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Original Article

Microbiological assessment at slaughter of chicken carcasses from commercial, backyard and semi-backyard production systems

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Abstract
Introduction: Smaller scale, alternative, chicken production systems are gaining popularity globally. However, this brings public health and market confidence concerns, especially where there are no established standards of production. The aim of this study was to carry out a microbiological analysis of chicken carcasses from the commercial, backyard and semi-backyard production systems, slaughtered in the same slaughterhouse.
Methodology: Samples of 102 chicken carcasses were taken in two steps of the slaughter (A: after bleeding; and B: after chiller tank) and were subjected to aerobic mesophilic, coliforms at 35 °C and coliforms at 45 °C counts, and *Salmonella* spp. detection. *Salmonella* spp. isolates were subjected to antimicrobial resistance analysis.
Results: At slaughter step A, carcasses from the backyard system had less contamination than carcasses from the commercial system, with a difference of 0.7 log_{10} CFU/mL. *Salmonella* was identified in carcasses of all production systems and in both slaughter steps. Nine chicken carcasses were positive for *Salmonella* and no significant difference was observed in the occurrence of *Salmonella* amongst the carcasses from different production systems. Two *Salmonella* isolates, that presented the highest resistance profiles (one isolate was resistant to eight and the other to six out of ten tested antibiotics), were identified on carcasses from the semi-backyard system.
Conclusions: Carcasses from the backyard system had a lower microbial count at the initial step of the slaughter process than the commercial production system. In addition, greater resistance to antimicrobials was observed in *Salmonella* isolates from semi-backyard system.

Key words: Carcass quality; broilers; *Salmonella*; free range; microbiology.

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Introduction
Brazil is the world's second largest producer and exporter of broiler meat, exporting to more than 131 countries [1]. This good performance reflects the quality, price and global confidence in the safety of products produced in this country [2].

Although most of the volume produced comes from the intensive system, there is growing interest in extensive systems such as backyard chicken production [3]. In Brazil, the backyard system has specific legislation that defines, for example, 70 days as the minimum animal age for slaughter [4]. Some geographic regions in Brazil work with variations that have not been legally characterized; these are called semi-commercial or semi-backyard, using slow-growing chicken in an intensive poultry production system [5].

The major concern linked to this scenario is the lack of data on the sanitary condition of backyard chickens (*e.g.* occurrence of zoonotic diseases, monitoring of asymptomatic carriers of zoonotic microorganisms) and the impact they might have on food safety [6-8]. Chicken meat is contaminated by pathogenic and spoilage microorganisms during all stages of the production chain [9-11]. Therefore, the search for hygiene indicator microorganisms (aerobic mesophilic, coliforms, *enterobactereaceae*, *Escherichia coli*), and the presence of pathogens (*Salmonella*, *Campylobacter*, *Shigella*, *Listeria*) is fundamental for monitoring hygiene practices in poultry slaughterhouses [5,10,12,13].

Among the foodborne pathogens, non-typhoidal *Salmonella* is one of the most important and, just in the United States, it is estimated to be associated with 1.2 million cases of disease, 23,000 hospitalizations and

450 deaths, being food the source of most of these illnesses [14]. In addition, *Salmonella* is associated with multidrug resistance (MDR), which has worsened the prognosis of salmonellosis and increased the hospitalization time for patients [15].

In Brazil, several studies have identified *Salmonella* spp. during chicken slaughtered process, with frequencies of positives ranging from 3.6 to 31.7% [16-18]. However, these studies aimed to identify and characterize microorganisms associated with carcasses from intensive system only, due to its greater economic relevance and volume production [1]. Besides, few industries carry out the slaughter of chickens from intensive and extensive systems concurrently and, for that reason, there is a knowledge gap on the influence of the rearing system on the microbiological quality of the carcass.

Thus, the objective of the study was to execute a microbiological assessment of chicken carcasses from the commercial (C), backyard (Bc) and semi-backyard (SB) production systems, slaughtered in the same slaughterhouse.

Methodology

Slaughtering facilities and sampling

The visits were carried out in a slaughterhouse registered under the Municipal Inspection Service (SIM) that on average slaughters 2,400 birds/week. The establishment receives poultry from commercial (C), backyard (Bc) and semi-backyard (SB) production systems. At the studied slaughterhouse, no pre-defined slaughtering order and operational hygiene procedures were performed between batches of chickens from each production system. For this reason, the batches order was taken randomly on each sampling visit.

Samples of 102 chicken carcasses (34 C, 34 Bc and 34 SB) were taken in two steps of the slaughter process (A and B). Step A corresponds to the end of the bleeding process and before the scalding tank, and step B refers to the exit from the chiller tank. Each carcass collected was identified by numbered seals and noted on a spreadsheet along with the production system and slaughter step. Samples for analysis were collected by superficial rinsing of each carcass in a sterile bag containing 500 mL of buffered peptone water (0.1%). The resulting homogenate was then packed in a thermal box until examination [19]. Conventional microbiological analyses were performed at the laboratory for inspection and technology of products of animal origin of the Faculty of Veterinary Medicine, at Federal University of Uberlândia (FAMEV/UFU) and molecular examination were carried out at the laboratory of molecular epidemiology-FAMEV/UFU.

Hygiene indicator microorganism enumeration

The post-rinse product was subjected to decimal serial dilutions in 9 mL of buffered peptone water (0.1%). For aerobic mesophilic (AM) counting, the samples were homogenized with PCA (Plate Count Agar) in sterile petri dishes and incubated at 37 °C for 48 hours. The most probable number (MPN) technique was used for coliforms at 35 °C (C35) and coliforms at 45 °C (C45) [18]. C35 were quantified by the multiple tube technique, using the presumptive test in lauryl sulfate tryptose broth (LST, Himedia) (35 °C for 24-48 hours) and confirmed in 2% brilliant green bile broth (BGLB, Himedia) (35 °C for 24-48 hours). Based on the C35 results, the C45 were detected using *Escherichia coli* broth (EC, Himedia) (44.5 °C for 24 hours in water bath).

For AM counting, plaques containing between 25 and 250 colonies were counted and the result expressed in $\log_{10}$ CFU/mL. The presence of coliforms at 35 °C and coliforms at 45 °C were confirmed by the turbidity and gas formation in Durham tubes of BGLB and EC Broth, respectively. Both results were expressed in $\log_{10}$ MPN/mL.

Detection of Salmonella spp.

Thirty millilitres of post-rinse product were transferred to Erlenmeyer flasks containing 30 mL of buffered peptone water (2%) and incubated at 37 °C for 24 hours according to ISO 6579-1 [20]. Samples with typical characteristics in LIA (Lysine Iron Agar – TMMedia) and / or TSI (Triple Sugar Iron Agar – TMMedia) were transferred to tryptic soya broth (TSB) and incubated at 37 ºC for 24 h, and then subjected to DNA extraction and purification, using the Wizard Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA). The extracted DNA was used detect the *ompC* gene (ompC-F 5”-ATCGCTGACTTATGCAATCG-3” and ompC-R 5”-CGGGTTGCGTTATAGGTCTG-3”) [21, 22].

The extracted DNA was used detect the *ompC* gene (ompC-F 5”- ATCGCTGACTTATGCAATCG-3” and ompC-R 5”-CGGGTTGCGTTATAGGTCTG-3”), which is typical of *Salmonella* spp. [21,22]. The following reagents were used for the reaction: 25 µL solution formed by 2 µL of sample DNA, 12.5 µL of GoTaq Green Master Mix (Promega), 8.5 µL of nuclease-free water (Promega) and 1 µL of each primer with a concentration of 10 pmol/µL. The conditions used for the reaction were: 95 °C for 2 minutes for

initial denaturation, 30 cycles of 95 °C for 30 seconds for denaturation, 57 °C for 1 minute for annealing, 72 °C for 1 minute for extension, and after the end of these 30 cycles, 72 °C for 5 minutes for final extension. The PCR products were subjected to horizontal electrophoresis on 1% agarose gel, stained with GelRed (Biotium, Inc., Hayward, CA, USA) and visualized with a transilluminator. PCR products of 204 bp were considered positive for *Salmonella*.

Antimicrobial resistance analysis

The antimicrobial resistance of *Salmonella* spp. isolates were assessed using the disk-diffusion technique, according to the recommendations of the "Clinical and Laboratory Standards Institute" [23,24]. Ten antimicrobials were used: ampicillin (AMP) 10 µg, meropenem (MER) 10 µg, cephalothin (CFL) 30 µg, neomycin (NEO) 30 µg, cephalexin (CFE) 30 µg, cefotaxime (CTX) 30 µg, ciprofloxacin (CIP) 5 µg, ceftriaxone (CRO) 30 µg, tobramycin (TOB) 10 µg and azithromycin (AZI) 15 µg. For the positive control, the standard strain *Escherichia coli* ATCC 25922 was used according to CLSI guidelines [23].

Each *Salmonella* isolate was inoculated in tubes containing 5 mL of saline (0.85%) and subjected to decimal serial dilutions in order to obtain 0.5 turbidity on the McFarland scale. Then the sample was swabbed on the surface of Muller-Hinton agar plates (MH, Kasvi). After this stage, antibiotic discs (Cecon, São Paulo, Brazil) were applied to the surface of the plates and incubated at 37 °C for 24 hours. The results were obtained by measuring the inhibition halos and comparing them to the reference values. The intermediate resistance was classified as resistant and isolates resistant to three or more antimicrobial classes were considered MDR [25].

Data analysis

All counts of hygiene indicator microorganisms were subjected to the Kolmogorov-Smirnov normality test. To compare the AM counts between points A and B, the paired *t test* was used, and to compare the counts of the different production systems, ANOVA ($p < 0.05$) was used. For the counts of C35 and C45, the Wilcoxon matched-pairs signed rank test was used to compare the slaughter steps and the Kruskal-Wallis test was used to compare production systems ($p < 0.05$).

The occurrence of *Salmonella* spp. was compared by Fisher's exact test ($p < 0.05$) and resistance to antimicrobials was analysed by frequency. All statistical analyses were performed using the GraphPad Prism5 program.

Results

The results of the AM counts indicated that total contamination declined during the slaughter process for all production systems analyzed, as indicated in Table 1 ($p < 0.05$). In step A, chicken carcasses from the backyard system had lower AM counts than the commercial system, with an average difference of 0.7 log_{10} CFU/mL ($p < 0.05$). On the other hand, at step B, there were no significant differences between the means of contamination by mesophilic aerobes in relation to the different production systems.

Coliform counts at 35 °C and 45 °C also indicated a reduction in contamination between steps A and B of slaughter process (Table 1). On the other hand, the production systems did not differ in terms of counts for these hygiene indicators and all carcasses showed similar counts at the beginning (Step A) and end of slaughter (Step B) ($p > 0.05$).

Table 1. Mean counts (log CFU/mL) of aerobic mesophilic, coliforms at 35 °C and coliforms at 45 °C in commercial, backyard and semi-backyard chicken carcasses, slaughtered in the same slaughterhouse.

Hygiene indicator microorganisms	Slaughter step *	n	Production Systems			*p* value**
			Backyard	Semi- Backyard	Commercial	
Aerobic mesophilic	A	34	5.3 (0.7)^{a,A}	5.9 (1.0)^{a,b,A}	6.0 (1.0)^{b,A}	0.0155
	B	34	3.9 (0.7)^{a,B}	3.6 (0.5)^{a,B}	3.8 (0.8)^{a,B}	0.2678
	p value **		< 0.0001	< 0.0001	< 0.0001	
	Δ (↓↑ %) ***		1,4 (↓ 26,4)	2,3 (↓ 39,0)	2,2 (↓ 36,7)	
Coliforms (35 °C)	A	34	3.5 (2.0)^{a,A}	4.9 (2.0)^{a,A}	3.6 (2.5)^{a,A}	0.0867
	B	34	2.0 (0.8)^{a,B}	2.3 (1.2)^{a,B}	1.4 (1.8)^{a,B}	0.2609
	p value **		0.0047	0.0011	0.0007	
	Δ (↓↑ %) ***		1,5 (↓ 42,9)	2,6 (↓ 53,1)	2,2 (↓ 61,1)	
Coliforms (45 °C)	A	34	2.8 (2.3)^{a,A}	3.6 (2.7)^{a,A}	3.3 (2.4)^{a,A}	0.5605
	B	34	1.6 (0.9)^{a,B}	1.8 (1.2)^{a,B}	1.6 (1.1)^{a,B}	0.8699
	p value **		0.0285	0.0040	0.0015	
	Δ (↓↑ %) ***		1,2 (↓ 42,9)	1,8 (↓ 50,0)	1,7 (↓ 51,5)	

* Step A: After bleeding; Step B: after chiller tank; ** Small superscript letters indicate differences between production system at same slaughter step, and upper case letters indicate difference between the slaughter steps at the same production system ($p < 0.05$); *** Δ = A-B, Percentage reduction (↓) or increase (↑)

Table 2. Occurrence of *Salmonella* spp. in commercial, backyard and semi-backyard chicken carcasses, slaughtered in the same slaughterhouse.

Slaughter step *	Production Systems			Total
	Backyard	Commercial	Semi-Backyard	
A	1/34 (2.94%)	0/34 (0.00)	1/34 (2.94%)	2/102 (1.96%)
B	4/34 (11.76%)	2/34 (5.88%)	1/34 (2.94%)	7/102 (6.86%)
Total	5/68 (7.35%)	2/68 (2.94%)	2/68 (2.94%)	

* Step A: After bleeding; Step B: after chiller tank; $p > 0.05$ for comparisons between production systems and between slaughter steps.

It was also observed that for all hygiene indicator microorganisms, the reduction in the average counts between step A and B of slaughter process was lower in the carcasses from the backyard system (Table 1).

Salmonella spp. was identified in carcasses of all evaluated production systems, with no significant difference between systems or between slaughter steps A and B ($p > 0.05$) (Table 2). In absolute numbers, step B had the highest frequency of positivity (6.86%) and chickens originating from a commercial system were the only ones that did not show positivity in step A.

The nine positive carcasses for *Salmonella* gave rise to 10 isolates that were assessed for their antimicrobial resistance (Table 3). The highest percentage of resistance was observed against ciprofloxacin (50%), tobramycin (40%), cephalothin (40%) and azithromycin (40%). On the other hand, the most efficient antimicrobial was ceftriaxone, since only one isolate presented resistance.

The resistance pattern of the isolates shows that five (50%) were classified as MDR, one of which was resistant to five classes of antimicrobials (Table 4). Furthermore, two *Salmonella* isolates with the highest resistance (S10 and S9) were identified on carcasses from the semi-backyard production system. Three isolates were sensitive to all tested antimicrobials, two from the backyard (S3 and S4) and one from the commercial production system (S5).

Table 3. Frequencies of *Salmonella* spp. isolates (n = 10) obtained from commercial, backyard and semi-backyard chicken carcasses with resistance to different antibiotics.

Class * / Antibiotic	Resistance (%)
Aminoglycosides	
Neomycin	2 (20%)
Tobramycin	4 (40%)
Carbapenems	
Meropenem	2 (20%)
Cephalosporins	
Cephalexin	2 (20%)
Ceftriaxone	1 (10%)
Cefotaxime	2 (20%)
Cephalothin	4 (40%)
Fluoroquinolones	
Ciprofloxacin	5 (50%)
Macrolides	
Azithromycin	4 (40%)
Penicillins	
Ampicillin	3 (30%)

* Concentrations evaluated according to references [21,22].

Discussion

Food safety has always been a challenge in the poultry industry, since microorganisms are present from the primary production stage and can be influenced by practices adopted at all stages of the production chain [13,26]. In our study, chicken carcasses from the backyard system (Bc) had a lower bacterial count (AM) than animals from the commercial system (C). In this case, the high initial contamination of the C carcasses can be attributed to the higher stocking density, greater deposition of organic matter in the poultry shed and higher level of stress experienced by the animals [10].

Because there are so many variables during the steps prior to the slaughterhouse, it is difficult for the industry to definitively control microbiological contamination [12]. Therefore, the microbial load that

Table 4. Antibiotic resistance profiles of *Salmonella* spp. isolates obtained from commercial, backyard and semi-backyard chicken carcasses slaughtered in the same slaughterhouse.

ID (Production Systems *)	Visit	Animal	Step	Multiple resistance: Antimicrobial class	Multiple resistance: Antimicrobial agents	Resistance pattern **							
S10 (SB) ***	6	8	B	5	8	CIP	CFL	TOB	AMP	CTX	NEO	MER	CRO
S9 (SB) ***	6	7	A	4	6	CIP	CFL	AMP	CTX	NEO	CFE		
S2 (C) ***	3	1	B	4	5	CIP	CFL	TOB	AMP	CFE			
S6 (Bc) ***	6	5	A	3	3	AZI	TOB	MER					
S7 (Bc) ***	6	5	B	3	3	AZI	CFL	TOB					
S8 (Bc)	6	6	B	2	2	CIP	AZI						
S1 (C)	3	1	B	2	2	CIP	AZI						

* SB: Semi-Backyard; C: Commercial; Bc: Backyard; ** MER (Meropenem); CFE (Cephalexin); CTX (Cefotaxime); CIP (Ciprofloxacin); NEO (Neomycin); CFL (Cephalothin); AZI (Azithromycin); CRO (Ceftriaxone); TOB (Tobramycin) and AMP (Ampicillin); *** Multidrug-resistant (MDR): resistant to three or more antimicrobial classes [23].

the birds arrive at the industry is a cause of concern for the self-control programmes of the slaughterhouse, which through good manufacturing practices (GMP) must monitor and reduce this contamination throughout the slaughter process [13]. In the present study, the slaughter process was efficient at reducing the hygiene indicator microorganisms (AM, C35 and C45) between slaughter steps A and B. Althaus *et al.* [27] analysed the different processes in a slaughterhouse and observed that the chiller step reduced the *Escherichia coli* count by 3.4 log UFC/g. Although *E. coli* was not analysed in the present study, a reduction of between 1.2 and 1.8 $\log_{10}$ CFU/mL was observed for C45 counts between steps A and B.

As a relationship between the production system and the initial contamination of the carcasses was identified, it would be worth considering which production system should be slaughtered first, since the possibility of cross-contamination has already been demonstrated in several studies [12,28]. The ideal approach would be to slaughter animals from Bc first and then animals from C. This would depend on GMP and all the industry's self-control programs [9].

No significant difference was observed between rearing systems regarding the contamination of the carcass in step B of the slaughter process. However, it should be noted that, for all the hygiene indicator microorganisms, the carcasses of the backyard system showed a lower reduction in contamination between the slaughter steps. A possible explanation of this observation is that only backyard chickens reach the minimum age to achieve full plumage and consequently have a larger follicle diameter, which could hinder the removal of microbiological contamination [4,29]. This observation reinforces the importance of standardizing the slaughter order according to the production system.

In relation to *Salmonella* spp., the results indicated that this bacterium may be present in animals from all production systems, having been identified in 7.35% of the samples from the backyard system and 2.94% of samples from the commercial and semi-backyard systems. Many studies have already shown the association between chicken meat and *Salmonella*; however, evidence of this specific relationship with animals raised in extensive systems remains scarce [12]. As in the present study, the few studies conducted have also identified the presence of this bacterium in backyard chicken carcasses, with frequencies ranging between 3.5% and 16% [30-32]. Therefore, even though production systems that cause less stress to animals are beneficial for reducing *Salmonella*, as noted by Iannetti *et al.* [10], we must maintain all the necessary steps for the control of this pathogen within the poultry production chain.

The absence of a difference between the presence of *Salmonella* spp. in stages A and B should be treated with caution, since despite its low occurrence, the slaughter process is not effective at reducing this microorganism. When *Salmonella* spp. are carried into slaughter plants, they can be spread during the different slaughter steps, compromising the safety of the final product and endangering consumer health [9,27].

An additional concern, besides the presence of this pathogen in poultry products, is the growing resistance of pathogens to antimicrobials and their impacts on global public health [7,33]. In the present study, only three *Salmonella* isolates were sensitive to all tested antimicrobials and the rest were resistant to at least two drugs. The drugs that were least effective were ciprofloxacin, tobramycin, cephalothin and azithromycin. Several studies have evaluated resistance to tobramycin and cephalothin, finding very variable results ranging from 100% resistant isolates to less than 18% resistant isolates [34-37].

Regarding ciprofloxacin, studies show a high frequency of *Salmonella* resistance in isolates collected from animals, food and humans [38,39]. This drug is frequently used in the treatment of salmonellosis and for this reason, the resistance observed is a cause of great concern for public health [40]. The widespread use of this drug in human medicine and its analogue, enrofloxacin, in veterinary medicine, may have exerted selective pressure that has culminated in the increase in drug-resistant strains [41,42].

With increased resistance to cephalosporins and quinolones, azithromycin has been adopted as a therapeutic option for the treatment of cases of bacterial enteritis [43]. However, as observed in the present study, other studies have also identified resistance to this drug [44,45]. In 2019, the identification of 255 cases of salmonellosis and 60 hospitalizations caused by *Salmonella* with low sensitivity to azithromycin drew the attention of the CDC (Centers for Disease Control and Prevention), which issued a national alert to reduce risks to consumers [46].

Another important result in the current study was the identification of five MDR isolates (50%). MDR bacteria have been identified in several studies and, for this reason, assume a prominent position on “One Health” perspective [47]. Antimicrobial resistance is a major public health concern worldwide and could be the cause of 10 million deaths per year by 2050 if nothing is done to change this scenario [48].

Given the low frequency of isolates found in the present study, it was not possible to reach any conclusion about the impact of production systems on *Salmonella* resistance. However, the two isolates resistant to the greatest number of drugs were found in carcasses from the semi-backyard system, which does not have a legal definition of production patterns. This type of production specific to some Brazilian geographic regions means that this system does not follow the strict biosafety rules of the industrial system nor the standards defined by law for the backyard system [4,6]. Thus, this scenario may be contributing to the inappropriate use of drugs and the high resistance observed.

According to the literature, Enterobacteriaceae isolates from backyard and organic systems tend to be sensitive to a wider range of antimicrobials [7,47,49]. This can be attributed to the less stressful conditions for animals in these systems, as well as specific rules on the use of drugs, as in the case of organic products [49]. Despite these observations, Kamboh *et al.* [7], identified Enterobacteriaceae isolates from livers of backyard chickens that were more resistant to chloramphenicol and oxytetracycline than isolates from the commercial system. Therefore, regional animal breeding characteristics and public policies regarding the use of antimicrobials can influence these resistance patterns [47].

In conclusion, the occurrence of *Salmonella* was not influenced by the production system, but the largest resistant profile was observed at isolates from the semi-backyard system. Also, chicken from the backyard system had a lower microbial count at earlier slaughter stages than those from the commercial production system, but presented smaller reduction in contamination throughout the process.

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