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Original Articles

Highly Pathogenic and Multidrug Resistant Avian Pathogenic Escherichia Coli in Free-Range Chickens from Brazil

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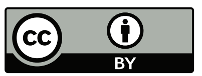
■Keywords

APEC, backyard chickens, colibacillosis, pathogenicity, virulence factors.



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ABSTRACT

Avian pathogenic *Escherichiacoli* (APEC) virulence mechanism has been continuously studied and it is believed to be multifactorial and because of this, this work aimed to characterize potentially APEC strains isolated from free-range hens. Isolates were submitted to PCR for the detection of virulence genes, which were of high prevalence. *In vivo* inoculation of day-old chicks revealed that 49 of these strains were of high and intermediate pathogenicity. In addition, isolates were submitted to antimicrobials susceptibility test with the majority of the strains presenting multiresistance. Phylogenetic analysis showed a greater presence of potentially APEC isolates in-group B2. In addition, high heterogeneity was detected among the isolates byXbaI enzyme. Fifteen serogroups were identified, being the O8 the most frequent. These results strengthen the fact that a combination of diverse factors are associated with the pathogenicity APEC strains, as well as to highlight its importance to public health and that free-range hens can act as a reservoirs of potentially zoonoticbacteria.

INTRODUCTION

Small producers in the word performmainly free-range hens raising, with precarious installations and minimal management often being its main limiting factors. These practices may contribute to the spread of diseases to both birds and consumers (Thekisoe*et al.*, 2003). In this regard, the avian pathogenic *Escherichia coli* (APEC) is a major agent with increasing interest among avian sanity, being associated with a series of extra-intestinal systemic infections, collectively referred as colibaciloses (Kaper *et al.*, 2004) and it is responsible for economic losses on poultry industry (Dho-moulin & Fairbrother, 1999).

E. coli strains pathogenicity are related to virulence factors that are used to differentiate between pathogenic and non-pathogenic strains (Rodriguez-Siek *et al.*, 2005). In this regard, a large number of potential virulence factors have been detected; however, virulence mechanisms have not yet been fully elucidated and, thus, require further studies. There is still no consensus in the literature as to which genes would be the ideal virulence markers. So far, it has been shown that APEC strains present virulence genes, which can be translated into adhesins, toxins, siderophores, colicin, serum resistance and others (Barbieri *et al.*, 2013).

However, it is known that the zoonotic potential of APEC strains is evidenced when common virulence factors of APEC are found in *E. coli*strains, thus resulting in extra intestinal diseases in humans. This characterize a positive relationship between the APEC, UPEC and NMEC (Johnson *et al.*, 2008). Hens and humans often share the same environment; there, these birds may present an important source of human infection, as well as acquire human strains. Glimpsing the difficulty to define APEC pathotype, this work aims to evaluate possible

genes related to APEC virulence and analyze the phenotype of isolates obtained from free-range hens raising in Brazil.

MATERIAL AND METHODS

This experiment was approved by the Committee on Ethics for the Use of Animals of the São Paulo State University (Unesp), School of Agricultural and Veterinary Sciences, São Paulo, Brazil, under protocol number 05749/14.

Population analyzed and Collection of samples

Samples were collected from 250 hens of unknown genetic origin and different age from seven small farms within Ribeirao Preto region, Sao Paulo state, Brazil, from January to April 2014. Five hundred samples were obtained, being 250 from the cloaca and 250 from the oropharynges. After collection, samples were placed in tubes containing 5 mL of BHI broth and kept on ice until arrival at the laboratory.

Detection of pathotypes and virulence genes

In order to detect APEC, a PCR screening was performed for the *cva*C, *iro*N, *iss*, *iut*A, *omp*T and *hly*F genes. Samples that were positive for at least five of the genes were used to detect *E. coli* isolates as recommended by Kemmett *et al.* (2013). DNA template preparation as well as PCR procedures and primers were used according to the EcL protocol available at http://www.apzec.ca/en/APZEC/Protocols/APZEC_PCR_en.aspx. In addition, all isolates were evaluated for 11 additional virulence genes as follow: *sit*A, *tsh*, *tra*T, *vat*, *ast*A, *iuc*C, *iuc*D, *pap*C, *irp*2, *fim*H and *fyu*A; also following the EcL protocol as cited above.

Serological identification and antimicrobial susceptibility test

Serotyping was performed by plate agglutination procedure according to Orskov *et al.* (1977) at the "*E. coli* Reference Center "(ECRC) at the Pennsylvania State University - USA. Serology was carried out using serogroups O1-O181 antisera with the exceptions of O31, O47, O72, O93, O94, and O122. Additionally, isolates were submitted, by the disc diffusion method (CLSI, 2010), to antimicrobial susceptibility testing against the following: ampicillin (10µg), cephalothin (30µg), streptomycin (10µg), gentamicin (10µg), ciprofloxacin (5µg), chloramphenicol (30µg), tetracycline (30 µg), nitrofurantoin (300µg), sulfamethoxazole + trimethoprim (25µg), ceftiofur (30µg), ceftriaxone (30µg), amoxicillin + clavulanic acid (30µg), norfloxacin (10µg) and fosfomycin (50µg).

Phylogenetic Typing and Pulsatile Field Electrophoresis (PFGE)

Identification of *chu*A and *yja*A genes and *Tsp*E4. C2 DNA fragment was performed with the primers proposed by Clermont *et al.* (2000). Genomic DNA digestion with XbaI and plug preparation was done as described by Ribot*et al.* (2006) with modifications and Salmonella strain Braenderup H9812 was used as a molecular weight reference. Migration was performed on 1% Pulsifield certified agarose gel with an initial time of 2.2 seconds and final time of 54.2 seconds on a 6 V cm-1 gradient and 120° angle for 23h at a temperature of 14°C. Similarity analysis was performed using Dice coefficients with 1% band position tolerance and 0,5% optimization. In addition, a dendrogram was obtained by UPGMA. These analysis were performed with the BioNumerics software version 7.1 (Applied Maths, Sint -Martens-Latem, Belgium).

Pathogenicity test

A 0.1 ml of bacterial culture were inoculated into the left thoracic air sac of 1 day-old chicks as described by Monroy *et al.* (2005). For inoculum preparation, a colony of each bacterial strain was seeded in 10 ml of BHI broth, incubated for 18 hours at 37 °C and subsequently diluted to a 1:10 ratio. Inoculum concentration was standardized to 10^7 CFU/mL. The *E. coli* (serogroup O1) belonging to the Laboratory of Ornithopathology of USP, was used as a positive control. Negative control birds were inoculated with BHI broth only. For each strain, as well as for the negative and positive control groups, ten male chicks from a commercial lineage were used. The strains were classified due to its mortality as follow: high (≥ 80%), intermediate (> 50% and <80%), low pathogenicity (≤ 50%) and non-pathogenic (zero mortality).

RESULTS AND DISCUSSION

Detection of additional pathotypes and virulence genes

From the 500 samples screened by PCR, 139 (27.8%)samples were positive for at least five of the APEC related genes (*cva*C, *iro*N, *iss*, *iut*A, *omp*T and *hly*F), of these, 75 were from the cloaca and 64 from the oropharynx. Of these positive samples, 69 (49.6%) strains (36 cloaca and 33 oropharynx) were isolated. The frequency of the referred genes observed in *E. coli* isolates and other virulence genes is shown in Table 1.

Table 1 – Frequency of each gene associated with virulence in the potentially 69 isolates of APEC from free-range hens.

Function	Genes	Frequency (%)
Adhesion	*fimH*	82.6
	papC	1.5
	tsh	30.4
Iron acquisition	*fyuA*	39.1
	iroN	87.0
	iucC	58.0
	iucD	74.0
	irp2	76,8
	iutA	88.4
	sitA	89.8
Haemolysin	*hlyF*	100
Serum resistance	*iss*	100
	traT	82.6
Toxins	*astA*	15.9
	vat	17.4
Multiple functions	*cvaC*	66.7
	ompT	100

High percentages (75.4%) of all six genes were observed, and according to Rodriguez-siek *et al.* (2005), *tra*T gene associated with the *cva*C gene in the APEC strains make part of the serum effects resistance mechanism and are usually associated with septicemia. In accordance with the definition of Ewers *et al.* (2007) for an isolate to be considered pathogenic the presence of at least one adhesion factor is necessary, one of iron acquisition and one of serum resistance. The present study observed that 64 (92.7%) isolates from free-range chicken harbored at least one adhesion-related virulence factor, one iron acquisition factor and a serum resistance factor. Serum resistance, is mainly encoded by the *iss* gene (Monroy *et al.*, 2005), and was found in 100.0% of the isolates and according to Tivendale *et al.* (2004) the iss gene was related to high levels of virulence and some authors report it is of high prevalence. In an interesting manner, the present study detected a frequency of 15.9% of the astA gene, with a similar frequency of the study of Won *et al.* (2009) of 17.8%. However, it is lower than the 20.0% frequency observed by Ewers *et al.* (2004). These differences may be, according to this author, due to this gene being present in a pathogenicity island.

Serological identification

In this study, fifteen serogroups were identified: O2 (8.7%), O5 (1.5%), O8 (23.2%), O9 5%), O11 (1.5%), O20 (1.5%), O38 (1.5%), O64 (1.5%), O88 (1.5%), O109 (1.5%), O117 (1.5%), O119 (2.9%), O120 (1.5%), O149 (2.9%), O158 (4.4%) (Table 3). According to Silveira *et al.* (2002), a diversity of serogroups are involved with colibacillosis, and have a relationship with the geographic and temporal factors, resulting in variations of prevalence of different clonal groups. Of the 69 samples, 30 (43.8%) were not typable for the O antigen, which is expected because the frequency non-typical samples could vary between 14.0% and 39.0% (Menão *et al.*, 2002).

Table 3 – Serogroup distribution within the 69 potentially APEC isolate.

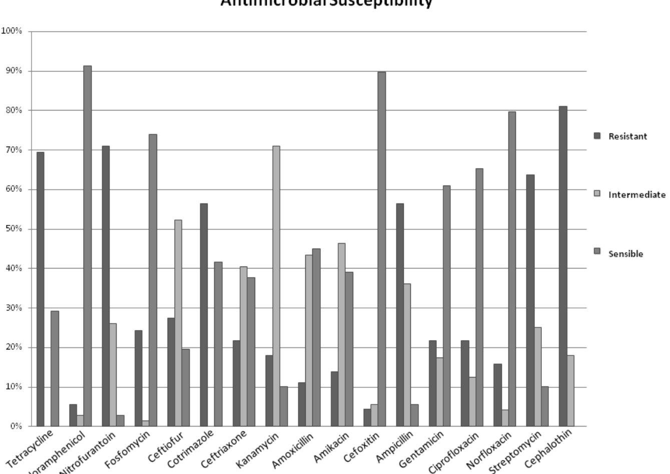
Serogroup	Number of samples	Percentage (%)*
O2	6	8.7
O5	1	1.5
O8	16	23.2
O9	1	1.5
O11	1	1.5
O20	1	1.5
O38	1	1.5
O64	1	1.5
O88	1	1.5
O109	1	1.5
O117	1	1.5
O119	2	2.9
O120	1	1.5
O149	2	2.9
O158	3	4.4
NT**	30	43.5

*Percentage of 69 isolates.

**Nottyphable.

Antimicrobial susceptibility test

All 69 tested isolates demonstrated resistance to at least one antimicrobial agent and most of them showed a multi-drug resistance profile with 59 (85.5%) isolates simultaneous resistant to three or more antimicrobials (Figure 1). It is often common that birds *E. coli* isolates present resistance to more than one antimicrobial, being the main reasons the indiscriminate and prolonged use of sub-therapeutic concentrations and inadequate use antimicrobial therapies (Mellata *et al.*, 2013). Some studies carried out in Brazil report that APEC strains resist to all classes of drugs, with sulfonamides and tetracyclines having the highest indexes, ranging from 50.0% to 90.0% (Zanatta *et al.*, 2004). Accordingly, the present study verified isolates with high resistance for tetracycline (69.5%) and sulfonamides (58.3%). In addition, among the aminoglycosides tested, streptomycin presented a higher index (63.8%). An explanation for these high levels of resistance resides in the fact that 100% of the strains were *iss* positive. This gene, in addition to the increased serum resistance, may lead to resistance to various antimicrobials (Abreu *et al.*, 2010) and can be transferred, by conjugation, to other nonvirulent bacteria, including ones of different species thus they become more pathogenic and resistant (Johnson *et al.*, 2006).



Phylogenetic Typing

The analysis revealed that most of the isolates in this study belong to phylogenetic group B2 (37/69), followed by group A (17/69), group B1 (13/69) and group D (2/69), as shown in Table 2. Studies of phylogenetic analyzes have shown that *E.coli* isolates can be grouped into four main phylogenetic groups: A, B1, B2 and D. Extra-intestinal pathogenic samples with a large variety of virulence factors are concentrated in groups B2 and D. Meanwhile, commensal samples are concentrated in groups A and B1 (Le Gall *et al.*, 2007). Isolates of phylogenetic groups B2 and D were associated with a greater number of virulence factors, presenting a mean of 11.5 and 13.5 virulence factors per isolate, respectively. Phylogenetic groups A and B1 presented 9.9 and 10.8 virulence factors per isolate, respectively. Studies suggest that virulent clonal groups are mainly derived from phylogenetic group B2 and, to a lesser extent, from group D, explaining the predominance of groups B2 and D, among clinical isolates (Johnson & Russo, 2002).Based on these results, it can be inferred that there is no group that comprise exclusively pathogenic or non pathogenic isolates, but rather that groups present combinations of commensal and pathogenic isolates, reinforcing the hypothesis that APEC should be considered a potential zoonotic agent.

Table 2 – Distribution of the 69 potentially APEC isolate in correlation with the phylogenetic group.

Phylogenetic group (nº - %)	VF mean/isolate*
A (17 – 24.6%)	9.9
B1 (13 – 18.8%)	10.8
B2 (37 – 53.6%)	11.5
D (2 – 2.9%)	13.5

*Represents a sum total of the virulence factors of the group and divided by the number of isolates.

Pulsatile Field Electrophoresis (PFGE)

Of the 69 potentially APEC isolates, only one was not typable by the XbaI enzyme with the remaining 68 isolates generating 59 pulse types. All other isolates were grouped into single pulse types, demonstrating that a high degree of heterogeneity is present among the APECs examined with the generated dendrogram by PFGE presenting three large clusters (Figure 2). Six pulse types were shared by more than one isolate, with 100% similarity. The pulse types 1Ca3 and 5Ca1, 21Fa4 and 71Fa1, 37Ca1 and 71Fa5 belonged to the same property. This could be explained by the fact that the birds were in constant contact in almost all the properties visited facilitating the transmission of clones. The pulse types 52Ca1 and 81Ca3, 52Ca5 and 82Fa1, 56Ca4 and 88Ca4from free-range hens from different locations present different genotypic profiles, pathogenicity and phylogenetic typing with only the last pulsetype presenting different serogroups, and the first two being non-typable for the O antigen. Pulsetypes with similarity greater than 95.0% were also found within the same bird. The isolates were from samples collected from the oropharynx, being: 149Fa3 and 149Fb2, 175Fa1 and 175Fb1, and 236Fa1 and 236Fb1, presenting genotype and phenotype similarities, except for pulse 149Fa3 and 149Fb2, which although were from the same bird, presented in the *in vivo* test, quite distinct results, being of low and high pathogenicity. Differences between the presence and absence of virulence genes between these pulses could be explained by the presence of a capsule that are found in virulent APEC strains (Moulin-schouleur *et al.*, 2006) and to genetic changes either caused by mutation (chromosome alteration) or by genetic transfer (Skyberg *et al.*, 2003).

Pathogenicity

Forty-three (62.3%) strains were highly pathogenic, six (8.7%) strains were intermediate pathogenic, 16 (23.2%) strains were low pathogenic, and four (5.8%) were non-pathogenic. All positive control birds deceased, while negative control birds remained alive. Clinical signs and macroscopic lesions were observed in higher frequency among birds inoculated with the high and intermediate pathogenic strains. Guastalli *et al.* (2013) in a study with commercial laying hens that showed signs of colibacillosis obtained approximately 50.0% of their isolates with high or intermediate pathogenicity, frequency below our result. This can be explained by the fact that free-range chickens have a higher genetic variability and greater rusticity, which

Isolate	Phylogeny	cvaC	hlyF	iroN	ompT	iss	iutA	astA	fimH	fyuA	irp2	iucC	iucD	papC	astA	traT	tsh	vat	Pathogenicity	Serogroup
189Cb2	B2	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	-	-	High	NT
210Fa2	B2	-	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	Low	NT
16Ca2	B2	-	+	+	+	+	+	-	+	+	+	-	+	-	+	+	-	+	High	O119
13Ca1	A	-	+	+	+	+	+	+	+	-	+	-	+	-	+	+	-	-	High	NT
10Ca1	B2	+	+	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	Intermediate	NT
11Ca2	B2	+	+	+	+	+	+	-	+	-	+	-	+	-	+	-	-	-	Intermediate	O8
58Cb2	B2	-	+	+	+	+	+	-	+	+	+	+	+	-	-	+	-	+	High	NT
88Ca5	B1	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	+	-	High	NT
13Fa1	B2	-	+	+	+	+	+	-	+	+	+	-	+	-	+	+	-	+	High	NT
70Fa1	B2	+	+	+	+	+	-	-	+	-	+	-	-	-	+	+	-	-	High	O8
156Fa1	B2	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	-	Low	NT
237Cb4	B2	+	+	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+	High	NT
77Fb1	B2	-	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	High	O8
113Cb1	B1	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	Low	O11
113Cb5	B1	+	+	-	+	+	+	+	+	+	-	-	+	-	+	-	-	-	Nonpathogenic	NT
1Ca3	B2	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	-	-	Low	O8
5Ca1	B2	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	-	High	O8
4Ca1	B2	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	-	Intermediate	O8
8Cb3	B2	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	-	Low	O8
9Fa1	B2	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	-	-	Low	O8
2Fa4	A	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	Nonpathogenic	O158
236Fa1	B1	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	High	O158
236Fb1	B2	+	+	+	+	+	+	-	+	+	-	-	+	-	+	-	+	+	High	O158
236Fb2	B1	+	+	-	+	+	+	-	-	+	-	-	+	-	+	-	+	+	High	NT
16Fa1	B2	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	High	NT
64Ca3	B2	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-	Low	NT
2Ca1	B2	+	+	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	Intermediate	NT
193Fb3	A	+	+	+	+	+	-	-	+	-	+	-	-	-	+	+	-	-	Low	O20
202Ca1	A	+	+	+	+	+	-	-	+	-	+	-	-	-	+	+	-	-	High	NT
52Ca1	A	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+	-	-	Nonpathogenic	NT
81Cb3	B1	+	+	+	+	+	+	+	-	+	-	-	+	-	+	+	-	-	High	NT
153Fa1	A	-	+	+	+	+	+	+	-	-	-	+	+	-	+	-	+	-	High	O5
212Fb4	B2	-	+	+	+	+	+	-	+	-	+	-	-	-	+	+	-	-	High	O149
52Ca5	D	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	Low	NT
82Fa1	B2	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	High	NT
86Ca1	A	-	+	+	+	+	+	+	-	-	+	+	+	-	+	-	+	-	High	NT
52Fa4	D	-	+	+	+	+	+	-	+	+	+	+	+	-	-	+	-	+	High	O119
145Fa3	B2	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	+	Low	NT
211Fb2	A	+	+	+	+	+	-	-	+	-	+	-	-	-	+	+	-	-	Low	O8
169Ca4	B1	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	High	O2
182Fa4	A	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	High	O2
116Fa4	B1	+	+	-	+	+	+	-	+	-	+	+	+	-	+	+	+	-	Low	NT
168Fa3	B1	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	-	High	NT
16Ca3	B2	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	+	High	O120
61Ca1	B2	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	High	O109
247Fb2	A	+	+	-	+	+	+	-	+	+	+	+	-	-	+	+	-	-	High	O9
56Ca4	B2	+	+	+	+	+	-	-	+	-	-	-	+	-	+	+	+	-	Intermediate	O149
88Ca4	B1	+	+	-	+	+	+	-	+	-	-	-	-	-	+	+	+	-	High	O2
21Fa4	A	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+	-	-	Intermediate	O8
71fa1	B2	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	-	High	O88
175Fa1	A	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	-	High	NT
175Fb1	A	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	-	High	O2
132Ca2	A	+	+	-	+	+	+	-	+	-	-	+	+	-	+	+	-	-	High	NT
239Fa2	B2	+	+	+	+	+	+	-	+	+	+	-	+	-	+	+	-	-	High	O8
88Fa2	B2	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	-	-	High	NT
133Ca1	B2	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	-	-	High	O8
133Ca5	B2	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	-	-	High	O8
179Cb4	B2	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	-	Low	NT
143Fa4	B2	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	-	High	O8
188Cb1	B2	-	+	+	+	+	+	-	-	-	+	-	-	-	+	+	-	-	High	O64
149Fa3	B1	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	Low	O2
149Fb2	B1	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-	High	O2
46Ca2	A	+	+	+	+	+	+	+	-	-	-	+	-	-	+	+	+	-	High	NT
77Ca1	B1	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	Low	O38
135Ca1	A	-	+	+	+	+	+	+	-	-	+	+	+	-	+	-	+	-	High	NT
37Ca1	A	+	+	+	+	+	-	-	+	-	+	-	-	-	+	-	-	-	Low	O8
71Fa5	B2	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	-	High	O8
157Fa1	B2	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	-	Nonpathogenic	NT

gives them resistance to diseases, adverse climate and food conditions (Albino *et al.*, 2001). Interestingly, in this study, 29.0% of the low or non-pathogenic isolates presented a high number of genes related to APEC virulence. According to Ikuno *et al.* (2006), the presence of a virulence gene in commensal *E. coli*strains can be used as an indicator of potential risks, but it is necessary to investigate beyond the presence of the genes and look for their expression. Thus, it can be concluded that, even if healthy, the "backyard chickens" are carriers and can disseminate pathogenic *E. coli* strains, of which can be transferred to other birds and/or animals, thus representing an important source of infection or reservoir.

Although APEC is not pathogenic to humans, it is of concern that poultry samples presents similarities to those of humans and that most virulence genes are similar to the ones identified in extra-intestinal strains causing diseases in humans, thus representing zoonotic risk (Johnson & Russo, 2002). The results found in the present study showed that hens act as reservoirs of multi-drug resistant and highly pathogenic ExPEC, representing a risk to the consumer, mainly because these birds live in close proximity to humans and other animals. It is important to emphasize that animals with virulence factors are an important source of infection, since the bacteria can be excreted along with feces or expelled by the respiratory tract. This is reinforced by the fact that although the isolates were obtained from samples of apparently healthy hens, and that, in principle, should be non-pathogenic; more than 70.0% were of high or intermediate pathogenicity by the test performed in 1 day-old chicks. Therefore, APEC studies in free-range hens, that can act as reservoirs and disseminators of this pathogen, contribute to improve methods for diagnosis, control, prevention and treatment of the diseases caused by this bacterium.

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