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Microbiological evaluation of inspected and non-inspected fresh sausages by the dry sheet medium culture plate method

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Abstract

Fresh sausage is the most consumed type of sausage in Brazil, owing to its high consumer acceptance and low cost. Despite its popularity, it can pose public health risks if contaminated with pathogens. This study aimed to evaluate the microbiological quality of inspected and non-inspected fresh sausages sold in the region of Bauru, São Paulo, Brazil. A total of 40 samples (30 inspected and 10 non-inspected) were analyzed using the dry sheet medium culture plate method (Compact Dry®) to detect *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, and total coliforms, according to the manufacturer's instructions. The results indicated a high level of contamination, particularly by *Salmonella* spp. (8/40, 20.00%) and *L. monocytogenes* (5/40, 12.50%). These findings highlight the need for stricter inspection procedures by regulatory agencies in the interior regions of São Paulo.

Keywords: public health; food safety; *Salmonella* spp.; *Listeria monocytogenes*.

Practical Application: Microbiological analysis reveals health hazards in non-inspected sausages.

1 INTRODUCTION

Sausage is a meat product made from animals raised and slaughtered under veterinary inspection. It may include fat and other ingredients, and it is typically stuffed into casings using appropriate technological procedures (Brasil, 2000). Among various types of sausages available on the market, fresh sausage, composed of raw meat, is the most commonly consumed type in Brazil. Its popularity is attributed to both its affordability and high level of consumer acceptance (Araújo et al., 2021).

However, despite its widespread consumption, fresh sausages can present a significant risk of microbiological contamination. This is due to several contributing factors, including inadequate hygienic and sanitary conditions of the raw materials, equipment, and utensils used in processing, as well as improper practices by food handlers (Lehto et al., 2011; Lopes et al., 2020).

Foodborne diseases are those that result from the ingestion of contaminated food or water, and are primarily caused by bacteria and their toxins, as well as viruses and parasites. These illnesses represent a major concern for public health (Brasil, 2024). Therefore, the microbiological monitoring of food products is essential to ensure consumer safety and reduce the risk of outbreaks.

In light of these concerns, the present study aimed to assess the microbiological quality of fresh sausage samples that were either inspected or not inspected by official regulatory agencies and that were commercially available in the Bauru Mesoregion, São Paulo, Brazil. The analysis was conducted using the dry sheet medium culture plate method, specifically to detect the presence of *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, and total coliforms.

1.1 Relevance of the work

This study demonstrated the presence of pathogens in fresh sausages sold in the Bauru region of São Paulo, particularly in products lacking official inspection. The findings highlight public health risks, including the presence of *Salmonella* spp. and *Listeria monocytogenes*, and reinforce the need for more rigorous sanitary control. The use of a practical and accessible methodology (Compact Dry®) also supports efficient microbiological monitoring and can aid regulatory efforts.

2 MATERIAL AND METHODS

2.1 Samples

The samples analyzed in this study consisted of fresh sausages that were either inspected or non-inspected by official regulatory agencies. These fresh sausages were sold in bulk in

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supermarkets and butcher shops located in the Bauru area, São Paulo, Brazil. Three groups (brands) of inspected sausages and one group of non-inspected sausages were analyzed. The sample codes were as follows: (a) inspected sausages from brand A (ISA; $n = 10$), (b) inspected sausages from brand B (ISB; $n = 10$), (c) inspected sausages from brand C (ISC; $n = 10$), and (d) non-inspected sausages (NIS; $n = 10$).

In total, 40 fresh sausage samples commercialized in the interior of São Paulo state were collected. These samples were transported in thermal boxes maintained at 4°C to the Microbiology Laboratory of the Public Food Guidance Service (SOAP), within the Department of Animal Production and Preventive Veterinary Medicine, School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP – Botucatu Campus).

2.2 Listeria monocytogenes

A 25 g portion of the sausage sample was weighed and placed in a sterile plastic bag. Then, 225 mL of Listeria Enrichment Broth (LEB, 222220, Difco) was added. The mixture was homogenized in an automatic homogenizer for 120 s and incubated at 30 ± 1°C for 24 ± 2 h. For the selective enrichment step, 0.1 mL aliquots were transferred to tubes containing 10 mL of Fraser Broth (Fraser Broth Base, CM0895-ISO), which were then incubated at 35 ± 1°C for 24–48 h. Tubes with presumptive positive results (darkened appearance) were plated. One milliliter of the enriched broth was transferred to Compact Dry LM® plates (dry sheet medium culture plate method). The plates were incubated at 35 ± 1°C for 24–48 h. For result interpretation, colonies with blue or light blue coloration were considered positive. Results were reported as the presence or absence of *L. monocytogenes* in 25 g of the sample.

2.3 Salmonella *spp.*

A 25 g portion of the sample was weighed into a sterile plastic bag, and 225 mL of previously sterilized buffered peptone water (BPW, CM0509, OXOID) was added. The mixture was homogenized for approximately 120 s and incubated at 36 ± 1°C for 22 ± 2 h. Then, 1 mL of the pre-enriched BPW with the sample was transferred to 10 mL of tetrathionate broth (TT, CM0029, OXOID), supplemented with 0.2 mL of iodine–iodide solution, and 0.1 mL was inoculated into 10 mL of Rappaport–Vassiliadis broth (RV, CM0669, OXOID). The tubes were incubated at 41 ± 2°C for 24 ± 2 h. Subsequently, 0.1 mL of the enriched broth was placed on one end of a Compact Dry SL® plate (dry sheet medium culture plate method), while 1 mL of sterile distilled water was placed on the opposite end of the plate. The plates were incubated at 41 ± 2°C for 24 ± 2 h. For positive result interpretation, the following were considered: (a) isolated or merged colonies ranging from black to greenish in color; (b) surrounding medium turning yellow; and (c) motility-associated spreading. Results were reported as the presence or absence of *Salmonella* spp. in 25 g of the sample.

2.4 Staphylococcus aureus

A 25 g portion of each sample was aseptically weighed into a sterile plastic bag, followed by the addition of 225 mL of pre-sterilized 0.85% saline solution. The mixture was homogenized for approximately 120 s. Subsequently, 1 mL of the homogenate was plated onto a Compact Dry XSA® plate, based on the dry sheet medium culture method. The plates were incubated at 35°C ± 2°C for 24 ± 2 h. For result interpretation, blue or light blue colonies were enumerated, and results were expressed as colony-forming units (CFUs) per gram of sample.

2.5 Escherichia coli *and total coliforms*

A 25 g of each sample was aseptically weighed and homogenized with 225 mL of pre-sterilized 0.85% saline solution for approximately 120 s. A 1 mL aliquot of the homogenate was inoculated onto a Compact Dry EC® plate, utilizing the dry sheet medium culture method. The plates were incubated at 35°C ± 2°C for 24 ± 2 h. For *E. coli*, blue colonies were enumerated. For total coliforms, colonies displaying blue, red, purple, or pink pigmentation were counted. Results were reported as CFU per gram of sample.

2.6 Statistical analysis

The quantitative data obtained from total coliform counts (CFU/g) were subjected to statistical analysis using analysis of variance (ANOVA) based on a completely randomized design. Tukey's multiple comparison test was employed for post hoc analyses, with significance set at $p < .05$ (Montgomery, 2012). The detection frequencies of *L. monocytogenes*, *Salmonella spp.*, *S. aureus*, and *E. coli* were analyzed using absolute frequency (AF) and relative frequency (RF).

2.7 Biological and chemical waste management

All biological materials and waste generated during the study were autoclaved at 121°C for 30 min prior to disposal, following established biosafety protocols. Chemical waste was managed by the Conservation and Maintenance Section of the General Administration at UNESP, Botucatu Campus, São Paulo, Brazil, which is responsible for the technical and analytical decisions regarding the disposal of all chemical waste generated at the institution.

3 RESULTS

3.1 Listeria monocytogenes

Among the inspected sausage samples, one from the brand ISB tested positive for *L. monocytogenes* (1/10, 10.00%; or 1/30, 3.33% among all inspected samples). In contrast, a higher positivity rate was observed in the NIS, with 4 out of 10 samples testing positive (40.00%; or 4/40, 10.00% of the total samples), as detailed in Table 1.

3.2 Salmonella *spp.*

Regarding *Salmonella spp.* detection, all groups except brand ISA exhibited some level of contamination. Specifically, ISB had one positive sample (1/10, 10.00%; or 1/30, 3.33% among

Table 1. Absolute frequency, relative frequency, and relative percentage of the presence/absence of *Listeria monocytogenes* in 25 g of sausage samples from groups inspected sausages from brand A, inspected sausages from brand B, inspected sausages from brand C, and non-inspected sausages.

Group	Result	AF	RF	RF (%)
ISA	Positive/25 g	0	0.00	0
(n = 10)	Negative/25 g	10	0.25	25
ISB	Positive/25 g	1	0.03	3
(n = 10)	Negative/25 g	9	0.22	22
ISC	Positive/25 g	0	0.00	0
(n = 10)	Negative/25 g	10	0.25	25
NIS	Positive/25 g	4	0.10	10
(n = 10)	Negative/25 g	6	0.15	15
Total		40	1.00	100%

AF: absolute frequency; RF: relative frequency; %: relative percentage; ISA: inspected sausages from brand A; ISB: inspected sausages from brand B; ISC: inspected sausages from brand C; NIS: non-inspected sausages.

Table 2. Absolute frequency, relative frequency, and relative percentage of the presence/absence of *Salmonella* spp. in 25 g of sausage samples from groups inspected sausages from brand A, inspected sausages from brand B, inspected sausages from brand C, and non-inspected sausages.

Group	Results	AF	RF	RF (%)
ISA	Positive/25 g	0	0.00	0
(n = 10)	Negative/25 g	10	0.25	25
ISB	Positive/25 g	1	0.03	3
(n = 10)	Negative/25 g	9	0.22	22
ISC	Positive/25 g	2	0.05	0
(n = 10)	Negative/25 g	8	0.20	25
NIS	Positive/25 g	5	0.125	12.5
(n = 10)	Negative/25 g	5	0.125	12.5
Total		40	1.00	100%

AF: absolute frequency; RF: relative frequency; %: relative percentage; ISA: inspected sausages from brand A; ISB: inspected sausages from brand B; ISC: inspected sausages from brand C; NIS: non-inspected sausages.

inspected samples), while ISC presented the highest contamination among inspected products (2/10, 20.00%; or 2/30, 6.67%). Among NIS, contamination was notably higher, with 5 out of 10 samples testing positive (50.00%; or 5/40, 12.50% of the total samples), as shown in Table 2.

3.3 Staphylococcus aureus

All sample groups exhibited detectable levels of *S. aureus* contamination. Among the inspected products, brand ISA had 4 out of 10 samples (40.00%; or 4/30, 13.33%) with counts ≥ 10 CFU/g. Brand ISB showed the highest contamination rate among inspected groups (6/10, 60.00%; or 6/30, 20.00%), followed by ISC (2/10, 20.00%; or 2/30, 6.67%). The NIS displayed the highest overall contamination, with 9 out of 10 samples (90.00%; or 9/40, 22.50%) exceeding ≥ 10 CFU/g (Table 3).

3.4 Escherichia coli

For *E. coli* enumeration, all inspected brands except ISA showed some level of contamination. Specifically, ISB and ISC each had 1 out of 10 samples (10.00%; or 1/30, 3.33%) with counts ≥ 10 CFU/g. The NIS group demonstrated the highest contamination rate, with 3 out of 10 samples (30.00%; or 3/40, 7.50%) exceeding this threshold (Table 4).

3.5 Total coliforms

All groups exhibited some level of total coliform contamination. Among inspected brands, ISA had the highest proportion with 9 out of 10 samples (90.00%; or 9/30, 30.00%) showing counts ≥ 10 CFU/g, followed by ISB (8/10, 80.00%; or 8/30, 26.67%) and ISC (6/10, 60.00%; or 6/30, 20.00%). The NIS group also had 9 out of 10 samples contaminated (90.00%; or 9/40, 22.50%) (Table 5).

Regarding mean CFU counts, ISA exhibited the highest levels (1790 ± 1656 CFU/g), followed by ISC (1581 ± 3490 CFU/g), ISB (1374 ± 3912 CFU/g), and NIS (433 509 CFU/g) (Table 6).

Table 3. Absolute frequency, relative frequency, and relative percentage of *Staphylococcus aureus* colony-forming unit counts per gram in samples from groups inspected sausages from brand A, inspected sausages from brand B, inspected sausages from brand C, and non-inspected sausages.

Group	Result	AF	RF	RF (%)
ISA	< 10 CFU/g	6	0.15	15
(n = 10)	≥ 10 CFU/g	4	0.10	10
ISB	< 10 CFU/g	4	0.10	10
(n = 10)	≥ 10 CFU/g	6	0.15	15
ISC	< 10 CFU/g	8	0.20	20
(n = 10)	≥ 10 CFU/g	2	0.05	5
NIS	< 10 CFU/g	1	0.03	3
(n = 10)	≥ 10 CFU/g	9	0.22	22
Total		40	1.00	100%

AF: absolute frequency; RF: relative frequency; %: relative percentage; CFU: colony-forming unit; ISA: inspected sausages from brand A; ISB: inspected sausages from brand B; ISC: inspected sausages from brand C; NIS: non-inspected sausages.

Table 4. Absolute frequency, relative frequency, and relative percentage of *Escherichia coli* colony-forming unit counts per gram in samples from groups inspected sausages from brand A, inspected sausages from brand B, inspected sausages from brand C, and non-inspected sausages.

Group	Result	AF	RF	RF (%)
ISA	< 10 CFU/g	10	0.25	25
(n = 10)	≥ 10 CFU/g	0	0.00	0
ISB	< 10 CFU/g	9	0.23	23
(n = 10)	≥ 10 CFU/g	1	0.02	2
ISC	< 10 CFU/g	9	0.23	23
(n = 10)	≥ 10 CFU/g	1	0.02	2
NIS	< 10 CFU/g	7	0.18	18
(n = 10)	≥ 10 CFU/g	3	0.07	7
Total		40	1.00	100%

AF: absolute frequency; RF: relative frequency; %: relative percentage; CFU: colony-forming unit; ISA: inspected sausages from brand A; ISB: inspected sausages from brand B; ISC: inspected sausages from brand C; NIS: non-inspected sausages.

Table 5. Absolute frequency, relative frequency, and relative percentage of total coliform colony-forming unit counts per gram in samples from groups inspected sausages from brand A, inspected sausages from brand B, inspected sausages from brand C, and non-inspected sausages.

Group	Result	AF	RF	RF (%)
ISA	< 10 CFU/g	1	0.03	3
(n = 10)	≥ 10 CFU/g	9	0.22	22
ISB	< 10 CFU/g	2	0.05	5
(n = 10)	≥ 10 CFU/g	8	0.20	20
ISC	< 10 CFU/g	4	0.10	10
(n = 10)	≥ 10 CFU/g	6	0.15	15
NIS	< 10 CFU/g	1	0.03	3
(n = 10)	≥ 10 CFU/g	9	0.22	22
Total		40	1.00	100%

AF: absolute frequency; RF: relative frequency; %: relative percentage; CFU: colony-forming unit; ISA: inspected sausages from brand A; ISB: inspected sausages from brand B; ISC: inspected sausages from brand C; NIS: non-inspected sausages.

Table 6. Mean ± standard deviation of total coliform colony-forming unit counts per gram in samples from groups inspected sausages from brand A, inspected sausages from brand B, inspected sausages from brand C, and non-inspected sausages. Statistical analysis (ANOVA) with Tukey's test at 5% significance.

Group	n	Mean ± standard deviation
ISA	10	1790 CFU/g ± 1656 CFU/g[a(1)]
ISB	10	1374 CFU/g ± 3912 CFU/g[a]
ISC	10	1581 CFU/g ± 3490 CFU/g[a]
NIS	10	433 CFU/g ± 509 CFU/g[a]
Total	40	

CFU: colony-forming unit; ISA: inspected sausages from brand A; ISB: inspected sausages from brand B; ISC: inspected sausages from brand C; NIS: non-inspected sausages. Statistical analysis (ANOVA) with Tukey's test at 5% significance.

[(1)] p = .70468 and CV = 213.284%.

This means that there was no statistically significant difference between the groups analyzed (p > .05) according to Tukey's test.

Legend: Different letters in the same column indicate a significant difference between groups according to Tukey's test at 5% significance. Identical letters indicate that there is no statistically significant difference (p > .05).

4 DISCUSSION

4.1 Listeria *spp.*

L. monocytogenes is the causative agent of listeriosis, a severe disease affecting both humans and animals. The infection is associated with high hospitalization and mortality rates, making it one of the most serious foodborne illnesses (European Food Safety Authority [EFSA] & European Centre for Disease Prevention and Control [ECDC], 2019). Major outbreaks have been linked to contaminated meat products, such as a significant outbreak in South Africa involving Bologna-type sausages, which resulted in 937 cases and 216 fatalities (Thomas et al., 2020). Clinical manifestations include central nervous system disorders like encephalitis and meningitis, as well as endocarditis, peritonitis, pneumonia, and osteomyelitis (Farber & Peterkin, 1991). In pregnant women, infection can lead to miscarriage, preterm birth, neonatal meningitis, septicemia, or fetal death (Rocourt et al., 2003). Although no confirmed cases of foodborne listeriosis have been reported in Brazil (Destro, 2006), the pathogen is frequently isolated from food products (Lima, 2021; Silva et al., 2004).

In the present study, brand ISB had one positive sample (1/10, 10.00%), while non-inspected sausages exhibited a much higher prevalence (4/10, 40.00%). These findings are concerning, given the pathogen's significance to public health. The presence of *L. monocytogenes* in inspected products is unacceptable, as it endangers consumer safety. Brazilian regulations mandate the absence of *L. monocytogenes* in 25 g of food products (Brasil, 2022).

4.2 Salmonella *spp.*

Salmonella spp. is responsible for salmonellosis, a leading foodborne illness of major public health concern. It is primarily contracted through the consumption of contaminated animal-derived products. In Brazil, *Salmonella* ranks as the third most common cause of foodborne outbreaks between 2014 and 2023. Clinical symptoms typically include gastrointestinal disturbances such as diarrhea, vomiting, nausea, and abdominal pain; severe cases may result in dehydration and even death (Brasil, 2024).

Our findings identified the pathogen in inspected brands–– ISB (1/10, 10.00%) and ISC (2/10, 20.00%), with an even higher prevalence in non-inspected sausages (NIS: 5/10, 50.00%). The detection of *Salmonella spp.* in inspected products underscores potential shortcomings in inspection protocols. According to Brazilian legislation, *Salmonella* spp. must be absent in 25 g of food samples (Brasil, 2022).

4.3 Staphylococcus aureus

The genus *Staphylococcus* represents the second leading cause of foodborne outbreaks in Brazil (Brasil, 2024). This opportunistic pathogen colonizes approximately 20–30% of the human population, primarily in the nasal passages (Wertheim et al., 2005), as well as on skin, throat, axillae, groin, and intestines (Williams, 1963). Foodborne illnesses associated with *S. aureus* typically result from the ingestion of pre-formed enterotoxins, leading predominantly to gastrointestinal symptoms such as projectile vomiting (Moriconi et al., 2020).

In this study, all groups showed counts ≥ 10 CFU/g: the NIS had the highest contamination (9/10, 90.00%), followed by ISB (6/10, 60.00%), ISA (4/10, 40.00%), and ISC (2/10, 20.00%). Although Brazilian regulations for fresh sausages do not specify criteria for coagulase-positive *Staphylococcus* detection, primarily represented by *S. aureus*, its presence is of public health relevance, reflecting deficiencies in hygiene practices, particularly by food handlers during production (Souza, et al., 2014).

4.4 Escherichia coli

E. coli is considered the most significant foodborne pathogen in Brazil (Brasil, 2024). While generally a commensal inhabitant of human and animal intestinal tracts, certain strains are diarrheagenic and capable of causing disease (Llorente et al., 2023).

Brazilian regulations set a maximum permissible limit of 5×10^3 CFU/g for *E. coli* in fresh sausages (Brasil, 2022). In this study, only ISA samples were free from *E. coli* contamination. All other groups showed samples with counts ≥ 10 CFU/g: NIS (3/10, 30.00%) and both ISB and ISC (1/10, 10.00% each). Although these counts are within legal limits, their presence indicates fecal contamination at some point in the production chain, reflecting lapses in hygiene practices, particularly during food handling.

4.5 Total coliforms

Coliform bacteria encompass both intestinal inhabitants of humans and animals and environmental species. Their presence in food and water serves as an indicator of hygienic and sanitary conditions (Conte et al., 2004).

All analyzed groups exhibited contamination: ISA had the highest mean count (1790 ± 1656 CFU/g), followed by ISC (1581 ± 3490 CFU/g), ISB (1374 ± 3912 CFU/g), and NIS (433 ± 509 CFU/g).

Although Brazilian regulations do not specify microbiological standards for total coliforms in fresh sausages, these results reveal significant contamination even among inspected products, further indicating hygiene failures during sausage preparation in retail establishments.

5 CONCLUSIONS

The microbiological quality of fresh sausages was unsatisfactory, with the presence of public health-relevant microorganisms even in inspected products. Although concerning, the findings demonstrated that non-inspected sausages posed a greater risk compared to inspected ones, with higher prevalence of *L. monocytogenes* and *Salmonella spp.* Regulatory agencies in the Bauru area, São Paulo State, must intensify their inspection procedures to ensure food safety.

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