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Original Aticles

Detection of enterobacteria in broiler carcasses for sale in the market

Pesquisa de enterobactérias em carcaças de frangos à venda no comércio

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A R T I C L E I N F O

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A B S T R A C T

This study aimed to analyze the microbiological quality of broiler marketed in Teresina, PI. The study was performed in the from September to November 2018, to which three groups of broiler carcasses produced in the city of Teresina were analyzed: without refrigeration, labeling and inspection (n=8); originated from local production, refrigerated, labeled, and inspected (n=8); and originated from other states, frozen, packed, labeled, and inspected (n=8). The collected samples were analyzed regarding the determination of the Most Probable Number (NMP) of total and thermotolerant coliforms, *Escherichia coli*, and detection of *Salmonella* spp., besides the measurement of the temperature of the carcasses. The logarithmic means of thermotolerant and total coliforms in the broiler carcasses varied from 0.70 to 4.66 NMP/g and from 0.73 to 4.66 NMP/g, respectively. The bacteria *E. coli* and *Salmonella* spp. were also detected in samples with refrigeration and inspection (25 % and 12.5 %, respectively). As for the marketing temperature, only the broiler carcass samples which were chilled and frozen were within the standard of the Brazilian legislation. The results observed in this study indicate the need for improvement in the processing, handling, and storage of the broiler meat marketed in Teresina, PI.

Palavras-chave:
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R E S U M O

Objetivou-se analisar a qualidade microbiológica de carcaças de frangos comercializadas em Teresina, PI. O estudo foi realizado no período de setembro a novembro de 2018, para o qual foram analisadas carcaças de frango produzidas no município de Teresina, sem refrigeração, rotulagem e inspeção (n=8); de produção local refrigeradas, embaladas com rotulagem e inspecionadas (n=8); e oriundas de outros estados congeladas, embaladas com rotulagem e inspecionadas (n=8). As amostras coletadas foram analisadas quanto à determinação do Número Mais Provável (NMP) de coliformes totais e termotolerantes, *Escherichia coli* e pesquisa de *Salmonella* spp., além de aferição da temperatura das carcaças. As médias logarítmicas de coliformes termotolerantes e totais nas carcaças de frango analisadas variaram de 0,70 a 4,66 NMP/g e de 0,73 a 4,66 NMP/g, respectivamente. Também foram encontradas bactérias *E. coli* e *Salmonella* spp. em amostras com refrigeração e com inspeção (25 % e 12,5 %, respectivamente). Quanto à temperatura de comercialização apenas as amostras de carcaça de frango resfriadas e as congeladas mostraram-se dentro do padrão da legislação brasileira. Os resultados observados nesse estudo indicam a necessidade de melhoria no processamento, manipulação e armazenamento da carne de frango comercializado em Teresina, PI.

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INTRODUCTION

The poultry industry is considered an important segment of agriculture in the whole world (UBABEF, 2012). In this aspect, Brazil is the third-largest chicken producer, especially of broilers (SCHMIDT; SILVA, 2018), exporting to 141 countries in 2016, among which are Saudi Arabia, China, Japan, United Arab Emirates, and Hong Kong (CNA, 2017).

Broiler meat production ranks among the predominant food sectors, presenting noticeable technical and sanitary advances, being characterized for a significant expansion in the market of "healthy" products (SILVA et al., 2018). The consumption of this type of meat has considerably increased due to factors such as the good image of the product related to its low-fat content, high protein content, besides the availability of its processed products and low cost. However, this food may contain pathogenic microorganisms that cause foodborne diseases to humans (PIMENTEL; GODOT; FIGUEIREDO, 2019).

Among the factors that influence the multiplication of microorganisms in food products are the state of the package, temperature, personal hygiene, and the pattern of food storage (MASOUMBEIGI et al., 2017). Thus, broiler meat contamination can occur during various stages of the production and marketing chain, during processing, packing, storage, and transportation (RAJAN; SHI; RICKE, 2017).

The main microorganisms related to the deterioration of meat derivatives are bacteria of the genera *Pseudomonas*, *Acinetobacter*, *Shewanella*, *Brochotrix*, and *Lactobacillus*, as well as yeasts and filamentous fungi. Some species of the family Enterobacteriaceae are also included, especially the coliforms *Escherichia, Enterobacter, Citrobacter*, and *Klebsiella* (FRANCO; LANDGRAFF, 2005).

When there is a high enumeration of total coliforms, it may be an indicator of contamination due to failures during food processing, inadequate cleaning, or even inadequate thermal treatment. When there is a high number of thermotolerant coliform bacteria, this can be interpreted as an indicator of the presence of pathogenic microorganisms of intestinal origin on the food (FRANCO; LANDGRAFF, 2005).

Escherichia coli is considered as the main indicator of fecal contamination, although it may also be introduced on foods from other non-fecal sources (FERNANDES; GOIS, 2015). These commensal bacteria, resistant to antimicrobials, and the products of animal origin and their antimicrobial resistance genes can be transmitted to humans when contaminated food is not properly heated (ZHU et al., 2017).
Among the relevant enterobacteria for broiler meat, those of the genus *Salmonella* are highlighted, especially the serotype *S. Enteritides*. These bacteria firmly adhere to the external surface of broiler carcasses, featuring a difficult removal by washing (SOUZA et al., 2014).

Infections by *Salmonella* are commonly associated with the consumption of contaminated food and water, as well as with direct contact with infected animals. Eggs and poultry products are among the main transmitting agents of human salmonellosis, being responsible for most of the outbreaks of foodborne diseases. An important fact that can be verified in epidemiological surveys is that *Salmonella* bacteria appear as the second main cause of foodborne diseases (CONNOR; SCHARTZ, 2005).

Broiler carcasses marketed in Brazil can be found either chilled or frozen, according to the specific norms and regulations (BRASIL, 2016). It is known that cooling does not prevent the presence of bacteria, such as those of the genus *Salmonella*. With freezing, however, the reduction or absence of viable bacterial cells is expected (SANTOS et al., 2000).

In Brazil, studies were developed to confirm the contamination of broiler meat by *E. coli* (SILVA et al., 2012), total coliforms (MENEZES et al., 2018), and *Salmonella* sp. responsible for great outbreaks of foodborne diseases (KOTTWITZ et al., 2009). This evidences the need for inspection of this type of food with the application of measures that tend to minimize contamination and, consequently, foodborne diseases, aiming at the quality control of these products (SOUZA et al., 2014).

The early detection of pathogenic microorganisms, as well as those that cause food spoilage, can help to control outbreaks and avoid the loss of food products (LORENZO et al., 2018). In this perspective, this study aimed to investigate the microbiological quality of broiler carcasses marketed in Teresina – PI, through the presence of indicator microorganisms, such as total coliforms, thermotolerant coliforms, *Escherichia coli*, and *Salmonella* spp.

MATERIAL AND METHODS

The present study was performed in the city of Teresina, PI, from September to November 2018, in randomly selected markets and supermarkets, as long as they met the following inclusion criterion: marketing of whole broilers (chilled or frozen).

Three broiler carcass groups were compared to perform the experiment: A) those obtained clandestinely in the city of Teresina and marketed without refrigeration and authorization of the Municipal Inspection Service (SIM) (Figure 1); B) those from local production, refrigerated, packed, labeled, and with authorization of the Federal Inspection Service (SIF); and C) those originated from other states, packed, labeled, and with authorization of the SIF. Eight broiler carcasses were collected from each classification, group totaling 24 analyzed samples.

During the collections, factors related to the hygienic-sanitary quality of the analyzed products were observed, such as the hygiene of the handler, equipment, and contact surfaces with the foods. Soon after the collection of the carcasses, their internal temperature was measured in the region of the chest muscle. These data were collected with the aid of a handheld digital thermometer with a probe sensor (Model Tp 3001, with a measuring range from -50 to 300ºC). The samples were stored in their own closed sale package, which were then placed in an isothermal box containing reusable ice, and taken to the Laboratory of Microbiological Food Control, belonging to the Nucleus of Food Study, Research and Processing (NUEPPA), of the Center of Agricultural Sciences of the Federal University of Piauí.

In the laboratory, the samples were removed from their packages and placed on a stainless-steel tray, previously disinfected with a 70% alcohol solution, in order to be analyzed. All analyses were performed according to the methodology recommended by Normative Instruction nº 30, of August 26, 2018, of the Ministry of Agriculture, Livestock, and Supply, and the Manual of Methods for Microbiological Analysis of Food and Water (SILVA et al., 2017).

In order to perform the microbiological analyses, 25g of skin and muscles were aseptically removed from the neck, wings, and periocloacal regions (Figure 2) and added into 225 mL of buffered peptone water at 0.1%, thus obtaining the first dilution (10^{-1}). From this dilution, the second dilution was obtained (10^{-2}) by transferring 1.0 mL from the previous dilution and inoculating it into 9.0 mL of peptone water at 0.1% until reaching the last dilution (10^{-3}). From the three dilutions obtained, the microbiological analyses were performed for total and thermotolerant coliforms, *E. coli*, and *Salmonella* sp.

For the determination of the Most Probable Number (NMP) of total and thermotolerant coliforms and detection of *Escherichia coli*, the decimal dilutions were prepared by inoculating 1.0 mL of each sample into three series of test tubes containing Lauryl Sulfate Tryptose Broth (LST), which contained inverted Durham tubes in their interior. The tubes were incubated in a bacteriological incubator at 35ºC for a period from 24 to 48 hours, considering positive in the presumptive evidence those that exhibited turbidity and gas production.

For the confirmation of total coliforms, aliquots from the positive cultures of the LST Broth were transferred to tubes containing Brilliant Green Bile Broth at 2.0% (VB), and these were incubated in a bacteriological incubator at 35°C for a period from 24 to 48 hours; the tubes that exhibited turbidity and gas formation were considered positive. For the confirmation of thermotolerant coliforms, aliquots from the positive cultures in the LST Broth were transferred to tubes containing *Escherichia coli* Broth (EC) and incubated in a water bath at 45.5ºC for a period from 24 to 48 hours, following the same positivity criterion.

For the detection of *E. coli,* the positive cultures in the EC Broth were spread in plates containing Eosin Methylene Blue Agar (EMB) by the streak plate method, followed by incubation in a bacteriological incubator at 35°C for 24h. After this period, from three to five suggestive colonies were selected (dark blue with metallic shine), transferred to Nutrient Agar tubes (AN), and incubated at 37°C/24h. Afterward, smears were made and stained by Gram's method for the verification of their morphology. After the confirmation of the presence of Gram-negative bacilli, these were subjected to biochemical confirmation by performing the following tests: indole production (I), methyl red (MV), Voges-Proskauer (VP), and citrate (C) (Vanderzant; Splittsoesser, 1992).

For the detection of *Salmonella* spp. in the dilution (10^{-1}) pre-enriched in buffered peptone water at 37ºC for 24 h, 1.0 mL was transferred to tubes containing 10 mL of Selenite Cystine Broth (SC), and 0.1 mL was transferred to tubes containing 10 mL of Rappaport-Vassiliadis Broth (RVS), being then incubated in a bacteriological incubator at 37ºC for a period from 18 to 24 hours. From the growth in the enrichment media, inoculation was performed with a smear loop on the surface of the *Salmonella Shigella* Agar (SS) and Hektoen Enteric Agar (HE) media for the selective plating, and the plates were incubated inverted in a bacteriological incubator at 37ºC for 24 hours. After this period, biochemical tests were performed by sowing the typical colonies in Triple Sugar Iron Agar (TSI) and Lysine Agar (LIA), incubated at 37ºC for 24 hours. The cultivations in positive TSI and LIA media were subjected to tests of urease, phenylalanine, indole, methyl red, Voges-Proskauer, and citrate for later performing of the rapid slide agglutination test, using somatic polyvalent and flagellar serums for the identification of the genus *Salmonella* spp.

The statistical analysis was performed using the SigmaStat software (version 3.5; Systat, Richmond, CA, USA). The results were transformed into logarithms for the analysis of variance and test of correlation by the Kruskal-Wallis test, with a significance level of $p<0.001$.

RESULTS AND DISCUSSION

The results obtained in the microbiological analyses of the 24 broiler carcass samples, analyzed regarding the total and thermotolerant coliforms and *E. Coli* are expressed in Table I. Besides these parameters, the temperature of the carcasses in their marketing environment was also measured. It can be verified that there was a difference ($P<0.001$) for the coliforms between the studied groups. The initial contamination of the carcasses in group A was probably favored by the exposure to the ambient temperature of 30°C, which favored the multiplication of mesophilic bacteria.

Another factor that may have favored the initial contamination was the obtainment of group A samples originated from clandestine slaughter and marketed in

the public market without refrigeration, whereas groups B and C were slaughtered under inspection and exposed to sale with proper refrigeration, according to the category of the product.

The RDC Resolution nº 12/2001 of ANVISA (revoked by the RDC Resolution nº 331, of December 23, 2019, and complemented by Normative Instruction N°60 of December 23, 2019) establishes the upper limit of 4.00 NMP/g in log 10 for thermotolerant coliforms in chilled or frozen *in natura* poultry meat (whole, fractioned, or cut carcasses) (BRASIL, 2001; BRASIL, 2019a, 2019b). The logarithm means of thermotolerant coliforms in the broiler carcasses analyzed varied from 0.70 to 4.66 NMP/g in log 10, variations that put only the groups B and C in conformity with the established by the legislation (Table II). These results reinforce the need for continuous procedures of inspection and quality control in chicken carcasses, aiming at reducing the potential risk for consumption of the multiplication of microorganisms during the exposure.

Table 1 – Mean results and standard deviation of the most probable number of total and thermotolerant coliforms, *Escherichia coli*, and mean values of sale exposure temperature at the moment of collection of broiler carcasses marketed in Teresina, PI

Groups	Total coliforms (1)	Thermotolerant coliforms (1)	*Escherichia coli* (1)	Sale exposure temperature (°C)
A	$4.66^{a} \pm 0.74$	$4.66^{a} \pm 0.74$	$0.38^{a} \pm 1.08$	30.3
B	$2.26^{b} \pm 1.68$	$1.71^{b} \pm 1.57$	$0.76^{a} \pm 1.41$	3.5
C	$0.73^{b} \pm 0.60$	$0.70^{b} \pm 0.55$	$0.00^{a} \pm 0.00$	-10.0

(1) most probable number per gram in base 10 logarithms (NMP/g in log 10); means followed by the same letter in the column do not differ statistically ($p<0.001$ of significance) by the Kruskal-Wallis test.

Table 2 – Percentage of broiler carcasses marketed in Teresina, PI, in disconformity with the legislation

Groups	Origin of the sample	Package origin	Thermotolerant coliforms (%)(1)	*Escherichia coli* (%)	*Salmonella* spp (%)(2)
A	Clandestine slaughter	Without package	75.0	12.5	25.0
B	With Federal Inspection	With package	0	25.0	12.5
C	Without Federal Inspection	With package	0	0	0

(1)BRASIL (2001); (2)BRASIL (2016); (2)BRASIL (2019b)

The sanitary hygienic conditions of the environment, of the food handler, storage temperature, hygiene, and conservation of utensils and equipment are critical and danger points for meat quality, even if these prerequisites are not observed in many marketing places, especially at open-air markets (DINIZ et al. 2013).

Microorganisms found in broiler carcasses, such as *Escherichia coli* (Table II), indicate the occurrence of direct or indirect fecal contamination from the obtainment until the preparation of the food. This species is part of the intestinal microbiota of humans and homeothermic animals (ALVES, 2012), being eliminated in large quantities via feces, so that its presence in foods indicate that other microorganisms of fecal origin might be present, thus representing dangers to the health of the consumer.

Sommers et al. (2018) concluded that the temperature of 4.0 ºC is efficient in inhibiting the multiplication of *E. coli* in ground chicken meat. Brazilian legislation establishes the following temperature parameters for the commercialization of *in natura* broiler carcasses: 0.0 to 4.0 ºC for chilled carcasses, and below -12.0 ºC (± 2.0 ºC)

for frozen carcasses (BRASIL, 1998). As for the marketing temperature observed in this experiment (Table I), only the group A samples were not exposed to sale at an appropriate temperature, and the remainder were in conformity with the current legislation, being efficient in inhibiting the multiplication of *E. coli.*

Generally, the poultry intestinal tract can be a natural reservoir of pathogenic microorganisms, such as *Salmonella* spp. (SHINOHARA et al., 2008), and for this reason, the Ministry of Agriculture, Livestock, and Supply (MAPA) established, through Normative Instruction (IN) n°20, of 2016, the control and monitoring of this bacteria in broilers and turkeys in facilities that commercialize and slaughter these animals under inspection, aiming at decreasing the prevalence of this microorganism to a proper safety level in order to protect the consumer (BRASIL, 2016). The musculature of healthy poultries must be free of microorganisms, and in this manner, the contamination present in broiler carcasses may come from feathers, skin, airways, and intestinal tract of these animals, as well as from the environment, handling, and processing in general.

In this study, *E. coli* and *Salmonella* spp. were present (Tables I and II) both in broiler carcasses from clandestine slaughter (group A) as well as in those that were inspected (group B). Considering that the group B samples were acquired in the market in their original packages, this contamination may have occurred due to hygiene issues in the several steps of carcass processing (PACHOLEWICZ et al., 2015). *Salmonella* can be present in the broiler immersion water at 61.1 °C, in the slaughterhouse, four at least three hours. However, during slaughter, the presence of this bacteria on the carcasses is variable, as it may or may not occur (PEREIRA et al., 2009). In this manner, the control of quality must observe the monitoring of products, especially when establishing prerequisite programs and identifying the critical control points.

Microorganisms such as *E. coli* and *Salmonella* spp., when present in the gastrointestinal tract, are capable of causing ulcerative lesions and, eventually, systemic inflammatory response syndrome (SIRS), which can be associated to intestinal inflammatory disease, ulcerative colitis, and Crohn's disease (MIRSEPASI-LAURIDSEN et al., 2016). In this research, it was evident that the broiler carcass samples may be contaminated by these bacteria, and in this manner, these results can serve as an alert for the control efficiency of prerequisite programs, the Hazard Analysis plan, and the determination of Critical Control Points. It should also be emphasized that the control of the exposure temperature of these foods, for marketing, must be efficient in order to avoid the multiplication or preexisting microorganisms, thus reducing biological hazards and guaranteeing the health of the consumer.

Conversely, Zhu et al. (2014) found a significant prevalence of *Salmonella* in both refrigerated (55.1%) and frozen broiler carcasses (33.5%). Even so, it is evidenced in the literature that freezing reduces the bacterial metabolism in broiler meat (TOZZO et al., 2018), with a consequence on multiplication, although after defrosting, if these bacteria remain at ambient temperature, they might recover from the injury caused by the cold and resume their multiplication ability.

According to the observed at the moment of collection, issues of personal hygiene, equipment, and contact surface hygiene were verified, besides the marketing temperature outside the recommended standards (group A). The inactivation of *Salmonella* present in broiler meat can occur in thermal processing methods that combine heating temperatures from 55 to 65 °C, gallic acid, and eugenol (LOPEZ-ROMERO et al., 2018). The results obtained in this study indicate that *Escherichia coli* and *Salmonella* spp. can be present on *in natura* broiler carcasses and, therefore, consumers should be alerted to the potential dangers of this food, which should contain on the package labeling instructions that orient the prevention of cross-contamination during domestic preparation and regarding the consumption without proper cooking.

CONCLUSIONS

The microbiological quality of broiler carcasses marketed in Teresina, PI, depends on the form of obtainment and commercialization, as the carcasses may contain total and thermotolerant coliforms, *Escherichia coli*, and *Salmonella* spp.

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