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Monitoring of contamination sources of *Listeria monocytogenes* in a poultry slaughterhouse

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ABSTRACT

This study evaluated the incidence and identify the *Listeria monocytogenes* potential contamination in a poultry slaughter plant and assessed the effectiveness of the sanitation process used by the company. A total of 920 breast and 774 thigh samples were analyzed, and *L. monocytogenes* was found in 8.64 and 44.19% of the samples, respectively. The thigh samples presented more contamination than the breast, throughout the study. The third work shift showed the highest thigh contamination (51%). The contamination percentage was lowest in summer and greater in winter. *L. monocytogenes* contamination in whole carcasses was absent in samples collected before the chilling process, while 5% of the samples were contaminated after this process. After the automated cutting process, the *L. monocytogenes* contamination percentage increased linearly, reaching 25 and 70% after 5 h for breast and thighs, respectively. The percentage of contamination on the equipment and utensils used on thigh samples was high during the processing (86.67%) and after the cleaning operation (66.87%). After pre-operational sanitation of equipment and utensils a lower incidence of *L. monocytogenes* in the production line was observed.

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1. Introduction

Listeria monocytogenes is the etiologic agent of listeriosis, an infection caused by contaminated food that can cause meningitis and abortions. It is among the major foodborne diseases (Scallan, Griffin, Angulo, Tauxe, & Hoekstra, 2011), often manifesting as sepsis, central nervous system infections and maternal and fetal infections. The risk groups are mostly elderly, pregnant women and people with compromised immune systems (Centers for Disease and Prevention, 2013). This bacterium can survive at pH 4.4–9.4 and water activity >0.92 (Chen, Wu, Zhang, Yan, & Wang, 2014; USFDA, 2013), with resistance to many disinfectants and temperature growth range of −0.4–45 °C (Wałecka-Zacharska, Kosek-Paszkowska, Bania, Karpiskova, & Stefaniak, 2012).

The etiologic agent of listeriosis is often found in contaminated food ready for consumption, such as milk, meat, fish, vegetables and derivatives (Chen et al., 2014), and also in the environment, equipment and utensils (Chiarini, Tyler, Farber, Pagottoy, & Destro, 2009; Lakicevic, Nastasijevic, & Raseta, 2015). In addition to water and the raw material, the employees themselves can re-contaminate the workplace through their clothes and shoes, and via individuals who are asymptomatic carriers (Hellström, 2011).

L. monocytogenes can resist industrial sanitizers, to colonize the whole environment, forming biofilms that make it even more resistant. Hence, it may remain in the processing environment for months and cause cross-contamination (Carpentier & Cerf, 2011). In industries, it is common to find other factors that benefit the permanence of *L. monocytogenes* in the environment, such as the inappropriate design of structures that hinder efficient cleaning and sanitization, cracks, grooves and loose welds that improve grip and favor the emergence of biofilms (Skovager et al., 2013).

Ready-to-eat chicken has been considered a principal agent involved in *L. monocytogenes* outbreaks (Carpentier & Cerf, 2011; Elmali, Can, & Yaman, 2015). Although many authors have studied the occurrence of *L. monocytogenes* in the final product (Brizio &

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Prentice, 2015; Pelisser, Mendes, Sutherland, & Batista, 2001), a smaller number of studies evaluate the incidence and identify the potential of *L. monocytogenes* contamination in poultry slaughter plants.

Given this microorganism is an hazard to human health, to improve the microbiological conditions of poultry meat, and serve as technical support for other food industries, the current study aimed to evaluate the incidence and identify the potential of *L. monocytogenes* contamination in a poultry slaughter plant and to assess the sanitation process efficacy.

2. Material and methods

The experiments were performed at a large poultry slaughter plant, located in the West of Santa Catarina, Brazil, that slaughters approximately 200,000 chickens per day, which has HACCP and Good Hygiene Practice implemented.

2.1. Incidence of L. monocytogenes in chicken cuts

The monthly incidence of *L. monocytogenes* was evaluated over 1 year in chicken cuts (breast n = 920; boned thighs n = 774) before the packaging process, in three work shifts (with 6 h each shift). The samples were collected once a month, always in the first week. The first work shift was after the pre-operational sanitation, while each of the following shifts relied only on an operational cleaning. The other tests were done with collection in a single day.

2.2. Identification of L. monocytogenes contamination sources in products at different processing steps

Based on the results of the *L. monocytogenes* incidence, we sought to identify the main contamination sources to define the highest percentages of prevalence in the products.

Whole carcasses were collected before (n = 40) and after (n = 40) passing through the cooling process (chiller), in a single collection, to identify the *L. monocytogenes* contamination at this processing step, and possible cross-contamination in the chiller. Analyses were performed with Fraser broth (Merck, Darmstadt, Germany) resulting from rinsing of the whole carcass.

Samples of breast and thigh were collected shortly after the cutting stage and on the mat before bone detection, in three daily work shifts. Thigh (n = 2/h) and breast (n = 2/h) samples were collected every hour, during 5 h in a single work shift (third shift immediately after cleaning), to verify a possible cross-contamination and to understand how the level of contamination changes during the production day. These samples were collected immediately after obtaining the respective cut and on three independent days of the same month, always on the same shift (total n = 60).

2.3. Determination of equipment and utensil contamination sources

Equipment and utensil samples were collected immediately after pre-operational sanitation (n = 297) and randomly during the production process (n = 276). The sampling points were the chiller, at the entrance of industrial cutter machine of the breast and thighs, breast chute, breast selection mat, thighs chute, thighs conveyor mat, thigh classifier, and thigh weighing bench. The knives, steel gloves and cutting boards used in boning thighs were evaluated. Samples were collected with swab in 20 cm^2 delimited area.

2.4. Effectiveness of pre-operational sanitation and operational cleaning to control L. monocytogenes

Based on the results obtained, two points after the cutter machine were selected, namely the chute from which the breast is removed, and chute where the thigh is boned. The cleaning effectiveness was assessed during production (n = 50), as well as during pre-operational process after line rinsing, and before (n = 50) and after sanitization (n = 50) in the breast and thigh chutes.

The pre-operational process performed by the company consists of waste collection and hot water (minimum 35 °C) passed over all surfaces and equipment of the production line. Then, a alkali detergent is used, and manual scrub is performed, where necessary. Next, the whole line, including the surfaces, equipment, and utensils, are rinsed with pressurized water (minimum 40 °C). Then, sanitization is performed; time and concentration of sanitizer (peracetic acid) are based on the supplier's recommendations (0.5%). Finally, one final rinse occurs before the equipment is assembled for organization and release of the production line. During the production, an operational cleaning occurs that consists of rinsing with pressurized water (minimum 40 °C).

2.5. Analysis of L. monocytogenes

A 25 g aliquot of the sample (breast and thigh samples with skin) was pre-enriched in 225 mL of half-strength Fraser broth (Merck) and incubated at 30 °C for 24 h. Next, 1 mL of pre-enriched half-strength Fraser broth was combined with 10 mL of Fraser broth (Merck) and incubated at 30 °C for 24 h. After, 1 mL of Fraser broth was boiled for 15 min and 0.5 mL was transferred to a VIDAS® LIS (Biomérieux, Marcy l'Étoile, France) reagent strip and screened for the presence of *Listeria* spp. Presumptive *L. monocytogenes* positive samples were also identified by applying 0.5 mL of the non-heated Fraser broth to the VIDAS® LMO2 (Biomérieux) reagent strip. Presumptive results with value < 0.10 to VIDAS® LIS and <0.05 to VIDAS® LMO2 tests are considered negative. If test values were above the threshold value recommended by the manufacturer into presumptive tests, confirmation was achieved by plating the remaining non-heated Fraser broth onto Chromoplate® Listeria selective-agar and Oxford agar (both Merck). Both selective agars were incubated at 37 °C for 24–48 h. Presumptive *L. monocytogenes* colonies were subcultured on tryptone soya yeast agar (Merck) and identified biochemically using API Listeria test strips (Biomérieux). In the confirmative test, the VIDAS® LIS and the VIDAS® LMO2 results were classified as negative when showed test values below the threshold value specified by the manufacturer (<0.23) and positive with test values above this threshold (>0.23). The VIDAS LMX method, with the optional ALOA agar confirmation method, was adopted as Official First Action status for the detection of *L. monocytogenes* in a variety of foods (Crowley et al., 2014).

Surfaces of carcasses and equipments were sampled randomly using a swab and a sterile 100 cm^2 square metal template. The used swabs were placed in individual sterile plastic bags with 25 mL of 0.1% sterile peptone water (Difco). For environmental samples following pre-operational sanitation, the residual present in the sample was neutralized with 1% sodium thiosulfate and 1% Tween 80.

2.6. Statistical analysis

The results presented normal distribution and were statistically analyzed by analysis of variance (ANOVA) followed by Tukey's or Student's test using Statistica 5.0 software. The 0.05 level of significance was used for all data analyses. The linear correlations were performed by Microsoft Excel software.

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