H., Lira, D.P.A., Mamizuka, E.M., Lincopan, N., 2016b. First report of the globally disseminated IncX4 plasmid carrying the *mcr*-1 gene in a colistin-resistant *Escherichia coli* sequence type 101 isolate from a human infection in Brazil. Antimicrob. Agents Chemother. 60, 6415-6417. https://doi.org/10.1128/AAC.01325-16

Ferreira, J.C., Penha Filho, R.A.C., Andrade, L.N., Berchieri Junior, A., Darini, A.L.C., 2014. IncI1/ST113 and IncI1/ST114 conjugative plasmids carrying *bla*$_{\text{CTX-M-8}}$ in *Escherichia coli* isolated from poultry in Brazil. Diagn. Microbiol. Infect. Dis. 80, 304–306. https://doi.org/10.1016/j.diagmicrobio.2014.09.012

Fitch, F.M., Carmo-Rodrigues, M.S., Oliveira, V.G.S., Gaspari, M.V., dos Santos, A., de Freitas, J.B., Pignatari, A.C.C., 2015. β-Lactam Resistance Genes: Characterization, Epidemiology, and First Detection of *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CTX-M-14}}$ in *Salmonella* spp. Isolated from Poultry in Brazil—Brazil Ministry of Agriculture's Pathogen Reduction Program . Microb. Drug Resist. 22, 164–171. https://doi.org/10.1089/mdr.2015.0143

Jalier, V., Nicolas M.H., Fournier G., Philippon A., 1988. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. 10, 867-878.

Johnson, T.J., Logue, C.M., Johnson, J.R., Kuskowski, M.A., Sherwood, J.S., Barnes, H.J., DebRoy, C., Wannemuehler, Y.M., Obata-Yasuoka, M., Spanjaard, L., Nolan, L.K., 2012. Associations Between Multidrug Resistance, Plasmid Content, and Virulence Potential Among Extraintestinal Pathogenic and Commensal *Escherichia coli* from Humans and Poultry. Foodborne Pathog. Dis. 9, 37–46. https://doi.org/10.1089/fpd.2011.0961

Johnson, T.J., Wannemuehler, Y., Doetkott, C., Johnson, S.J., Rosenberger, S.C., Nolan, L.K., 2008. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J. Clin. Microbiol. 46, 3987–3996. https://doi.org/10.1128/JCM.00816-08

Jouini, A., Vinué, L., Slama, K. Ben, Sáenz, Y., Klibi, N., Hammami, S., Boudabous, A., Torres, C., 2007. Characterization of CTX-M and SHV extended-spectrum β-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. J. Antimicrob. Chemother. 60, 1137–1141. https://doi.org/10.1093/jac/dkm316

Kaper, J.B., Nataro, J.P., Mobley, H.L.T., 2004. Pathogenic *Escherichia coli*. Nat. Rev. Microbiol. 2, 123-40. https://doi.org/10.1038/nrmicro818

Kariyawasam, S., Han, J., 2019. Avian Pathogenic *Escherichia coli*: Link to Foodborne Urinary Tract Infections in Humans, Food Safety in Poultry Meat Production. https://doi.org/10.1007/978-3-030-05011-5_12

Koga, V.L., Tomazetto, G., Cyoia, P.S., Neves, M.S., Vidotto, M.C., Nakazato, G., Kobayashi, RKT., 2014. Molecular screening of virulence genes in extraintestinal pathogenic *Escherichia coli* isolated from human blood culture in Brazil. Biomed Res. Int. 2014. https://doi.org/10.1155/2014/465054

Koga, V.L, de Brito, K.C.T., de Brito, B.G., Nakazato, G., Kobayashi, R.K.T., Vespero, E.C., Scandorieiro, S., Oba, A., 2015. Comparison of Antibiotic Resistance and Virulence Factors among *Escherichia coli* Isolated from Conventional and Free-Range Poultry. Biomed Res. Int. 2015, 1–8. https://doi.org/10.1155/2015/618752

Koga, V.L., Rodrigues, G.R., Scandorieiro, S., Vespero, E.C., Oba, A., de Brito, B.G., de Brito, K.C.T., Nakazato, G., Kobayashi, R.K.T., 2015. Evaluation of the Antibiotic Resistance and Virulence of *Escherichia coli* Strains Isolated from Chicken Carcasses in 2007 and 2013 from Paraná, Brazil . Foodborne Pathog. Dis. 12, 479–485. https://doi.org/10.1089/fpd.2014.1888

Leverstein-van Hall, M.A., Dierikx, C.M., Cohen Stuart, J., Voets, G.M., van den Munckhof, M.P., van Essen-Zandbergen, A., Platteel, T., Fluit, A.C., van de Sande-Bruinsma, N., Scharinga, J., Bonten, M.J.M., Mevius, D.J., 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin. Microbiol. Infect. 17, 873–880. https://doi.org/10.1111/j.1469-0691.2011.03497.x

Li, Q., Sherwood, J.S., Logue, C.M., 2007. Characterization of antimicrobial resistant *Escherichia coli* isolated from processed bison carcasses. J. Appl. Microbiol. 103, 2361–2369. https://doi.org/10.1111/j.1365-2672.2007.03470.x

Liao, W., Jiang, J., Xu, Y., Yi, J., Chen, T., Su, X., Pan, S., Wei, X., Li, Y., 2010. Survey for B-lactamase among bacterial isolates from Guangzhou, China hospitals between 2005-2006. J. Antibiot. (Tokyo). 63, 225–229. https://doi.org/10.1038/ja.2010.23

Liu, H., Zhou, H., Li, Q., Peng, Q., Zhao, Q., Wang, J., Liu, X., 2018. Molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* isolated from the rivers and lakes in Northwest China. BMC Microbiol. 18, 1–12. https://doi.org/10.1186/s12866-018-1270-0

Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L.F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.H., Shen, J., 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. Lancet Infect. Dis. 16, 161–168. https://doi.org/10.1016/S1473-3099(15)00424-7

Logue, C.M., Wannemuehler, Y., Nicholson, B.A., Doetkott, C., Barbieri, N.L., Nolan, L.K., 2017. Comparative analysis of phylogenetic assignment of human and avian ExPEC and fecal commensal *Escherichia coli* using the (previous and revised) clermont phylogenetic typing methods and its impact on avian pathogenic *Escherichia coli* (APEC) classification. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.00283

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect.18, 268-81.

Manges, A.R., 2016. *Escherichia coli* and urinary tract infections: The role of poultry-meat. Clin. Microbiol. Infect. 22, 122–129. https://doi.org/10.1016/j.cmi.2015.11.010

Marshall, B.M., Levy, S.B., 2011. Food animals and antimicrobials: Impacts on human health. Clin. Microbiol. Rev. 24, 718–733. https://doi.org/10.1128/CMR.00002-11

Mellata, M., 2013. Human and Avian Extraintestinal Pathogenic *Escherichia coli* : Infections, Zoonotic Risks, and Antibiotic Resistance Trends. Foodborne Pathog. Dis. 10, 916–932. https://doi.org/10.1089/fpd.2013.1533

Müller, A., Stephan, R., Nüesch-Inderbinen, M., 2016. Distribution of virulence factors in ESBL-producing *Escherichia coli* isolated from the environment, livestock, food and humans. Sci. Total Environ. 541, 667–672. https://doi.org/10.1016/j.scitotenv.2015.09.135

Nordstrom, L., Liu, C.M., Price, L.B., 2013. Foodborne urinary tract infections: A new paradigm for antimicrobial-resistant foodborne illness. Front. Microbiol. 4, 1–6. https://doi.org/10.3389/fmicb.2013.00029

Peirano, G., Asensi, M.D., Pitondo-Silva, A., Pitout, J.D.D., 2011. Molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* from Rio de Janeiro, Brazil. Clin. Microbiol. Infect. 17, 1039–1043. https://doi.org/10.1111/j.1469-0691.2010.03440.x

Pérez-pérez, F.J., Hanson, N.D., 2002. Detection of Plasmid-Mediated AmpC β - Lactamase Genes in Clinical Isolates by Using Multiplex PCR. J. Clin. Microbiol. 40, 2153-2162. https://doi.org/10.1128/jcm.40.6.2153-2162.2002

Pitout, J.D.D., 2012. Extraintestinal pathogenic *Escherichia coli*: A combination of virulence with antibiotic resistance. Front. Microbiol. 3, 1–7. https://doi.org/10.3389/fmicb.2012.00009

Rocha, F.R., Pinto, V.P.T., Barbosa, F.C.B., 2015. The Spread of CTX-M-Type Extended-Spectrum β-Lactamases in Brazil: A Systematic Review. Microb. Drug Resist. 22, 301–311. https://doi.org/10.1089/mdr.2015.0180

Sabaté, M., Moreno, E., Pérez, T., Andreu, A., Prats, G., 2006. Pathogenicity island markers in commensal and uropathogenic *Escherichia coli* isolates. Clin Microbiol Infect. 12, 880-886. https://doi.org/10.1111/j.1469-0691.2006.01461.x

Saladin, M., Ould-Hocine, Z., Verdet, C., Delisle, F., Arlet, G., Cao, V.T.B., Lambert, T., Donay, J.-L., Herrmann, J.-L., Philippon, A., 2002. Diversity of CTX-M β-lactamases and their promoter regions from Enterobacteriaceae isolated in three Parisian hospitals. FEMS Microbiol. Lett. 209, 161–168. https://doi.org/10.1016/S0378-1097(02)00484-6

Sampaio, J.L.M., Gales, A.C., 2016. Antimicrobial resistance in Enterobacteriaceae in Brazil: focus on β-lactams and polymyxins. Brazilian J. Microbiol. 47, 31–37. https://doi.org/10.1016/j.bjm.2016.10.002

Sato, N., Kawamura, K., Nakane, K., Wachino, J.-I., Arakawa, Y., 2013. First Detection of Fosfomycin Resistance Gene *fos*A3 in CTX-M-Producing *Escherichia coli* Isolates from Healthy Individuals in Japan . Microb. Drug Resist. 19, 477–482. https://doi.org/10.1089/mdr.2013.0061

Sellera, F.P., Fernandes, M.R., Sartori, L., Carvalho, M.P.N., Esposito, F., Nascimento, C.L., Dutra, G.H.P., Mamizuka, E.M., Pérez-Chaparro, P.J., McCulloch, J.A., Lincopan, N., 2017. *Escherichia coli* carrying IncX4 plasmid-mediated *mcr*-1 and *bla* $_{\text{CTX-M}}$ genes in infected migratory Magellanic penguins (Spheniscus magellanicus). J. Antimicrob. Chemother. 72, 1255–1256. https://doi.org/10.1093/jac/dkw543

Valentin, L., Sharp, H., Hille, K., Seibt, U., Fischer, J., Pfeifer, Y., Michael, G.B. renne., Nickel, S., Schmiedel, J., Falgenhauer, L., Friese, A., Bauerfeind, R., Roesler, U., Imirzalioglu, C., Chakraborty, T., Helmuth, R., Valenza, G., Werner, G., Schwarz, S., Guerra, B., Appel, B., Kreienbrock, L., Käsbohrer, A., 2014. Subgrouping of ESBL-producing *Escherichia coli* from animal and human sources: an approach to quantify the distribution of ESBL types between different reservoirs. Int. J. Med. Microbiol. 304, 805–816. https://doi.org/10.1016/j.ijmm.2014.07.015

Vicent, C., Boerlin, P., Daignault, D., Dozois, C.M., Dutil, L., Galanakis, C., Reid-Smith, R.J., Tellier, P.-P., Tellis, P.A., Ziebell, K., Manges, A.R., 2010. Food reservoir for *Escherichia coli* causing urinary trat infections. Emerg Infect Dis. 16, 88-95. https://doi.org/10.3201/eid1601.091118

Warren, R.E., Ensor, V.M., O'Neill, P., Butler, V., Taylor, J., Nye, K., Harvey, M., Livermore, D.M., Woodford, N., Hawkey, P.M., 2008. Imported chicken meat as a potential source of quinolone-resistant *Escherichia coli* producing extended-spectrum β-lactamases in the UK. J. Antimicrob. Chemother. 61, 504–508. https://doi.org/10.1093/jac/dkm517

Woodford, N., Fagan, E.J., Ellington, M.J., 2006. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases [4]. J. Antimicrob. Chemother. 57, 154–155. https://doi.org/10.1093/jac/dki412

Table 1 - Oligonucleotides used for amplification in the PCR

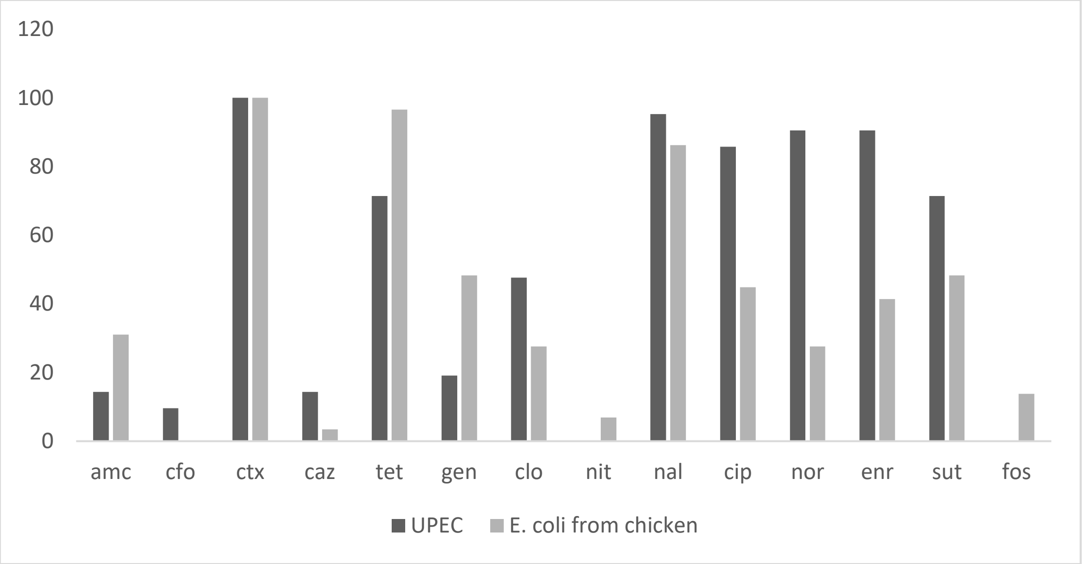
Target	Gene	Oligonucleotide sequence (5' to 3')	References
ESBL	*bla*$_{\text{CTX-M-1}}$ group	F- AAA AAT CAC TGC GCC AGT TC R- AGC TTA TTC ATC GCC ACG TT	Woodford et al., 2006
	bla$_{\text{CTX-M2}}$ group	F- CGA CGC TAC CCC TGC TAT T R- CCA GCG TCA GAT TTT TCA GG	
	bla$_{\text{CTX-M8}}$ group	F- TCG CGT TAA GCG GAT GAT GC R- AAC CCA CGA TGT GGG TAG C	
	bla$_{\text{CTX-M9}}$ group	F- CAA AGA GAG TGC AAC GGA TG R- ATT GGA AAG CGT TCA TCA CC	
	bla$_{\text{CTX-M25}}$ group	F- GCA CGA TGA CAT TCG GG R- AAC CCA CGA TGT GGG TAG C	
	bla$_{\text{CTX-M-1}}$ group	F-GGT TAA AAA ATC ACT GCG TC R-TTG GTG ACG ATT TTA GCC	Saladin et al., 2002
	bla$_{\text{CTX-M2}}$ group	F-ATG ATG ACT CAG AGC ATT CG R- TGG GTT ACG ATT TTC GCC GC	
	bla$_{\text{CTX-M8}}$ group	F-TGA GA GAC ATC GCG TTA AG R-TAA CCG TCG GTG ACG ATT TT	Jouini et al., 2007
	bla$_{\text{CTX-M9}}$ group	F- CAA AGA GAG TGC AAC GGA TG R-TGA TTC TCG CCG CTG AAG	Dallenne et al., 2010; Woodford et al., 2006
	bla$_{\text{TEM}}$	F- CAT TTC CGT GTC GCC CTT ATT C R- CGT TCA TCC ATA GTT GCC TGA C	Dallenne et al., 2010
	bla$_{\text{SHV}}$	F- CAC TCA AGG ATG TAT TGT G R- TTA GCG TTG CCA GTG CTC G	Jouini et al., 2007
pAmpC	*bla*$_{\text{MOX-1}}$, *bla*$_{\text{MOX-2}}$, *bla*$_{\text{CMY-1}}$, bla$_{\text{CMY-8}}$ to *bla*$_{\text{CMY-11}}$	F- GCT GCT CAA GGA GCA CAG GAT R- CAC ATT GAC ATA GGT GTG GTG C	Pérez-Pérez and Hanson, 2002
	bla$_{\text{LAT-1}}$ to *bla*$_{\text{LAT-4}}$, *bla*$_{\text{CMY-2}}$ to *bla*$_{\text{CMY-7}}$, *bla*$_{\text{BIL-1}}$	F- TGG CCA GAA CTG ACA GGC AAA R- TTT CTC CTG AAC GTG GCT GGC	

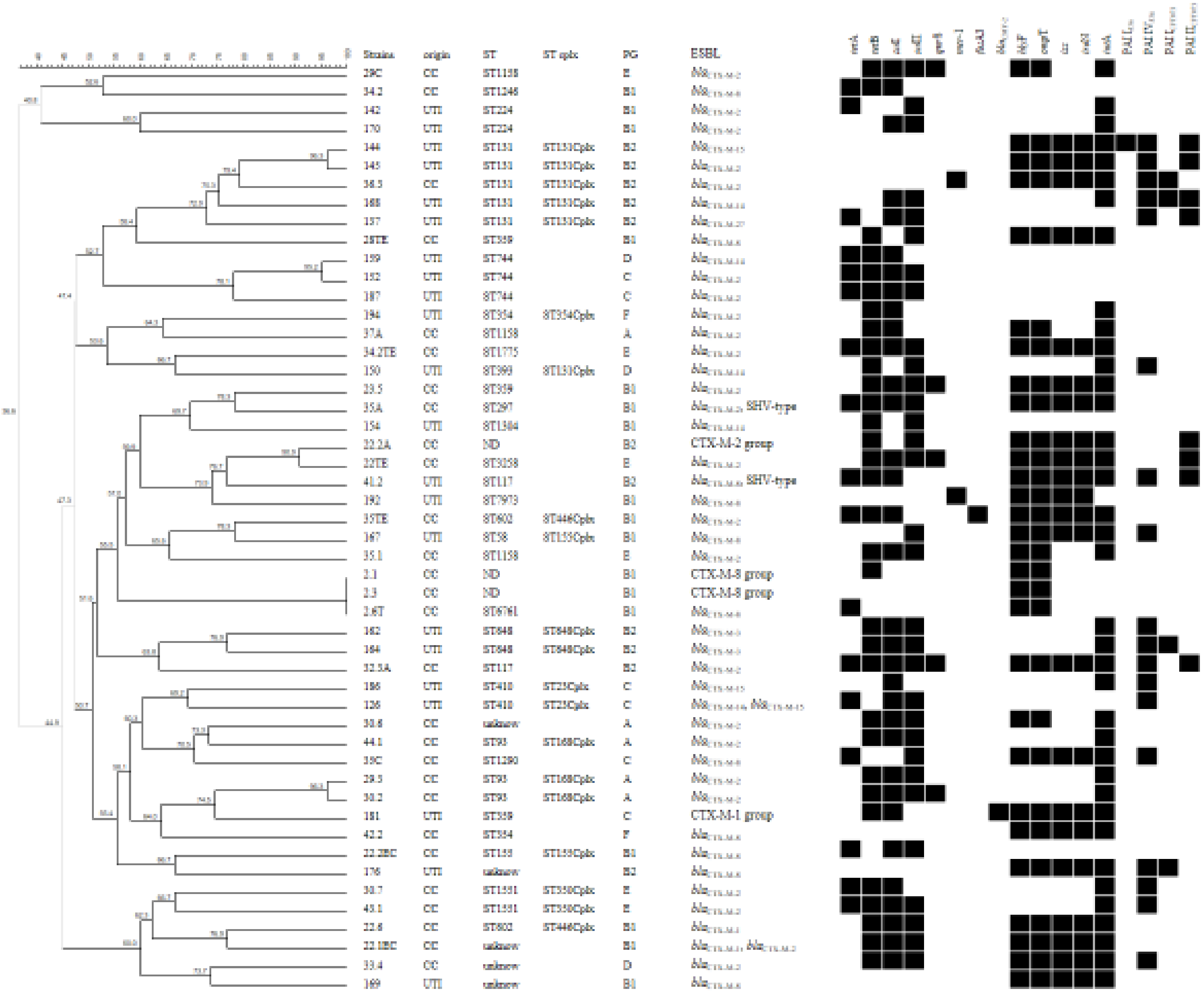
	bla$_{\text{DHA-1}}$ and *bla*$_{\text{DHA-2}}$	F- AAC TTT CAC AGG TGT GCT GGG T	
		R- CCG TAC GCA TAC TGG CTT TGC	
	bla$_{\text{ACC}}$	F- AAC AGC CTC AGC AGC CGG TTA	
		R- TTC GCC GCA ATC ATC CCT AGC	
	bla$_{\text{MIR-1T}}$, *bla*$_{\text{ACT-1}}$	F- TCG GTA AAG CCG ATG TTG CGG	
		R- CTT CCA CTG CGG CTG CCA GTT	
	bla$_{\text{FOX-1}}$ to *bla*$_{\text{FOX-5b}}$	F- AAC ATG GGG TAT CAG GGA GAT G	
		R- CAA AGC GCG TAA CCG GAT TGG	
	bla$_{\text{CMY-2}}$, *bla*$_{\text{CMY-4}}$, *bla*$_{\text{CMY-6}}$, *bla*$_{\text{CMY-7}}$, *bla*$_{\text{CMY-12}}$, *bla*$_{\text{CMY-13}}$, *bla*$_{\text{CMY-14}}$, *bla*$_{\text{CMY-18}}$, *bla*$_{\text{LAT-3}}$	F- ATG ATG AAA AAA TCG TTA TGC TGC	Dierikx et al., 2010
		R- GCT TTT CAA GAA TGC GCC AGG	
PMQR	*qnr*A	F- AGA GGA TTT CTC ACG CCA GG	Cattoir et al., 2007
		R- TGC CAG GCA CAG ATC TTG AC	
	*qnr*B	F- GGM ATH GAA ATT CGC CAC TG	
		R- TTT GCY GYY CGC CAG TCG AA	
	*qnr*S	F- GCA AGT TCA TTG AAC AGG GT	
		R- TCT AAA CCG TCG AGT TCG GCG	
Tetracycline resistance	*tet*A	F- GCC TTT CCT TTG GGT TCT CT	Li et al., 2007
		R- TGT CCG ACA AGT TGC ATG AT	
	*tet*B	F- GCT TTC AGG GAT CAC AGG AG	
		R- GAC CAA GAC CCG CTA ATG AA	
Sulfonamide resistance	*sul*I	F- ACG AGA TTG TGC GGT TCT TC	Li et al., 2007
		R- GGT TTC CGA GAT GGT GAT TG	
	*sul*II	F- CCG TCT CGC TCG ACA GTT AT	
		R- GTG TGT GCG GAT GAA GTC AG	
Plasmid-mediated	*mcr*-1	F-CGG TCA GTC CGT TTG TTC	
		R-CTT GGT CGGG TCT GTA GGG	

colistin			
Plasmid-mediated fosfomycin	*fos*A3	F-GGC ATT TTA TCA GCA GT R-AGA CCA TCC CCT TGT AG	Sato et al., 2013
Virulence factors	*hly*F	F-GGC CAC AGT CGT TTA GGG TGC TTA CC R-GGC GGT TTA GGC ATT CCG ATA CTC AG	Johnson et al., 2008
	*iro*N	F-AAT CCG GCA AAG AGA CGA ACC GCC T R-GTT CGG GCA ACC CCT GCT TTG ACT TT	
	*omp*T	F-TCA TCC CGG AAG CCT CCC TCA CTA CTA T R-TAG CGT TTG CTG CAC TGG CTT CTG ATA C	
	*iut*A	F-GGC TGG ACA TCA TGG GAA CTG G R-CGT CGG GAA CGG GTA GAA TCG	
	iss	F-CAG CAA CCC GAA CCA CTT GAT G R-AGC ATT GCC AGA GCG GCA GAA	
Pathogenic islands	PAI I_{536}	F-TAA TGC CGG AGA TTC ATT GTC R-AGG ATT TGT CTC AGG GCT TT	Sabaté et al., 2006
	PAI II_{536}	F-CAT GTC CAA AGC TCG AGC C R-CTA CGT CAG GCT GGC TTT G	
	PAI IV_{536}	F-AAG GAT TCG CTG TTA CCG GAC R-TCG TCG GGC AGC GTT TCT TCT	
	PAI $\mathrm{I}_{\mathrm{CFT073}}$	F-GGA CAT CCT GTT ACA GCG CGC A R-TCG CCA CCA ATC ACA GCG AAC	
	PAI $\mathrm{II}_{\mathrm{CFT073}}$	F-ATG GAT GTT GTA TCG CGC R-ACG AGC ATG TGG ATC TGC	
	PAI $\mathrm{I}_{\mathrm{J96}}$	F-TCG TGC TCA GGT CCG GAA TTT R-TGG CAT CCC ACA TTA TCG	

Table 2 – Number of PAIs and virulence factors genes according phylogenetic groups within UPEC strains and *E. coli* isolated from CC

		CC (n= 29)							**UTI (n= 21)**						
Pylogenetic group		A (n=5)	B1 (n=11)	B2 (n=4)	C (n=1)	D (n=1)	E (n=6)	F (n=1)	A (n=0)	B1 (n=6)	B2 (n=7)	C (n=5)	D (n=2)	E (n=0)	F (n=1)
Number of PAIs	3 PAIs	-	-	-	-	-	-	-	-	-	2	-	-	-	-
	2 PAIs	-	-	3	-	-	-	-	-	-	4	-	-	-	-
	1 PAI	-	-	1	1	1	3	-	-	1	1	2	1	-	-
	0 PAI	5	11	-	-	-	3	1	-	-	5	3	1	-	1
Number of virulence factors	5 genes	-	6	4	1	1	2	1	-	2	3	1	-	-	-
	4 genes	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	3 genes	2	-	-	-	-	2	-	-	-	-	-	-	-	-
	2 genes	-	3	-	-	-	-	-	-	-	-	-	-	-	-
	1 gene	3	-	-	-	-	2	-	-	2	3	1	1	-	1
	0 gene	-	2	-	-	-	-	-	-	1	1	3	1	-	-


120
100
80
60
40
20
0
amc
cfo
ctx
caz
tet
gen
clo
nit
nal
cip
nor
enr
sut
fos
UPEC
E. coli from chicken



Strains	origin	ST	ST cplx	PG	ESBL
29C	CC	ST1158		E	$bla_{CTX-M-2}$
34.2	CC	ST1248		B1	$bla_{CTX-M-8}$
142	UTI	ST224		B1	$bla_{CTX-M-2}$
170	UTI	ST224		B1	$bla_{CTX-M-2}$
144	UTI	ST131	ST131Cplx	B2	$bla_{CTX-M-15}$
145	UTI	ST131	ST131Cplx	B2	$bla_{CTX-M-2}$
36.3	CC	ST131	ST131Cplx	B2	$bla_{CTX-M-2}$
168	UTI	ST131	ST131Cplx	B2	$bla_{CTX-M-14}$
157	UTI	ST131	ST131Cplx	B2	$bla_{CTX-M-27}$
28TE	CC	ST359		B1	$bla_{CTX-M-8}$
159	UTI	ST744		D	$bla_{CTX-M-14}$
152	UTI	ST744		C	$bla_{CTX-M-2}$
187	UTI	ST744		C	$bla_{CTX-M-2}$
194	UTI	ST354	ST354Cplx	F	$bla_{CTX-M-2}$
37A	CC	ST1158		A	$bla_{CTX-M-2}$
34.2TE	CC	ST1775		E	$bla_{CTX-M-2}$
150	UTI	ST393	ST131Cplx	D	$bla_{CTX-M-14}$
23.5	CC	ST359		B1	$bla_{CTX-M-2}$
35A	CC	ST297		B1	$bla_{CTX-M-2}$, SHV-type
154	UTI	ST1584		B1	$bla_{CTX-M-14}$
32.2A	CC	ND		B2	CTX-M-2 group
22TE	CC	ST3058		E	$bla_{CTX-M-2}$
41.2	UTI	ST117		B2	$bla_{CTX-M-8}$, SHV-type
192	UTI	ST7973		B1	$bla_{CTX-M-8}$
35TE	CC	ST602	ST446Cplx	B1	$bla_{CTX-M-2}$
167	UTI	ST58	ST155Cplx	B1	$bla_{CTX-M-8}$
35.1	CC	ST1158		E	$bla_{CTX-M-2}$
2.1	CC	ND		B1	CTX-M-8 group
2.3	CC	ND		B1	CTX-M-8 group
2.6T	CC	ST6761		B1	$bla_{CTX-M-8}$
162	UTI	ST648	ST648Cplx	B2	$bla_{CTX-M-9}$
164	UTI	ST648	ST648Cplx	B2	$bla_{CTX-M-9}$
32.5A	CC	ST117		B2	$bla_{CTX-M-2}$
186	UTI	ST410	ST23Cplx	C	$bla_{CTX-M-15}$
126	UTI	ST410	ST23Cplx	C	$bla_{CTX-M-14}$, $bla_{CTX-M-15}$
30.6	CC	unknow		A	$bla_{CTX-M-2}$
44.1	CC	ST93	ST168Cplx	A	$bla_{CTX-M-2}$
39C	CC	ST1280		C	$bla_{CTX-M-8}$
29.3	CC	ST93	ST168Cplx	A	$bla_{CTX-M-2}$
30.2	CC	ST93	ST168Cplx	A	$bla_{CTX-M-2}$
181	UTI	ST359		C	CTX-M-1 group
42.2	CC	ST354		F	$bla_{CTX-M-8}$
22.2BC	CC	ST155	ST155Cplx	B1	$bla_{CTX-M-8}$
176	UTI	unknow		B2	$bla_{CTX-M-8}$
30.7	CC	ST1551	ST350Cplx	E	$bla_{CTX-M-2}$
45.1	CC	ST1551	ST350Cplx	E	$bla_{CTX-M-2}$
22.8	CC	ST602	ST446Cplx	B1	$bla_{CTX-M-1}$
22.1BC	CC	unknow		B1	$bla_{CTX-M-1}$, $bla_{CTX-M-2}$
33.4	CC	unknow		D	$bla_{CTX-M-2}$
169	UTI	unknow		B1	$bla_{CTX-M-8}$

5.2.ARTIGO CIENTÍFICO II

O artigo II foi submetido e aprovado pela revista “BMC Microbiology”, e tem como título: **Characterization of CMY-2-type beta-lactamase-producing *Escherichia coli* isolated from chicken carcasses and human infection in a city of South Brazil.**

Koga *et al. BMC Microbiology* (2019) 19:174
https://doi.org/10.1186/s12866-019-1550-3

BMC Microbiology

RESEARCH ARTICLE Open Access

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

Vanessa L. Koga[1], Renato P. Maluta[2], Wanderley D. da Silveira[2], Renan A. Ribeiro[3], Mariangela Hungria[3], Eliana C. Vespero[4], Gerson Nakazato[1] and Renata K. T. Kobayashi[1*]

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*$_{\text{CMY-2}}$ 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 *tet*A, *tet*B, *sul*I and *sul*II. No strain was considered an extended-spectrum beta-lactamases (ESBL) producer, and the *bla*$_{\text{TEM-1}}$ 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 IS*Ecp1*-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*$_{\text{CMY-2}}$ linked to IS*Ecp1* 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

---

* Correspondence: kobayashirkt@uel.br
[1]Basic and Applied Bacteriology Laboratory, Department of Microbiology, State University of Londrina (UEL), Londrina, PR, Brazil
Full list of author information is available at the end of the article

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, cefpirome, and carbapenems, which is an important clinical concerns 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 *amp*R 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 *amp*C genes originated from chromosomal *amp*C 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-1like, 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 betalactamases 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 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 I'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), cefoxitin (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
bla$_{\text{ESBL}}$	*bla*$_{\text{CTX-M-1}}$ group	F- AAA AAT CAC TGC GCC AGT TC R- AGC TTA TTC ATC GCC ACG TT	[17]
	bla$_{\text{CTX-M2}}$ group	F- CGA CGC TAC CCC TGC TAT T R- CCA GCG TCA GAT TTT TCA GG	
	bla$_{\text{CTX-M8}}$ group	F- TCG CGT TAA GCG GAT GAT GC R- AAC CCA CGA TGT GGG TAG C	
	bla$_{\text{CTX-M9}}$ group	F- CAA AGA GAG TGC AAC GGA TG R- ATT GGA AAG CGT TCA TCA CC	
	bla$_{\text{CTX-M25}}$ group	F- GCA CGA TGA CAT TCG GG R- AAC CCA CGA TGT GGG TAG C	
	bla$_{\text{TEM}}$	F- CAT TTC CGT GTC GCC CTT ATT C R- CGT TCA TCC ATA GTT GCC TGA C	[18]
	bla$_{\text{SHV}}$	F- CAC TCA AGG ATG TAT TGT G R- TTA GCG TTG CCA GTG CTC G	[19]
bla$_{\text{pAmpC}}$	*bla*$_{\text{MOX-1}}$, *bla*$_{\text{MOX-2}}$, *bla*$_{\text{CMY-1}}$, bla$_{\text{CMY-8}}$ to *bla*$_{\text{CMY-11}}$	F- GCT GCT CAA GGA GCA CAG GAT R- CAC ATT GAC ATA GGT GTG GTG C	[7]
	bla$_{\text{LAT-1}}$ to *bla*$_{\text{LAT-4}}$, *bla*$_{\text{CMY-2}}$ to *bla*$_{\text{CMY-7}}$, *bla*$_{\text{BIL-1}}$	F- TGG CCA GAA CTG ACA GGC AAA R- TTT CTC CTG AAC GTG GCT GGC	
	bla$_{\text{DHA-1}}$ and *bla*$_{\text{DHA-2}}$	F- AAC TTT CAC AGG TGT GCT GGG T R- CCG TAC GCA TAC TGG CTT TGC	
	bla$_{\text{ACC}}$	F- AAC AGC CTC AGC AGC CGG TTA R- TTC GCC GCA ATC ATC CCT AGC	
	bla$_{\text{MIR-1T}}$, *bla*$_{\text{ACT-1}}$	F- TCG GTA AAG CCG ATG TTG CGG R- CTT CCA CTG CGG CTG CCA GTT	
	bla$_{\text{FOX-1}}$ to *bla*$_{\text{FOX-5b}}$	F- AAC ATG GGG TAT CAG GGA GAT G R- CAA AGC GCG TAA CCG GAT TGG	
	bla$_{\text{CMY-2}}$, *bla*$_{\text{CMY-4}}$, *bla*$_{\text{CMY-6}}$, *bla*$_{\text{CMY-7}}$, *bla*$_{\text{CMY-12}}$, *bla*$_{\text{CMY-13}}$, *bla*$_{\text{CMY-14}}$, *bla*$_{\text{CMY-18}}$, *bla*$_{\text{LAT-3}}$	F- ATG ATG AAA AAA TCG TTA TGC TGC R- GCT TTT CAA GAA TGC GCC AGG	[20]
PMQR	*qnr*A	F- AGA GGA TTT CTC ACG CCA GG R- TGC CAG GCA CAG ATC TTG AC	[21]
	*qnr*B	F- GGM ATH GAA ATT CGC CAC TG R- TTT GCY GYY CGC CAG TCG AA	
	*qnr*S	F- GCA AGT TCA TTG AAC AGG GT R- TCT AAA CCG TCG AGT TCG GCG	
Tetracycline resistance	*tet*A	F- GCC TTT CCT TTG GGT TCT CT R- TGT CCG ACA AGT TGC ATG AT	[22]
	*tet*B	F- GCT TTC AGG GAT CAC AGG AG R- GAC CAA GAC CCG CTA ATG AA	
Sulfonamide resistance	*sul*I	F- ACG AGA TTG TGC GGT TCT TC R- GGT TTC CGA GAT GGT GAT TG	[22]
	*sul*II	F- CCG TCT CGC TCG ACA GTT AT R- GTG TGT GCG GAT GAA GTC AG	
Insertion sequence	ISE*cp1* - 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*$_{\text{CMY}}$ 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 Labora- tory 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*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$, *bla*- $_{\text{TEM}}$), plasmid mediated quinolone resistance (PMQR) determinants (*qnr*A, *qnr*B and *qnr*S), sulfonamide resistance genes (*sul*I and *sul*II) and tetracycline resistance genes (*tet*A and *tet*B) [7, 17–22]. To define the mechan- ism of transmission of the pAmpC gene, we screened *bla*$_{\text{ampC}}$ for the insertion sequence IS*Ecp*1 by IS*Ecp*1- *bla*$_{\text{CMY}}$ linkage PCR [23]. Strains positive for this PCR were sequenced as previously described. Details regard- ing oligonucleotide primers and references are illustrated in Table 1. The PCR mixture was prepared using Pro- mega 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 (LPixIma- geHE). 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 sequen- cing the PCR amplificon *adk*, *fum*C, *gyr*B, *icd*, *mdh*, *pur*A and *rec*A. Sequencing was performed as previously described in this manuscript.

Pulsed-field gel electrophoresis

Genomic relationships were analyzed by *Xba*I restriction digestion followed by pulsed-field gel electrophoresis (PFGE) using the CHEF DR III PFGE System (BioRad, Hercules, CA, USA). Electrophoresis conditions con- sisted 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% similar- ity 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*$_{\text{CMY-2}}$ gene in all pAmpC-pro- ducing *E. coli* strains.

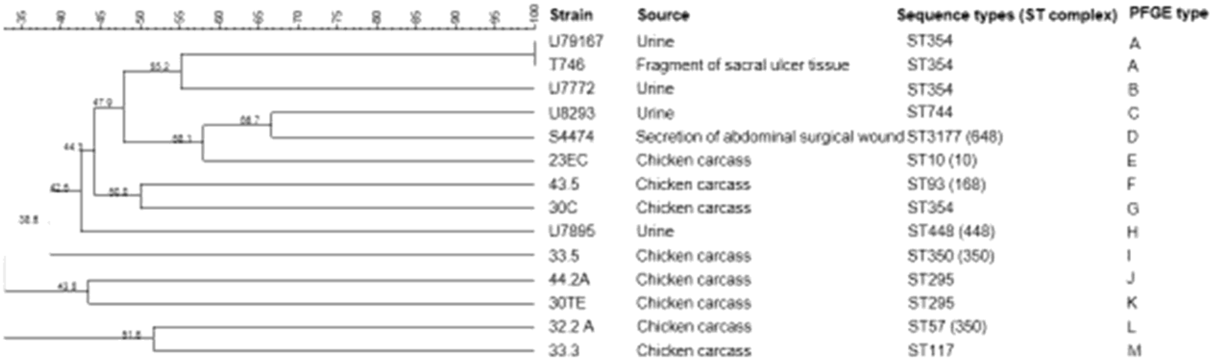
All the strains were resistant to amoxicillin-clavu- lanic acid, and 92.86% of the strains were resistant to cefoxitin. According to the antimicrobial sus- ceptibility profile for non-beta-lactam antimicrobials, the strains presented a high frequency of resistance for mainly tetracycline (92.86%), nalidixic acid (92.86%) and sulfamethoxazole-trimethoprim (78.57%) (Table 2). Among the non-beta-lactamase genes, the strains showed *tet*A (7 from chickens and 2 from humans), *tet*B (6 from chickens and 3 from humans), *sul*I (8 from chickens) and *sul*II (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*$_{\text{TEM-1}}$ gene was found in 2 strains isolated from human.

To detect whether IS*Ecp1* is upstream of *bla*$_{\text{CMY-2}}$, PCR with a forward primer targeting the IS*Ecp1* element and a reverse primer targeting the *bla*$_{\text{CMY}}$ 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*$_{\text{CMY-2}}$ genes are linked to an upstream IS*Ecp1*-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).



Strain	Source	Sequence types (ST complex)	PFGE type
U79167	Urine	ST354	A
T746	Fragment of sacral ulcer tissue	ST354	A
U7772	Urine	ST354	B
U8293	Urine	ST744	C
S4474	Secretion of abdominal surgical wound	ST3177 (648)	D
23EC	Chicken carcass	ST10 (10)	E
43.5	Chicken carcass	ST93 (168)	F
30C	Chicken carcass	ST354	G
U7895	Urine	ST448 (448)	H
33.5	Chicken carcass	ST350 (350)	I
44.2A	Chicken carcass	ST295	J
30TE	Chicken carcass	ST295	K
32.2 A	Chicken carcass	ST57 (350)	L
33.3	Chicken carcass	ST117	M

Fig. 1 Relationship among pAmpC-producing *E. coli* strains from chicken carcasses and humans infections by PFGE and MLST

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 $bla_{\text{CMY-2}}$ gene by sequencing. According to the literature, $bla_{\text{CMY-2}}$ 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 IS*Ecp1* in AmpC beta-lactamase positive *E. coli* strains

Strains	Antimicrobial resistance profile to non beta-lactams	Betalactamase genes	Non beta-lactam resistance genes	IS*Ecp1*
Chicken carcass				
23EC	tet, gen, nal, sut	$bla_{\text{CMY-2}}$	*tet*A, *tet*B, *sul*I, *sul*II	+
30C	tet, gen, clo, nal, cip, nor, enr, sut	$bla_{\text{CMY-2}}$	*tet*B, *sul*I, *sul*II	+
30TE	tet, nal, sut	$bla_{\text{CMY-2}}$	*tet*A, *tet*B, *sul*I, *sul*II	+
32.2 A	tet, nal, sut	$bla_{\text{CMY-2}}$	*tet*A, *tet*B, *sul*I, *sul*II	+
33.3	tet, nit, nal, cip, enr	$bla_{\text{CMY-2}}$	*tet*A, *tet*B, *sul*I, *sul*II	+
33.5	tet, nal, cip, nor, enr, sut	$bla_{\text{CMY-2}}$	*tet*A, *tet*B, *sul*I, *sul*II	–
43.5	tet, gen, clo, nit, sut	$bla_{\text{CMY-2}}$	*tet*A, *sul*I, *sul*II	–
44.2 A	tet, nal, sut	$bla_{\text{CMY-2}}$	*tet*A, *sul*I	+
Human samples				
U79167	tet, nal, cip, nor, enr, sut	$bla_{\text{CMY-2}}$; $bla_{\text{TEM-1}}$	*tet*A	+
U7895	tet, nal, cip, nor, enr, sut	$bla_{\text{CMY-2}}$	*tet*A	–
T746	tet, nal, cip, nor, enr, sut	$bla_{\text{CMY-2}}$; $bla_{\text{TEM-1}}$	*tet*B	–
U7772	tet, gen, nal,cip, nor, enr, sut	$bla_{\text{CMY-2}}$	*tet*B	+
S8293	tet, nit, nal, nor, enr	$bla_{\text{CMY-2}}$	*tet*B, *sul*II	+
S4474	nal, cip, nor, enr	$bla_{\text{CMY-2}}$	–	+

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*$_{\text{CMY-2}}$ 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*$_{\text{CMY-2}}$ genes harbored on different plasmids in *E. coli* from poultry [13, 14]. In Brazil, da Silva and collaborators (2017) [16] found *bla*$_{\text{CMY-2}}$ 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, cefpirome, 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 *tet*A, *tet*B, *sul*I and *sul*II (Table 2), which corroborates the high frequency of phenotypic resistance for tetracycline and sulfamethoxazole-trimethoprim. In addition, two strains from humans have the *bla*$_{\text{TEM-1}}$ gene, which encodes a beta-lactamase with a lower spectrum of action. The *bla*$_{\text{pAmpC}}$ 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*$_{\text{ampC}}$ genes onto plasmids. It has been reported that beta-lactamase genes can be genetically linked to an upstream insertion elements, as ISE*cp1*. Many studies have shown that *bla*$_{\text{AmpC}}$- type genes are associated with mobile genetic elements, including insertion sequences such as ISE*cp1*, most of which are carried on transferable plasmids [5, 31, 32]. In Brazil, only one study reported the presence of the ISE*cp* 1-*bla*$_{\text{CMY-2}}$ 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*$_{\text{CMY-2}}$ gene linked to an upstream ISE*cp* 1-like element. This insertion element can be responsible for the transposition of *bla*$_{\text{CMY-2}}$ 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*$_{\text{CMY-2}}$ 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 ISE*cp1* upstream of *bla*$_{\text{CMY-2}}$ 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 resist- ance 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 asso- ciated with *in ovo* vaccination on the 18th day of incuba- tion because the vaccine can also select antimicrobial resistant bacteria in poultry [33].

Further research about the dissemination of resist- ant 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* $_{\text{CMY-2}}$ gene linked with an ISE*cp1*-type insertion element in both chicken carcasses and in human infection in a re- stricted 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 healthcaree, 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*$_{\text{CMY-2}}$, linked to the ISE*cp1* element, was present both in chicken meat and human infection, suggesting that mobile genetic elements can be respon- sible 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

Acknowledgements

The authors would like to thank all colleagues of department of microbiology for their laboratory cooperation.

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. RKTK coordinated the manuscript and the overall study. All authors have read and approved the manuscript.

Funding

This work was supported by “Programa Institucional de Pesquisa Básica e Aplicada, da Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná (Chamada Pública CP 09/2016 – Protocol 10748)” and “Coordenação de Aperfeiçoamento do Pessoal de Ensino Superior” (CAPES). These funding bodies have the role of pay the consumption materials used in this study and pay the scholarships to students. None of these funding bodies had any in role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

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 obtaine 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.

Author details

[1]Basic and Applied Bacteriology Laboratory, Department of Microbiology, State University of Londrina (UEL), Londrina, PR, Brazil. [2]Bacterial Molecular Biology Laboratory, Department of Genetics, Evolution and Bioagents, Institute of Biology, State University of Campinas (UNICAMP), Campinas, SP, Brazil. [3]Soil Biotechnology Laboratory, Brazilian Agricultural Research Corporation (Embrapa), Londrina, PR, Brazil. [4]Department of Pathology and Clinical and Toxicological Analysis, State University of Londrina (UEL), Londrina, PR, Brazil.

Received: 13 December 2018 Accepted: 19 July 2019

References

1. Cyoia PS, Koga VL, Nishio EK, Houle S, Dozois CM, de Brito KCT, de Brito BG, Nakazato G, Kobayashi RKT. Distribution of ExPEC virulence factors, *bla*$_{\text{CTX-M}}$, *fos*A3, and *mcr*-1 in *Escherichia coli* isolated from commercialized chicken carcasses. Front Microbiol. 2019;9:1–9.
2. Lazarus B, Paterson DL, Mollinger JL, Rogers BA. Do human extraintestinal *Escherichia coli* infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. Clin Infect Dis. 2015;60(3):439–52.
3. Liebana E, Carattoli A, Coque TM, Hasman H, Magiorakos AP, Mevius D, Peixe L, Poirel L, Schuepbach-Regula G, Torneke K, Torren-Edo J, Torres C,

Threlfall J. Public health risks of enterobacterial isolates producing extended-spectrum β-lactamases or AmpC β-lactamases in food and food-producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control options. Clin Infect Dis. 2013;56(7):1030–7.

4. Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect. 2012;18(7):646–55.
5. Jacoby GA. AmpC β-lactamases. Clin Microbiol Rev. 2009;22(1):161–82.
6. Da Silva Dias RC, Borges-Neto AA, D'Almeida Ferraiuoli GL, de Oliveira MP, Riley LW, Moreira BM. Prevalence of AmpC and other β-lactamases in enterobacteria at a large urban university hospital in Brazil. Diagn Microbiol Infect Dis. 2008;60(1):79–87.
7. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC β-lactamases genes in clinical isolates by using multiplex PCR. J Clin Microbiol. 2002;40(6):2153–62.
8. Rensing KL, Abdallah HM, Koek A, Elmowalid GA, Vandenbroucke-Grauls CMJE, Al Naiemi N, van Dijk K. Prevalence of plasmid-mediated AmpC in Enterobacteriaceae isolated from humans and from retail meat in Zagazig. Egypt Antimicrob Resist Infect Control. 2019;8:1–8.
9. Meini S, Tascini C, Cei M, Sozio E, Rossolini GM. AmpC β-lactamase-producing Enterobacterales: what a clinician should know. Infection. 2019;47:363-75.
10. Castanheira M, Pereira AS, Nicoletti AG, Pignatari ACC, Barth AL, Gales AC. First report of plasmid-mediated *qnr*A1 in a ciprofloxacin-resistant *Escherichia coli* strain in Latin America. Antimicrob Agents Chemother. 2007;51(4):1527–9.
11. Pavez M, Neves P, Dropa M, Matté MH, Grinbaum RS. Elmor de Araújo MR, Mamizuka EM, Lincopan N. emergence of carbapenem-resistant *Escherichia coli* producing CMY-2-type AmpC β-lactamase in Brazil. J Med Microbiol. 2008;57(Pt12):1590–2.
12. Koga VL, Rodrigues GR, Scandorieiro S, Vespero EC, Oba A, de Brito BG, de Brito KCT, Nakazato G, Kobayashi RKT. Evaluation of the antibiotic resistance and virulence of *Escherichia coli* strains isolated from chicken carcasses in 2007 and 2013 from Paraná. Brazil Foodborne Pathog Dis. 2015;12(6):479–85.
13. Casella T, Haenni M, Madela NK, de Andrade LK, Pradela LK, de Andrade LN, Darini ANC, Madec J-Y, Nogueira MCL. 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. J Antimicrob Chemother. 2018;73(12):3293–7.
14. Ferreira JC, Penha Filho RAC, Andrade LN, Berchieri Junior A, Darini ALC. Diversity of plasmids harbouring *bla*$_{\text{CMY2}}$ in multidrug-resistant *Escherichia coli* isolated from poultry in Brazil. Diagn Microbiol Infect. 2017;88(4):361–4.
15. Botelho LAB, Kraychete GB. Costa e Silva JL, Regis DVV, Picão RC, Moreira BM, Bonelli RR. Widespread distribution of CTX-M and plasmid-mediated AmpC β-lactamases in *Escherichia coli* from Brazilian chicken meat. Mem Inst Oswaldo Cruz. 2015;110(2):249–54.
16. Da Silva KC, Cunha MP, Cerdeira L, de Oliveira MG, de Oliveira MC, Gomes CR, Lincopan N, Knöbl T, Moreno AM. High-virulence CMY-2- and CTX-M2-producing avian pathogenic *Escherichia coli* strains isolated from commercial turkeys. Diagn Microbiol Infect Dis. 2017;87(1):64–7.
17. Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases. J Antimicrob Chemother. 2005;57(1):154–5.
18. Dallenne C, da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J Antimicrob Chemother. 2010;65(3):490–5.
19. Jouini A, Vinué L, Slama KB, Sáenz Y, Klibi N, Hammami S, Boudabous A, Torres C. Characterization of CTX-M and SHV extended-spectrum β-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. J Antimicrob Chemother. 2007;60(5):1137–41.
20. Dierikx C, van Essen-Zandbergen A, Veldman K, Smith H, Mevius D. Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. Vet Microbiol. 2010;145(3–4):273–8.
21. Cattoir V, Poirel L, Rotimi V, Soussy C-J, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. J Antimicrob Chemother. 2007;60(2):394–7.
22. Li Q, Sherwood JS, Logue CM. Characterization of antimicrobial resistant *Escherichia coli* isolated from processed bison carcasses. J Appl Microbiol. 2007;103(6):2361–9.
23. Liao W, Jiang J, Xu Y, Yi J, Chen T, Su X, Pan S, Wei X, Li Y. Survey for β-lactamase among bacterial isolates from Guangzhou, China hospitals between 2005–2006. J Antibiot (Tokyo). 2010;63(5):225–9.
24. Clinical Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals; 3rd edition, CLSI document M31-A3. Wayne, Pennsylvania (PA): CLSI; 2008.
25. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 23th informational supplement M100-S23. Wayne, Pennsylvania (PA): CLSI; 2013.
26. Jarlier V, Nicolas M-H, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev Infect Dis. 1988;10(4):867–78.
27. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.
28. Rocha DA, Campos JC, Passadore LF, Sampaio SC, Nicodemo AC, Sampaio JL. Frequency of plasmid-mediated AmpC β-lactamases in *Escherichia coli* isolates from urine samples in São Paulo. Brazil Microb Drug Resist. 2016; 22(4):321–7.
29. Campana EH, Barbosa PP, Fehlberg LCC, Gales AC. Frequency of plasmid-mediated AmpC in Enterobacteriaceae isolated in a Brazilian teaching hospital. Braz J Microbiol. 2013;44(2):477–80.
30. Moura Q, Fernandes MR, Silva KC, Montes DF, Esposito F, Dropa M, Noronha C, Moreno AM, Landgraf M, Negrão FJ, Lincopan N. Virulent nontyphoidal *Salmonella* producing CTX-M and CMY-2 β-lactamases from livestock, food and human infection. Brazil Virulence. 2018;9(1):281–6.
31. Guo YF, Zhang WH, Ren SQ, Yang L, Lü DH, Zeng ZL, Liu YH, Jiang HX. IncA/C plasmid-mediated spread of CMY-2 in multidrug resistant *Escherichia coli* from food animals in China. PLoS One. 2014;9(5):1-7.
32. Fernández-Alarcón C, Singer RS, Johnson TJ. Comparative genomics of multidrug resistance-encoding IncA/C plasmids from commensal and pathogenic *Escherichia coli* from multiple animal sources. PLoS One. 2011;6(8):1-9.
33. Ferreira JC, Penha Filho RAC, Kuaye APY, Andrade LN, Chang YF, Darini ALC. Virulence potential of commensal multidrug resistant *Escherichia coli* isolated from poultry in Brazil. Infect Genet Evol. 2018;65:251–6.
34. Sellera FP, Fernandes MR, Moura Q, Carvalho MPN, Lincopan N. Extended-spectrum-β-lactamase (CTX-M)-producing *Escherichia coli* in wild fishes from a polluted area in the Atlantic Coast of South America. Mar Pollut Bull. 2018;135:183–6.
35. Pietsch M, Eller C, Wendt C, Holfelder M, Falgenhauer L, Fruth A, Grössl T, Leistner R, Valenza G, Werner G, Pfeifer Y, RESET Study Group. Molecular characterization of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates from hospital and ambulatory patients in Germany. Vet Microbiol. 2017;200:130–7.
36. de Souza da-Silva AP, de Sousa VS, Martins N, da Silva Dias RC, Bonelli RR, Riley LW, Moreira BM. *Escherichia coli* sequence type 73 as a cause of community acquired urinary tract infection in men and women in Rio de Janeiro. Brazil Diagn Microbiol Infect Dis. 2017;88(1):69–74.
37. Müller A, Stephan R, Nüesch-Inderbinen M. Distribution of virulence factors in ESBL-producing *Escherichia coli* isolated from the environment, livestock, food and humans. Sci Total Environ. 2016;541:667–72.
38. Maluta RP, Logue CM, Casas MR, Meng T, Guastalli EA, Rojas TC, Montelli AC, Sadatsune T, de Carvalho RM, Nolan LK, da Silveira WD. Overlapped sequence types (STs) and serogroups of avian pathogenic (APEC) and human extra-intestinal pathogenic (ExPEC) *Escherichia coli* isolated in Brazil. PLoS One. 2014;9(8):1-11.
39. Ben Sallem R, Ben Slama K, Rojo-Bezares B, Porres-Osante N, Jouini A, Klibi N, Boudabous A, Sáenz Y, Torres C. IncI1 plasmids carrying *bla*$_{\text{CTX-M-1}}$ or *bla*$_{\text{CMY-2}}$ genes in *Escherichia coli* from healthy humans and animals in Tunisia. Microb Drug Resist. 2014;20(5):495–500.
40. Voets GM, Fluit AC, Scharringa J, Schapendonk C, van den Munckhof T, Leverstein-van Hall MA, Stuart JC. Identical plasmid AmpC beta-lactamase genes and plasmid types in *E. coli* isolates from patients and poultry meat in the Netherlands. Int J Food Microbiol. 2013;167(3):359–62.
41. Pitondo-Silva A, Minarini LA, Camargo IL, Darini AL. Clonal relationship determined by multilocus sequence typing among enteropathogenic *Escherichia coli* isolated in Brazil. Can J Microbiol. 2009;55(6):672–9.
42. Hasan B, Sandegren L, Melhus A, Drobni M, Hernandez J, Waldenström J, Alam M, Olsen B. Antimicrobial drug-resistant *Escherichia coli* in wild birds and free-range poultry. Bangladesh Emerg Infect Dis. 2012;18(12):2055–8.

43. De Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, Du Y, Hu J, Lei Y, Li N, Tooming-Klunderud A, Heederik DJ, Fluit AC, Bonten MJ, Willems RJ, de la Cruz F, van Schaik W. Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. PLoS Genet. 2014;10(12):e1004776.
44. Börjesson S, Ny S, Egervän M, Bergström J, Rosengren A, Englund S, Löfmark S, Byfords S. Limited dissemination of extended-spectrum β-lactamase- and plasmid-encoded AmpC-producing *Escherichia coli* from food and farm animals. Sweden Emerg Infect Dis. 2016;22(4):634–40.
45. Wang H, Ren L, Yu X, Hu J, Chen Y, He G, Jiang Q. Antibiotic residues in meat, milk and aquatic products in Shanghai and human exposure assessment. Food Control. 2017;80:217–25.
46. Dutil L, Irwin R, Finley R, Ng LK, Avery B, Boerlin P, Bourgault AM, Cole L, Daignault D, Desruisseau A, Demczuk W, Hoang L, Horsman GB, Ismail J, Jamieson F, Maki A, Pacagnella A, Pillai DR. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans. Canada Emerg Infect Dis. 2010;16(1):48–54.

Publisher’s Note

6. CONCLUSÃO

De acordo com os resultados obtidos neste trabalho pode-se concluir que:

- CTX-M-2 e CMY-2, foram as enzimas do tipo ESBL e pAmpC, respectivamente, mais frequentemente encontrada em amostras clínicas humanas e em carcaças de frango;

- A maioria das amostras foram classificadas como multirresistentes, sendo *tet*A, *tet*B, *sul*I e *sul*II os genes de resistência antimicrobiana mais associada tanto as amostras produtoras de ESBL quanto as produtoras de pAmpC;

- Nosso trabalho evidencia que o gene *mcr*-1 pode estar circulando entre amostras de ITUs no Brasil desde o ano de 2013;

- As amostras de *E. coli* isoladas de amostras clínicas humanas e de carcaças de frango apresentam um perfil de virulência e resistência semelhantes, indicando uma possível risco zoonótico;

- ST131, ST354, ST359 foram encontrados tanto em amostras isoladas de ITUs quanto em carcaças de frango, indicando que a carne de frango pode ser um reservatório de patógenos humanos, associados a multirresistência a importantes antimicrobianos ao homem

- ST131 foi o mais encontrado entre as amostras estudadas, no entanto, não estão relacionados a um tipo de ESBL específico;

- As amostras de *E. coli* isoladas de amostras clínicas humanas e de carcaças de frango não apresentaram similaridades por técnicas moleculares, demonstrando que na nossa região não há um clone que dissemina as betalactamases, mas indica que presença de elementos genéticos móveis como ISE*cp1* podem estar envolvidos na disseminação dessas resistências, principalmente em pAmpC.