

Original Article

Avian pathogenic *Escherichia coli* (APEC) and uropathogenic *Escherichia coli* (UPEC): characterization and comparison

Daniela Tonini da Rocha¹, Felipe de Oliveira Salle¹, Karen Apellanis Borges¹, Thales Quedi Furian¹, Vladimir Pinheiro do Nascimento¹, Hamilton Luiz de Souza Moraes¹, Carlos Tadeu Pippi Salle¹

¹ Centro de Diagnóstico e Pesquisa em Patologia Aviária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Abstract

Introduction: Avian pathogenic *E. coli* (APEC) and uropathogenic *E. coli* (UPEC) are responsible for avian colibacillosis and human urinary tract infections, respectively. There are genetic similarities between the APEC and UPEC pathotypes, suggesting the APEC strains could be a potential reservoir of virulence and antimicrobial-resistance genes for the UPEC strains. This study aimed to characterize and compare APEC and UPEC strains regarding the phylogroup classification, pathogenicity and antimicrobial susceptibility.

Methodology: A total of 238 APEC and 184 UPEC strains were selected and characterized. The strains were assayed for antimicrobial susceptibility and classified into phylogenetic groups using a multiplex-PCR protocol. In addition, the APEC strains had previously been classified according to their *in vivo* pathogenicity.

Results: The results showed that both pathotypes had variation in their susceptibility to most of the antimicrobial agents evaluated, with few strains classified as multidrug resistant. The highest resistance rate for both pathotypes was to amoxicillin. Classifying the APEC and UPEC strains into phylogenetic groups determined that the most frequently frequencies were for groups D and B2, respectively. These results reflect the pathogenic potential of these strains, as all the UPEC strains were isolated from unhealthy patients, and most of the APEC strains were previously classified as pathogenic.

Conclusions: The results indicate that distribution into phylogenetic groups provided, in part, similar classification to those of *in vivo* pathogenicity index, as it was possible to adequately differentiate most of the pathogenic and commensal or low-pathogenicity bacteria. However, no relationship could be found between the specific antimicrobial agents and pathogenicity or phylogenetic group for either pathotype.

Key words: APEC; UPEC; pathogenicity; antimicrobial susceptibility; phylogenetic groups.

J Infect Dev Ctries 2021; 15(7):962-971. doi:10.3855/jidc.14217

(Received 21 October 2020 – Accepted 15 December 2020)

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Introduction

Escherichia coli is a part of both the animal and human commensal flora and can be found in several environments. *E. coli* is also a well-known pathogen that can be divided into two major groups: diarrheagenic *E. coli* (DEC) and extraintestinal pathogenic *E. coli* (ExPEC). The DEC strains are responsible for gastrointestinal infections, while ExPEC strains are responsible for diseases outside the intestinal tract such as sepsis, urinary tract infections and meningitis [1,2]. Avian pathogenic *E. coli* (APEC) are also ExPEC strains and are responsible for causing colibacillosis in poultry [3]. It is known that ExPEC strains comprise many lineages, but only a specific portion is responsible for the majority of infections [4]. Manges *et al.* [4] analyzed 217 studies carried out between 1995 and 2018 that performed multilocus

sequence typing (MLST) or whole-genome sequencing (WGS) to characterize ExPEC strains and observed that 20 major sequence types accounted for more than 85% of *E. coli* isolates.

Avian colibacillosis refers to any localized or systemic infection caused by an APEC strain. It is characterized as a multifaceted syndrome, which may include respiratory disease, septicemia, swollen head syndrome, yolk-sac infection, omphalitis, and cellulitis [5]. It is also an economically important disease that threatens food safety and avian welfare worldwide. Economic losses result from mortality and reduced productivity in the affected birds, including decreased hatching rates and egg production, increased carcass condemnation at slaughter, and significant costs associated with treatment and prophylaxis [6,7]. In Brazil, lesions associated with colibacillosis are among

the leading causes of poultry condemnation during slaughtering process [8-10].

Urinary tract infections (UTI) are the third most common type of infections in humans worldwide, after those involving the respiratory and alimentary tracts [11]. In addition to significant annual economic costs, these infections also result in decreased workforce productivity and high patient morbidity [12]. According to the National Kidney Foundation (New York, USA), 80% to 90% of UTIs are caused by uropathogenic *E. coli* (UPEC) strains. It is estimated that 6 to 8 million cases are diagnosed each year in the United States. The most common signs are increased urinary frequency, burning urination, pelvic pain and strong smelling and cloudy urine, and a change in urine color [13].

Both avian colibacillosis and human UTIs can be treated with antibiotics. However, antibiotic resistance is increasing, and treatment has been complicated by a rise in both the number of antibiotic-resistant strains and the prevalence of antibiotic-resistance mechanisms [12,14]. The number of multidrug-resistant *E. coli* strains has increased considerably in the last decade, limiting treatment options in both humans and animals [15,16].

E. coli strains usually present with a broad spectrum of lifestyles, phenotypes, and genotypes. This species has marked genome plasticity, which results in large variations that make it difficult to classify the strains, including differentiating among pathogenic and commensal bacteria [17]. WGS technologies have provided an improved discriminatory power to study the complete genomes of several bacterial pathogens [18]. However, WGS remains expensive in developing countries, where it represents a major cost that cannot be supported by all laboratories [19].

The genetic similarity between APEC and UPEC suggests a common ancestral origin and the possibility of potentially pathogenic strains compromising human health. If the pathotypes are genetically similar, the APEC strains can be considered potential reservoirs of virulence and antimicrobial-resistance genes for human ExPEC, including UPECs [20,21]. In Brazil, close similarities (serogroup, virulence factors, phylogenetic group, and sequence type) have been shown between APEC and human ExPEC, suggesting the important role of APEC strains associated with human ExPEC infections [1]. Nevertheless, the unambiguous distinction between ExPECs and commensal strains is difficult, as the strains that can cause extraintestinal infection are facultative pathogens and belong to the normal flora of many healthy individuals [17]. The molecular classification of APEC and UPEC strains

into phylogenetic groups proposed by Clermont *et al.* [20] can help in the identification of pathogenic or commensal strains [21].

Previous studies carried out in Brazil, characterized ExPEC strains and obtained variable results. The characterization of ExPEC isolates from UTIs has shown that virulence genes are present in both ExPEC and commensal strains, but some are more frequent in ExPEC isolates. Also, B2 pathogenic phylogroup are highly prevalent among ExPEC strains. For some antimicrobials, ExPEC presents higher resistance profiles, when compared to commensal isolates [22]. In relation to APEC isolates, highly pathogenic and multidrug resistant strains were isolated from several avian species, including broilers, laying hens, helmeted guineafowl, turkeys, and urban pigeons [16,23-27].

The aim of this study was to characterize and to compare APEC and UPEC strains regarding the phylogroup classification and antimicrobial susceptibility. The frequency of phylogenetic groups and non-susceptibility strains were compared to an *in vivo* pathogenicity index (PI) for APEC that was previously determined by our group.

Methodology

Bacterial strains

A total of 238 APEC and 184 UPEC strains isolated between 2002 and 2014 were randomly selected from our stock collection. The APEC strains were previously isolated from three different broiler sources (cellulitis lesions, broiler bedding material, and respiratory diseases) from poultry companies in the state of Rio Grande do Sul (RS, Brazil). The UPEC strains were provided by the microbiology laboratory of a public hospital in Porto Alegre (RS, Brazil) and were isolated from patients who had confirmed UTIs. All strains were previously confirmed as *E. coli* through a biochemical profile. The bacterial isolates were kept frozen at -80 °C in brain-heart-infusion (BHI) broth (Oxoid, Basingstoke, United Kingdom) supplemented with 15% glycerol (Synth, Diadema, Brazil). The bacteria were retrieved from frozen stocks and cultured overnight at 37°C in BHI broth (Oxoid, Basingstoke, United Kingdom). All strains were subsequently transferred to eosin-methylene blue (EMB) agar (Oxoid, Basingstoke, United Kingdom) and incubated at 37 °C for further 24 hours to confirm the purity of the stocks. Typical *E. coli* colonies on EMB agar were selected and biochemically identified through their production of urease and hydrogen sulfide (H₂S), methyl-red and Voges-Proskauer reactions, citrate utilization, and indole production [28].

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by the disk-diffusion technique according to the Clinical and Laboratory Standards Institute method [29]. The interpretation was based on the criteria described in the approved standards VET01-S2 [30] and M100-S26 [31], as appropriate. An *E. coli* (ATCC 25922) strain was selected to ensure the validity of the test. The following antibiotic disks (Oxoid) were used: amikacin, 30 µg; ampicillin, 10 µg; gentamicin, 10 µg; cefuroxime, 30 µg; and norfloxacin, 10 µg. All strains classified as intermediate were considered non-susceptible. Strains that presented resistance to all classes of antimicrobials tested (beta-lactams, aminoglycosides, and fluoroquinolones) were considered multidrug resistant [32]. Multiple antibiotic-resistance (MAR) indexes were defined as described by Krumperman [33].

Phylogenetic groups

An aliquot of 1 mL of BHI broth from an overnight incubation was selected for DNA extraction using a commercial kit (NucleoSpin Tissue - Macherey-Nagel, Düren, Germany). The DNA was stored at -20 °C until the analyses were performed. The APEC and UPEC strains were classified into four phylogenetic groups (A, B1, B2, D) according to the discrimination scheme developed by Clermont *et al.* [20], which is based on the detection of the *chuA*, *yjaA* and *TspE4.C2* DNA fragments through multiplex-PCR. The reaction mix (Invitrogen/Thermo Fisher Scientific, Carlsbad, USA) was composed of 2.5 µL of 10× buffer solution (50 mM KCl, 10 mM Tris-HCl; pH 8.3), 2 µL of deoxynucleoside triphosphates (2 mM), 2 µL of each primer or oligonucleotide (20 pmol), 1.5 U Go Taq Hot

Start Polymerase, 1.5 mM MgCl₂, 5 µL DNA template, and ultrapure water to a final volume of 25 µL. The sequences of the primers and the expected amplicon sizes are described in Table 1 [20]. The amplification reactions were performed in a Thermal Cycler 2720 Applied Biosystems thermocycler (Life Technologies, Singapore) under the following conditions: initial denaturation for 5 minutes at 94 °C; 30 cycles of 30 seconds at 94 °C, 30 seconds at 55 °C, and 30 seconds at 72 °C; and a final extension of 5 minutes at 72 °C. An *E. coli* (ATCC 25922) strain was used as a positive control. The amplified products were separated by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and transilluminated with UV light. The strains were classified into four phylogenetic groups, and the Shannon diversity index was determined. This index is calculated as the natural logarithm of the proportion of individuals of one particular group divided by the total number of individuals. Populations with higher indexes are considered more diverse [34].

Pathogenicity index for avian pathogenic *Escherichia coli*

An *in vivo* pathogenicity index (PI) for APEC strains was previously determined by our group [35]. Briefly, a group of 10 one-day-old chicks was intraperitoneally inoculated with 0.1 mL of a bacterial inoculum containing 10⁸ CFU/mL. First, an individual pathogenicity index (IPI) was determined, which corresponds to the PI for each bird inoculated with a strain. The inoculated chicks were evaluated for the presence of five macroscopic lesions (perihepatitis, PH; pericarditis, PC; peritonitis, PT; cellulitis, C; and airsacculitis, A) and their time to death (TD) over a

Table 1. PCR reactions for determining the phylogenetic groups: primer sequences and amplicon sizes.

Gene	Primers sequences (5'-3')	Amplicon size (pb)
<i>chuA</i>	F: GACGAACCAACGGTCAGGAT R: TGCCGCCAGTACCAAAGACA	279
<i>yjaA</i>	F: TGAAGTGTCAGGAGACGCTG R: ATGGAGAATGCGTTCCTCAA	211
<i>TspE4C2</i>	F: GAGTAATGTCGGGGCATTCA R: CGCGCCAACAAAGTATTACG	152

Table 2. *In vivo* pathogenicity index: classification and distribution of avian pathogenic *Escherichia coli* (APEC) strains selected for this study.

<i>In vivo</i> pathogenicity index ¹		Total of strains (n)	Relative frequency (%)
Classification	Index range		
non-pathogenic	0	23	9.7
low	1 to 3	91	38.2
intermediate	4 to 6	47	19.7
high	7 to 10	77	32.4

¹Previously described by Souza *et al.* [35].

period of 7 days. For the IPI calculation, which ranges from zero to ten, the following formula has been used: $IPI = (TD \times 5) + PC + PH + A + C$. The presence of each lesion type corresponded to one point, and the total time until death corresponded to the remaining 5 points. To obtain the time-to-death value, an index of 1, corresponding to the maximum value, was divided by the number of days that the birds were evaluated (7 days), resulting in a value of 0.1428, which is a survival-bonus factor. Thus, every day that the birds survived was discounted 0.1428 of the time-to-death value total. The PI for each strain was determined as the mean of the IPIs for 10 inoculated chicks from the same group. The *E. coli* strains were classified into 4 pathogenicity groups according to this PI: high pathogenicity (PI ranging from 7 to 10), intermediate pathogenicity (PI ranging from 4 to 6), low pathogenicity (PI ranging from 1 to 3), and non-pathogenic (PI equal to zero). The *in vivo* PI classifications of the APEC strains used in this study and their distribution [35] are described in Table 2.

Statistical analysis

The obtained data were subjected to statistical analysis using the GraphPad Prism software (San Diego, US) and the Statistical Package for Social Sciences® (SPSS) (Armonk, US). Descriptive statistics were used to determine the frequencies of the samples according to phylogenetic group and antimicrobial resistance. The non-parametric Fisher exact test was used for the comparison of the frequencies in contingency tables of categorical and independent variables (antimicrobial susceptibility, phylogenetic groups, pathogenicity classification) between pathotypes (APEC and UPEC) and among variables. As the variable pathogenicity index were not normally distributed (Kolmogorov–Smirnov test); the non-parametric Kruskal–Wallis test and Dunn's *post hoc* test were used for comparison of the mean of pathogenicity index for the phylogenetic group. Significance was defined at $p < 0.05$. Bonferroni correction was applied to adjust confidence intervals for multiple hypothesis testing.

Results

Antimicrobial susceptibility testing

The resistant strains to the five antimicrobials tested, mean MAR, and total number of multi-drug resistant strains are described in Table 3. Comparing the APEC and UPEC strains, the statistical analysis indicated higher resistance rates among UPEC strains for ampicillin, cefuroxime, and norfloxacin ($p < 0.05$);

among APEC strains, resistance was higher for gentamicin ($p < 0.05$). Comparing resistance within pathotypes, UPEC strains presented the highest resistance rate to ampicillin ($p < 0.010$). Among APEC strains, the relative frequency of ampicillin-resistant strains was higher, but did not differ ($p > 0.010$) from norfloxacin. A total of five APEC strains (2.1%) and eight UPEC strains (4.3%) were considered multidrug-resistant strains, with no statistical difference ($p > 0.05$) between the groups. Similarly, the MAR index was low for both pathotypes.

Phylogenetic groups

The multiplex-PCR protocol was able to differentiate 100% of the APEC and UPEC strains into the four proposed phylogenetic groups. The relative and absolute frequencies of the phylogenetic groups observed are described in Table 4. Comparing the APEC and UPEC pathotypes, frequencies were significantly higher ($p < 0.05$) in APEC for phylogenetic groups A and B1; and Group B2 were more frequent ($p < 0.05$) among UPEC strains. Phylogenetic Group B2 presented the lower frequency among the APEC strains, but did not differ from Group A ($p > 0.012$).

Pathogenicity index by phylogenetic groups

The PIs of the APEC strains according to their phylogenetic group distribution are described in Table 5. The mean PI was significantly higher ($p < 0.05$) for the phylogenetic groups B2 and D, but B2 and B1 did not present significant difference ($p > 0.05$). Corroborating this finding, 73.9% (17/23) of non-pathogenic and 68.1% (62/91) of low pathogenicity strains were classified in commensal groups (A and B1) ($p < 0.05$). 78.1% (52/77) of high pathogenicity strains were classified in pathogenic groups (B2 and D) ($p < 0.05$). Intermediate strains did not show differences between commensal and pathogenic groups ($p > 0.05$). However, individual analyzes of pathogenicity index classification according to the phylogenetic groups have shown similarities ($p > 0.012$) among groups (Table 5).

Pathogenicity index by antimicrobial susceptibility

The distribution of the APEC strains according to their antimicrobial resistance and pathogenicity classification is described in Table 6. The resistance rates were similar ($p > 0.012$) among all phylogenetic groups, regardless the antimicrobial agent. It was not possible to determine a relationship between specific antimicrobial agents and the pathogenicity groups.

Table 3. Antimicrobial susceptibility in *Escherichia coli* strains according to the pathotype: relative frequency of non-susceptible strains, MAR, and total number of multidrug-resistant strains.

Pathotype	Total (N)	Antimicrobial susceptibility: non-susceptible strains % (n)					MAR average index	Total MDR strains (n)
		AMK	AMP	CXM	GEN	NOR		
APEC	238	2.9 (7) ^a	28.6 (68) ^a	4.6 (11) ^a	16.8 (40) ^a	20.6 (49) ^a	0.1	5 ^a
UPEC	184	1.6 (3) ^a	67.4 (124) ^b	13 (24) ^b	8.2 (15) ^b	19.6 (36) ^a	0.2	8 ^a

Avian pathogenic *Escherichia coli* (APEC) and uropathogenic *Escherichia coli* (UPEC); Amikacin (AMK), ampicillin (AMP), cefuroxime (CXM), gentamicin (GEN), norfloxacin (NOR); Multidrug resistant (MDR); Different lowercase letters in the same column indicate that there is statistical difference ($p < 0.05$) between pathotypes within the same antimicrobial agents (Fisher's exact test).

Table 4. *Escherichia coli* phylogenetic groups: relative frequency (%) of the four phylogenetic groups (A, B1, B2, and D) and Shannon diversity index, according to pathotype.

Pathotype	Total strains (N)	Phylogenetic groups - Percentage (n)				Shannon diversity index
		A	B1	B2	D	
APEC	238	24.4 (58) ^a	27.7 (66) ^a	17.2 (41) ^a	30.7 (73) ^a	1.3652
UPEC	184	15.8 (29) ^b	6 (11) ^b	51.6 (95) ^b	26.6 (49) ^a	1.1533

Avian pathogenic *Escherichia coli* (APEC) and uropathogenic *Escherichia coli* (UPEC); different lowercase letters in the same column indicate that there is statistical difference ($p < 0.05$) between pathotypes within the same phylogenetic groups (Fisher's exact test).

Table 5. Pathogenicity index of avian pathogenic *Escherichia coli* (APEC) strains according to their distribution into phylogenetic groups (absolute frequencies).

Phylogenetic group	Mean pathogenicity index for the phylogenetic group ¹	Pathogenicity index ^{2,3}			
		Non-pathogenic (n = 23)	Low (n = 91)	Intermediate (n = 47)	High (n = 77)
A (n = 58)	2.6 ^a	15 ^a	26 ^{ab}	9 ^{ab}	8 ^a
B1 (n = 66)	3.9 ^{ab}	2 ^b	36 ^b	13 ^{ab}	15 ^{ab}
B2 (n = 41)	5.5 ^{bc}	4 ^b	11 ^c	5 ^a	21 ^{bc}
D (n = 73)	5.7 ^c	2 ^b	18 ^{ac}	20 ^b	33 ^c

¹Different lowercase letters on the same column indicate that there is statistical difference ($p < 0.05$) in the mean pathogenicity index among the phylogenetic groups (Kruskal-Wallis test and Dunn's *post hoc* test). ²Classification according to pathogenicity index: non-pathogenic (0), low pathogenicity (1 to 3), intermediate pathogenicity (4 to 6) or high pathogenicity (7 to 10) [35]. ³Different lowercase letters in the same column indicate that there is statistical difference ($p < 0.012$) among frequencies of phylogenetic groups within the same pathogenicity index classification (Fisher's exact test; adjusted p -value).

Table 6. Relative (%) and absolute (n) resistance frequencies of the avian pathogenic *Escherichia coli* (APEC) strains to the evaluated antimicrobials and their pathogenicity classifications.

Antimicrobial agent	Pathogenicity classification ¹			
	Non-pathogenic % (n = 23)	Low % (n = 91)	Intermediate % (n = 47)	High % (n = 77)
Amikacin	0	3.3 (3) ^a	6.4 (3) ^a	1.3 (1) ^a
Ampicillin	17.4 (4) ^a	33 (30) ^a	31.9 (15) ^a	24.7 (19) ^a
Cefuroxime	4.4 (1) ^a	5.5 (5) ^a	4.3 (2) ^a	3.9 (3) ^a
Gentamicin	4.4 (1) ^a	14.3 (13) ^a	27.7 (13) ^a	16.9 (13) ^a
Norfloxacin	4.4 (1) ^a	25.3 (23) ^a	31.9 (15) ^a	13 (10) ^a

¹Classification according to pathogenicity index: non-pathogenic (0), low pathogenicity (1 to 3), intermediate pathogenicity (4 to 6) or high pathogenicity (7 to 10) [35]. Different lowercase letters in the same line indicate that there is statistical difference ($p < 0.012$) among frequencies within the same antimicrobial agent (Fisher's exact test; adjusted p -value).

Phylogenetic groups by antimicrobial susceptibility

The APEC and UPEC strains distributions are described in Tables 7 and 8, respectively, according to their antimicrobial susceptibility and phylogenetic classifications. Partially similar results were found for both pathotypes. Regarding APEC strains, there were no significant differences ($p > 0.012$) in susceptibility, regardless the antimicrobial agent. Among UPEC strains, significant differences ($p < 0.012$) were found only between phylogroups B1 and B2 for norfloxacin resistance.

Discussion

The APEC and UPEC pathotypes are considered pathogenic and are responsible for avian colibacillosis and human UTIs, respectively [1,2]. Due to their close similarities with respect to the serogroup, virulence factors, phylogenetic group, and sequence types, it has been suggested that APEC strains can act as a source of genetic material supplying virulence genes to other ExPEC or even in the transmission of strains [1]. However, the phylogenetic relationship between the two pathotypes (APEC and UPEC) has not been completely elucidated and requires further investigation [36].

E. coli strains are known to have marked genome plasticity and great variation, which makes it difficult to differentiate between pathogenic and commensal

bacteria [17]. Thus, in recent years, increased attention has been directed toward analyzing the phylogenetic characterization of *E. coli* strains. Differentiating among commensal and pathogenic strains could help us determine the sources of such pathogenic strains and limit the spread of multidrug resistance [37]. Clermont *et al.* [20] proposed a multiplex-PCR protocol to differentiate strains into phylogenetic groups. In this study, all strains were differentiated and classified by this method. Comparing phylogenetic group frequencies between the pathotypes, the UPEC and APEC strains had similar frequencies in Group D. However, phylogenetic group B2 was more frequent in the UPEC strains, and less frequent in APEC strains. In the literature, the distribution of APEC strains into phylogenetic groups has varied depending on several factors such as source of isolation and region or country [38-40] and has also been related to their *in vivo* pathogenicity. The distribution of our UPEC strains into phylogenetic groups was similar to those in previous reports [39,41-43].

Previous phylogenetic analyses have demonstrated that virulent extraintestinal *E. coli* strains usually belong to Group B2 and, to a lesser extent, Group D. In contrast, most commensal strains are associated with groups A and B1 [20]. This was clearly evident for the UPEC strains in this study, with more than 50% of strains classified in Group B2, and almost 27% in

Table 7. Relative (%) and absolute (n) resistance frequencies of the avian pathogenic *Escherichia coli* (APEC) strains to the evaluated antimicrobials and their classifications according to phylogenetic group.

Antimicrobial agent	Phylogenetic groups			
	A % (n = 58)	B1 % (n = 66)	B2 % (n = 41)	D % (n = 73)
Amikacin	1.7 (1) ^a	3 (2) ^a	0	5.5 (4) ^a
Ampicillin	19 (11) ^a	37.9 (25) ^a	31.7 (13) ^a	24.7 (18) ^a
Cefuroxime	5.2 (3) ^a	6.1 (4) ^a	0	5.5 (4) ^a
Gentamicin	17.6 (10) ^a	15.2 (10) ^a	14.6 (6) ^a	19.8 (14) ^a
Norfloxacin	13.8 (8) ^a	30.3 (20) ^a	19.5 (8) ^a	17.8 (13) ^a

Different lowercase letters on the same line indicate that there is statistical difference ($p < 0.012$) among phylogenetic groups for the same antimicrobial agent (Fisher’s exact test; adjusted p -value).

Table 8. Relative (%) and absolute (n) resistance frequencies of the uropathogenic *Escherichia coli* (UPEC) strains to the evaluated antimicrobials and their classifications according to phylogenetic group.

Antimicrobial agent	Phylogenetic groups			
	A % (n = 29)	B1 % (n = 11)	B2 % (n = 95)	D % (n = 49)
Amikacin	0	0	3.2 (3)	0
Ampicillin	58.6 (17) ^a	63.6 (8) ^a	71.6 (68) ^a	67.4 (33) ^a
Cefuroxime	20.7 (6) ^a	18.2 (2) ^a	9.5 (9) ^a	14.3 (7) ^a
Gentamicin	3.5 (1) ^a	0	9.5 (9) ^a	10.2 (5) ^a
Norfloxacin	27.6 (8) ^{ab}	54.6 (6) ^b	11.6 (11) ^a	22.5 (11) ^{ab}

Different lowercase letters on the same line indicate that there is statistical difference ($p < 0.012$) among phylogenetic groups in the same antimicrobial agent (Fisher’s exact test; adjusted p -value).

Group D. Although more than 30% of APEC strains were classified in Group D, only 17.2% were in Group B2. These results can be partially explained by our selection of analyzed strains. While the APEC strains were randomly selected from a stock collection, and thus had varying degrees of pathogenicity [35], all the UPEC strains were collected from patients with confirmed UTIs. It is noteworthy that in Clermont *et al.* [20] study, most ExpEC strains used were isolated from human sources. This scheme is probably not as accurate for APEC as it is for human ExPEC, and it may be biased towards human ExPEC.

The Shannon diversity index is a statistic index that assumes all groups are represented in a sample and that they are randomly sampled. Populations with higher indexes are considered more diverse [21]. In this study, the APEC strains had a slightly higher Shannon diversity index, indicating a more diversified population. This result was corroborated by the lack of significant differences among the APEC strains distributions into phylogenetic groups. Moreover, while all UPEC strains originated from unhealthy patients, the APEC strains were isolated from three different broiler sources. Similarly, Coura *et al.* [44] reported a higher Shannon index for *E. coli* isolated from poultry than other animal species.

We also analyzed the relationship between the *in vivo* pathogenicity and assigned phylogenetic group for the APEC strains. Based on the strains' PIs, we were able to calculate mean PIs for each phylogenetic group, which were similar for groups B2 (5.5) and D (5.7) and higher than those observed in Group A. Comparing the phylogenetic groups, we found that most of the non-pathogenic strains were in Group A, and the majority of low pathogenic strains were in groups A and B1. Groups B2 and D included the majority of high pathogenicity strains. These results are in line with expectations, as these groups are generally known to be pathogenic phylogenetic groups [20], and confirm our previously proposed PI [35]. It is noteworthy that APEC strains were selected from our stock collection in a partially random manner following a criterion to balance the proportion between the non-pathogenic/low pathogenicity and intermediate/high pathogenicity strains. An equal number of strains from each classification category was not possible due to the unavailability of equal proportions in our stock collection. Regarding the previously determined *in vivo* PI, our results indicate that distribution into phylogenetic groups provided, in part, similar classification to those of pathogenicity. However,

future studies with biological models for determining a PI for the UPEC strains are still needed.

Antibiotic resistance is one of the biggest threats to global health, and it leads to higher medical costs, prolonged hospital stays, and increased mortality [45], and the intensive use of antibiotics in animals may raise the risk of transmitting drug-resistant microorganisms to humans [46]. The antimicrobial susceptibility data in this study show that the resistance rates were higher for ampicillin and cefuroxime among UPEC, while resistance for gentamicin was higher among APEC strains. Ampicillin resistance was high for both groups, and it is currently one of the most commonly used antibiotics and the first broad-spectrum penicillin for the treatment of infections due to *Enterobacteria* [47], which probably contributes to the increased levels of resistance. This result is similar to previous reports in humans [48,49] and poultry [50]. The statistical analysis showed no differences between the APEC and UPEC multidrug-resistant strain frequencies. MAR indexes were similar for both pathotypes. Although these drugs represent the main antimicrobial classes, a wider range of antimicrobials could demonstrate more similarities between the APEC and UPEC isolates.

To determine if antimicrobial resistance is related to pathogenicity, we compared the susceptibility rates among the phylogenetic groups and the PIs. The analyses showed that no group could be considered the most drug-resistant, as no differences were found among APEC strains and only one difference was found in UPEC strains. Previous studies have shown a relationship between phylogenetic background and antibiotic resistance [39], which could be explained by two main factors. First, the extensive use of antibiotics to promote weight gain and for prophylaxis purposes has led to higher levels of resistance in commensal organisms. Second, pathogenic strains usually have more virulence, resistance, and plasmid-mediated resistance genes [39]. Like previous studies, which have shown that biofilm formation is not related to *in vivo* pathogenicity [51], in the present study it was not possible to determine a relationship between specific antimicrobial agents and the pathogenicity classification groups of the APEC strains. Although antibiotic resistance is not a virulence factor, it is a key factor in the development of infection and may be considered a virulence-like factor in the specific ecological niches that antibiotic-resistant bacteria colonize [52].

The study has important outcomes about the characterization of APEC and UPEC strains. However, it is noteworthy that it has limitations. In our study, we

did not carry out the MLST, which is used to identify clinically significant sequence type (ST) lineages [53]. Some specific sequences are frequently associated with pathogenic groups of ExPEC. One of the most prevalent lineages is ST131, which belongs to the B2 phylogenetic group [54,55]. This ST is usually associated with UTIs caused by *E. coli* strains [55,56,57], and it has been associated with increased antimicrobial resistance [56,57,58,59]. The sequencing typing would probably show a high frequency of ST131 among UPEC strains and explain the increased frequency of group B2 among these pathotype.

Conclusion

In this study, the APEC and UPEC strains showed variation in their resistance rates to most of the antimicrobial agents evaluated. However, the MAR index was low, and for both pathotypes, less than 5% of strains were classified as multidrug resistant. The pathotypes also varied in their phylogenetic group classifications, with groups D and B2 being the most common in the APEC and UPEC strains, respectively. The results indicate that distribution into phylogenetic groups provided, in part, similar classification to those of *in vivo* pathogenicity index, as it was possible to adequately differentiate most of the pathogenic and commensal or low-pathogenicity bacteria. However, it was not possible to determine a relationship between specific antimicrobial agents and pathogenicity or phylogenetic group in either pathotype.

Authors' contributions

V.P.N., H.L.S.M. and C.T.P.S are the leaders of this project. D.T.R., F.O.S., K.A.B., T.Q.F. V.P.N., H.L.S.M. and C.T.P.S. conceived and designed the experiments. D.T.R., F.O.S., K.A.B., T.Q.F. performed the experiments. K.A.B. and T.Q.F. analyzed the data. D.T.R., K.A.B. and T.Q.F. wrote and prepared the manuscript text. All authors (D.T.R., F.O.S., K.A.B., T.Q.F., V.P.N., H.L.S.M. and C.T.P.S.) critically reviewed and approved the manuscript.

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Corresponding author

Karen Apellanis Borges, PhD
Universidade Federal do Rio Grande do Sul, 9090 Avenida Bento Gonçalves.
Phone: +55 51 3308-6138
Fax: +55 51 3308-6138
Email: karen.borges@ufrgs.br

Conflict of interests: No conflict of interests is declared.