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Virulence genes and antibiotic resistance assessment of the *blaCTX-M-15* gene in ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* from poultry food chain and human clinical cases in Brazil

Avaliação de genes de virulência e resistência a antimicrobianos em *Escherichia coli* e *Klebsiella pneumoniae* produtoras de beta-lactamase de espectro estendido na cadeia produtiva avícola e em fezes humanas no Brasil

Natália Pereira[1]*; Marita Vedovelli Cardozo[2]; Elisabete Aparecida Lopes Guastalli[3]; Luis Fernando dos Santos[4]; Mylena Karoline Valmorbida[5]; Lucas José Luduvério Pizauro[6]; Camila Chioda de Almeida[7]; Caio Roberto Soares Bragança[2]; Fernando Antônio de Ávila[8]

Highlights

Resistant *E. coli* and *K. pneumoniae* isolated from poultry and human samples.

Presence of virulence-related genes in isolates.

High pathogenicity of *Escherichia coli* demonstrated in vivo.

Potential risks to animal and human health.

[1] Doctorate Student in Agropecuary Microbiology Post Graduate Program, Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias UNESP/FCAV, Jaboticabal, SP, Brazil. E-mail: nataliavet16@gmail.com

[2] Profs. Drs. in Veterinary Medicine Undergraduate Program, Universidade do Estado de Minas Gerais, UEMG, Passos, MG, Brazil. E-mail: maritavedovelli@gmail.com; caio.braganca@uemg.br

[3] Researcher in Biological Institute, Centro Avançado de Pesquisa Tecnológica do Agronegócio Avícola, CAPTAA, São Paulo, SP, Brazil. E-mail: beteguastalli@hotmail.com

[4] Researcher in Center of Bacteriology, Instituto Adolfo Lutz, São Paulo, SP, Brazil. E-mail: luis.santos@ial.sp.gov.br

[5] Master in Agropecuary Microbiology Post Graduate Program, UNESP/FCAV, Jaboticabal, SP, Brazil. E-mail: mylikarolyne15@gmail.com

[6] Researcher in Department of Technology, UNESP/FCAV, Jaboticabal, SP, Brazil. E-mail: lucaspizauro@gmail.com

[7] Doctorate in Agropecuary Microbiology Post Graduate Program, UNESP/FCAV, Jaboticabal, SP, Brazil. E-mail: milachioda@gmail.com

[8] Prof. Dr. in Agropecuary Microbiology Post Graduate Program, UNESP/FCAV, Jaboticabal, SP, Brazil. E-mail: f.avila@unesp.br

* Author for correspondence

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Abstract

Brazil is one of the world's largest exporters of chicken-derived products. Consumer concerns regarding food contamination by multidrug-resistant bacteria capable of causing diseases have increased steadily over the years as they pose a significant public health risk. This study aimed to characterize strains of beta-lactam-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from the poultry production chain and human clinical samples (chicken cloaca, chicken meat, human feces). A total of 36 isolates were examined, including 28 *Escherichia coli* and 8 *Klebsiella pneumoniae* isolates carrying the *blaCTX-M-15* gene. These isolates were obtained from chicken cloaca and meat from poultry farms and slaughterhouses, respectively, as well as from human fecal samples from a clinical laboratory. All the establishments were located in São Paulo, Brazil. The isolates were characterized for their virulence genes by polymerase chain reaction, antimicrobial resistance by the disc diffusion method, serotyping by an agglutination test, and pathogenicity by an *in vivo* day-old chicken mortality test. The isolates exhibited a high frequency of *Escherichia coli* associated virulence genes such as *iutA, iss, hlyF, ompT* and *iroN.* In addition, *mrkD* was detected in the isolates. All isolates exhibited resistance to at least three different antimicrobial classes, and 21.4% (n = 6) of the *Escherichia coli* strains exhibited high pathogenicity in the day-old chicken assay *in vivo.* These results indicate a potential increase in the *blaCTX-M-15* gene associated with virulence genes and antimicrobial resistance in *Escherichia coli* and *Klebsiella pneumoniae.* Both of these Enterobacteriaceae can be found in poultry feces and possibly contaminate poultry products, thus posing a risk of infection to other animals and humans, raising an alert about the sanitary aspects of food production in Brazil.

Key words: Avian pathogenic *Escherichia coli.* Foodborne disease. *Klebsiella pneumoniae.* Poultry. Public health. Virulence genes.

Resumo

O Brasil é um dos maiores exportadores de produtos derivados de frango para o mundo e a preocupação de consumidores em relação à contaminação de alimentos com bactérias multiresistentes e capazes de causar doenças vêm aumentanto com os anos, sendo um alerta à saúde púbica. O estudo teve como objetivo caracterizar cepas de *Escherichia coli* e *Klebsiella pneumoniae* resistentes a Beta Lactâmicos isoladas da cadeia produtiva de frango e amostras clínicas humanas (cloaca, carne de frango e fezes humanas). Foram utilizados 36 isolados, sendo 28 *Escherichia coli* e 8 *Klebsiella pneumoniae* portadores do gene *blaCTX-M-15*, obtidos de amostras de fezes e carne de aves de corte, provenientes de criadouros e abatedouros avícolas, respectivamente, e amostras de fezes humanas de laboratório de análises clínicas humano. Todos os estabelecimentos eram localizados no Estado de São Paulo, Brasil. A caracterização de genes de virulência foi realizada por meio de PCR (Reação em Cadeia da Polimerase), a resistência a antimicrobianos pelo método de difusão em disco, sorotipagem por teste de aglutinação e patogenicidade por meio de teste de mortalidade *in vivo* em pintainhos de um dia de vida. Os isolados demonstraram uma alta frequência de genes de virulência relacionados à *Escherichia coli*, como *iutA, iss, hlyF, ompT* e *iroN*. O gene *mrkD* também foi encontrado em grande parte dos isolados. Todos os isolados foram resistentes a pelo menos três diferentes classes de antimicrobianos e 21.4% (6) das cepas de *Escherichia coli* demonstraram alta patogenicidade no teste *in vivo.* Esses resultados evidenciaram

o potencial aumento do gene *blaCTX-M-15* associado a genes de virulência e multiresistência em *Escherichia coli* e *Klebsiella pneumoniae,* ambas enterobactérias podem ser encontradas em fezes de aves e possivelmente contaminar produtos como a carne, aumentando o risco de infecção em humanos. Isso destaca a necessidade de cuidados sanitários rigorosos na produção de animais e alimentos no Brasil.

Palavras-chave: *Escherichia coli* Patogênica para aves. Doenças transmitidas por alimentos. *Klebsiella pneumoniae.* Saúde pública. Genes de virulência.

Introduction

As Brazil is the world's largest exporter of chicken meat and meat derivatives, European consumers have expressed concerns about the potential role of Brazilian chicken products in the transfer of resistance genes from food to humans (Botelho et al., 2015; Casella et al., 2017; Ferreira et al., 2016). Although it has been shown that human-to-human transmission within the community is the main source of ESBL transmission, concerns about food origin are attributed to the fact that genetic determinants, such as plasmids encoding CTX-M enzymes, could be transmitted to humans via the food chain or direct contact with animals or the environment (Dierikx et al., 2013).

South America exhibits widespread distribution of *blaCTX-M-2* and *blaCTX-M-8,* with a low reported frequency of the *blaCTX-M-15* gene to date. The spread of *blaCTX-M-15* in Brazil has only been documented since 2010 in clinical isolates, and its occurrence in meat, particularly chicken, has been sporadically reported (Botelho et al., 2020). Furthermore, in addition to being ESBL producers, these strains can harbor virulence factors and have a high potential for pathogenicity, such as Avian Pathogenic *Escherichia coli* (APEC), which causes colibacillosis in poultry (Monroy et al., 2005). The most sought virulence genes include those associated with adhesins (*fimH, pap* and *tsh*), iron acquisition system (*iucABCD, iutA, iroN, sitA* and *irp2*), serum resistance (*ompT, iss* and *traT*), and toxin production (*vat, astA, hlyF* and *cvaC*) (Jørgensen et al., 2017).

Therefore, based on the risk of transmission of resistance genes and the potential of enterobacteria to cause disease in both animals and humans, the present study aimed to characterize isolates of beta-lactam-resistant *Escherichia coli* and *Klebsiella pneumoniae* from the poultry production chain and human clinical cases (chicken cloaca, chicken meat, and human feces).

Material and Methods

Sample origins

For this study, were utilized 36 *blaCTX-M-15* gene-positive isolates stored in a freezer at -80 °C in the Sao Paulo State University laboratory. These isolates consisted of 28 *E. coli* and eight *K. pneumoniae* strains that had previously been identified by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (Cardozo et al., 2021). The isolates

tested positive for *blaCTX-M-15* by PCR. The laboratory team obtained these isolates from 300 samples, including 100 samples of poultry feces, chicken meat, and clinical samples (human feces). A total of 100 poultry fecal samples were collected from two farms (50 from each farm). One hundred chicken meat samples were collected from two different slaughterhouses (50 from each slaughterhouse), and 100 human feces samples were obtained from two different clinical laboratories (50 from each laboratory). All establishments in São Paulo were located within an 80 km radius of Ribeirao Preto, São Paulo.

Virulence gene detection by PCR

The genomic DNA preparations, PCR, and primers were as described by Ewers et al. (2005), T. J. Johnson et al. (2008), and Schouler et al. (2012). The PCR was performed with solutions containing 2 mM of each dNTP, 3.6 µL of 10X DreamTaq™ Green Buffer (containing 20 mM MgCl2), 1.25 pmol of each primer, 1 unit of Dream TaqGreen™ DNA Polymerase, 4 µL of template DNA, and water to complete the 20 µL volume. The following genes associated with APEC virulence were investigated: *iutA, hlyF, iss, iroN, ompT, sitA, tsh, traT, iucC, iucD, papC, fimH, vat, cvaC, astA* and *irp2* (Ewers et al., 2004; , J. R. Johnson & Stell, 2000; Maurer et al., 1998; Rodriguez-Siek et al., 2005; Siqueira et al., 2009). In addition, the *K. pneumoniae* virulence genes *rmpA, magA, Kfu, Uge, WabG* and *mrkD* were investigated (Brisse et al., 2009; Shah et al., 2017).

Serological identification of serogroups

E. coli samples were identified using somatic antigen O (serogroup O1-O188) and flagellar H (H1-H56). The serology was determined by agglutination tests using specific antisera as described by Orskov and Orskov (1992).

in vivo pathogenicity test

All *in vivo* experiments were approved by the Animal Ethics Commission (CEUA), Protocol No. 015560/18). To determine the pathogenicity of the *E. coli blaCTX-M-15* isolates, ten commercial, white variety, male, day-old chicks were inoculated with 0.1 mL of each strain, as well as the same amount of the positive and negative bacterial culture controls, into the left thoracic air sac, as described by Monroy et al. (2005). For the inoculum preparation, a single colony of each bacterial strain was seeded in 10 mL of BHI broth, incubated for 18 hours at 37 °C, and then diluted at 1:10 or until standardized at 10^7 CFU mL. *E. coli* EC 55 of serogroup O1 with *cva-cvi, tsh, iucD* and *iss* virulence genes from the Ornithopathology Laboratory Culture Collection (USP) was used as a positive control. As a negative control, was used the *E. coli* K12 strain from the Laboratory of Bacterial Antigens II, Department of Microbiology and Immunology, Institute of Biology, University of Campinas. The isolates were classified based on their pathogenicity according to the day-old chicken mortality rate evaluated after ten days as high (≥ 80%), intermediate (> 50% and < 80%), low pathogenicity (≤ 50%), or non-pathogenic (zero mortality).

Antimicrobial susceptibility testing

The susceptibility test was performed by the disk diffusion method following standard guideline CLSI (2023) for the antimicrobials: ampicillin (10 µg), streptomycin (10 µg), gentamicin (10 µg), tetracycline (30 µg), nitrofurantoin (300 µg), sulfamethoxazole + trimethoprim (25 µg), amikacin (30 µg), cefoxitin (30 µg), cephalothin (30 µg), ceftazidime (30 µg), amoxicillin + clavulanic acid (30 µg), norfloxacin (10 µg), and fosfomycin (50 µg). The isolates were inoculated in tubes containing 3 mL of BHI broth and incubated at 35 °C until a broth turbidity of MacFarland 0.5 standard was reached. Thereafter, cultures were plated on Mueller-Hinton agar plates. After drying, discs containing antimicrobials were placed. Inhibition halo readings were performed after 18 h of incubation at 35 °C using a millimeter ruler, and the obtained diameters were compared with the disk manufacturer standards.

Statistical analysis

In this study, we employed a comprehensive statistical analysis approach to effectively summarize and present our data. This involved utilizing descriptive statistics to provide a concise summary, encompassing measures such as means, medians, standard deviations, and quartiles for the continuous variables and generating frequency tables and corresponding percentages for the categorical variables. All statistical analyses were performed using R language for statistical computing (version 4.0.1).

Results and Discussion

Table 1 presents the results of the methodology. A group of virulence-associated genes, including *iutA, hlyF, iss, iroN* and *ompT* is typically associated with APEC strains responsible for causing colibacillosis in poultry (T. J. Johnson et al., 2008). However, despite our results showing that 24 of 28 (85.7%) *E. coli* strains tested positive for these genes simultaneously, the samples were obtained from animals with no apparent signs of colibacillosis. Therefore, our *E. coli* samples cannot be considered to be APEC strains, although they can be potentially pathogenic and cause illness in poultry production.

Table 1
Isolate characterization with description of species, origin, APEC-related genes, *K. pneumoniae* virulence-related genes, pathogenicity, *E. coli* serotypes, and antibiogram

Isolate	Specie	Origin	APEC*	Other APEC genes	K. pneumoniae	Pathogenicity	Serotype	Antibiogram	
								Resistant	Susceptible
1	*K. pneumoniae*	Cloaca	-	-	*mrkD*	-	-	NOR, AMP, NIT, CFL, CAZ, TET, SUT	EST, AMC, GEN, AMI, FOS, CFO
2	*K. pneumoniae*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, iucC, sitA, traT*	*mrkD*	-	-	EST, AMC, NOR, GEN, AMP, CFO, NIT, CFL, CAZ, TET, SUT	AMI, FOS
3	*E. coli*	Cloaca	*hlyF, iss, iroN, ompT, iutA*	*astA, iucC, sitA, traT*	*mrkD*	Low	ONT:H32	EST, NOR, GEN, AMP, CFL, CAZ, TET, SUT	AMC, AMI, FOS, CFO, NIT
4	*E. coli*	Cloaca	*hlyF, iss, iroN, ompT, iutA*	*astA, fimH, iucC, sitA, traT*	*mrkD*	Intermediary	ONT:H32	EST, AMC, NOR, FOS, AMP, CFO, NIT, CFL, CAZ, TET, SUT	GEN, AMI
5	*E. coli*	Cloaca	*hlyF, iss, iroN, ompT, iutA*	*astA, iucC, sitA, traT*	*mrkD*	High	ONT:H32	EST, AMC, NOR, AMP, NIT, CFL, CAZ, TET, SUT	GEN, AMI, FOS, CFO
6	*E. coli*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, fimH, iucC, sitA, traT*	-	Intermediary	ONT:H32	EST, AMC, NOR, AMI, AMP, NIT, CFL, CAZ TET, SUT	GEN, FOS, CFO
7	*E. coli*	Cloaca	*hlyF, iss, iroN, ompT, iutA*	*astA, iucC, sitA, traT*	-	Low	ONT:H32	EST, AMC, NOR, AMI, AMP, NIT, CFL, CAZ, CFZ, TET, SUT	GEN, FOS, CFO

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8	*E. coli*	Cloaca	*hlyF, iss, iroN, ompT, iutA*	*astA, fimH, iucC, iucD, sitA, traT*	-	High	ONT:H32	EST, AMC, NOR, GEN, AMP, NIT, CFL, CAZ TET, SUT	AMI, FOS, CFO
9	*E. coli*	Cloaca	*hlyF, iss, iroN, ompT, iutA*	*astA, iucC, sitA, traT*	-	Intermediary	ONT:H32	EST, AMC, NOR, AMP, NIT, CFL, CAZ, TET, SUT	GEN, FOS, AMI, CFO
10	*E. coli*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, iucC, papC, sitA, traT*	-	Low	ONT:H32	EST, AMC, NOR, GEN, AMI, AMP, CFL, CAZ, TET, SUT	FOS, CFO, NIT
11	*E. coli*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, iucC, papC, sitA, traT*	-	Low	ONT:H32	EST, AMC, NOR, GEN, AMI, AMP, NIT, CFL, CAZ, TET, SUT	FOS, CFO
12	*E. coli*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, iucC, sitA, traT*	-	Non-pathogenic	ONT:H32	EST, AMC, NOR, GEN, AMP, NIT, CFL, CAZ, TET, SUT	AMI, FOS, CFO
13	*K. pneumoniae*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, iucC, sitA, traT*	*mrkD*	-	-	EST, AMC, NOR, GEN, AMP, CFO, NIT, CFL, CAZ, TET, SUT	AMI, FOS
14	*K. pneumoniae*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, iucC, sitA, traT*	*mrkD*	-	-	EST, AMC, NOR, GEN, AMP, NIT, CFL, CAZ, TET, SUT	AMI, FOS, CFO
15	*E. coli*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, sitA, traT*	*mrkD*	Non-pathogenic	ONT:H32	EST, AMC, NOR, GEN, FOS, AMP, NIT, CFL, CAZ, TET, SUT	AMI, CFO

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16	*E. coli*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, iucC, sitA, traT, tsh*	*mrkD*	Intermediary	ONT:H32	EST, AMC, NOR, GEN, AMI, AMP, NIT, CFL, CAZ, TET, SUT	FOS, CFO
17	*E. coli*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, iucC, sitA, traT, tsh*	-	Intermediary	OR:H16	EST, AMC, NOR, GEN, AMI, AMP, NIT, CFL, CAZ, TET, SUT	FOS, CFO
18	*E. coli*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, sitA, traT, tsh*	*mrkD*	Low	ONT:H32	EST, AMC, NOR, GEN, AMP, NIT, CFL, CAZ, TET, SUT	AMI, FOS, CFO
19	*K. pneumoniae*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, cvaC, fimH,iucC, sitA, traT, tsh*	*mrkD*	-	-	EST, AMC, NOR, GEN, AMP, NIT, CFL, CAZ, TET, SUT	AMI, FOS, CFO
20	*K. pneumoniae*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, cvaC, sitA*	*mrkD*	-	-	EST, AMC, NOR, GEN, AMI, FOS, AMP, NIT, CFL, CAZ, TET, SUT	CFO
21	*E. coli*	Cloaca	-	*sitA, traT*	-	Non-pathogenic	O139:H19	EST, AMC, NOR, GEN, FOS, AMP, NIT, CFL, CAZ, TET, SUT	AMI, CFO
22	*E. coli*	Cloaca	*hlyF, iss, ompT, iutA*	*astA, iucC, sitA, traT*	-	Non-pathogenic	ONT:H32	EST, AMC, NOR, GEN, AMI, FOS, AMP, CFL, CAZ, TET, SUT	CFO, NIT

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23	*E. coli*	Cloaca	*iutA*	*iucC, sitA, traT*	-	Non-pathogenic	O153:H34	EST, AMC, NOR, GEN, AMI, AMP, CFL, CAZ, TET, SUT	FOS, CFO, NIT
24	*E. coli*	Cloaca	*iutA*	*iucC, sitA, traT*	-	Non-pathogenic	O153:H34	EST, AMC, NOR, GEN, AMI, AMP, CFO, NIT, CFL, CAZ, TET, SUT	FOS
25	*E. coli*	Human feces	*hlyF, iss, iroN, ompT, iutA*	*astA, iucC, sitA, traT*	*mrkD*	Intermediary	ONT:H32	EST, AMC, NOR, GEN, AMI, AMP, NIT, CFL, CAZ, TET, SUT	FOS, CFO
26	*E. coli*	Human feces	*hlyF, iss, iroN, ompT, iutA*	*astA, iucC, sitA, traT*	*mrkD*	Intermediary	ONT:H32	EST, AMC, NOR, GEN, FOS, AMP, CFO, NIT, CFL, CAZ, TET, SUT	AMI
27	*E. coli*	Human feces	*hlyF, iss, ompT, iutA*	*cvaC, iucC, sitA, traT, tsh*	*mrkD*	Non-pathogenic	O139:H19	EST, AMC, NOR, AMI, AMP, NIT, CFL, CAZ, TET, SUT	GEN, FOS, CFO
28	*K. pneumoniae*	Human feces	*hlyF, iss, ompT, iutA*	*cvaC, sitA, traT, vat*	*mrkD*	-	-	EST, AMC, NOR, GEN, FOS, AMP, CFO, NIT, CFL, CAZ, TET, SUT	AMI
29	*E. coli*	Human feces	*hlyF, iss, iroN, ompT, iutA*	*astA, cvaC, papC, sitA, traT, vat*	*mrkD*	Low	O87:H15	EST, AMC, NOR, GEN, FOS, AMP, CFO, NIT, CFL, CAZ, TET, SUT	AMI

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30	*E. coli*	Chicken meat	*hlyF, iss, iutA*	*astA, fimH, iucC, iucD, sitA, traT*	*mrkD*	Intermediary	ONT:H32	EST, AMC, NOR, GEN, AMI, FOS, AMP, CFO, NIT, CFL, CAZ, TET, SUT	-
31	*E. coli*	Chicken meat	*hlyF, iss, iroN, ompT, iutA*	*astA, fimH, iucC, sitA, traT*	-	High	O139:H19	EST, AMC, NOR, GEN, AMP, CFO, NIT, CFL, CAZ, TET, SUT	AMI, FOS
32	*E. coli*	Chicken meat	*hlyF, iss, ompT, iutA*	*astA, iucC, papC, sitA, traT*	*mrkD*	High	ONT:H32	EST, NOR, GEN, AMP, CFO, NIT, CFL, CAZ, TET, SUT	AMC, AMI, FOS
33	*E. coli*	Chicken meat	*hlyF, iss, ompT, iutA*	*astA, sitA, traT*	-	Non-pathogenic	ONT:H32	EST, AMC, NOR, GEN AMI, AMP, NIT, CFL, CAZ, TET, SUT	FOS, CFO
34	*K. pneumoniae*	Chicken meat	*hlyF, iss, ompT, iutA*	*astA, sitA, traT*	*mrkD*	-	-	EST, AMC, NOR, GEN, AMP, NIT, CFL, CAZ, TET, SUT	AMI, FOS, CFO
35	*E. coli*	Chicken meat	*hlyF, iss, ompT, iutA*	*astA, iucC, sitA, traT*	*mrkD*	High	ONT:H32	EST, NOR, GEN, AMP, CFO, NIT, CFL, CAZ, TET, SUT	AMC, AMI, FOS
36	*E. coli*	Chicken meat	*hlyF, iss, iroN, ompT, iutA*	*astA, iucC, sitA, traT*	-	High	ONT:H32	EST, AMC, NOR, GEN, AMP, CFO, NIT, CFL, CAZ, TET, SUT	AMI, FOS

*Classification based on Johnson et al. (2008) for potential APEC strains (pathogenic avian *Escherichia coli*) with positive results for at least four of five genes: *hlyF, iroN, iss, iutA* and *ompT*. AMC: Amoxycillin + clavulanic acid; MIL: amikacin; AMP: ampicillin; CAZ: ceftazidime; CFL: cephalothin; CFO: cefoxitin; EST: streptomycin; FOS: fosfomycin; GEN: gentamicin; NIT: nitrofurantoin; NOR: norfloxacin; TET: tetracycline; SUT: sulfamethoxazole + trimethoprim

Although South America has a widespread distribution of *blaCTX-M-2* and *blaCTX-M-8,* the high frequency of *blaCTX-M-15* was unexpected because it is not detected as often and its occurrence in poultry meat in Brazil is sporadic (Botelho et al., 2020). The presence of *blaCTX-M-15*-carrying isolates in this study is important, as it may be associated with the fact that our strains exhibited a high frequency of virulence-associated genes, which indicates the potential to cause disease with no efficient alternatives for treatment. Some authors (T. J. Johnson et al., 2008; Schouler et al., 2012) have argued that the presence of at least four of the five plasmid virulence-related genes, *iss, iroN, iutA, ompT* and *hlyF,* can characterize a strain as a potentially pathogenic APEC and distinguish it from opportunistic strains. In our study, among the 28 *E. coli* strains, four (14.3%) were positive for all five of these plasmid virulence-related genes, and six (21.4%) exhibited high levels of *in vivo* pathogenicity.

Furthermore, when we analyzed *K. pneumoniae* strains from different sources (feces, chicken meat, and human feces), seven out of eight strains (87.5%) harbored four out of five genes (*iss, iroN, iutA, ompT,* and *hlyF*). Some of these genes have also been detected in ESBL-producing *K. pneumoniae* strains in previous studies, indicating their non-specificity for APEC and their importance in the pathogenicity of different bacterial species (Candan & Aksöz, 2015; Gharrah et al., 2017). Regarding the other genes associated with *E. coli* virulence, our study demonstrated a low frequency of *fimH, papC* and *tsh* genes, corresponding to 16.6%, 11.1%, and 13.9%, respectively. These genes encode fimbriae (*fimH* and *papC*) and non-fimbriae (*tsh*) adhesins, which facilitate bacterial adhesion to the avian respiratory epithelial system and internal organs (Dziva & Stevens, 2008). Notably, they may occur at a higher frequency in potential APEC strains, as demonstrated in previous studies (Barbieri et al., 2013; Borzi et al., 2018).

In contrast to adhesin-related genes, our isolates exhibited high frequencies of *ompT* (86.1%), *iss* (88.9%), and *traT* (94.4%). These findings are consistent with those of Cummins et al. (2019), who identified the presence of these genes in different APEC lineages in Australia. These genes demonstrate the potential of these isolates to resist the host organism because of their ability to produce outer membrane proteins that inhibit complement-induced phagocytosis (Hejair et al., 2017). Additionally, we assessed the presence of *iucC* (75%), *iucD* (5.6%), *iutA* (94.4%), *iroN* (30.6%), *sitA* (97.2%), and *irp2* (0%) genes, which are associated with the iron acquisition system and metabolism. According to Rodriguez-Siek et al. (2005), these genes and operons are widely distributed among APEC strains and may be related to pathogenicity, such as colibacillosis, in poultry. It is also important to note that in this study, we observed the presence of other genes (Table 1), such as *vat* (5.6%), *astA* (83.3%), *hlyF* (88.9%), and *cvaC* (13.9%). These genes encode a vacuolating protein, thermostable toxin, α-hemolysin, and colicin V protein, respectively. These virulence factors can enhance the pathogenicity of the strain by improving competition for nutrients within the host organism and causing damage to host cells, which can lead to diarrhea (Murase et al., 2015; Paixão et al., 2015; Zhao et al., 2019).

Furthermore, 36 isolates were assessed for genes associated with *K. pneumoniae* virulence, and only the *mrkD* gene was detected in 58.3% (21/36) of the isolates, including both *K. pneumoniae* and *E. coli.* The *mrk*ABCDF operon encodes type 3 fimbriae adhesins, initially identified in *K. pneumoniae* strains but has now been identified in bacteria from different genera, including *E. coli,* possibly acquired through lateral gene transfer (Stahlhut et al., 2013). Specifically, the *mrkD* gene is responsible for biofilm formation on abiotic surfaces, with its alleles present in *Klebsiella* spp. strains. It provides specificity for fimbrial binding to materials such as collagen, thus conferring the ability to adhere to urinary and respiratory cells as well as to materials such as catheters (Murphy & Clegg, 2012). The presence of this gene in 58.3% of the samples studied, including strains of *K. pneumoniae* and *E. coli,* indicates that these isolates may have virulence potential and zoonotic capabilities, as they were identified in isolates from animal and human nosocomial infections in hospitals (Bakhtiari et al., 2021).

Regarding the serological tests, it was observed that the most prevalent serotypes among the *E. coli* samples were O139:H19 (10.7%, three isolates), O153:H34 (7.1%, two isolates), and O87:H15 (3.6%, one isolate). Most of the samples were considered to be non-typeable for the somatic antigen (O), but 75% (21 isolates) exhibited the H32 flagellar antigen. Only one strain (3.6%) was considered rough, and typing of the O antigen was impossible even though it contained the flagellar antigen H16.

In the serotyping analysis, three isolates of the O139:H19 serotype had four of five genes associated with APEC virulence and exhibited high pathogenicity. In this regard, Wang et al. (2018) identified a relationship between serogroup O139 and verotoxigenic *E. coli* (VTEC) strains isolated from fecal samples of bovine species. Therefore, relying solely on serotyping may not be adequate for determining the virulence of a strain and may not be sufficient for classifying pathotypes. The serogroups most associated with pathogenic APEC are O1, O2, and O78 (Ewers et al., 2005), whereas in the present study, the majority of the isolates were non-typeable. However, serotype O139: H19 isolates can be classified as virulent *Escherichia coli* based on their virulence profiles. However, the strains classified as serotype O153:H34 exhibited no pathogenicity and had only one of the five APEC-related genes; thus, they could not be categorized as the APEC pathotype. Balière et al. (2016) demonstrated a relationship between serogroup O153 and *enteropathogenic E. coli* (EPEC) strains isolated from mollusks and their respective breeding areas. In addition, the H32 antigen was the most common among *E. coli* strains and was related to both virulent and non-virulent strains. Although the literature lacks specific data regarding this antigen, it has previously been found in strains of enterohemorrhagic *E. coli* (EHEC) associated with the O26 antigen in a group of avirulent strains and is related to non-adherent properties (Piazza et al., 2013).

In the pathogenicity test, six (21.4%) of the *E. coli* isolates exhibited high pathogenicity in the mortality test of day-old chicks (Table 1). Eight isolates (28.5%) exhibited intermediate pathogenicity, six (21.4%) exhibited low pathogenicity,

and eight (28.5%) were considered non-pathogenic. Notably, six *Escherichia coli* isolates were classified as highly pathogenic, with two isolates originating from the cloaca and four from chicken meat. None of the strains isolated from human feces exhibited high pathogenicity in the *in vivo* day-old chicken assay. Furthermore, all of the isolates exhibited diverse genetic profiles with variations in the presence or absence of certain genes. However, it is important to highlight that all of them were classified as multidrug-resistant. According to Barbieri et al. (2013), virulence genes capable of encoding different virulence factors may influence the pathogenicity of bacterial strains. Moreover, the highly pathogenic and multidrug-resistant isolates detected in chicken meat and cloaca may pose a risk to individuals who handle and consume these contaminated food products. Additionally, this study revealed the presence of bacteria with low and intermediate pathogenicity in the cloaca, chicken meat, and human feces. This suggests the existence of different strains with similar genetic and phenotypic characteristics in avian production. This observation aligns with previous studies indicating that pathogens isolated from human infections share genetic relatedness with strains found in animals, such as poultry and swine (Manges, 2016; Projahn et al., 2019), thus reinforcing the role of these animals as potential sources of infection by these microorganisms.

In the present study, all of the isolates (*E. coli* and *K. pneumoniae*) were resistant to at least six antimicrobials, mainly cephalothin, ceftazidime, tetracycline, ampicillin, norfloxacin, sulfamethoxazole, and trimethoprim. They were also resistant to at least three different antimicrobial classes, which characterizes them as multidrug-resistant, as detailed in Table 1. This suggests that these strains harbor other resistance genes. While antimicrobial-resistant enterobacterial strains have previously been identified in human and food samples (Antunes et al., 2019), the findings of this study, along with the literature (Abreu et al., 2023), suggest that there may be indiscriminate and incorrect use of antimicrobials in animal production. Such practices can potentially contribute to an increase in microbial resistance through the selective pressure exerted by already resistant strains (Tamhankar & Lundborg, 2019).

Conclusion

This study revealed that enterobacteria isolated from poultry can carry different virulence- and resistance-related genes and exhibit different serotypes and levels of pathogenicity. These characteristics result in highly pathogenic microorganisms that are capable of causing diseases in animals and humans. Antimicrobial resistance can further exacerbate the challenge of treating these infections, as it not only complicates therapy but also promotes the survival and dissemination of these pathogens. These findings demonstrate potential risks to animal and human public health. They also highlight the increasing prevalence of pathogenic *blaCTX-M-15* in Brazil, serving as a warning to authorities regarding the importance of proper sanitary management in animal production and the responsible use of antimicrobials.

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Ethical Declaration

All *in vivo* experiments were performed in accordance with the guidelines and regulations approved by the Animal Ethics Commission (CEUA; Protocol No. 015560/18).

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