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Total and Thermo-Tolerant Coliforms, *Salmonella spp.*, *Escherichia coli, Pseudomonas spp* and *Listeria monocytogenes* in Broilers Chicken Meat Processing Chain in Southern Brazil

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Abstract Sixty-six samples were collected from a broiler chicken abattoir. Eighteen samples were collected from the retail market in Southern Brazil, where the slaughtered broilers were commercialized, so that the microbiological assessment of the samples in the broiler industrial chain could be undertaken. And eighteen samples submitted to clients´ common home procedures were similarly assessed. All samples were evaluated by quantifying total and thermotolerant coliforms, psycrotrophic bacteria, *Pseudomonas* spp, *Escherichia coli* and research for *Salmonella* spp and *Listeria monocytogenes.* Results on counts and on the occurrence of microorganisms in samples from specific sites in the broiler processing chain demonstrate the need for a re-assessment of hygiene practices throughout the whole process and educational activities should be direct to all people involved, including consumers.

Keywords: *pathogenic microorganisms, chicken, industrial hygienic, Listeria monocytogenes*

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1. Introduction

In Brazil the slaughterhouse of broiler chickens is nationwide, spread throughout all the states and regions. There are chicken meat producing regions and several types of products, comprising special meat cuts, offal and whole processed chickens are offered on the market. The amount of indicator microorganisms evaluates the product´s hygiene and sanitary conditions in the abattoir´s industrial process in the handling of the carcass and in the distribution of the final product. Further, direct research for pathogenic microorganisms is also basic to analyze food safety [1]. Total coliforms evaluate the product´s hygiene conditions since their high number is an indication of contamination from failures during processing, inadequate cleaning or insufficient thermal treatment. Further, a high number of thermo-tolerant coliforms indicates the presence of intestine pathogens such as *Escherichia coli* [2].

The number of psycrotrophic microorganisms, such as *Pseudomonas* spp., predominant in carcasses and chilled meat cuts is highly relevant because pathogens may gradually multiply themselves at temperatures equal to or lower than 0°C. They can cause most modification in products, making the meat shelf life depends on the conservation and on the number of microorganisms after the post-conservation period [2]. *Pseudomonas* spp. members are known to be the most common microbiota involved in spoilage of many kinds of foods, due to their very simple nutritional requirements and their metabolic versatility that allows them to thrive in various environments [3].

Outbreaks caused by *Salmonella* spp. and *Escherichia coli* are known and they involved several types of food, especially food of animal origin [2]. Furthermore, *Listeria monocytogenes* is the etiological agent of listeriosis, a serious disease with high lethal rates between 20% to30% for risk groups. Due to such high indexes, listeriosis ranks second as one of the most frequent death causes by food contamination [4,5].

There are several studies about contamination by these microorganisms in broilers´ carcasses during the processing stages as in meat cuts available on supermarket shelves. The contamination level by degrading and pathogenic indicator microorganisms, such as *Salmonella* spp. and *Listeria monocytogenes* is still a fact that should be set right in broiler abattoirs and in selling outlets where these products and their derivatives are commercialized [6,7,8].

It should be underscored that microbial contamination can occurs during consumers´ handling procedures such as storage in home refrigerators, lack of hygiene during handling and even cross contamination by contact with

other contaminated food. There is often a false impression that flaws occurred during the processing of carcasses in the abattoir. However, data on the hygiene and sanitary quality of chicken meat cuts in this environment are rare and non-conclusive [9]. since precise sites of contamination are largely unknown and efficacious measures in microbial reduction or elimination at this point in the industrial chain are impaired.

The aim of this study was evaluates the microbiological quality of samples within broilers chicken meat processing chain in Southern Brazil, by quantifying total and thermotolerant coliforms, psycrotrophic bacteria, *Pseudomonas* spp, *Escherichia coli* and research for *Salmonella* spp and *Listeria monocytogenes.*

2. Materials and Methods

2.1. Sample Collection

Sixty-six samples were collected from a broiler chicken abattoir in and 18 samples from the retail market from Southern Brazil, where the slaughtered broilers were commercialized so that the microbiological assessment of the samples in the broiler industrial chain could be undertaken. Eighteen samples submitted to clients´ common home procedures were similarly assessed.

2.1.1. Abattoir

With support from the inspection service of the Department of Agriculture of Rio Grande do Sul state, was selected the largest slaughterhouse in the region south of this state to carry out this study. Samples were collected in a broiler industrial plant with an approximate slaughter of 22,000 broilers/day. Six collections with nine carcasses per collection were performed in the abattoir.

Sampling was performed at three sites in the slaughter process: prior to scalding (PS), prior to chilling (PC) and after chilling (AC) with three broiler carcasses for each sampling site, retrieving 300mL aliquots in sterile glass flasks.

Carcass samples were treated by washing, immersion and friction in sterile polyethylene bags containing 450mL of saline water 0.85% for the first sampling site and 225mL of saline water 0.85% for the other two sites. Solutions were later placed in sterile glass flasks and placed in isothermal box for transference to the laboratory for analysis.

2.1.2. Retail Outlets and Home Environment

Six trays with chicken cuts (drumsticks) from the same sample lots collected from the abattoir were used to evaluate retail and consumer samples (simulating procedures by consumers). The drumsticks were bought from the retail market in Pelotas Southern Brazil, two days after being brought for commercialization (in according the research of Department of Commerce, two days is a mean time of permanence of this product in markets of this city). The drumsticks from three trays were immediately sent for analysis and the other three trays were taken to the consumer´s home and stored in a refrigerator at 7°C for two days to simulate normal storage conditions by consumers (also according the research of Department of Commerce).

Chicken drumsticks collected from commercial outlet and stored at consumer´s home were similarly treated as above (washing by immersion and friction in 225 mL of saline water 0.85%). Two cuts (one chicken tight and one drumstick) were placed in a sterile polyethylene bag, respectively shamming samples from outlet and client. Three samples from outlet cuts and three from consumer cuts were analyzed for each collection.

2.2. Microbiological Analyses

Samples were analyzed at the Food Microbiological Laboratory of the School of Nutrition, Federal University of Pelotas, Pelotas, Brazil, following procedures by Downes & Ito [10] for quantification of total and thermotolerant coliforms, psycrotrophic microorganisms, *Escherichia coli* and *Pseudomonas* spp. and research of *Salmonella* spp.; and procedures by International Organization for Standardization [11] for research of *Listeria monocytogenes*.

2.2.1. Quantification of Total and Thermotolerant Coliforms

The Most Probable Number (MPN) technique was employed to tally the number of total and thermotolerant coliforms. Coliform analysis was performed in a Sodium Lauryl Sulfate Broth incubated at 35°C for 48 hours. Total coliforms were counted in Brilliant Green Bile Lactose Broth incubated at 35°C for 48 hours. Thermotolerant coliforms were counted in *Escherichia coli* broth incubated at 45.5°C for 48 hours and results were given in $MPN.g^{-1}$.

2.2.2. Quantification of *Escherichia coli*

The Most Probable Number (MPN) technique was employed for *E. coli* from positive tubes with sowing on plates with Eosin Methylene Blue agar, incubated at 37°C for 24 hours. Colonies with characteristic morphology were identified as *E. coli* by Indol production test, Methyl Red and Voges-Proskauer Reactions, and Citrate test and results were given in $MPN.mL^{-1}$.

2.2.3. Quantification of Psycrotrophic Microorganisms

Psycrotrophic microorganisms were counted by pour-plate technique of dilutions with the addition of standard plate count agar. Plates were incubated at 7°C for 10 days and results given in $CFU.g^{-1}$.

2.2.4. Quantification of *Pseudomonas* spp.

Pseudomonas spp. were counted by surface plating in pseudomonas isolation agar, incubated during 48 hours at 30°Candresults were given in $CFU.g^{-1}$.

2.2.5. Analysis of *Salmonella* spp.

Salmonella spp. was isolated by pre-enrichment in peptone water buffered 0.85% at 37°C for 24 hours and by selective enrichment in Rappaport Vassiliadis broth at 42°C for 24 hours and Tetrationate Broth at 37°C for 24 hours. Seeding was performed in Xylose Lysine Desoxycolate agar and Hektoen Enteric agar incubated for 24h at 37°C. Typical colonies were biochemically identified in Triple Iron Agar, Iron Lysine Agar and

Urease Agar at 37°C for 24 hours. Samples with characteristic biochemical reaction underwent serum identification with polyvalent somatic and flagellar anti-salmonella serum.

2.2.6. Analysis of Listeria Monocytogenes

Research on *L.monocytogenes* followed methodology by the International Organization for Standardization, ISO 11.290-1-Detection method (ISO, 1996) with modifications. The pre-enrichment stage was undertaken in a Listeria Enrichment Broth, incubated at 30°C for 24 hours, followed by incubation of a Fraser Broth aliquot at 35°C for 48 hours. Seeding was then performed in Oxford and Palcam agars at 35°C for 48 hours. Purified isolates underwent motility phenotypic tests, fermentation of carbohydrates (dextrose, xylose, rhamnose and mannitol) and the presence of catalase and β-hemolysine.

2.3. Statistical Analysis

Data retrieved in the quantifications of total and thermotolerant coliforms, *Escherichia coli*, psycrotrophics and *Pseudomonas* spp. underwent analysis of variance, followed by Tukey´s test ($p < 0.05$).

3. Results

3.1. Total and Thermo-tolerant Coliforms

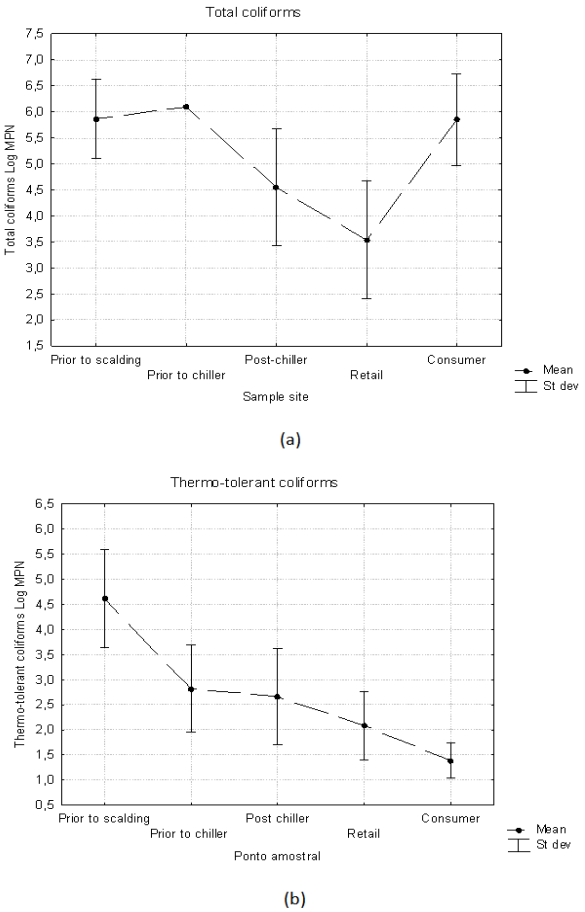

Figure 1. Means and standard deviation of concentrations of total (a) and thermotolerant (b) coliforms in broilers meat processing chain in Southern Brazil

Figure 1 shows results of analyses of total and thermo-tolerant coliforms in samples from specific sites in broiler processing chain of an abattoir in the Southern Brazil.

Brazilian law does not establish any limits for total coliforms in broilers´ carcasses or meat cuts but establishes a maximum limit of 4Log MPN.g^{-1}for thermo-tolerant coliforms in chilled or frozen meats, “in natura”, broilers (whole carcasses, fractioned or cuts) [12]. However, Chambers [13] underscores in case of total coliforms, counts over 2 Log CFU.mL^{-1}reveal lack of hygiene in prime matter or during processing. According with these limits, 88.9% (16/18) of the samples in the PS stage, 100% in AC stage (18/18) and 72.2% (13/18) in PC stage exceeded limit. In other words, 87% (47/54) of samples analyzed during processing had total coliform concentrations above the maximum limit described by Chambers [13]. Further, 72.2% (13/18) and 94.4% (17/18) of the samples in the stages retail and consumer, respectively, exceeded this maximum limits. Results indicate lack of hygiene in processing and storage within the whole industrial chain.

3.2. *Escherichia coli*

Figure 2 demonstrates results for the quantification of *Escherichia coli (E.coli)* in samples from specific sites in the broiler industrial chain in the Southern Brazil.

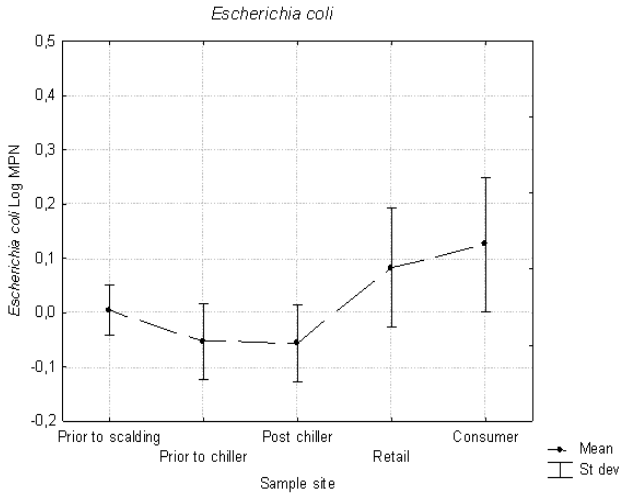

Figure 2. Means and standard deviation in *Escherichia coli* counts in samples from specific sites in broilers meat processing chain in Southern Brazil

E.coli occurred in all the sites evaluated within the broiler industrial chain, except water samples. Contamination averages < 3.0 MPN.mL^{-1} were obtained for two sampling sites in water samples from the scalding and pre-chiller stages.

Contamination averages ranged between -0.09 and 0.89 Log MPN.g^{-1}. In fact, the consumer sampling site had the highest microbial concentration average. Contamination at this site was significantly higher than that of concentrations in the PC and AC stages, with no significant difference between the other sites under analysis.

3.3. Psycrotrophic Microorganisms and *Pseudomonas* spp.

Figure 3 exhibits counts of psycrotrophic microorganisms and *Pseudomonas* spp. in samples retrieved from specific

sites in the broiler industrial chain in Southern Brazil. Although counts of psycrotrophic aerobic microorganisms reveal the degree of deterioration of chilled food, Brazilian law does not determine maximum microbial concentration rates for such micro-organisms.

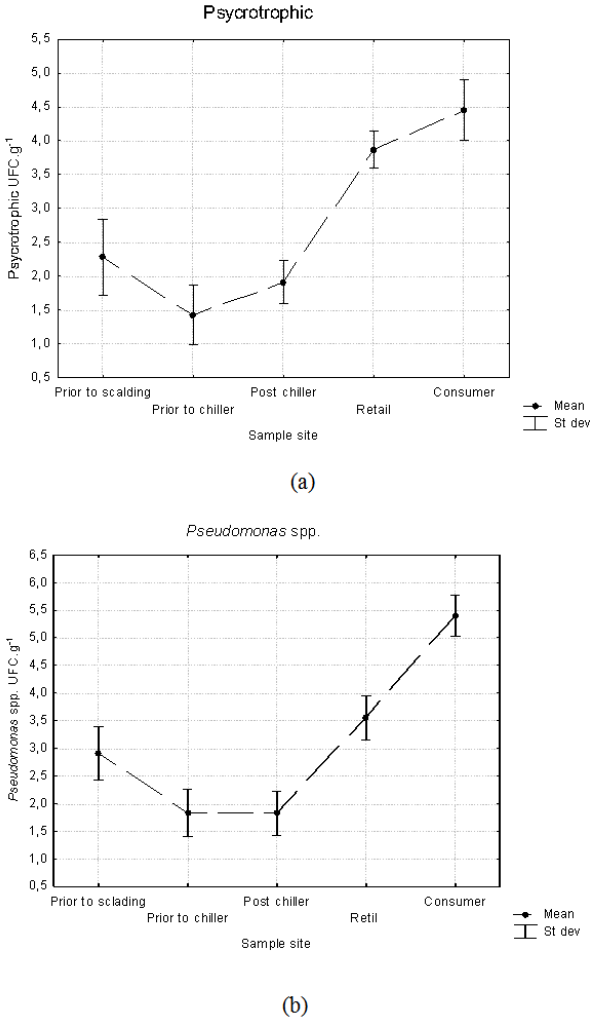

Figure 3. Means and standard deviation of counts of psycrotrophic microorganisms (a) and *Pseudomonas* spp.(b) in samples from specific sites in broilers meat processing chain in Southern Brazil

In the case of the quantification of *Pseudomonas* spp. there was a significant decrease in counts for carcasses at the pre-chiller site when compared to those for the first sampled site, prior to scalding. The above shows that the scalding stage (contamination average 2.3 log CFU.mL^{-1}) triggered the reduction of the genus´s microbiome, as suggested by Andrew and Ron [14].

3.4. Analysis of *Salmonella* spp.

Salmonella spp. occurred in all the sampled sites from the abattoir. Bacteria could be isolated in 5.5% (1/18) of the samples at the pre-scalding site; in 16.6% (3/18) at the pre-chiller site and 5.5% (1/18) of samples at the post-chiller site.

Salmonella spp. was not reported in samples collected on the retail market and in samples obtained by sham management and storage conditions practiced by consumers. In this case, refrigeration temperature was efficient to inhibit or inactivate the bacteria and no contact between cuts and the bacteria occurred during storage.

3.5. Analysis of Listeria Monocytogenes

Listeria spp. was isolated in 10 (9.8%) out of the 102 samples analyzed in the broiler industrial chain, or rather, six (60%) were *L. monocytogenes*; three (30%) were *L. ivanovii* and one (10%) was *L. grayi*. Table 1 discriminates results of *Listeria* spp. for each stage.

Table 1. Species of *Listeria* spp. isolated in broilers meat processing chain in Southern Brazil

Stage in the chicken industrial chain	Isolated species of *Listeria* spp. (positive samples/total samples/%)
Pre-scalding stage	*L. monocytogenes* (3/18 - 16.7%)
Water used in scalding	ND
Pre-chiller stage	*L. grayi* (1/18- 5.6%)
Water used in the pre-chiller stage	*L. monocytogenes* (1/18 - 5.6%)
Post-chiller stage	ND
Retail market	*L. ivanovii* (1/18 - 5.6%) *L. monocytogenes* (1/18 - 5.6%)
Consumer	*L. ivanovii* (2/18 - 11.1%) *L. monocytogenes* (1/18 - 5.6%)

ND - not detected

At the abattoir, *L. monocytogenes* was isolated from water in the pre-scalding and in the pre-chiller stages. The detection of the pathogen at the last site was highly important since all the broiler carcasses underwent the chilling system and might have been contaminated by the water.

In samples from retail and consumer, the pathogen was isolated in one sample in each stage and indicated that most probably crossed contamination occurred in the cuts while stored in the market or in the refrigerator at home. In fact, *L. monocytogenes* did not occur in samples at the post-chiller sampling site (the last site sampled in the industrial chain).

4. Discussion

There was no significant decrease in counts of total coliforms in the samples collected in the abattoir. It means that the stages in industrial processing were not efficient in the decrease of coliform concentrations as expected. Or rather, broilers´ carcasses were exposed to inadequate hygiene conditions during processing.

This may be corroborated by the fact that water samples from the scalding and pre-chilling processing also evidenced mean total coliform counts of 2.5 and 3 log MPN.mL^{-1}, respectively. Although a decrease in mean counts occurred in the post-chiller site when compared to the pre-chiller sample site, reduction failed to be significant ($p < 0.05$). However, a significant increase in counts at this spot is reported when only the retail and consumers´ samples are compared.

The thermotolerant coliforms counts (Figure 1 b) demonstrates a decrease in the average concentration

throughout the industrial chain and corroborates the statistical analysis. There was a significant decrease of counts in PC and AC stages when compared to the PS stage, whilst there was no significant difference between stages PC and AC. Consequently, the scalding stage was more efficient that the pre-chilling one on the microbial decrease of bacteria of the thermotolerant coliform group. Count averages for these bacteria in the analysis of water for scalding and pre-chilling were respectively 2.4 and 3.04 log MPN.mL^{-1}. There was no significant difference between retail and consumer samples.

The results also shows that 27.8% (5/18) and 5.6% (1/18) of samples respectively at the post-chiller and retail sample sites do not comply with Brazilian law since they are above the 3 Log MPN.g^{-1} dead mark. On the other hand, the samples from the consumer sample site failed to provide a microbial concentration above the thermo-tolerant coliform limit [12].

Similar results on total and thermo-tolerant coliforms counts indicate the need for corrective measures from the hygiene and sanitary point of view during slaughter, processing and commercialization of broilers.

The concentration of total coliforms in cuts from the domestic environment as significantly higher ($p < 0.05$) to those obtained in counts for retail cuts indicated that the former cuts, stored in conventional refrigerators, had been exposed to conditions that increased the concentration of bacteria of this group. Although hygiene conditions were not adequate, there was no contact between stored cuts and potential sources of thermotolerant coliform bacteria.

On the other hand, about *E. coli* Northcutt *et al.* [15] had different results. According to these authors, the chilling system maintained its capacity in reducing contamination by *E. coli* in broilers´ carcasses even with the passing of time and increase in organic load. This was not being reported in current assay. Figure 2 shows that there is a reduction trend in the contamination by *E.coli* as the industrial process occurs and an increase in contamination during the retail and consumer stages. As in the case of total coliforms, this fact indicated that there were favorable conditions for the increase in the microbial concentration of *E. coli* in chicken cuts during their home storage and in the conventional refrigerator.

Svobodov *et al.* [8] obtained similar results when they counted bacteria in broiler carcasses during the several stages in the industrial process, and contamination by *E.coli* was verified in decreased with each subsequent stage during the industrial process. Alvarez *et al.* [16] quantified *E. coli* in chicken cuts bought in shops in León, Spain, and obtained averages between 2.6 and 4.3 log CFU.g^{-1}, higher than those reported in current assay.

Psycrotrophic microorganisms showed a trend towards microbial decrease as the broiler industrial process occurred even though reduction was significant only between the AC site and the previous evaluated stage (PS) (Figure 3) Coupled to averages of counts of water, 2.5 and 2.4 log CFU.mL^{-1}, respectively, in the scalding and pre-chilling stages, this fact indicated that the scalding stage reduced contamination by the microbial group, very different from that in the chilling stage.

Although there was no significant difference between retail and consumer samples, the averages of these sampling sites were significantly higher than those obtained in the counts of sampled sites during the industrial process. This is probably due to the fact that sampled cuts from retail and consumer were stored at refrigeration temperatures, respectively between 4°C and 8°C. This group of microorganisms is actually capable of developing and multiplying in such storage conditions.

In Spain, Alvarez *et al.* [16] also counted the group´s microorganisms in chicken cuts obtained from the retail market and registered averages (between 5.96 and 7.87 log CFU.g^{-1}) which were higher than those in current assay, exceeding the guidelines of their country for chicken meat.

There was no significant decrease in *Pseudomonas* spp. count averages between the second and third sampling site, in the pre-chiller and post-chiller stages, perhaps due to the fact that psycrotrophic bacteria may survive and develop in low temperatures (0 to 15°C), what is confirmed by the contamination average obtained from pre-chiller water samples (3 log CFU.mL^{-1}).

Counts from retail samples were significantly higher than those obtained from carcasses at PC site in the abattoir and lower than those obtained in counts from consumer cuts. Count average of consumer cuts was more than two fold than that of the post-chiller sampling site.

Pseudomonas spp. counts, indicative of the end of useful life, lie between 6 and 7 logCFU.g^{-1} [17]. According with this, all the samples were adequate for consumption with regard to the microbial genus.

In case of *Salmonella* spp. detection, the results of were provided at different spots and suggested that cross contamination occurred between broiler carcass, handlers, and badly hygienized surfaces or equipment. Cross contamination was not caused by water used in intermediary washings since *Salmonella* spp. was not isolated in any water sample from the scalding and pre-chiller stages. Since a high occurrence of *Salmonella* spp. was detected in carcasses sampled in the pre-chiller stage, contamination may be attributed to the previous stage, or evisceration, when a mistake in the rupture of the intestines would cause high contamination by enteric microorganisms [18].

Lillard [19] stated that in a research by the US Food Safety and Inspection Service 5% of the broilers at the abattoir were already contaminated by *Salmonella* spp. and contamination increased to 36% in broiler carcasses after the final processing stage. The author also registered that there was a significant increase in *Salmonella* spp. after the immersion of the carcasses in the chilling tank. In other words, the latter may be an important site for cross contamination of broiler carcasses in processing plants.

L. monocytogenes is a pathogenic bacterium largely disseminated in the environment and has been isolated in soil, water, sewage, vegetables, silages, healthy human and animal feces [20]. Other authors have also detected *L. monocytogenes* in broiler carcasses during the meat processing stages [21,22,23].

Contrastingly to current study, Barbalho *et al.* [21], analyzing broiler carcasses in an abattoir in northeastern Brazil, detected *L. monocytogenes* only in wrapped chicken samples. This fact suggested that carcasses were contaminated during or after the chiller process, corroborated by Miettinen *et al.* [24] in their analysis of chicken carcasses in a Finnish abattoir.

Chiarini *et al.* [22] isolated *L. monocytogenes* in 20% of sampling sites in an abattoir with automatic evisceration

in the southeastern region of Brazil, and in 6.4% of sampling sites in an abattoir with mechanical evisceration. In Spain, López *et al.* [6] detected a 31% occurrence of *L. monocytogenes* in chicken carcasses during the processing stages.

Svobodová *et al.* [8] assessed chicken carcasses during the processing stages in an abattoir in the Czech Republic and isolated 14 *Listeria* spp. strains in 12 carcasses. The most frequent isolation occurred after the plucking and evisceration stages, although no isolate was identified as *L. monocytogenes*. Research work has been undertaken to identify this pathogen in chicken cuts or products exposed on the market [7,25,26].

In China, Yan *et al.* [26] reported occurrence of the bacterium in 6.28% (46/733) of raw meat products, while in Malaysia, Goh et al. [7] isolated *L. monocytogenes* in 20% of analyzed chicken cuts.

Results in current study on the occurrence of *L. monocytogenes* are of great concern since the pathogen was isolated throughout the processing stages including in samples tagged as consumer´s. Chicken carcasses from this stage are cuts stored in conventional refrigerators. The cuts actually come in contact with other food which may contaminate them. It should be underscored that *L. monocytogenes* is an etiological agent of listeriosis, a disease that inflicts severe infections such as septicemias, encephalitis, meningitis and abortion, with high hospitalization and death rates.

Results showed that problems related to the microbiological quality of the broiler processing chain are evident and this fact facilitates preventive and corrective actions. The implementation of specific actions for consumers´ guidance and conscience-raising are clearly required with regard to good practices related to the management and storage of food in the home. Besides that, there are only rare reports in scientific literature on microbiological studies performed at consumers´ home or simulating home storage, as in current assay.

5. Conclusion

Analysis results for total coliforms reveal that deficiency in hygiene occurs in the processing and storage throughout the entire broiler industrial processing. In the case of thermo-tolerant coliforms, there was a decrease in contamination during processing, although 11% of the samples for consumption registered counts above the legal limits. *E. coli*was detected in all the sites evaluated in the processing, except in samples of water for scalding and pre-chiller. Count averages for psycrotrophic microorganisms and bacteria of the genus *Pseudomonas* spp. highly increased in the retail and consumer stages.

Salmonella spp. was reported in all the abattoir´s sampled sites, although the pathogen was absent in the retail and consumer sites. Bacteria of the genus *Listeria* spp. occurred throughout the whole processing, with a high occurrence of *L. monocytogenes*, observed in the pre-scalding and consumer stages.

Results about occurrence of microorganisms in samples from specific sites in the broiler processing chain in Southern Brazil demonstrate the need for a re-assessment of hygiene practices throughout the whole process and educational activities should be directed to all people involved, including consumers.

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