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Antimicrobial resistance profiles in *Escherichia coli* isolated from whole-chicken carcasses from conventional, antibiotic-free, and organic rearing systems

Perfil de resistência em *Escherichia coli* isoladas de carcaças de frango provenientes de sistemas de produção convencional, livre de antibióticos e orgânico

Tatiana Regina Vieira[1]*; Esther Ferrazza Cavinatto de Oliveira[2]; Samuel Paulo Cibulski[3]; Mauro Riegert Borba[4]; Marisa Cardoso[4]

Highlights

Low resistance to antimicrobials in *Escherichia coli* isolated from chicken carcasses.
The highest frequencies of resistance against tetracycline and trimethoprim.
Chicken from the organic system presented a lower frequency of MDR *Escherichia coli.*
Chicken from the organic system presented a higher frequency of TSUS *Escherichia coli.*

Abstract

Antimicrobial resistance (AMR) is a growing concern in human and animal health. Public discussions on these issues have contributed to an increased demand for antibiotic-free food. Studies comparing the antimicrobial resistance profiles of bacteria in foodstuffs originating from farming systems with restrictions on the use of antimicrobials are scarce. This study aimed to assess the antimicrobial resistance profiles of generic *Escherichia coli* isolated from whole chickens originating from farming systems with and without restrictions on the use of antimicrobials. For this purpose, three groups of *E. coli* strains were formed: (GC) from chickens reared in conventional production systems, without restriction on the use of antimicrobials (n=72); (GL) from chickens reared in farming systems certified as free of any antibiotic use (n=72); and (GO) from chickens from an organic farming system (n=72). Whole chicken units were individually rinsed as recommended by ISO 17604:2015, and *E. coli* was isolated from the rinse suspension. To evaluate the resistance profile, *E. coli* strains were tested against 12 antimicrobials using broth microdilution or disk diffusion tests. Eighty strains

[1] Pos-Doc, Dr[a], Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, RS, Brazil. E-mail: tatianareginavieira@gmail.com
[2] Student of the Biology Undergraduate Course, UFRGS, Porto Alegre, RS, Brazil. E-mail: esthercavinatto@gmail.com
[3] Prof. Dr., Universidade Federal da Paraíba, UFPB, João Pessoa, PB, Brazil. E-mail: spcibulski@gmail.com
[4] Profs. Drs., Faculdade de Veterinária, UFRGS, Porto Alegre, RS, Brazil. E-mail: mauro.borba@ufrgs.br; mcardoso@ufrgs.br
* Author for correspondence

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(40.7%) were found to be fully susceptible to the tested antimicrobials, and 23.6% were multidrug resistant. The highest frequencies of resistance were observed to tetracycline (GC=37,5%; GL=34,7%; GO=25%) and trimethoprim (GC=27,8%; GL=34,7%; GO=22,2%). In the case of multidrug resistant strains, GC presented 32% (n=23) of strains with multidrug resistance characteristics whereas the GL and GO groups presented 22% (n=16) and 17% (n=12), respectively. As for the totally susceptible strains, a frequency of 56% of Tsus strains was observed in the organic group, whereas this frequency was 33% in the GC and GL groups. Using GC as a reference, the Poisson regression model showed a higher occurrence of fully susceptible *E. coli* strains, as well as lower frequencies of multidrug resistance and resistance to ampicillin and nalidixic acid in GO. The GL group exhibited the lowest frequency of ampicillin resistance. These observations suggest that the lower selection pressure for antimicrobial use in the farming system may be reflected in the resistance profile of bacteria present in foodstuffs purchased by consumers.
Key words: Alternative production system. Antibiotic restriction. Food-production animals. Public health.

Resumo

A resistência antimicrobiana (AMR) é uma preocupação crescente para a saúde humana e animal. A discussão pública dessas questões tem contribuído para o aumento da demanda por alimentos produzidos sem o uso de antibióticos. No entanto, estudos que comparem os perfis de resistência antimicrobiana de bactérias em alimentos oriundos de sistemas agrícolas com restrição ao uso de antimicrobianos ainda são escassos. O objetivo deste estudo foi avaliar o perfil de resistência antimicrobiana em *Escherichia coli* genérica isolada de carcaças de frangos inteiros oriundos de sistemas de criação com e sem restrições ao uso de antimicrobianos. Para tanto, três grupos de cepas de *E. coli* foram formados: (GC) isolados de carcaças de frangos criados no sistema convencional - sem restrição de uso de antimicrobianos (n=72); (GL) isolados de frangos de sistemas certificados sem uso de antimicrobianos (n=72); (GO) de frangos originados de produção orgânica (n=72). As unidades de frango inteiro foram submetidas à lavagem conforme recomendado pela ISO 17604: 2015 e *E. coli* foi isolada da suspensão de enxágue. Para avaliar o perfil de resistência, as cepas de *E. coli* foram testadas frente à 12 antimicrobianos pelos testes de microdiluição em caldo ou difusão em disco. Oitenta cepas (40,7%) foram totalmente suscetíveis aos antimicrobianos testados e 23,6% multirresistentes. As maiores frequências de resistência foram observadas frente a tetraciclina (GC=37,5%; GL=34,7%; GO=25%) e trimetoprima (GC=27,8%; GL=34,7%; GO=22,2%). No caso de cepas multirresistentes, GC apresentou 32% (n=23) das cepas com características de multirresistência enquanto os grupos GL e GO apresentaram 22% (n=16) e 17% (n=12), respectivamente. Quanto às cepas totalmente suscetíveis, foi observada uma frequência de 56% de cepas Tsus no grupo orgânico enquanto tal frequência foi de 33% nos grupos GC e GL. Utilizando GC como referência, o modelo de regressão de Poisson demonstrou maior ocorrência de cepas de *E. coli* totalmente suscetíveis, bem como menores frequências de multirresistência e resistência à ampicilina e ácido nalidíxico no GO. Em GL, apenas a frequência mais baixa de resistência à ampicilina pôde ser demonstrada. Essas observações sugerem que a menor pressão de seleção do uso de antimicrobianos no sistema de cultivo pode se refletir no perfil de resistência das bactérias presentes nos alimentos adquiridos pelo consumidor.
Palavras-chave: Restrição de antibióticos. Animais de produção. Saúde pública. Sistemas de produção alternativa.

Introduction

Chicken meat is globally the main source of animal protein with consumption surpassing 98 million tons in 2019 (Organisation for Economic Cooperation and Development [OECD], 2020). The success of poultry production has resulted from decades of genetic selection, nutrition, vaccines, new technologies, and improvements in sanitary management. However, the use of antimicrobials is assumed to have played a role in bringing industrial poultry farming to the current level of production.

Antimicrobial resistance is one of the top concerns in human and animal health. Among the players involved in the emergence and spread of antimicrobial resistance, intensive animal production systems are considered an important link in the selection of resistant bacteria (World Health Organization [WHO], 2015, 2019). Concerns regarding the spread of resistant bacteria have led many countries to adopt policies to control the use of antimicrobials in animal production, and in several cases, a complete ban has been placed on their use in prophylaxis and growth promotion (Agyare et al., 2018). However, initiatives towards the withdrawal of antimicrobial use in food animals have raised questions related to their effectiveness and the time span needed to reduce or eradicate antimicrobial-resistant populations (Millman et al., 2013; Davis et al., 2018; Roth et al., 2019). Investigations of resistant bacteria in animal production systems, in which antimicrobials have been withdrawn, can contribute to determining the frequency of resistance, especially with regard to antimicrobials classified as critically important to human health (WHO, 2019).

In this scenario, the livestock industry has looked for alternatives to reduce the use of antimicrobials; among them, organic and antibiotic-free breeding systems have emerged as options. In alternative farming systems, among other premises, is the withdrawal of antimicrobials used as growth promoters, prophylaxis, and restriction of therapeutic administration (Diaz-Sanchez et al., 2015; Demattê & Pereira, 2017). Consumers' perception of foodstuffs from alternative systems, such as organics, is that they are inherently safer than conventional systems. However, studies comparing the antimicrobial resistance profiles of bacteria in foodstuffs produced by alternative systems and those available to consumers are scarce. Specifically, there is a lack of understanding of how restriction on the use of antimicrobials on farm may be reflected in the resistance profile of bacteria in retail food. Therefore, the present study aimed to compare patterns of antimicrobial resistance in generic *Escherichia coli* isolated from frozen whole-chicken originating from conventional production systems and those from farming systems certified as having restricted use of antimicrobials.

Materials and Methods

Study design

A descriptive comparative study of the phenotypic profile of resistance to antimicrobials was carried out in generic *Escherichia coli* (*E. coli*) isolated from frozen whole chickens acquired in the Brazilian retail market. Based on the results of a pilot study, the sample size in each of the three groups was determined to be 72 *E. coli* strains in order to detect a difference in proportions characterized by a variance of 0.01 with an average proportion of 0.66, assuming a 0.05 level Chi-square test and a power of 80%. The group of *E. coli* strains was formed according to the origin of isolation in terms of the whole-chicken carcass package information, as follows: (GC) from chickens reared in conventional production systems, without restriction on the use of antimicrobials as growth promoters, prophylaxis or therapeutic; (GL) from chickens reared in farming systems certified as free of any antibiotic use; (GO) from chickens from an organic farming system, in which the use of antimicrobials is not allowed in any of the production stages; however, diseased animals can be treated once in their lives, and the withdrawal period set by the organic regulation must be followed before slaughter. Antibiotic-free and organic farming was considered only when a certification seal was provided on the package. To include intra-sample diversity, three *E. coli* strains per whole chicken sample were included in each group. Therefore, 24 frozen whole chickens from each conventional, antibiotic-free, and organic farming system were purchased to obtain the number of *E. coli* strains.

All purchased products were frozen whole chickens, slaughtered under federal inspection, packaged in the industry, with or without giblets, and available in commercial establishments selling directly to the consumer. Retail markets were selected by convenience, considering the availability of products and access to points of sale. The purchased whole-chicken units belonged to different batches from five brands of the conventional production system, two brands of the antibiotic-free system, and one brand of the organic system.

Isolation of Escherichia coli and grouping of strains according to the origin

Frozen whole-chicken samples were transported in cooling boxes to the laboratory and stored under refrigeration until they were completely thawed. The samples were then transferred to sterile plastic bags, weighed, and washed with 1% buffered peptone water (BPW), as recommended by ISO 17604:2015.

The enumeration of *E. coli* was performed after serial decimal dilutions (10^{-1} to 10^{-6}) of the liquid obtained from carcass washing. From the dilutions, 1mL aliquots were transferred, in duplicate, to plates containing chromogenic agar for *E. coli* (Chromocult® Merck Millipore), following the manufacturer's instructions. After incubation at 36 °C ± 1 °C for 24-hour, typical colonies (dark blue to violet) were counted, and the number of colony-forming units (CFU) per gram was calculated according to ISO 17604:2015 and ISO 6887-1:2017. In addition, 30mL of washing suspension was added to 30mL of BPW 1% for enrichment. After incubation at 36 °C ± 1 °C for 18-24h, serial dilutions of the enrichment broth were performed, followed by isolation on CHROMagar™ *E. coli.* After incubation at 36 °C ± 1 °C for 18-24h, typical colonies of *E. coli* were selected in different quadrants of the plate.

The *E. coli* strains were formed from three colonies of each chicken sample obtained from Chromocult® or CHROMagar™ agar plates. In both cases, each *E. coli* typical colony were transferred to tryptic soy agar plates (TSA, Merck Millipore), and phenotypic confirmation of the species was performed as described by Quinn et al. (2011) or by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-ToF).

After confirmation, 72 strains were assigned according their isolation origin to each GC, GL, or GO, and were stored in skim milk with glycerol at -20 °C until the performance of susceptibility tests.

Antimicrobial susceptibility tests

Antimicrobial susceptibility was assessed by determining the minimum inhibitory concentration (MIC) or by agar diffusion test. The selection of antimicrobials was based on the importance of their use in human and veterinary medicine, following the panel determined by the Commission Implementing Decision 2013/652/EU (European Center for Disease Prevention and Control, European Food Safety Authority and Europe Medicines Agency [ECDC, EFSA, EMA], 2017). The minimum inhibitory concentration (MIC) was determined by the broth microdilution method in plastic 96-well plates, as recommended by the Clinical and Laboratory Standards Institute [CLSI] (2020) for the following antimicrobials (Sigma-Aldrich): nalidixic acid (NAL), ampicillin (AMP), cefotaxime (CTX), ciprofloxacin (CIP), colistin (COL), gentamicin (GEN), and tetracycline (TET). The tested concentration range and cutoff points used in the interpretation of the results followed the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI (2020). After the incubation period (24- hour at 36 °C ± 1 °C), the plates were examined visually, and the lowest concentration of an antimicrobial, in which no bacterial growth was observed, was considered the MIC of the antimicrobial for the tested isolate. Discs (Thermo Scientific Oxoid) of ceftazidime-CAZ (30 µg), chloramphenicol-CLO (30 µg), meropenem - MER (10 µg), tigecycline - TIG (15 µg), and trimethoprim (TRI; 5 µg) were used in the agar diffusion test of the *E. coli* strains, performed as recommended (CLSI, 2018, 2020). *Escherichia coli* ATCC 25922 was used as quality control.

Data analysis

The results were tabulated in a Microsoft Excel spreadsheet and exported to statistical software when necessary. The difference in the average of total antimicrobial-resistant isolates in the GC, GL, and GO groups was calculated using analysis of variance (ANOVA), as described by Millman et al. (2013). To verify the relationship of resistance, to each antimicrobial evaluated, and the three different groups (assuming GC as the basis), prevalence ratios (PR) were estimated using a *Poisson* regression model. This calculation was performed using R software (R Core Team [R], 2013), with the packages Epitools and Stat.

Results and Discussion

There was no significant difference (p=0.72) in *E. coli* counts (CFU.g^{-1}) on carcasses from different types. All whole-chicken samples had *E. coli* counts per gram within the microbiological limits (5x10^{3}) determined by the Brazilian legislation (Ministério da Saúde [MS], 2019a,b). Besides, 27 (37.5%) samples had counts lower than 1 CFU.g^{-1} of *E. coli,* demonstrating that all products were suitable for consumption based on this indicator of hygienic quality.

The distribution of the MIC values of the isolated *E. coli* strains showed that MIC_{50} in all groups of strains was much lower than the resistance breakpoint for all tested antimicrobials (Table 1). Moreover, no statistically significant difference (p>0.05) was observed between the mean MIC values for any of the antimicrobials among the three groups (p>0.05). Comparisons between group pairs also showed no statistical difference: GC × GL (p=0.96), GC × GO (p=0.06), and GL × GO (p=0.07). Considering that the selection pressure is supposed to benefit the multiplication of resistant bacteria (WHO, 2015; European Centre for Disease Prevention and Control, European Food Safety Authority & Europe Medicines Agency [ECDC, EFSA & EMA], 2017), it could be expected that in production systems with restricted use of antimicrobials, a modulation towards the reduction in antimicrobial MIC against bacterial populations will take place. In the present study, no significant difference in the mean MIC values between the groups was detected. However, it must be taken into account that the average MIC levels were not particularly high even in the GC group, which encompassed *E. coli* strains isolated from chicken carcasses originating from rearing systems that were administered antimicrobials. Considering the importance of Brazil in the global market and the growing restrictions on the use of antimicrobials in animal production worldwide, it is possible to speculate that, even in conventional farming, the use of antibiotics might have been reduced in recent times. Despite the low diversity of brands available in the market for the GL (two brands) and GO (one brand) groups, this apparently did not influence the results obtained, because even with different management, there was no difference in the resistance averages between the groups. In addition, the focus of this study was to demonstrate the profile of bacterial resistance that reaches every consumer.

Table 1
Minimum Inhibitory Concentration values of antimicrobials for *Escherichia coli* strains isolated from whole-chicken carcasses originated from conventional (GC), antibiotic-free (GL) and organic (GO) farming systems

Ampicillin (mg/L)	0.5	1	2	4	8	16	32	64	128	256	512	>512		MIC_{50}
GC	0	4	13	28	0	0	0	0	0	7	16	4		4
GL	0	6	22	29	0	0	0	1	2	1	4	7		4
GO	0	0	33	27	0	0	0	0	0	4	3	5		4
Ciprofloxacin (mg/L)	0.004	0,008	0,015	0,03	0,06	0,125	0,25	0,5	1	2	4	8	>8	MIC_{50}
GC	0	2	40	7	1	2	8	6	4	0	0	1	1	0,015
GL	0	0	44	9	4	3	5	4	2	0	0	0	1	0,015
GO	0	1	53	8	1	0	0	4	1	0	0	1	3	0,015
Colistin (mg/L)	0.06	0.125	0.25	0.5	1	2	4	8	16					MIC_{50}
GC	0	0	0	49	23	0	0	0	0					0,5
GL	0	0	1	26	45	0	0	0	0					1
GO	0	0	0	14	53	5	0	0	0					1
Cefotaxime (mg/L)	<0.015	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	MIC_{50}
GC	0	0	4	39	22	2	0	0	0	0	1	0	0	0,06
GL	8	0	18	32	5	0	0	0	0	3	0	0	1	0,06
GO	0	0	10	47	6	3	0	0	0	0	1	2	0	0,06
Gentamicin (mg/L)	0.125	0.25	0.5	1	2	4	8	16	32	64	>64			MIC_{50}
GC	1	0	47	18	2	0	0	0	3	1	0			0,5
GL	0	0	29	21	4	0	0	0	12	5	1			1
GO	0	2	23	36	0	0	0	8	1	1	1			1
Nalidixic acid (mg/L)	0.5	1	2	4	8	16	32	64	128	256	512	>512		MIC_{50}
GC	0	0	6	33	11	3	4	0	3	12	0	0		4
GL	0	4	29	17	7	3	3	0	0	6	3	0		4
GO	0	0	7	51	5	0	1	0	0	0	8	0		4
Tetracycline (mg/L)	0.25	0.5	1	2	4	8	16	32	64	128	256			MIC_{50}
GC	0	1	26	17	1	0	0	0	5	19	3			2
GL	0	0	16	31	0	0	0	1	13	8	3			2
GO	0	0	8	33	13	0	0	5	0	6	7			2

GC: conventional, GL: antibiotic-free, GO: organic.
Gray-filled cells represent resistance breakpoint values according to CLSI.

Regarding the profile of resistance or susceptibility to antimicrobials among the 216 *E. coli* strains analyzed, 88 (40.7%) were fully susceptible to 12 tested antimicrobials. All the strains were susceptible to colistin, meropenem, and tigecycline. These results are quite encouraging from a public health perspective, since these antimicrobials are on the list of last-resort drugs for the treatment of life-threatening infections in humans (WHO, 2019). Resistance to meropenem and tigecycline has seldom been reported in enterobacteria originating from animal production (Osman et al., 2018; European Food Safety Authority & European Centre for Disease Prevention and Control [EFSA & ECDC], 2019), which corroborates the results of our study. Although resistance to tigecycline has been reported in 29.3% of *E. coli* strains isolated from organic chicken farms (Musa et al., 2021), the authors did not rule out the possibility of an environmental origin for the resistant strains. In contrast, resistance to colistin has been detected in several investigations in the poultry production chain, including in Brazil (Lopes et al, 2020; Lentz et al., 2021), with frequencies ranging from 1.8% (Perrin-Guyomard et al., 2016; Pesciaroli et al., 2020) to as high as 57.94% (Barbieri et al., 2021) and 79.8% (Irrgang et al., 2016). In addition, food animals have been suggested as possible reservoirs and spreaders of genes that confer resistance to this class of antimicrobials (Irrgang et al., 2016; Perrin-Guyomard et al., 2016; Guenther et al., 2017; Monte et al., 2017; Yassin et al., 2017; Bitrus et al., 2018; Zhang et al., 2018). The absence of strains resistant to colistin observed in our study may be related to the ban on the use of this antibiotic as a growth promoter in Brazil (Ministério da Agricultura Pecuária e Abastecimento [MAPA], 2016). This fact may have led to the phasing out of its use for all purposes in poultry farming, resulting in lower selection pressure in recent years. Supporting this hypothesis, a study in China also demonstrated that the prevalence of colistin-resistant *E. coli* decreased from 18% to 5% a few years after the ban on colistin as a poultry growth promoter (Wang et al., 2020).

Among the 128 generic *E. coli* strains that showed resistance, 76 (59.4%) were resistant to up to two antimicrobials tested (Table 2), indicating a relatively low level of resistance in the strains analyzed. Overall, the highest frequencies of resistant strains were found against tetracycline (32.4%), trimethoprim (28.2%), ampicillin (25.0%), and nalidixic acid (18.5%). Similar patterns of resistance to tetracycline, ampicillin, and nalidixic acid were observed in all groups, with a predominance of resistant strains in GC (Figure 1). Despite the fact that tetracyclines and beta-lactams have been prohibited as growth promoters since 2009 in Brazil [MAPA], 2009), these antibiotics are still used therapeutically and are included, along with quinolones and ionophores, among the antimicrobials commonly used in the treatment of sick birds (Hofacre et al., 2013; Landoni & Albarellos, 2015; Cardoso, 2019; Rabello et al, 2020). This may contribute to the persistence of resistance to these antimicrobials. Studies carried out with *E. coli* isolated from the poultry production chain have demonstrated variable frequencies of resistance to antimicrobials. However, ampicillin, tetracycline, and nalidixic acid have often been reported to have the highest resistance rates (Elumba et al., 2018; Abraham et al., 2019; Varga et al., 2019; Jahantigh et al., 2020; Musa et al., 2020; Pesciaroli et al., 2020; Rabello et al., 2020). In addition, this study demonstrates a much less critical scenario than that reported between 2007 and 2013 in Brazil and listed by Rabello et al. (2020). In this study, relatively higher frequencies of resistance against tetracycline (70-91%), nalidixic acid (61-78%), and ampicillin (20-67%) were observed in *E. coli* isolated from chicken.

Table 2
Frequencies of resistant strains by the number of resistant antimicrobial categories

Group	Number of resistant antimicrobial categories							Total resistant strains
	1	2	3	4	5	6	7	
Conventional (GC)	17	8	16	2	4	1	0	48
Antibiotic-free (GL)	15	16	9	3	3	0	2	48
Organic (GO)	4	16	7	3	0	2	0	32
Total	36	41	32	7	7	3	2	128

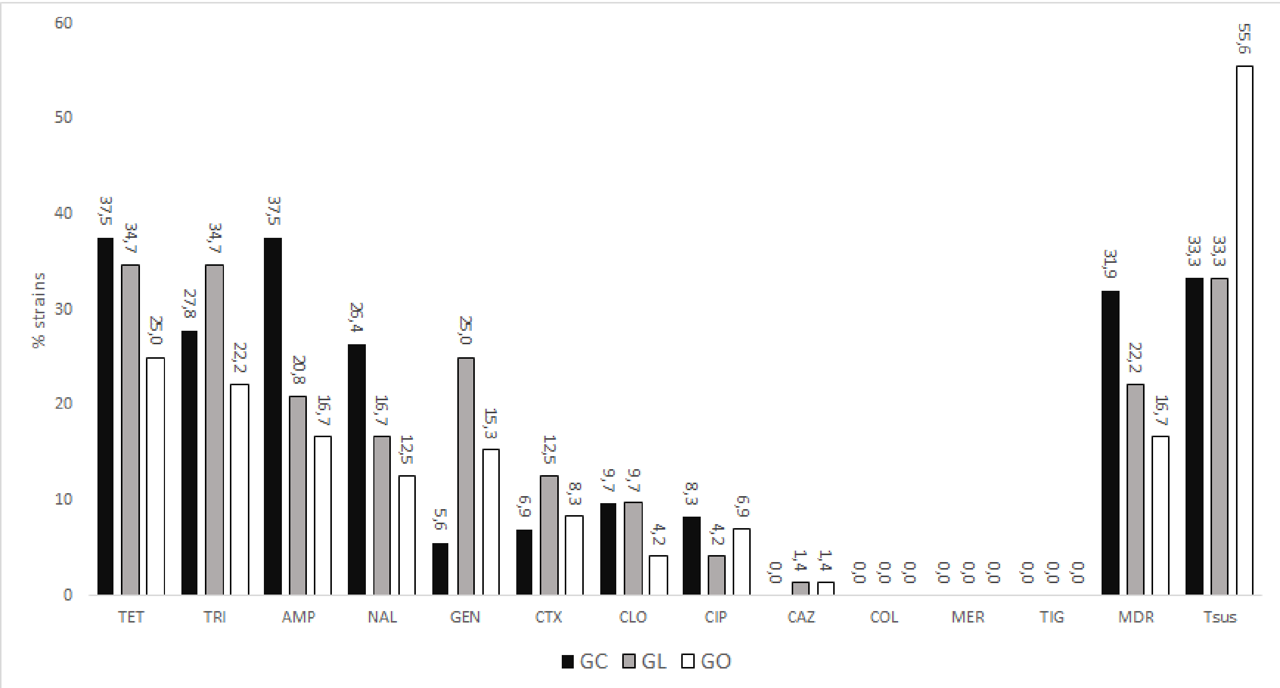

Figure 1. Relative frequency of antimicrobial resistant, multidrug resistant and fully susceptible *Escherichia coli* strains isolated from whole-chicken samples originated from conventional (GC), antibiotic-free (GL) and organic (GO) farming systems.
TET, tetracycline; TRI, trimethoprim; AMP, ampicillin; NAL, nalidixic acid; GEN, gentamicin; CTX, cefotaxime; CLO, chloramphenicol; CIP, ciprofloxacin; CAZ, ceftazidime; COL, colistin; MER, meropenem; TIG, tigecycline; MDR, multidrug resistance; Tsus, fully susceptible.

Differences between groups in the prevalence ratios of resistance were estimated using the Poisson regression model (Table 3). A statistically significant difference in the prevalence of resistance among the groups was observed to ampicillin, nalidixic acid, and gentamicin, as well as in the prevalence of fully susceptible and MDR strains. For ampicillin, the prevalence of resistance in GL was 0.56 times and in GO 0.44 times compared to that in GC. For nalidixic acid, the prevalence of GO was 0.47 times the prevalence of GC. However, a discrepant scenario occurred in relation to gentamicin, as in GL, 25% of the strains were resistant to this antimicrobial compared to 5% in GC. The Poisson model estimated a prevalence rate of 4.5 of resistant strains in GL in relation to GC. Because resistant strains were identified in different samples of frozen chicken from different batches and slaughterhouses, any possibility of being a resistant clone can be ruled out. In agreement with the results found in GC, the reported resistance to gentamicin is usually lower than 10% in commensal *E. coli* (EFSA & ECDC, 2018; Much et al., 2019; Sanchez et al., 2020). Higher gentamicin resistance has also been reported in laying hens of organic keeping systems in comparison to conventional systems in Germany, but no hypothesis has been raised for these findings (Schwaiger et al., 2008). Although the use of antimicrobials is an important factor in the selection of resistant strains (Xiong et al., 2018), this result may be related to other factors that might be involved in the persistence of antimicrobial-resistant strains in farms. Among these, the carriage of resistant strains by other animal species (Literak et al., 2009), long-term persistence in the environment (Schwaiger et al., 2008) and exposure to biocides or heavy metals (Kampf, 2019; Puangseree et al., 2021) have been suggested. All of these factors may be present even in organic farming.

The most relevant differences among the groups of *E. coli* strains were found between GO and GC regarding MDR, defined as non-susceptibility to at least one agent in three or more antimicrobial categories, and full susceptible strains (Magiorakos et al., 2012). In this sense, GO presented frequencies estimated at 1.67 times and 0.52 times of those observed in GC, respectively, for full susceptibility and MDR. In general, data from the literature consistently report higher resistance patterns in strains from conventional and antimicrobial-free groups when compared to organic groups (Pesciaroli et al., 2020; Sanchez et al., 2020; Musa et al., 2020). Moreover, and consistent with our results, MDR rate is often smaller in strains from organic production systems (Davis et al., 2018; Much et al., 2019; Pesciaroli et al., 2020; Musa et al., 2020). These data suggest that the restriction on antimicrobial use reflects first in the selection of MDR strains and allow the enhancement of full susceptible ones in the population, probably as result of decreasing the selection of some resistance phenotypes (for instance, ampicillin and nalidixic acid in our study). On the contrary, changes in other resistance markers, such as tetracycline, might need a longer time span to become observable. The predominance of tetracycline-resistant strains in *E. coli* of chickens and chicken meat has been reported (EFSA & ECDC, 2018), and the fact that chickens are raised free or in organic farming does not seem to change this profile (Much et al., 2019).

Table 3
Poisson regression model to estimate the association between *Escherichia coli* resistance of strains originated from farming systems with restricted antibiotic use for each evaluated antimicrobial and assuming GC as basis

Antimicrobial	Farming system	Prevalence Ratio	95% CI
Ampicillin	GL	0.56*	0.32-0.95
	GO	0.44*	0.24-0.80
Cefotaxime	GL	1.80	0.63-5.10
	GO	1.20	0.38-3.75
Chloramphenicol	GL	1	0.36-2.70
	GO	0.43	0.11-1.59
Ciprofloxacin	GL	0.50	0.13-1.92
	GO	0.83	0.26-2.60
Gentamicin	GL	4.50*	1.60-12.64
	GO	2.75	0.91-8.23
Nalidixic acid	GL	0.63	0.33-1.2
	GO	0.47*	0.22-0.97
Tetracycline	GL	0.93	0.59-1.43
	GO	0.67	0.40-1.09
Trimethoprim	GL	1.25	0.76-2.03
	GO	0.80	0.45-1.41
Multidrug resistant (MDR)	GL	0.70	0.40-1.20
	GO	0.52*	0.28-0.96
Fully susceptible (Tsus)	GL	1	0.63-1.58
	GO	1.67*	1.13-2.45

CI, confidence interval; GC, strains isolated from whole-chicken samples originating from conventional farming; GL, strains isolated from whole-chicken samples originating from antibiotic-free farming; GO, strains isolated from whole-chicken samples originating from organic farming.
(*) Statistical difference at a significance level of 0.05.

In our study, the association of the frequency of resistance in whole-chicken *E. coli* with the type of production may be limited by the fact that the samples analyzed were collected from retail sources instead of taking them from live birds in the production system itself. Even considering this limitation, the results suggest that the administration regime of antibiotics on farms may be reflected in retail products. In this case, the hazard to public health might be diminished, since food is the most consistent link in terms of the transfer of bacteria-carrying resistance genes between farm animals and humans.

Conclusions

There are still many knowledge gaps regarding the association of the use of antimicrobials in animal production and the increase and dissemination of resistant strains. The results of the present study corroborate the complexity of this theme and the need of an approach involving not only the animal and human health sectors, but also the environmental, to tackle this problem. The observations indicate that the rate of antimicrobial resistance in generic *E. coli* strains isolated from frozen whole-chicken is in general not high in Brazil, especially against the critically important antimicrobials for humans. The lower prevalence of multidrug resistance and the higher full-susceptibility among *E. coli* strains from organic-raised chicken suggest that there is a lower selective pressure towards resistance in the organic farming.

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