Research Article

Comparison of Antibiotic Resistance and Virulence Factors among Escherichia coli Isolated from Conventional and Free-Range Poultry

Vanessa L. Koga,1 Sara Scandorieiro,1 Eliana C. Vespero,2 Alexandre Oba,3 Benito G. de Brito,4 Kelly C. T. de Brito,4 Gerson Nakazato,1 and Renata K. T. Kobayashi1

1Department of Microbiology, Laboratory of Basic and Applied Bacteriology, State University of Londrina (UEL), Rodovia Celso Garcia Cid, Caixa Postal 6001, 86051-980 Londrina, PR, Brazil
2Department of Pathology and Clinical and Toxicological Analysis, State University of Londrina (UEL), Avenida Robert Koch, No. 60, Vila Operária, 86038-350 Londrina, PR, Brazil
3Department of Zootechnia, State University of Londrina (UEL), Rodovia Celso Garcia Cid, Caixa Postal 6001, 86051-980 Londrina, PR, Brazil
4Laboratory of Bird Health, Fepagro Animal Health, Veterinary Research Institute Desidério Finamor (IPVDF), Estrada do Conde, No. 6000, 92990-000 Eldorado do Sul, RS, Brazil

Correspondence should be addressed to Renata K. T. Kobayashi; kobayashirkt@uel.br

Received 1 April 2015; Revised 28 September 2015; Accepted 30 September 2015

Academic Editor: Miguel Prieto

Microbiological contamination in commercial poultry production has caused concerns for human health because of both the presence of pathogenic microorganisms and the increase in antimicrobial resistance in bacterial strains that can cause treatment failure of human infections. The aim of our study was to analyze the profile of antimicrobial resistance and virulence factors of E. coli isolates from chicken carcasses obtained from different farming systems (conventional and free-range poultry). A total of 156 E. coli strains were isolated and characterized for genes encoding virulence factors described in extraintestinal pathogenic E. coli (ExPEC). Antimicrobial susceptibility testing was performed for 15 antimicrobials, and strains were confirmed as extended spectrum of β-lactamases-(ESBLs-)producing E. coli by phenotypic and genotypic tests. The results indicated that strains from free-range poultry have fewer virulence factors than strains from conventional poultry. Strains from conventionally raised chickens had a higher frequency of antimicrobial resistance for all antibiotics tested and also exhibited genes encoding ESBL and AmpC, unlike free-range poultry isolates, which did not. Group 2 CTX-M and CIT were the most prevalent ESBL and AmpC genes, respectively. The farming systems of poultries can be related with the frequency of virulence factors and resistance to antimicrobials in bacteria.

1. Introduction

Resistance to antimicrobial agents has become a major concern both for human health and in veterinary medicine.Antimicrobial agents are being used in many countries in veterinary practice for therapy and prophylaxis of infectious diseases and for growth promotion in food animals. However, the indiscriminate use of antimicrobials can result in bacterial selection pressure of the intestinal microbiota of animals [1–3]. Because multiresistant bacteria are frequently found in poultry meat [4–6], chicken products are suspected to be a source of foodborne pathogen and/or antimicrobial resistance bacteria for humans [1–3, 7, 8].

Escherichia coli have an important role within resistant bacteria populations, being widely used as a bioindicator of antimicrobial resistance and being pathogenic to humans and animals. Extraintestinal pathogenic Escherichia coli (ExPEC) can cause many human infections, such as septicemia, meningitis, and urinary tract infections, and can also cause disease in birds, being responsible for significant economic
losses in poultry industry [1, 9]. ExPECs are characterized by the possession of many virulence factors including adhesins, toxins, iron acquisition systems, and serum resistance factors and, in phylogenetic classification, belong mainly to group B2 and occasionally to group D, whereas commensal E. coli belong to groups B1 and A [10, 11].

β-lactamase production is the most common mechanism of resistance for β-lactam in Gram-negative bacteria and is increasing in occurrence in humans, becoming a major public health problem [9]. However, β-lactamases of community and environmental origin have been discovered, for example, in food animals. Poultry are recognized as important carriers of β-lactamase-producing E. coli, and extended-spectrum β-lactamase (ESBL)/AmpC-producing bacteria in birds have been reported in many countries [12–14].

ESBL production confers resistance to 3rd- and 4th-generation cephalosporins but not to cephemycins (cefoxitin) and carbapenems and is inactivated by clavulanic acid. The AmpC enzymes confer resistance to 3rd-generation cephalosporins and cephemycins but are inhibited by β-lactamase inhibitors. Plasmid-mediated β-lactamases can carry multiple resistance genes non-β-lactam, and their indiscriminate use can lead to coselection and/or coexistence in bacteria populations [9, 13, 15].

Many studies reported that there is a genetic similarity among avian and human ExPEC, leading to the hypothesis that meat animals play a role as reservoirs for drug-resistant bacteria and pathogenic bacteria [1, 16]. Little is known regarding the microbiological quality of chicken meat from different systems of poultry farming and their potential antimicrobial resistance and/or pathogenic behavior upon consumption. The aim of this study was to analyze the profile of virulence factors and antimicrobial resistance, including searching for ESBL/AmpC groups genes, in strains of E. coli isolated from conventional and free-range poultry carcass.

2. Material and Methods

2.1. Bacterial Isolates. A total of 156 E. coli strains were isolated from commercial refrigerated chicken carcass, intended only for local consumption, sold in the city of Londrina (north region in Paraná, Brazil). Of these, 35 E. coli strains were isolated from 15 free-range poultry (commonly created by family agriculture) and 121 E. coli strains from 26 conventionally raised poultry (sold in markets in the region, obtained from groackers) [17]. Each chicken carcass was placed into the sterile packaging with 100 mL of Brain Heart Infusion (Himedia Laboratories Pvt. Ltd., Mumbai, India). After homogenization, 0.1 mL was smeared onto MacConkey agar (Neogen Corporation Lansing, Michigan) and crystal violet red neutro bile agar (Neogen Corporation Lansing, Michigan) by pour plate. Both were incubated at 37°C for 18–24 h. Colonies suspected to be E. coli were confirmed by biochemical tests such as EPM, MLi [18, 19], and Simons citrate agar (Merck, KGaA, Darmstadt, Germany). One-to-eight strains were collected from each chicken carcass. Only strains that showed different genotypic characteristics of virulence factors and phenotypic resistance were selected.

2.2. Phylogenetic Classification. E. coli strains were assigned to phylogenetic groups (A, B1, B2, or D), according to the method of Clermont and collaborators [10]. This method is based on analysis of presence of the chuA and yjaA genes and the DNA fragment (TSPE4.C2), as determined by Polymerase Chain Reaction (PCR). This PCR reaction contained 1.25 U Taq DNA polymerase (Life technologies, Rockville, MD) in 1x PCR buffer (Life technologies, Rockville, MD), 0.2 mM of each dNTP, 2.5 mM MgCl₂, and 1 μM of each primer. The conditions of PCR consisted of 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s with a final extension step at 72°C for 7 min. PCR amplicons were visualized on 2.0% agarose gels stained with GelRed (Biotium, Hayward, CA, USA). After gel electrophoresis, the images were captured using Image Capture Systems (LPixImageHE).

2.3. Virulence Factor Genes. Several virulence factors normally studied in ExPEC strains were surveyed. The selected genes were as follows: iutA (aerobactin siderophore receptor gene), hlyF (putative avian hemolysin), iss (episomal increased serum survival gene), iroN (salmochelin siderophore receptor gene), and ompT (episomal outer membrane protease gene) [11]. This PCR contained 1.25 U Taq DNA polymerase (Life technologies, Rockville, MD) in 1x PCR buffer (Life technologies, Rockville, MD), 0.2 mM of each dNTP, 2.5 mM MgCl₂, and 1 μM of each primer. The conditions of PCR consisted of 94°C for 2 min, followed by 25 cycles of 94°C for 30 s, 63°C for 30 s, and 68°C for 3 min, with a final extension step at 72°C for 10 min [11]. PCR amplicons were visualized on 2.0% agarose gels stained with GelRed (Biotium, Hayward, CA, USA). After gel electrophoresis, the images were captured using Image Capture Systems (LPixImageHE).

2.4. Antimicrobial Susceptibility Testing. Antimicrobial susceptibility was performed using the standard disk diffusion method recommended by the Clinical and Laboratory Standards Institute [20, 21]. Antimicrobials used included 5 μg of ciprofloxacin, 10 μg of each of ampicillin, gentamicin, norfloxacin, and enrofloxacin, 30 μg of each of cefazolin, cefotaxime, cefoxitin, ceftazidime, tetracycline, nalidixic acid, and chloramphenicol, 300 μg of nitrofurantoin, 1.25/23.75 μg of trimethoprim-sulfamethoxazole, and 20/10 μg of amoxicillin-clavulanic acid (Oxoid Ltd., Basingstoke, Hants, UK). Strains resistant to third-generation cephalosporins were confirmed for ESBL production by double-disc diffusion testing between amoxicillin/clavulinate and cefotaxime or cefazidime [22], or by using a combination disc test including cefotaxime, cefotaxime + clavulanic acid (Becton Dickinson, Sparks, MD), ceftazidime, and ceftazidime + clavulanic acid (Becton Dickinson, Sparks, MD), according to the CLSI recommendations. The strains positive in the phenotypic tests to ESBL production were screened to ESBL genes. Strains that showed cefoxitin and/or to 3rd-generation cephalosporins intermediate or resistance were tested by molecular screening of AmpC type genes. The E. coli isolate ATCC25922 was used as a quality control to antimicrobial susceptibility testing, and the results were interpreted as per CLSI criteria.
2.5. Characterization of β-Lactamase Genes of ESBL and AmpC Groups. ESBL-producing E. coli was characterized for ESBL genes encoding CTX-M (1, 2, 8, 9, and 25 groups), TEM, and SHV type by PCR [23–25]. All isolates suspected by phenotypic tests for the production of AmpC were tested by a multiplex PCR described by Pérez-Pérez and Hanson [26]. Six family-specific plasmid mediated AmpC genes (MOX, FOX, EBC, ACC, DHA, and CIT) were evaluated. PCR amplicons were visualized on 2% agarose gels stained with GelRed (Biotium, Hayward, CA, USA). After gel electrophoresis, the images were captured using Image Capture Systems (LPixImageHE).

2.6. Statistical Analysis. Comparisons of frequencies among different groups were made by Fisher’s exact test and Chi-square test. Findings were considered to be significant where \( p < 0.05 \). The test was performed with the statistical program R version 3.1.0.

3. Results

According to phylogenetic classification, the most prevalent group in strains from free-range poultry was the group A (54.3%), whereas the strains from conventionally raised poultry most frequently belonged to group B1 (37.2%), although no statistically significant differences were observed between them and groups B1, B2, and D (Table 1).

Regarding the search for virulence factors, we found significant difference for the majority of the genes studied between strains from free-range and conventional poultry, with the exception of the \( iss \) gene (\( p > 0.05 \)) (Table 1). Few strains from free-range poultry were positive for virulence factors, with only 10 strains (28.6%) having at least one of the virulence factors studied. In contrast, 91 strains (75.2%) from conventionally raised poultry had at least one virulence factor.

According to the antimicrobial susceptibility test, strains from conventionally raised poultry showed a higher frequency of antimicrobial resistance than strains from free-range poultry for all antimicrobials tested (Figure 1). The frequency of antimicrobial resistance to strains from free-range poultry was low, except to tetracycline (60% of resistance), whereas the strains from conventional poultry showed a high frequency of resistance mainly to tetracycline, nalidixic acid, and ampicillin (Figure 1).

ESBL/AmpC genes appeared only in strains isolated from conventional poultry (42.1% of 121 strains from conventional poultry). Forty strains were ESBL-producing E. coli. The most prevalent group within these ESBL was the group 2 CTX-M (62.5% of ESBL-producing strains). Eleven strains showed only the CIT group of AmpC genes (9.1% of 121 strains from conventional poultry). No strain had ESBL and AmpC genes together (Table 2).

All ESBL/AmpC-producing strains showed resistance to one or more non-β-lactam antimicrobials, with resistance to tetracycline (98%) the most prevalent (Table 2).

We observed that ESBL/AmpC-producing strains were present in all four phylogenetic groups (A, B1, D, and B2), although there were few B2 strains. The majority of these strains were positive for at least one virulence factor.

4. Discussion

Many studies have demonstrated similarities between human and avian ExPEC, leading to the hypothesis that poultry products may serve as a source of ExPEC and are closely linked to human infections. Poultry meat exhibits the highest levels of E. coli contamination, and these are indicated as being more extensively antimicrobial-resistant than E. coli from other meats [27].
Table 2: Characteristics of β-lactamase genes and phenotypic antimicrobial resistance profile of strains of ESBL/AmpC-producing E. coli.

| Isolate number | Phenotypic resistance profile | β-lactamase genes |
|----------------|-------------------------------|-------------------|
| 1              | Amp, amc, cfz, ctx, tet, nal  | Group 1 CTX-M     |
| 2              | Amp, kz, ctx, cn, tet, nal    | Group 2 CTX-M     |
| 3              | Amp, kz, ctx, cn, tet, nal, cip, nor, enr, sut | Group 2 CTX-M |
| 4              | Amp, kz, ctx, cn, tet, nal, cip, sut | Group 2 CTX-M |
| 5              | Amp, kz, ctx, cn, tet, nal, cip, enr | Group 2 CTX-M |
| 6              | Amp, kz, ctx, cn, tet, nal    | Group 2 CTX-M     |
| 7              | Amp, kz, ctx, clo, cn, nal, sut | Group 2 CTX-M |
| 8              | Amp, kz, ctx, tet, nal, cip, nor, enr, sut | Group 2 CTX-M |
| 9              | Amp, kz, ctx, tet, nal, cip, nor, enr, sut | Group 2 CTX-M |
| 10             | Amp, kz, ctx, clo, tet, nal   | Group 2 CTX-M     |
| 11             | Amp, kz, ctx, cn, tet, nal, cip, enr, sut | Group 2 CTX-M |
| 12             | Amp, kz, ctx, cn, tet, nal, enr | Group 2 CTX-M |
| 13             | Amp, kz, ctx, cn, tet, nal    | Group 2 CTX-M     |
| 14             | Amp, kz, ctx, clo, tet, nal, cip, nor, sut | Group 2 CTX-M |
| 15             | Amp, amc, kz, cn, tet, nal, sut | Group 2 CTX-M |
| 16             | Amp, amc, kz, ctx, cn, tet, nal | Group 2 CTX-M |
| 17             | Amp, amc, kz, ctx, cn, tet, nal, cip, nor, enr, sut | Group 2 CTX-M |
| 18             | Amp, kz, ctx, cn, tet, nal    | Group 2 CTX-M     |
| 19             | Amp, kz, ctx, cn, tet, nal    | Group 2 CTX-M     |
| 20             | Amp, kz, ctx, clo, tet, nal, cip, nor, sut | Group 2 CTX-M |
| 21             | Amp, kz, ctx, cn, tet, nal, cip, enr | Group 2 CTX-M |
| 22             | Amp, amc, kz, ctx, tet, nit, nal | Group 2 CTX-M |
| 23             | Amp, amc, kz, ctx, cn, tet, nal | Group 2 CTX-M |
| 24             | Amp, amc, kz, ctx, clo, tet, nit, nal, cip, nor, enr, sut | Group 8 CTX-M |
| 25             | Amp, kz, ctx, tet, cip, nor, enr | Group 8 CTX-M |
| 26             | Amp, amc, kz, ctx, tet, nit, sut | Group 8 CTX-M |
| 27             | Amp, amc, kz, ctx, tet, nal, sut | Group 8 CTX-M |
| 28             | Amp, amc, kz, ctx, tet, cip, nor, enr | Group 8 CTX-M |
| 29             | Amp, amc, kz, ctx, tet, cip, nor, enr | Group 8 CTX-M |
| 30             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 31             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 32             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 33             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 34             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 35             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 36             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 37             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 38             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 39             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 40             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 41             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 42             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 43             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 44             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 45             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 46             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 47             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 48             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |
| 49             | Amp, amc, kz, ctx, tet, nit, cip, nor, enr | Group 8 CTX-M |

SHV: Group 1 CTX-M, Group 2 CTX-M
CIT: Group 2 CTX-M, Group 8 CTX-M

Amp, amc, kz, ctx, tet, nal, cip, nor, enr, sut | Group 1 CTX-M, Group 2 CTX-M
Amp, kz, ctx, tet, nal, cip, nor, enr, sut | Group 2 CTX-M, Group 8 CTX-M
Amp, amc, kz, ctx, tet, nal, cip, nor, enr, sut | Group 2 CTX-M, Group 8 CTX-M
Amp, amc, kz, ctx, tet, nal, cip, nor, enr, sut | Group 8 CTX-M, SHV
Avian *E. coli* often possess virulence genes similar to those found in human ExPEC [27]. We measured 5 virulence genes carried by plasmids that are normally studied in human ExPEC [28, 29] and used by Johnson and collaborators [11] to distinguish avian pathogenic avian *E. coli* (APEC) from commensal *E. coli*. Our results demonstrated that strains from conventionally raised poultry have a greater number of virulence genes than the strains from free-range poultry, with the exception of the *iss* gene (*p > 0.05*). Furthermore, few strains from free-range poultry showed virulence factors, unlike strains from conventionally raised poultry, of which 75.2% had at least one virulence factor. These genes were also found in *E. coli* isolated from urinary tract infections [30], and some of these genes (*iss, iroN, ompT*, and *hlyF* genes) were found also in conjugative plasmid in human *E. coli* strains isolated from sepsis, in Brazil, indicating a possible zoonotic risks [28]. According to phylogenetic classification, our results showed most prevalence of group A in strains from free-range poultry and group B1 in strains from conventionally raised poultry. Thus, the majority of the strains show characteristics relative to commensal phylogenetic groups, although most strains from conventionally raised poultry were positive for virulence factors. These results can be related to the creation system because the conventional poultry are raised in larger groups in few areas, generating a high density, which facilitates the transmission of bacteria between them because there are many virulence genes carried by plasmids, whereas free-range poultry creation is in small groups, making it more difficult to transmit pathogens [13].

Antimicrobial resistance in bacteria isolated from food of animal origin is often associated with the use of antibiotics in livestock [2, 3, 8, 31]. Due to indiscriminate use of antimicrobials in poultry feeds, since 2006, in Europe, the use of antimicrobials as growth promoters is prohibited [31]. The use of several antibiotics including tetracyclines, β-lactams, systemic sulfonamides, and quinolones has been banned as growth promoters in many countries, for example, in Brazil [32, 33].

However, an interesting finding in our study was the low frequency of antimicrobial resistance in strains from free-range poultry except to tetracycline. It is known that the use of antimicrobials in family agriculture is restricted or even absent, being casually used for treating diseases [34]. Another hypothesis for the low observed frequency is that free-range poultry normally live in small groups, compared to conventionally raised poultry, leading to individual therapeutic interventions, whereas in the poultry industry, birds are kept in larger groups, so population-based therapeutics are mostly appropriate [13].

Tetracycline was the antimicrobial with the highest frequency of resistance in both rearing systems. The high frequency may be due to the easy access to and low price of these antimicrobials and poor monitoring by regulatory bodies in veterinary medicine in Brazil because these antimicrobials have prohibited use. Another explanation of the high frequency of resistance in strains from free-range poultry is its contact with environmental microorganisms, which produce natural antibiotics, or by soil contamination with the feces of wild animals that carry antibiotic-resistant microorganisms [8, 35].

β-lactam antimicrobials, especially the third-generation cephalosporins, are the most common treatment for human infections by Enterobacteriaceae. However, a large number of resistant bacteria have emerged worldwide. Among ExPEC, β-lactamases remain the most important mechanisms of β-lactam resistance. β-lactamases are hydrolytic enzymes that cleave the β-lactam ring. The emergence of β-lactamases is mainly linked to the spread of genes encoding ESBLs and/or plasmid-mediated AmpC β-lactamases [9]. However, ESBL/AmpC-producing bacteria are now being found in increasing numbers in food-producing animals, for example, in poultry meat [5, 13, 36].

One notable finding was the presence of ESBL/AmpC β-lactamases only in strains from conventional poultry, with group 2 CTX-M and CIT groups being the most prevalent ESBL and AmpC, respectively. The absence in strains from free-range poultry may indicate the low use of antimicrobials in its production.

**Table 2: Continued.**

| Isolate number | Phenotypic resistance profile | β-lactamase genes |
|----------------|-----------------------------|-------------------|
| 50             | **Amp, amc, kz, tet, nal**   | Group 8 CTX-M, SHV |
| 51             | **Amp, amc, kz, ctx, clo, tet, nal, cip, nor, enr, sut** | Group 2 CTX-M, Group 8 CTX-M, SHV |

Ampicillin (AMP); amoxicillin-clavulanic acid (AMC); cefazolin (KZ); ceftazidime (CAZ); cefotaxime (CTX); chloramphenicol (CLO); gentamicin (CN); tetracycline (TET); nitrofurantoin (NIT); nalidixic acid (NAL); ciprofloxacin (CIP); norfloxacin (NOR); enrofloxacin (ENR); trimethoprim-sulfamethoxazole (SUT); not found (NF).
and Klebsiella pneumoniae. Six families of plasmid-mediated AmpC β-lactamases have been identified [26]. Among AmpC, the CIT group was the most frequently observed in our results. Studies have related the presence of the CIT group in poultry in other countries [4, 12, 13]. In Brazil, the presence of plasmid-mediated AmpC-producing in human isolates has been sporadically reported [38, 39]. The presence of II AmpC-producing strains indicates the importance of studies both in human and in veterinary clinical practice.

Despite the increase of ESBL/AmpC-producing E. coli isolates in food-producing animals, little is known about the use of β-lactam because these are banned as growth promoters in Brazilian aviculture. One hypothesis is that the coselection and co-resistance have taken place because the gene encoding ESBL and other classes of non-β-lactam can be located in the same mobile genetic element, such as plasmid or transposon [15]. In our study, ESBL/AmpC-producing strains showed resistance to one or more non-β-lactam antibiotics, mainly to tetracycline (98% of the cases). The presence of ESBL and AmpC gene was not observed in the same strain. It is possible that there is a limit to the amount of β-lactamase that a bacterial cell can accommodate and still be a viable pathogen [26].

We also note that the β-lactamases may be present in strains belonging to phylogenetic groups from commensal groups A and B1, as well as virulent strains from group D. We note also that the majority of ESBL/AmpC-producing strains have one or more virulence genes tested. This can indicate that some strains harbor antimicrobial resistance genes mediated by plasmids and perhaps are harboring virulence factors encoding genes mediated by other plasmids too. Some studies have shown that virulence plasmids and multidrug resistance plasmid were not found in the same strains [8, 40]. However, Johnson and collaborators [41] found in some APEC strains hybrid resistance plasmids encoding multiple resistance to both antimicrobials and virulence-associated genes that were able to infect human cells and cause meningitis in rats.

In our results, it is clear that even with the prohibition of many antimicrobials there is still a high frequency of antimicrobial resistance in strains from conventional poultry. The low frequency of antimicrobial resistance in strains from free-range poultry may indicate that the low use of antimicrobials in this system rearing may be related to the low frequency of resistance and virulence, which can lead to a low risk of transmission of pathogens or resistance genes to humans through consumption of chicken meat. The monitoring of antimicrobial resistance frequencies in animal foods can aid in the detection of banned poultry farming practices.

5. Conclusion

The high frequency of antimicrobial resistance, associated with several virulence factors, made E. coli in a potential food problem, due to the possibility of horizontal transfer of virulence genes and antimicrobial resistance to the human resident microbiota and/or human pathogens. The absence or restricted use of antimicrobials in free-range poultry production may be contributing to the lower frequency of bacterial virulence factors and resistance to antimicrobials, leading to a lower risk of their transmission to humans.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

This study was supported by Conselho Nacional Desenvolvimento Científico e Tecnológico (CNPq) (Chamada MCTI/CNPq/ANVISA no. 23/2012). Thanks are also due to CAPES for the use of financial facilities.

References

[1] T. J. Johnson, C. M. Logue, J. R. Johnson et al., “Associations between multidrug resistance, plasmid content, and virulence potential among extraintestinal pathogenic and commensal Escherichia coli from humans and poultry,” Foodborne Pathogens and Disease, vol. 9, no. 1, pp. 37–46, 2012.
[2] B. M. Marshall and S. B. Levy, “Food animals and antimicrobials: impacts on human health,” Clinical Microbiology Reviews, vol. 24, no. 4, pp. 718–733, 2011.
[3] M. Mellata, “Human and avian extraintestinal pathogenic Escherichia coli: infections, zoonotic risks, and antibiotic resistance trends,” Foodborne Pathogens and Disease, vol. 10, no. 11, pp. 916–932, 2013.
[4] A. Ghodousi, C. Bonura, A. M. D. Noto, and C. Mammina, “Extended-spectrum β-lactamase, AmpC-producing, and fluoroquinolone-resistant Escherichia coli in retail broiler chicken meat, Italy,” Foodborne Pathogens and Disease, vol. 12, no. 7, pp. 619–625, 2015.
[5] C. Dierikx, J. van der Goot, T. Fabri, A. van Essen-Zandbergen, H. Smith, and D. Mevius, “Extended-spectrum β-lactamase, AmpC-producing, and extended-spectrum β-lactamase-producing Escherichia coli in Dutch broilers and broiler farmers,” The Journal of Antimicrobial Chemotherapy, vol. 68, no. 1, pp. 60–67, 2013.
[6] H.-X. Jiang, D.-H. Lü, Z.-L. Chen et al., “High prevalence and widespread distribution of multi-resistant Escherichia coli isolates in pigs and poultry in China,” Veterinary Journal, vol. 187, no. 1, pp. 99–103, 2011.
[7] T. Asai, M. Hiki, M. Ozawa et al., “Control of the development and prevalence of antimicrobial resistance in bacteria of food animal origin in Japan: a new approach for risk management of antimicrobial veterinary medicinal products in Japan,” Foodborne Pathogens and Disease, vol. 11, no. 3, pp. 171–176, 2014.
[8] L. Bélanger, A. Gareniaux, J. Hardé, M. Boulanne, E. Nadeau, and C. M. Dozois, “Escherichia coli from animal reservoirs as a potential source of human extraintestinal pathogenic E. coli,” FEMS Immunology and Medical Microbiology, vol. 62, no. 1, pp. 1–10, 2011.
[9] J. D. D. Pitout, “Extraintestinal pathogenic Escherichia coli: a combination of virulence with antibiotic resistance,” Frontiers in Microbiology, vol. 3, no. 9, pp. 1–7, 2012.
[10] O. Clermont, S. Bonacorsi, and E. Bingen, “Rapid and simple determination of the Escherichia coli phylogenetic group,” Applied and Environmental Microbiology, vol. 66, no. 10, pp. 4555–4558, 2000.
[11] T. J. Johnson, Y. Wannemuehler, C. Doetkott, S. J. Johnson, S. C. Rosenberger, and L. K. Nolan, "Identification of minimal predictors of avian pathogenic Escherichia coli virulence for use as a rapid diagnostic tool," Journal of Clinical Microbiology, vol. 46, no. 12, pp. 3987–3996, 2008.

[12] S. Börjesson, M. Egervärn, M. Lindblad, and S. Englund, "Frequent occurrence of extended-spectrum beta-lactamase- and transferable AmpC beta-lactamase-producing Escherichia coli on domestic chicken meat in Sweden," Applied and Environmental Microbiology, vol. 79, no. 7, pp. 2463–2466, 2013.

[13] C. Ewers, A. Bethe, T. Semmler, S. Guenther, and L. H. Wieler, "Extended-spectrum beta-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective," Clinical Microbiology and Infection, vol. 18, no. 7, pp. 646–655, 2012.

[14] R. E. Warren, V. M. Ensor, P. O'Neill et al., "Imported chicken meat as a potential source of quinolone-resistant Escherichia coli producing extended-spectrum beta-lactamases in the UK," Journal of Antimicrobial Chemotherapy, vol. 61, no. 3, pp. 504–508, 2008.

[15] D. M. Livermore, "Bacterial resistance: origins, epidemiology, and impact," Clinical Infections Diseases, vol. 36, supplement 1, pp. S1–S23, 2003.

[16] P. Bauchart, P. Germon, A. Brée, E. Oswald, J. Hacker, and U. Dobrindt, "Pathogenicomic comparison of human extraintestinal and avian pathogenic Escherichia coli—search for factors involved in host specificity or zoonotic potential," Microbial Pathogenesis, vol. 49, no. 3, pp. 105–115, 2010.

[17] V. L. Koga, G. R. Rodrigues, S. Scandoriero et al., "Evaluation of the antibiotic resistance and virulence of Escherichia coli strains isolated from chicken carcasses in 2007 and 2013 from Paraná, Brazil," Foodborne Pathogens and Disease, vol. 12, no. 6, pp. 479–485, 2015.

[18] M. R. F. Toledo, C. F. Fontes, and L. R. Trabulsi, "Um meio para a realização dos testes de motilidade, indol e lisina descarboxilase," Revista de Microbiologia, vol. 13, no. 3, pp. 230–235, 1982 (Portuguese).

[19] M. R. F. Toledo, C. F. Fontes, and L. R. Trabulsi, "EPM-Modificação do meio Rugai e Araújo para realização simultânea dos testes de produção de gás e partir de glicose, HIS2, urease e triptofano desaminase," Revista de Microbiologia, vol. 13, no. 4, pp. 309–315, 1982 (Portuguese).

[20] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Approved Standard, CLSI Document M31-A3, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, USA, 3rd edition, 2008.

[21] Clinical and Laboratory Standards Institute, "Performance standards for antimicrobial susceptibility testing: 23rd informational supplement," CLSI Document M100-S23, CLSI, Wayne, PA, USA, 2013.

[22] G. A. Jacoby and P. Han, "Detection of extended-spectrum beta-lactamases in clinical isolates of Klebsiella pneumoniae and Escherichia coli," Journal of Clinical Microbiology, vol. 34, no. 4, pp. 908–911, 1996.

[23] G. Arlet and G. Philippin, "Construction by polymerase chain reaction and use of intragenic DNA probes for three main types of transferable beta-lactamase (TEM, SHV, CARB) [corrected]," FEMS Microbiology Letters, vol. 66, no. 1, pp. 19–25, 1991.

[24] B. Bedenić, C. Randegger, E. Stobberingh, and H. Hächler, "Molecular epidemiology of extended-spectrum beta-lactamases from Klebsiella pneumoniae strains isolated in Zagreb, Croatia," European Journal of Clinical Microbiology & Infectious Diseases, vol. 20, no. 7, pp. 505–508, 2001.

[25] N. Woodford, E. J. Fagan, and M. J. Ellington, "Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum beta-lactamases," The Journal of Antimicrobial Chemotherapy, vol. 57, no. 1, pp. 154–155, 2006.

[26] F. J. Pérez-Pérez and N. D. Hanson, "Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR," Journal of Clinical Microbiology, vol. 40, no. 6, pp. 2153–2162, 2002.

[27] A. R. Manges and J. R. Johnson, "Food-borne origins of Escherichia coli causing extraintestinal infections," Clinical Infectious Diseases, vol. 55, no. 5, pp. 712–719, 2012.

[28] V. L. Koga, G. Tomazetto, P. S. Cyoia et al., "Molecular screening of virulence genes in extraintestinal pathogenic Escherichia coli isolated from human blood culture in Brazil," BioMed Research International, vol. 2014, Article ID 465054, 9 pages, 2014.

[29] Y. Luo, Y. Ma, Q. Zhao et al., "Similarity and divergence of phylogenies, antimicrobial susceptibilities, and virulence factor profiles of Escherichia coli isolates causing recurrent urinary tract infections that persist or result from reinfection," Journal of Clinical Microbiology, vol. 50, no. 12, pp. 4002–4007, 2012.

[30] P. S. Cyoia, G. R. Rodrigues, E. K. Nishio et al., "Presence of virulence genes and pathogenicity islands in Extraintestinal pathogenic Escherichia coli isolated in Brazil," The Journal of Infection in Developing Countries, In press.

[31] J. I. R. Castanon, "History of the use of antibiotic as growth promoters in European poultry feeds," Poultry Science, vol. 86, no. 11, pp. 2466–2471, 2007.

[32] Brasil Ministério da Agricultura, "Pecuária e Abastecimento. 27 de junho de 2003. Proíbe a fabricação, a manipulação, o fracionamento, a comercialização, a importação e o uso dos princípios ativos cloranfenicol e nitrofuranos e os produtos que contenham estes princípios ativos, para uso veterinário e susceptível de emprego na alimentação de todos os animais e insetos," Instrução Normativa, no. 9, 2003 (Portuguese), http://www.agricultura.gov.br/arg_editor/file/CRC/IN%2009 _2003%20%-%20Proib%20uso%20de%20cloranfenicol%20e% 20nitrofuranos.

[33] Ministério da Agricultura, Pecuária e Abastecimento, "Regulamento técnico para a fabricação, o controle de qualidade, a comercialização e o emprego de produtos antimicrobianos de uso veterinário," Instrução Normativa 26, Ministério da Agricultura, Pecuária e Abastecimento, Brasília, Brazil, 2009 (Portuguese), http://sistemaweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=visualizarAtoPortalMapa&chave=1984822284

[34] A. S. Obeng, H. Rickard, O. Ndi, M. Sexton, and M. Barton, "Antibiotic resistance, phylogenetic grouping and virulence potential of Escherichia coli isolated from the faeces of intensively farmed and free range poultry," Veterinary Microbiology, vol. 154, no. 3-4, pp. 305–315, 2012.

[35] L. S. Rossa, E. V. Stahlke, D. C. Diez, S. H. Weber, S. C. Stertz, and R. F. Macedo, "Resistência antimicrobiana e ocorrência de micro-organismos patogênicos e indicadores em frangos orgânicos e convencionais: estudo comparativo," Biotemas, vol. 26, no. 3, pp. 211–220, 2013 (Portuguese).

[36] F. Reich, V. Atanassova, and G. Klein, "Extended-spectrum beta-lactamase- and ampc-producing enterobacteria in healthy broiler chickens, Germany," Emerging Infectious Diseases, vol. 19, no. 8, pp. 1253–1259, 2013.
[37] S. A. Fernandes, D. L. Paterson, Â. C. Ghilardi-Rodrigues, J. M. Adams-Haduch, A. T. Tavechio, and Y. Doi, “CTX-M-2-producing *salmonella* typhimurium isolated from pediatric patients and poultry in Brazil,” *Microbial Drug Resistance*, vol. 15, no. 4, pp. 317–321, 2009.

[38] E. H. Campana, P. P. Barbosa, L. C. C. Fehlberg, and A. C. Gales, “Frequency of plasmid-mediated AmpC in enterobacteriaceae isolated in a Brazilian teaching hospital,” *Brazilian Journal of Microbiology*, vol. 44, no. 2, pp. 477–480, 2013.

[39] M. Pavez, P. Neves, M. Dropa et al., “Emergence of carbapenem-resistant *Escherichia coli* producing CMY-2-type AmpC β-lactamase in Brazil,” *Journal of Medical Microbiology*, vol. 57, no. 12, pp. 1590–1592, 2008.

[40] C. Bonnet, F. Diarrassouba, R. Brousseau, L. Masson, E. Topp, and M. S. Diarra, “Pathotype and antibiotic resistance gene distributions of *Escherichia coli* isolates from broiler chickens raised on antimicrobial-supplemented diets,” *Applied and Environmental Microbiology*, vol. 75, no. 22, pp. 6955–6962, 2009.

[41] T. J. Johnson, D. Jordan, S. Kariyawasam et al., “Sequence analysis and characterization of a transferable hybrid plasmid encoding multidrug resistance and enabling zoonotic potential for extraintestinal *Escherichia coli*,” *Infection and Immunity*, vol. 78, no. 5, pp. 1931–1942, 2010.