Antibiotic resistance and virulence factors among *Escherichia coli* isolates from avian organic fertilizer

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ABSTRACT: *Brazilian poultry industry generates large amounts of organic waste, such as chicken litter, which is often used in agriculture. Among the bacteria present in organic fertilizer are members of the Enterobacteriaceae family, such as Escherichia coli. The aim of this study was to analyze the profile of virulence factors and antimicrobial resistance of E. coli isolates from avian organic fertilizer. A total of 47 E. coli strains were tested through Polymerase chain reaction to detect virulence genes (hlyF, iss, ompT, iutA and iroN). Fourteen antimicrobials were used to test antimicrobial susceptibility in the strains. Genes characteristic of Avian Pathogenic E. coli (APEC) were reported among the strains, with the hlyF, iss and ompT genes being the most prevalent. The isolates showed high resistance (>50%) to tetracycline, gentamicin, cefotaxime, nitrofurantoin, trimethoprim-sulfamethoxazole and ampicillin. Multidrug resistance was reported in a great number of strains (>70%). The results showed the presence of APEC cells with virulence genes and antimicrobial resistance after 15 days of the windrowing process in poultry houses, it means this process should be improved to eliminate these cells.*
Key words: *poultry production, Enterobacteriacea, zoonotic risk, virulence factors.*

Resistência a antibióticos e fatores de virulência entre isolados de *Escherichia coli* provenientes de fertilizante orgânico aviário

RESUMO: *A indústria avícola brasileira gera grandes quantidades de resíduos orgânicos, como a cama de frango, utilizada frequentemente na agricultura. Entre as bactérias presentes neste fertilizante orgânico estão os membros da família Enterobacteriaceae, entre eles a Escherichia coli. O objetivo deste estudo foi analisar o perfil de fatores de virulência e resistência antimicrobiana de isolados de E. coli provenientes do fertilizante orgânico aviário. Um total de 47 cepas de E. coli foram testadas por meio da reação em cadeia da polimerase para detectar genes de virulência (hlyF, iss, ompT, iutA e iroN). Quatorze antimicrobianos foram utilizados para testar a susceptibilidade antimicrobiana nos isolados. Genes característicos de E. coli Patogênica Aviária (APEC) foram encontrados entre os isolados, sendo os genes hlyF, iss e ompT os mais prevalentes. Os isolados apresentaram alta resistência (>50%) à tetraciclina, gentamicina, cefotaxima, nitrofurantoína, trimetoprima-sulfametoxazole e ampicilina. Múltipla resistência a drogas antimicrobianas foi encontrada em grande número de isolados (>70%). Os resultados obtidos mostraram a presença de células APEC portando genes de virulência e resistência a antimicrobianos após 15 dias de processo de empilhamento nas granjas, indicando que o processo necessita de um aperfeiçoamento para eliminar estas células.*
Palavras-chave: *produção avícola, Enterobacteriaceae, risco zoonótico, fatores de virul*

INTRODUCTION

The poultry industry is an important part of the economy in Brazil which is one of the world's most important producers and the leading exporter of chicken meat (ABPA, 2018). Each year, billions of kilograms of poultry litter are produced in Brazil, so as a waste-saving measure, farms are encouraged to reuse poultry manure as fertilizer. Poultry litter is primarily composted for use in crops production.

The use of improperly composted or non-composted manure as organic fertilizer spread directly onto soil can cause microbiological foodborne illnesses associated with the consumption of fresh fruits or vegetables (OLAIMAT & HOLLEY, 2012).

In Brazil a normative instruction from government (BRASIL, 1999) indicated the necessity of a decomposition process of the poultry litter without a fixation of time for the process, before the application of this material to soil. SILVA et al.,

(2007) recommended at least 12 days of fermentative process for the poultry litter arranged in piles covered with polyethylene cover in the chicken houses. Due to economic reasons litter is often reutilized for several flocks, sometimes for 6 consecutives flocks (SONODA et al., 2012). During downtime between flocks the poultry litter is subjected to an in-house treatment aiming to reduce or inactivating residual microorganisms (VAZ et al., 2017). Studies have been indicated that manure-borne pathogens can survive in secondary habitats (i.e., manures, soils, and water) for weeks or even months, with their survival depending on the organism and soil biological, chemical, and physical conditions (ISHII et al., 2010).

E. coli is a normal inhabitant of the gastrointestinal tracts of humans and animals ;however, some strains are known to be pathogenic. *E. coli* strains that cause disease outside the intestine are known as extraintestinal pathogenic *E. coli* (ExPEC) and include human uropathogenic *E. coli* (UPEC) and avian pathogenic *E. coli* (APEC) that is a large infectious agent present in chickens which causes economic losses to the poultry industry worldwide (KOGA et al., 2015). Regarding of host of origin ExPEC strains share many traits (JOHNSON et al., 2012. Some studies have indicated a overlapping of the characteristics between APEC and human ExPEC, leading to the hypothesis of a zoonotic potential of poultry strains (CUNHA et al., 2017). The avian intestines have been considered as a reservoir of potential *E. coli* with zoonotic potential that could be transferred directly from birds to humans. (EWERS et al., 2009).

There is not a consensus on how to define the APEC pathotype regarding its virulence traits. However, most of the authors accepted that the presence of these five genes act as minimal predictors (*hly*F, *iss*, *iro*N, *omp*T and *iut*A), all of them are encoding plasmid-associated virulence factors and can indicate if an *E. coli* strain has a potential to cause extra-intestinal diseases in birds (JOHNSON et al., 2008, STELLA et al., 2016). According JOHNSON et al., (2007) in APEC strains the virulence and antibiotic resistance genes are often carried on the same genetic elements, plasmids like the Col V.

Antimicrobial agents have been used in food-producing animals for a long time. However, increases in antimicrobial–resistant bacteria, both in humans and animals, have generated significant concern regarding food safety (ASAI et al., 2014). Currently chicken products are suspected to be sources of foodborne pathogens and/or antimicrobial-resistant bacteria for humans (MARSHALL & LEVY, 2011). Multidrug-resistant bacteria are frequently

reported in poultry (JOHNSON et al., 2012). It is supposed that their presence was an answer to the selective pressure due to the indiscriminate use of antimicrobials in aviculture as feed additives or as therapy (MARSHALL & LEVY, 2011).

There are gaps in the knowledge about the survival of *E. coli* in avian organic fertilizer, such as the environmental risk for human heath for the use of improperly composted poultry litter, so the aim of this study was to analyze the profile of virulence factors and antimicrobial resistance of *E. coli* isolates from avian organic fertilizer.

MATERIALS AND METHODS

Reutilization of poultry litter (windrowing)

According to VAZ et al., (2017) after the removal of the poultry floc, the caked portions of the bedding material are eliminated and the remaining litter was windrowed at the center of the poultry house, forming a single pile, which was covered with a 200 μm non-breathable black tarpaulin for 10 days. Then the tarpaulin was removed, and the litter was redistributed throughout the poultry house for a new poultry flock housing. After each grow out the litter was windrowed and after that the material was ready for land apply.

Bacterial isolates

Poultry litter were obtained from two different farms located in Ituverava, SP, Brazil. The poultry litters came from the same grow out cycle (three) in both farms, and the bedding material used was peanut hulls. The litters were mixed and arranged in two 1,2 m^3 piles to deep stacking. Samples were gathered at 5, 10, 15, 30 and 60 days after the beginning of fermentative process (windrowing). In brief, 2 g of litter sample was inoculated in BHI broth (Brain Heart Infusion) and incubated for 24 hours at 37 ºC. After incubation, the content was streaked out on MacConkey agar and incubated for 24 hours at 37 ºC. The colonies (five per plate) with typical *E. coli* characteristics were biochemically identified through the indole production, methyl red and Voges-Proskauer reactions, citrate utilization, production of urease and hydrogen sulfide (H2S) after incubation for 24 and 72 hours at 37 °C (KONEMAN et al., 2001).

Poultry litter temperature and pH were measured every day for 8 days, and afterward, these parameters were checked with a lag 4 days.

Virulence factor genes

Five genes encoding virulence factors were investigated. The selected genes were *hly*F

(putative avian hemolysin), *iss* (episomal increased serum survival gene), *iro*N (salmochelin siderophore receptor gene), *omp*T (episomal outer membrane protease gene) and *iuc*A (aerobactin siderophore receptor gene). The microbial DNA template was obtained based on a thermal lysis technique (KESKIMAKI et al., 2001). Isolates identified as *E. coli* were subjected to polymerase chain reaction (PCR). The PCR reaction was done as described by JOHNSON et al., (2008). PCR amplicons were visualized on 2.0% agarose gel stained with ethidium bromide. After gel electrophoresis the images were captured using an Image Capture system.

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 (CLSI 2008, 2013). Isolates showing intermediate or full resistance were classified as resistant in this study because these drugs would no longer be useful in treating a human infection once the bacteria has intermediate resistance. Antimicrobials used included the following: nalidixic acid (NAL, 30 µg); ampicillin (AMP, 10 µg); amoxicillin-clavulanic acid (AMC, 20/10 µg); cefoxitin (CFO, 30 µg); cefazolin (CTZ, 30 µg); cefotaxime (CTX, 30 µg); ceftazidime (CEF, 30 µg); ciprofloxacin (CIP, 10 µg); chloramphenicol (CLO, 300 µg); gentamicin (GEN,10 µg); nitrofurantoin (NIT, 300 µg); norfloxacin (NOR, 10 µg); trimethoprim-sulfamethoxazole (SUT, 1.25/23.75 µg); tetracycline (TET, 30 µg). All the antimicrobial drugs tested were usually used in veterinary applications. The *E. coli* isolate ATCC 25922 was used as a quality control to antimicrobial susceptibility testing, and the results were interpreted as per CLSI criteria. Isolates were classified as multidrug resistance (MDR) according to previously described criteria (MAGIORAKOS et al., 2012).

Statistical analysis

Frequencies were compared among different groups by using the Fisher exact test and the chi-square test. Findings were considered significant for $p \leq 0.05$. The tests were performed with the statistical program R version 3.1.0 (R Foundation for Statistical Computing, Vienna Austria).

RESULTS

The temperature and the pH were monitored in the composting system at both heaps since the first day until the sixty day at two depth 30 and 60 centimeters. According figure 1 the temperature ranges from 38 ℃ to 26 ℃ in both heap and also the pH range from 9.3 to 8.2 with the most alkaline pH around the 18th day.
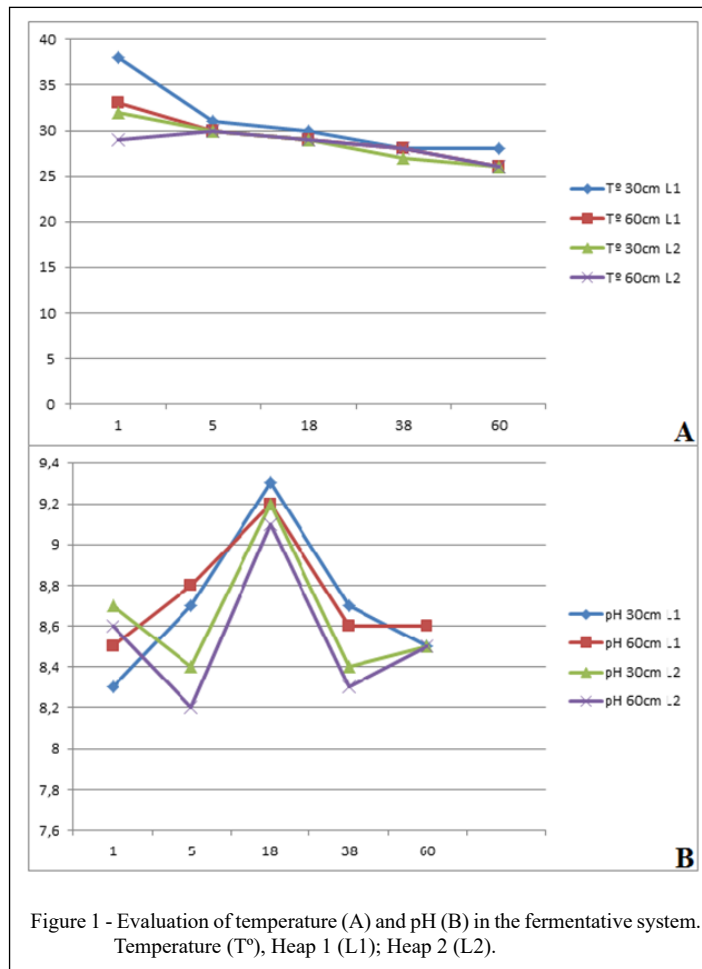
A total of 50 poultry litter samples were collected in the 5th, 10th, 15th, 30 and 60 days,;however only in the 5th day and the 15th day *E. coli* strains were isolated from the litter. In both the first and the second positive collection, the *hly* gene was the most prevalent and was present in 80.0% and 94.0% of isolates respectively, we alsoreported significant difference for most of the genes studied between strains from the first and second positive collection with the exception of *iut* gene (p>0.05) (Table 1). Few strains from the both collections were negative for virulence genes, with 5 strains in the first collection and only one strain from the second collection without any virulence genes present. Table 2 presents, that most of the strains harbored at least two of the targeted genes in both collections' positive days. The most frequent profile found in the first collection was *hly*F, *iss*, *omp*T, *iut*A while in the second collection was the *hly*F, *iss*, *omp*T.

According to the antimicrobial susceptibility test, strains from the second positive collection showed a higher frequency of antimicrobial resistance than strains from the first collection for most of the antimicrobial tested (Figure 2). Regarding multidrug resistance only two isolates in the first positive collection were sensitive to all the antimicrobial drugs tested while only two isolates in the second collection were resistant to eleven of the antimicrobial drugs tested (Table 3). Most of the isolates in both collections, 1ª (70.0%) and 2ª (94.0%) harbored resistance to three or more of the antimicrobial drugs.

DISCUSSION

The feasibility of chicken production depends on the economic and environmental sustainability of the management system of the poultry litter discard. In Brazil, reuse of poultry litter is considered when the previous flocks did not present clinical or subclinical infection during housing (VAZ et al., 2017) and the litter can be reused for six cycles of chicken growing (VAZ et al., 2017).

Composting is one of the most suitable technologies for disposing livestock manures. A correct process depends on temperature and pH for a sanitation effect, and both depending of a correct balance between the carbon and nitrogen (C/N)

Figure 1 - Evaluation of temperature (A) and pH (B) in the fermentative system. Temperature (T°), Heap 1 (L1); Heap 2 (L2).

1 to permit a thermophilic phase with temperatures
2 above 55-60 °C for at least 3 days (ISHII et al.,
3 2010). However, the organic waste may not always
4 be suitable for a correct composting, it means that

1 sometimes it is necessary to increase the carbon
2 disposable by adding an additional biodegradable
3 organic matter and active biomass to the occurrence
4 of the thermophilic phase (SONG et al., 2014).

Table 1 - Prevalence of virulence genes among strains of *Escherichia coli* isolated from poultry litter in Ituverava, SP, Brazil, 2018.

	First positive collection (n=30)	Second positive collection (n=17)
	N° of isolates (%)	N° of isolates (%)
Virulence genes		
*hly*F[*]	24 (80.0)	16 (94.0)
iss[*]	18 (54.0)	15 (88.2)
*omp*T[*]	18 (54.0)	15 (88.2)
*iro*N[*]	0 (0)	1 (5.8)
*iut*A[**]	19 (63.3)	7 (41.0)

[*]$P \leq 0.05$; [**] $p > 0.05$.

Table 2 - Genetic profiles of *Escherichia coli* strains isolated from poultry litter in Ituverava, SP, Brazil, 2018.

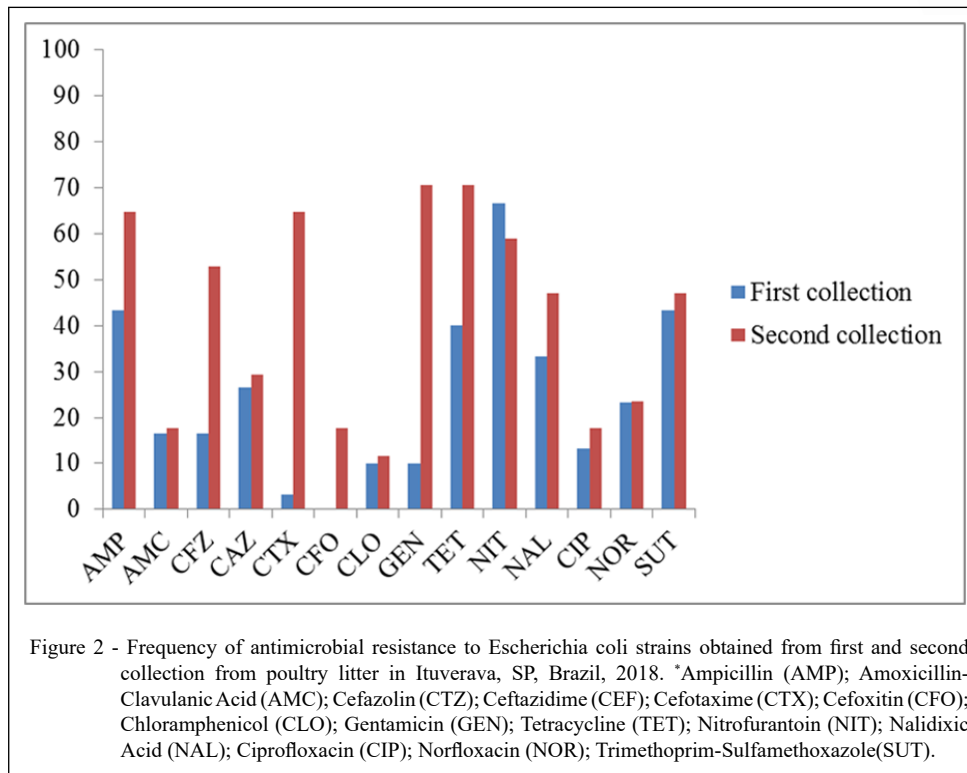| Pathotype genes	First positive collection Number of isolates (%)	Second positive collection Number of isolates (%)
*hly*F, *iss*, *omp*T	2 (6.6)	7 (41.1)
*hly*F, *iss*, *omp*T, *iut*A	10 (33.3)	6 (35.2)
*hly*F, *iss*, *iut*A	6 (20.0)	1 (5.8)
*hly*F, *omp*T	5 (16.6)	1 (5.8)
*hly*F, *omp*T, *iut*A	1 (3.3)	-------
*hly*F, *iss*, *omp*T, *iro*N	------	1 (5.8)
*iut*A	2 (6.6)	-------
None	4 (16.6)	1 (5.8)

In the windrowing technique only the poultry litter was piled and covered with a black tarpaulin promoting an incomplete composting process, actually a fermentative process with low temperatures (<50 ºC) depending on the bedding material used in the poultry houses (VAZ et al., 2017). In the present research the temperature reaches a maximum of 38 ºC what was not enough for a good thermophilic phase. Almost the same temperature 35 to 40 ºC range was reported by WILKINSON et al., (2011) in the static windrow during the period of 21 to 63 day in a trial with poultry litter. Also, SONG et al., (2014) described a composting of chicken manure in which the temperature was 38 to 40 ºC until the fourteen day of the trial. The low temperature in all the trials can be explained for the bad proportion of C/N in the bedding material used in the chicken house. To reach the correct temperature of composting should be necessary to increase the green material in the litter maybe adding to the peanut hulls some elephant grass also used in chicken litters to improve the C/N balance.

In this study the pH of the poultry litter rang for 8.2 to 9.3 during the trial. SONG et al., (2014) reported a pH of 8.7 in the fourteen day of trial, SONODA et al., (2012) reported a pH of 8.8 and VAZ et al., (2017) reported a mean value of pH around 9.0, all of them agreeing with the present study and using the same windrowing method. The increased pH was most likely the result of ammonia releases due to ammonification (BUSTAMANTE et al., 2008).

SILVA et al., (2007) reported that the number of *E. coli* isolates recovered from poultry litter after 3 flocks grow out decreased for 1/3 of the *E. coli* number recovered in the first flock grow out, this effect is kept in the 4th and 5th flocks grow out. They also reported a re-growing of the *E. coli* cells in poultry litter during the 6th flock grow out. Based on

this report we decided to use poultry litter from the 3th flock grow out in this trial, it means the trial begin with the smallest number of *E. coli* cells present in the poultry litter. In the present study *E. coli* cells were isolated only in two sampling days the 5th and the 15th. MARTIN et al., (1998) described that total coliforms were not detected in 94% of the Georgia poultry litter samples they tested. Likewise, BROOKS et al., (2015) demonstrated an inability to detect common bacterial pathogens in the litter, in the same line WINKLER et al., (2017) described that *E. coli* levels in poultry litter were often below the limit of detection (10 CFU g^{-1}) in samples collected at farms in Texas. In Brazil, GAZAL et al., (2015) reported an isolation of *E. coli* from 40% of the samples of avian organic fertilizer. So, it is not uncommon a low frequency of isolation of *E. coli* from avian organic fertilizer. However, we did not have an explication for the absence of *E. coli* isolates from the 10th day, it should be better investigated in other trial.

Earlier, the avian strains of *E. coli* were considered as not causing any important disease in man and animals, so were not of much zoonotic significance. But as APEC share not only identical serotypes but specific virulence factors also with human pathogens, their zoonotic potential is now under consideration. Some authors have reported that *E. coli* from poultry is the food animal source most closely linked to human Extra intestinal *E. coli* (ExPEC) suggested a potential zoonotic risk (MANGES & JOHNSON, 2012; MELLATA, 2013). A multiplex PCR targeting five genes (*hly*F, *iss*, *iro*N, *omp*T and *iut*A) has been identified as being the most significantly associated with highly pathogenic APEC (SYLVESTER & SING, 2002). These genes were also reported in *E. coli* isolated from urinary tract infection (CYOIA et al., 2015) and they were

Figure 2 - Frequency of antimicrobial resistance to Escherichia coli strains obtained from first and second collection from poultry litter in Ituverava, SP, Brazil, 2018. *Ampicillin (AMP); Amoxicillin-Clavulanic Acid (AMC); Cefazolin (CTZ); Ceftazidime (CEF); Cefotaxime (CTX); Cefoxitin (CFO); Chloramphenicol (CLO); Gentamicin (GEN); Tetracycline (TET); Nitrofurantoin (NIT); Nalidixic Acid (NAL); Ciprofloxacin (CIP); Norfloxacin (NOR); Trimethoprim-Sulfamethoxazole(SUT).

also reported in conjugative plasmid in human *E. coli* strains isolated from sepsis in Brazil, indicated a possible zoonotic risk (KOGA et al., 2014).

KOGA et al., (2015) examined *E. coli* strains isolated from chicken carcasses obtained from conventional and free-range poultry system. Among them the strains from the conventional system showed a lower frequency to four of the five APEC genes examined in the present study (*hly*F-47.1%; *iut*A-54.5%; *iss*-35.5%; *omp*T-52.9%; *iro*N-28.9%), except for *iro*N gene. Agreeing with KOGA et al., (2015), STELLA et al., (2016) examined 91 *E. coli* strains

isolated from feces of healthy broiler chickens and their results (*hly*F-49.4%; *iut*A-43.0%; *iss*-31.7%; *omp*T-46.0%; *iro*N-22.0%) also showed a higher frequency for the *iro*N gene than the present study.

DE CARLI et at (2015) examined 138 *E. coli* isolates from tissue and organs of chickens suspected to have colibacillosis in poultry flocks in Brazil, they reported high frequencies of APEC pathogenic genes, *hly*F-100%; *omp*T-100%; *iro*N-98.8%; *iss*-96.3% and *iut*A-81.5%. These results agree with those reported by JEONG et al., (2012) also from APEC strains from infected chickens *iro*N-

Table 3 - Prevalence of antimicrobial resistance among *Escherichia coli* strains isolated from poultry litter in Ituverava, SP, Brazil, 2018.

Prevalence of antimicrobial resistance	First positive collection	Second positive collection
	Number of isolates	Number of isolates
No resistance detected	2	----
Resistance to 1 or 2 antimicrobials	7	1
Resistance to 3 or 4 antimicrobials	7	3
Resistance to 5 or 10 antimicrobials	14	11
Resistance to 11 to 14 antimicrobials	----	2

100%; *omp*T-94.1%; *hly*F-87.1%; *iss*-78.2% and *iuc*D-73.3%. In another way CUNHA et al., (2017) examined *E. coli* isolates from chickens' cases of colibacillosis and GAZAL et al., (2015) examined *E. coli* from avian organic fertilizer, in both was found a great number of characteristic ExPEC genes found in human infection among the *E.coli* isolates from chicken, what reinforce an overlapping of characteristics between APEC and human ExPEC, leading to the hypothesis of a zoonotic potential risk of poultry strains (GAZAL et al., 2015).

The genetic profiles of *E. coli* strains isolated in the present trial showed different profiles distribution; however, more than 50% of the identified profiles were concentrate in two profiles *hly*F+*iss*+*omp*T+*iut*A (33.3%) and *hly*F+*iss*+*iut*A (20.0%) what agree with the results reported by STELLA et al., (2016) which indicated the most frequent profile as *iro*N+*omp*T+*iss*+*iut*A+*hly*F followed by *omp*T+*iut*A+*hly*F representing 50% of the genetic profiles recovered from *E. coli* strains isolated from feces of healthy broiler chickens. The virulence genes examined in the present study included those encoding plasmid-associated virulence genes (JOHNSON et al., 2006) associated with APEC strains and human ExPEC strains as well as antibiotic resistance genes often carried on the same genetic elements (SKYBERG et al., 2006). Also, there is a well-documented history of transfer of *E. coli* strains and their plasmids from poultry to humans (VAN DEN BOGAARD et al., 2001).

Antimicrobial resistance in bacteria isolated from food animals is often associated with the use of antibiotics in livestock (MARSHALL & LEVY 2011; MELLATA, 2013). Even though many antimicrobials such as tetracycline, β-lactams and quinolones are prohibited as growth promoters in Brazil (BRASIL 2003, 2009) the frequency of antimicrobial resistance among the *E. coli* strains isolated in the present study was high for all these antimicrobial drugs. According to KOGA et al., (2015) the *E. coli* strains from conventional poultry system showed a higher frequency of antimicrobial resistance for β lactams, chloramphenicol, tetracycline and quinolones drugs than the present study while the cephalosporins, aminoglycosides and nitrofurantoin showed a lower frequency of antimicrobial resistance when compared with the present study. STELLA et al., (2016) reported a frequency of 100% of resistance to cephalothin, erythromycin, streptomycin, neomycin, ampicillin and amoxicillin. Also, FURTULA et al., (2010) from Canada and ADELOWO et al., (2014) from Nigeria reported a high level of resistance for tetracycline,

sulphamethoxazole and aminoglycosides among *E. coli* from poultry showing a great dissemination the antimicrobial resistance in the poultry industry.

Tetracycline was the antimicrobial with the highest frequency of resistance in the second positive collection in the present study. The high frequency of resistance can be explained, in part, because it has a low price and is easily obtained, which leads to an indiscriminate and incorrect uses. According to KHAN et al., (2014) the use of tetracyclines in poultry production is very common, which is generating a significant increase in resistance findings what was also reported by KOGA et al., (2015) in Brazil and similar data were also provided for other countries (FURTULA et al., 2010; ADELOWO et al., 2014).

β lactam antimicrobials, especially the third generation cephalosporins are the most common treatment for human infections by Enterobacteriacea. However, many resistant bacteria to these drugs have emerged worldwide (FURTULA et al., 2010; PITOUT, 2012) what agree with the high frequency of antimicrobial resistance to cephalosporins reported among the *E. coli* isolates in the present study, and in STELLA et al., (2016).

Quinolones are broad-spectrum agents that are often used for enteric infections and human urinary tract therapy (PITOUT, 2012). However, the presence of quinolone-resistant *E. coli* in animals has increased (ADELOWO et al., 2014). Our results indicated a high frequency of resistance to nalidixic acid in strains isolated from poultry litter (50.0%). These results agree with KOGA et al., (2015) who reported a high frequency of resistance to nalidixic acid (70.0%) in *E. coli* isolates obtained from poultry in conventional system of production (farming system), and also with CARVALHO et al., (2015) who reported a nalidixic acid resistance of 75.0% from *E. coli* isolates obtained in broiler houses in Rio Grande do Sul, Brazil, showing a necessity of attention to the critical use of antimicrobials used in treatment of human infections.

GAZAL et al., (2015) examined sixty-four *E. coli* isolates from avian organic fertilizer in Brazil that was a final product of the poultry litter composting process, the windrowing process of a 10 days of a fermentative process. Most isolates were resistant to tetracycline (35.9%); amoxicillin (20.35); ampicillin (18.7%); streptomycin (17.1%) and trimethoprim-sulfamethoxazole (12.5¨%). Results of the present study showed a higher level of resistance for these antimicrobial drugs, likely due to the intensity of use of the antimicrobial drugs in the preventive treatment of the different chicken flocks. Most of these antimicrobial resistance genes are associated with

virulence genes present in the same APEC and ExPEC plasmids (JOHNSON et al., 2006). The great number of *E. coli* isolates showing multidrug resistance to 5 to 10 drugs in the present study reinforced this probably resistance linked to plasmid origin. Therefore, it seems very important to pay more attention to the spread of resistance through such poultry production systems and the necessity of a stricter regulation of antimicrobial usage in the production systems with close interaction between humans and animals.

SONODA et al., (2012) reported that the composting method, piled (windrowing) did not significantly reduced the pathogenic bacterial population present in the litter, it means that the period of 10-12 days of composting seemed not to be sufficient to eliminate the virulence factors and antimicrobial resistance genes linked to plasmids inside the pathogenic bacteria. Results in the present study agreed with those from SONODA et al., (2012), which showed that the sanitary gap period of 10 to 12 days among the flocks grow out used in the poultry house in Brazil is not enough to permit a correct composting process with the destruction of pathogenic *E.coli* cells. We reported APEC strains with virulence and antimicrobial resistance genes present in litter samples after 15 days of the windrowing process, it means that the process should have a amplified period of composting and when necessary a correction of the C/N balance by adding more green mass to the litter to permit a correct composting process.

CONCLUSION

The strains characterized in this research with a high frequency of antimicrobial resistance, associated with several virulence genes showed that *E. coli* strains may be present in organic fertilizers after fifteen days of the fermentative process usually used by Brazilian farmers what represents a risk for the dissemination of these genes in the environment, to avoid the period of composting of avian organic fertilizer must be amplify.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

JMAA,JMM conceived, designed and supervised the experiment. JMAA, MVC, MMB performed the experiments and data collection. JMAA, JMM prepared the draft of the manuscript. All authors critically revised the manuscript and approved the final version.

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