

Microbial contamination of luncheon meat sliced and packaged at supermarkets in Porto Alegre, Brazil*

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ABSTRACT

Background: Ready-to-eat (RTE) foods are considered a high risk food group, since they are often consumed without a cooking step. Luncheon meat, a RTE food widely consumed in Brazil, is traditionally produced as industrially vacuum-packaged loaves and afterwards is sliced and re-packaged at retail stores. Since this practice may pose an additional hazard of contamination, the purpose of this study was to evaluate total coliform counts (TCC), coagulase-positive staphylococci counts (CPS), and the occurrence of *Escherichia coli* and *Listeria* sp. in luncheon meat samples sliced and packaged at supermarkets.

Materials, Methods & Results: Three supermarket stores belonging to either regional or national chains located in Porto Alegre were intentionally chosen, and luncheon meat samples were purchased from the same sampled stores weekly during a 20-week period. In each sampling event, five store-packaged luncheon meat samples were obtained and analyzed. Individual samples (25 g) were homogenized, decimally diluted in buffered peptone water and submitted to TCC in Violet Red Bile Agar. Thermotolerant coliform (FC) were confirmed in EC broth incubated at 45°C. Confirmed tubes were streaked on McConkey agar and submitted to *E. coli* identification. Isolation and enumeration of coagulase-positive staphylococci (CPS) were performed on Baird-Parker agar. The presence of *Listeria* sp. was tested in pooled samples submitted to pre-enrichment in University of Vermont (UVM) *Listeria* Enrichment broth, followed by incubation in Fraser broth and isolation on tryptose agar with nalidixic acid and Palcam agar. TCC mean varied from 1.2 log₁₀ CFU.g⁻¹ (store B) to 5.5 log₁₀ CFU.g⁻¹ (store C), while CPS mean counts were similar (0.63; 0.65 and 0.86 log₁₀ CFU.g⁻¹) for samples purchased at the three stores. Considering Brazilian standards for FCC, in stores A (n=6) and C (n=8) samples considered unsafe (above 3log. g⁻¹) were found, while all samples purchased at store B are considered sound according to that standard. Moreover, three samples from each store (A and C) confirmed for the presence of *E. coli*. Samples contaminated with *Listeria* sp. (n=16) were also found. *Listeria monocytogenes* was isolated from six samples, and was found in all sampled supermarkets. A trend of *Listeria* sp. isolation frequency in samples with higher TCC was observed.

Discussion: The results demonstrated that bacteria may be introduced in luncheon meat during the slicing and packaging at supermarkets. Our data are in accordance with other reports that indicate slicing as a critical control point of contamination and transfer of pathogen, including *L. monocytogenes*, to foods during processing. Differences observed on extend of product contamination may have resulted from variable levels of cleanliness during handling and slicing procedures at the three supermarkets. Specifically, the cleaning of equipment surfaces represents a challenge for sanitation programs, since most equipment is not hygienically designed and must be unassembled prior to sanitization procedures. This fact may lead to the decrease of cleaning and disinfection frequency and to the hazard of biofilm formation. In conclusion, luncheon meat sliced and packaged in supermarkets may pose a hazard to the consumers, and the adoption of more rigorous disinfection protocols for equipment and surfaces in contact with ready-to-eat foods in these stores is advisable.

Keywords: Ready-to-eat (RTE) foods, luncheon meat, *Listeria*, Coliforms.

INTRODUCTION

Ready-to-eat (RTE) foods are a high risk food group, since they are often consumed without a cooking step. Among the food borne pathogens that may be transmitted by RTE foods, *Listeria monocytogenes* represent a major concern, because of its ability to form biofilm in the food processing environment and to survive and multiply in products at refrigerated temperatures [12]. In Brazil, *L. monocytogenes* has been reported as a cause of hospitalization of patients and has been isolated from food products like milk, cheese and salami [1,13,17,21].

Luncheon meat, a RTE food widely consumed in Brazil, is traditionally produced as industrially vacuum-packaged loaves, and afterwards is sliced and re-packaged at retail stores. Therefore, a product originally processed in a factory, may be exposed to recontamination hazard at the retail level. The slicing and re-packaging of RTE products are often conducted in large supermarket stores that offer a wide variety of products, process daily RTE food amounts above 400 kg, and serve more than 5,000 consumers per day [10]. Considering the amount of processed RTE foods and the number of consumers served, products manipulated in those stores may represent a hazard to public health if good hygienic practices were not adopted. In a previous study [11], a lack of standard cleaning and disinfection procedures was detected in supermarkets, pointing out the contamination hazard for foods processed in those stores. Thus, the purpose of this study was to evaluate microbiological contamination occurring in luncheon meat sliced and packaged at supermarkets to assess the hazard of this kind of RTE product for consumers.

MATERIALS AND METHODS

Sampled Stores

Three supermarket stores belonging to either regional or national chains located in Porto Alegre were intentionally chosen, according to the high number of customers served. The average number of customers served daily in each store, based on store manager's estimates, ranged from 6,000 on work days to more than 10,000, during the weekend. All stores purchased vacuum-packed luncheon meat loaves directly from factories under federal inspection and sliced and re-packaged them in the store. The amount of all RTE products processed ranged from 500 to 1,000 kg daily, at least half of them being represented by RTE meats (luncheon meat, salami and bologna).

The sampled stores processed RTE products in temperature-controlled areas and sliced RTE meats and cheeses in different equipment. The sanitation protocol of surfaces and equipment in all stores was composed of removal of food debris with a scrubber, followed by a cleaning and disinfection phase with chlorinate alkaline detergent (2% Sodium hypochlorite and 9.4% Sodium hydroxid) and a washing step with tap water.

Sampling Procedures

Luncheon meat was purchased from the same sampled stores weekly during a 20-week period. From each visited store, five store-packaged luncheon meat samples were obtained. During a 6-week period, three industry-packaged luncheon meat samples were also purchased from the same stores. All samples were transported to the laboratory on ice and processed immediately.

Sampling Processing and Bacteriological Isolations

Luncheon meat samples (25 g) were individually placed in a sterile plastic bag with 225 mL of buffered peptone water. The samples were homogenized in a stomacher¹ for 60 s, decimally diluted in buffered peptone water and 1 mL aliquots were pour-plated in Violet Red Bile Agar² for Total Coliform Counts (TCC). Thermotolerant coliform (FCC) were confirmed in EC broth² incubated at 45°C for 24 h. Tubes with gas production were considered positive for thermotolerant coliforms. Confirmed tubes were streaked on McConkey agar² and submitted to *E.coli* identification [20]. For the isolation of coagulase-positive staphylococci (CPS), aliquots (0.1 mL) were streaked on Baird-Parker agar² and typical colonies (black to dark gray, with an opaque zone surrounded by a clear halo) were selected and tested for coagulase production using rabbit plasma. Except for EC broth, all media were incubated at 37°C for 24-48 h.

The presence of *Listeria* was tested in pooled samples. Approximately 5 g of luncheon meat was removed aseptically from the package of five samples purchased at the same store in the same sampling event and put in a stomacher bag until a 25 g-aliquot was obtained. The presence of *Listeria* was tested according to the protocol proposed by [3]. In the pre-enrichment step, 225 mL of University of Vermont (UVM) *Listeria* Enrichment broth³ was added to 25 g of the pooled luncheon meat sample, and incubated at 30°C for 24 h. Aliquots (0.1 mL) of the UVM broth were transferred to tubes containing 9.9 mL of Fraser broth³ and incubated at 35°C for 24 h. Samples that presented darkening were

streaked on tryptose agar with nalidixic acid and Palcam agar³. After incubation at 35°C for 24 h, typical colonies were identified by biochemical tests and CAMP test on sheep blood agar, using *Staphylococcus aureus* (ATCC 49444) e *Rhodococcus equi* (ATCC 6939) as test strains.

Data Analyses

For data analysis, TCC, FCC and CPS were expressed as log₁₀ CFU g⁻¹. The number of *Listeria* sp. positive pools and the mean log TCC of the correspondent pool were compared by Logistic Regression, using the procedure PROCLOG of SAS software⁴.

RESULTS

A significant difference in overall contamination rates in luncheon meat purchased at the three supermarkets sampled in this study was observed (Table 1). In store A the mean log TCC found in the luncheon meat samples (5.5 CFU.g⁻¹) was at least 3-log greater than the mean log TCC found in samples purchased in stores B and C (1.2 CFU.g⁻¹ and 2.3 CFU.g⁻¹, respectively). A much lower median log of TCC (0.46 CFU.g⁻¹) and an overall lower TCC contamination were found in prepackaged luncheon meat samples (Table 2). Considering Brazilian standards [2] for FCC, in stores A (n=6) and C (n=8) samples considered unsafe (above 3log.g⁻¹) were found, while samples purchased at store B and prepackaged samples purchased at any of the stores presented FCC below 3log.g⁻¹ and are considered

sound according to that standard. Moreover, three samples from each store (A and C) confirmed for the presence of *E. coli*.

On the contrary, the level of contamination of samples with coagulase-positive staphylococci did not vary among stores. Luncheon meat samples presented CPS median log counts of 0.63 CFU.g⁻¹, 0.65 CFU.g⁻¹, and 0.86 CFU.g⁻¹ in store A, B and C, respectively. Similarly, prepackaged samples presented CPS median log counts of 0.73 CFU.g⁻¹.

L. monocytogenes was isolated in samples purchased at the three supermarkets, and predominated in samples from store A (Table 3). Moreover, a trend (P=0.12) of higher frequency of isolation of *Listeria* sp. in luncheon meat samples with higher TCC was observed.

DISCUSSION

The hazard that contaminated food poses to consumers will depend on extent and type of contamination, the potential of the food-stuff to foster growth of contaminant bacteria and the kind of preparation that food will undergo prior to consumption. In the case of the luncheon meat, the typical consumption will be in sandwiches, salads and other dishes that will not undergo a cooking step. Thus, the introduction of bacteria during the slicing and packaging of luncheon meat at supermarkets may represent an additional concern to the food safety.

Table 1. Total Coliforms, Thermotolerant coliforms and Coagulase-positive Staphylococci counts in luncheon meat samples sliced and packaged at three supermarkets (A, B, C) in Porto Alegre, RS, Brazil.

| Counts (log ₁₀ cfu.g ⁻¹) | Total Coliforms | | | Thermotolerant Coliforms | | | Coagulase-positive Staphylococci | | |
|--|-----------------|-----|-----|--------------------------|-----|-----|----------------------------------|-----|-----|
| | A | B | C | A | B | C | A | B | C |
| Negative | 2 | 65 | 32 | 94 | 99 | 89 | 89 | 84 | 79 |
| <3 | - | 15 | 18 | - | 1 | 3 | - | 2 | - |
| 3-4 | 2 | 15 | 33 | - | - | 3 | - | 6 | 11 |
| 4-5 | 26 | 2 | 13 | 1 | - | 3 | 10 | 3 | 9 |
| 5-6 | 36 | 3 | 4 | 3 | - | 2 | - | 5 | 1 |
| 6-7 | 28 | - | - | 2 | - | - | 1 | - | - |
| >7 | 6 | - | - | - | - | - | - | - | - |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Table 2. Total Coliforms, Thermotolerant coliforms and Coagulase-positive Staphylococci counts in industry-packaged luncheon meat samples purchased at three supermarkets (A, B, C) in Porto Alegre, RS, Brazil.

| Counts (log ₁₀ cfu.g ⁻¹) | Total Coliforms | | | Thermotolerant Coliforms | | | Coagulase-positive Staphylococci | | |
|--|-----------------|----|----|--------------------------|----|----|----------------------------------|----|----|
| | A | B | C | A | B | C | A | B | C |
| Negative | 12 | 9 | 14 | 18 | 17 | 18 | 11 | 11 | 13 |
| ≤3 | 6 | 8 | 2 | | 1 | | 1 | 4 | 2 |
| 3-4 | | 1 | 2 | | | | 5 | 3 | 3 |
| 4-5 | | | | | | | 1 | | |
| Total | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 |

Table 3. *Listeria* species recovered from luncheon meat sample pools* sliced and packaged at three supermarkets (A, B, C) in Porto Alegre, RS, Brazil.

| Listeria species | Store | | |
|-------------------------|-------------|-------------|-------------|
| | A (n=20) | B (n=20) | C (n=20) |
| <i>L. monocytogenes</i> | 4 (20%) | 2 (10%) | 1 (5%) |
| <i>L. innocua</i> | 2 (10%) | 2 (10%) | 4 (20%) |
| <i>L. grayi</i> | 1 (5%) | 0 | 0 |

*pool of five individual samples

The difference between the TCC counts observed on the luncheon meat samples analyzed in this study may have resulted from variable levels of exposition to contaminants during handling and slicing procedures at the three supermarkets. Cleaning and disinfection of surfaces in the food processing environment may affect the quality and safety of the processed food products [9]. Specifically, the cleaning of equipment surfaces represents a challenge for sanitation programs, since most equipment is not hygienically designed and must be unassembled prior to sanitization procedures. This fact may lead to the decrease of cleaning and disinfection frequency and to the hazard of biofilm formation. Consequently, products that touch or pass over

contaminated surfaces will potentially pick up microorganisms from the microbial consortium that may have developed on the surfaces [6].

Besides the improper cleaning and sanitizing of equipment and surfaces, the difference found on TCC counts may also reflect the processing practices adopted by each supermarket in terms of extent of manipulation and contact of products with the environment during processing. In spite of the fact that no standard is established for TCC in meat products in Brazil, the mean TCC counts found in our study can be considered high. In store-packaged pork and pork sausage, TCC means of 1.5 and 2.2 log CFU.g⁻¹, respectively, have been reported [7]. In the aforementioned study, store-ground

pork and prepackaged product were compared and a difference greater than 1-log CFU.g⁻¹ of TCC between these two kinds of products was reported. In this case, an initial bacterial load on pork trimmings used for grinding in the store was also pointed out as a possible explanation for the higher level of contamination. In our study, a cooked product purchased by the supermarkets as prepackaged loaves was sampled, thus the chance of a very high initial TCC load seems to be less probable. To check this hypothesis, luncheon meat samples sliced and packaged in pork fabrication plants and purchased at the same supermarkets were also included in the study. In spite of the fact that a direct comparison is not possible, a much lower TCC contamination were found in prepackaged luncheon meat samples, demonstrating that the product was probably delivered to the supermarket with a lower contamination level. Additionally, only in stores A and C samples with FCC unsound counts according to the Brazilian standards [2] were found and *E. coli* was identified in the sampled product. In these cases a bacteria was isolated, for which the primary habitat is the intestinal tract of man and animal. In this sense, a direct or indirect fecal contamination may have occurred and a general lack of cleanliness in handling can be suspected.

Concerning the coagulase-positive staphylococci counts, there was a lower discrepancy between samples in the analysis conducted. In store C, a higher percentage (21%) of samples with CPS counts above the Brazilian standards [2] was found, however unsound samples were also detected among those purchased at stores A (11%), B (14%), and among industry-packaged samples (13%). Those samples may represent a hazard to the consumer, since they may be contaminated by staphylococcal enterotoxins as well. Moreover, once enterotoxin has been produced on the food, further heat treatment employed in its processing or cooking will be able to inactivate staphylococci, although the preformed toxin may remain active [18]. Coagulase-positive staphylococci can enter the process on raw materials, but it can also be introduced into foods during processing from unclean hands and unsanitary utensils and equipment. Both humans and

animals may carry staphylococci in nostrils and throats [16], thus workers can transfer staphylococci on their hands to equipment and to the product during manipulation. In spite of being a pre-cooked product, luncheon meat's recontamination during slicing can be a concern at both industry and retail level.

Our data are in accordance with other reports that indicate slicing as a critical control point of contamination and transfer of pathogen to cooked meat products [4, 8]. Among the pathogens, *L. monocytogenes* has been one of the most often implicated in food borne disease caused by consumption of RTE foods [12]. Most studies agreed that environment is a potential source of *L. monocytogenes* contamination of RTE meat [12,14] and the persistence of *L. monocytogenes* in the processing environment has been demonstrated for periods of months until years [4].

As a general rule, it was proposed that a percentage of products positive for *L. monocytogenes* above 5% at the end of their shelf-life may indicate that the hygienic conditions at a RTE factory are unsuitable [14]. In our study, all supermarkets had frequencies of overall isolation higher than 5% in luncheon meat, indicating that manufacturing practices have to be revised and improved.

CONCLUSIONS

Our results suggest that luncheon meat may pose a potential hazard for consumers. In addition, retail stores need to implement or improve cleaning and disinfection procedures, as was reinforced by the higher TCC counts in sliced and packaged products at supermarkets compared to industry-packaged samples. The implementation of good manufacturing practices and the monitoring of procedures taken to assure food safety are also advisable.

SOURCES AND MANUFACTURERS

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