

Determination of pathogens and contamination indicators in fresh pork sausages in the state of São Paulo

Determinação de patógenos e indicadores de contaminação em linguiças frescas de origem suína no estado de São Paulo

Gabriela Terezinha Daniel¹, Alessandra Nassar², Aline Feola de Carvalho³,
Simone Miyashiro⁴, Vanessa Castro⁵, Sérgio Santos de Azevedo⁶,
Eliana Scarcelli Pinheiro⁷

Abstract

The highest cost of beef and chicken meat justifies higher consumption of fresh sausage products, especially *linguiça*, due to their easy preparation and affordability for consumers, making necessary an evaluation of sanitary hygienic conditions of these products. The objective was to investigate the presence of pathogens such as *Salmonella* spp., coagulase-positive *Staphylococcus*, thermotolerant coliforms, *Escherichia coli* and *Campylobacter* spp. in artisanal and inspected fresh pork sausages. It was found that 12% (6/50) of the artisanal sausage samples were contaminated with *Salmonella* spp., 58% (29/50) presented coagulase-positive *Staphylococcus* levels above the acceptable limits for consumption and 76% (38/50) presented thermotolerant coliform levels above the acceptable limits. In sausage samples produced under inspected conditions, 6% (3/50) were contaminated with *Salmonella* spp., 24% (12/50) presented thermotolerant coliform levels above the acceptable limits, 2% (1/50) presented enteropathogenic *E. coli*. None samples showed coagulase-positive *Staphylococcus* counts above the limits, or presence of *Campylobacter* spp. Sensitizing traders and consumers about the importance of inspection service in food of animal origin is urgent for a sanitary acceptable production, since foodborne diseases continue to be a public health problem.

Keywords: Sausages; *Salmonella*; *Staphylococcus*; Coliforms; *Campylobacter*.

¹ Mestrado em Sanidade Animal pelo Instituto Biológico de São Paulo, São Paulo, São Paulo, Brasil. Patologista Clínica do Hospital Veterinário da Universidade Cruzeiro do Sul, São Paulo, São Paulo, Brasil.

² Doutorado em Clínica Veterinária pela Universidade de São Paulo (USP), São Paulo, São Paulo, Brasil. Pesquisadora Científica do Instituto Biológico de São Paulo, São Paulo, São Paulo, Brasil. Responsável Técnica do Laboratório de Bacteriologia Geral do Instituto Biológico de São Paulo, São Paulo, São Paulo, Brasil. *E-mail:* afcnassar@sp.gov.br

³ Mestrado em Sanidade Animal, Segurança Alimentar e Ambiental pelo Instituto Biológico de São Paulo, São Paulo, São Paulo, Brasil.

⁴ Doutorado em Epidemiologia Experimental Aplicada às Zoonoses pela Universidade de São Paulo, São Paulo, São Paulo, Brasil. Pesquisadora Científica do Laboratório de Bacteriologia Geral do Instituto Biológico de São Paulo, São Paulo, São Paulo, Brasil.

⁵ Mestrado em Epidemiologia Experimental e Aplicada às Zoonoses pela Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, São Paulo, Brasil. Pesquisadora Científica do Laboratório de Doenças Bacterianas da Reprodução do Instituto Biológico de São Paulo, São Paulo, São Paulo, Brasil.

⁶ Doutorado em Epidemiologia Experimental Aplicada às Zoonoses pela Universidade de São Paulo, São Paulo, São Paulo, Brasil. Professor Titular da Unidade Acadêmica de Medicina Veterinária do Centro de Saúde e Tecnologia Rural da Universidade Federal de Campina Grande (UFCG), Campina Grande, Paraíba, Brasil.

⁷ Doutorado em Microbiologia pelo Instituto de Ciências Biomédicas da Universidade de São Paulo, São Paulo, São Paulo, Brasil. Pesquisadora Científica do Laboratório de Doenças Bacterianas da Reprodução do Instituto Biológico de São Paulo, São Paulo, São Paulo, Brasil.

Resumo

O alto custo das carnes de frango e bovina justifica maior consumo de produtos embutidos frescos, especialmente a linguiça, devido ao preço acessível ao consumidor e fácil preparo, tornando necessário estudos para avaliar as condições higiênico-sanitárias. Os objetivos foram verificar a presença de *Salmonella* spp., *Staphylococcus* coagulase positiva, coliformes termotolerantes, *Escherichia coli* patogênicas e *Campylobacter* spp. em linguiças suínas tipo frescal artesanais e fiscalizadas. Os resultados obtidos em linguiças artesanais foram de 12% (6/50) contaminadas com *Salmonella* spp., 58% (29/50) de *Staphylococcus* coagulase positiva e 76% (38/50) nas quantificações de coliformes termotolerantes, dados que apresentam níveis de contaminação superiores aos da legislação vigente. Nas linguiças produzidas sob fiscalização detectou-se 6% (3/50) de contaminação por *Salmonella* spp.; 24% (12/50) de quantificação de coliformes termotolerantes acima dos limites aceitáveis, e 2% (1/50) de *E. coli* enteropatogênica. Nenhuma amostra apresentou contagens de *Staphylococcus* coagulase positiva fora dos padrões, ou contaminação por *Campylobacter* spp. Sensibilizar comerciantes e consumidores sobre a importância do serviço de inspeção em alimentos de origem animal é premente para que haja uma produção sanitária aceitável, pois doenças transmitidas por alimentos continuam sendo um problema à saúde pública.

Palavras-chave: Embutidos; *Salmonella*; *Staphylococcus*; Coliformes; *Campylobacter*.

Introduction

Per capita consumption of meat pork in Brazil was 14.47 kg/inhabitant in 2016. Data from 2021 show that this has increased to 16.86 kg/inhabitant, according to the Brazilian Institute for Geography and Statistics (IBGE).⁽¹⁾ The highest proportion of this type of meat is consumed in the form of fresh sausages, followed by hot dog sausages, hams and smoked products.⁽²⁻³⁾ It is important to consider that consumers prefer processed products due to the easy preparation and affordable prices.⁽⁴⁾

Fresh products, especially fresh sausage, present high risk of contamination by microorganisms proliferation during preparation, manufacture and stocking of the product, because they do not undergo to any heat treatment. Moreover, they are subjected to intense handling, which does not always follow good manufacturing practices (GMP). Hence, sanitary and hygiene conditions control is necessary.⁽⁵⁾

Microbiological evaluation of food constitutes one of the parameters to determine the sanitary quality of the product and is equally important to verify if the microbiological standards and specifications are suitable.⁽⁶⁾

Salmonella spp. and *Staphylococcus aureus* stand out among pathogens that cause foodborne infections in several countries.⁽⁷⁻⁸⁾ *Salmonella* spp. detection, counting and enumeration of coagulase-positive *Staphylococcus* (CPS) have been used to assess the hygienic-sanitary conditions of foods.⁽⁹⁻¹⁰⁾

Detection of high number of thermotolerant coliforms in food, including processed foods, is interpreted as a possible presence of intestinal pathogens, mainly *Escherichia coli*.⁽¹¹⁾

Campylobacteriosis is another disease of great importance, and *Campylobacter jejuni* and *C. coli* species are responsible for most enteric infections.⁽¹²⁾ *Campylobacter* enteritis is well characterized as a zoonotic disease of food origin and several studies have demonstrated the significance of animal reservoirs in the epidemiology of *Campylobacter* spp. and food of animal origin is considered to be the main source of campylobacteriosis infection.⁽¹³⁾

Thus, the aim of the present study was to verify the presence of *Salmonella* spp., coagulase-positive *Staphylococcus*, most probable number of total and thermotolerant coliforms, pathogenic *Escherichia coli* and *Campylobacter* spp. in artisanal and inspected fresh pork sausages.

Material and Methods

From March 2013 to March 2014, 100 fresh pork sausages samples were collected in São Paulo, Bragança Paulista, São João da Boa Vista and Jundiaí (all municipalities in the state of São Paulo). Among these, 50 artisanal sausages samples were acquired from street fairs, supermarkets and butchers; and 50 sausages samples bearing the registration mark of municipal (SIM) or state (SISP) inspection services were also acquired. Samples were stored in disposable sterile bags and transported in isothermal boxes with recyclable ice, at a temperature of not more than 8 °C, to Biological Institute laboratories, São Paulo, SP, where they were processed. Methodologies used for *Salmonella* spp. isolation techniques, CPS counts and most probable number of total and thermotolerant coliforms were performed as described by Silva *et al.*⁽¹⁴⁾ For the microbial determinations, 25 ± 0.2 g of each samples was taken aseptically, diluted in 225 mL sterile buffered peptone water and homogenized for 2 min in a stomacher.

From each samples, three or four colonies showing typical *Salmonella* spp. morphology on MacConkey (Difco®) agar and *Salmonella-Shigella* (Difco®) agar were suspended in 20 µL of nuclease-free water and submitted to PCR technique with primers described by Cortez.⁽¹⁵⁾ For *S. Enteritidis*, *S. Typhimurium* and *S. Choleraesuis* characterization, PCR assays were performed according to Soumet *et al.*⁽¹⁶⁾ and Chiu *et al.*,⁽¹⁷⁾ respectively.

After confirmation of total and thermotolerant coliforms presence, colonies were isolated on EMB agar (eosin methylene blue). In order to detect virulence factors genes as *stx1*, *stx2*, *eae*, *bfpA*, *aggR*, *elt*, *esth*, *estp*, *invE* and *astA*, two to five colonies were suspended in 20 µL of nuclease-free water and submitted to PCR technique with primers described by Miyuki *et al.*⁽¹⁸⁾

For *Campylobacter* spp. detection, microbiological culturing techniques⁽¹³⁾ and PCR were used. DNA from sausage samples was extracted with the commercial reagent DNazol (Invitrogen®), as adapted by Chomczynski.⁽¹⁹⁾ Primers targeting

the *hip400F*, *HIP1134R*, *CC18F* and *CC519R* genes described by Linton *et al.*,⁽²⁰⁾ were used to detect the species *C. jejuni* and *C. coli*.

To compare the artisanal and inspected samples regarding the proportion of substandard samples, Fisher's exact test was calculated, with a significance level of 5%.⁽²¹⁾ The statistical software used in the analyses was BioEstat 5.03.

Results and Discussion

Although it is known that *Salmonella* spp. can be inactivated through thermal processing of food, the possibility of cross-contamination with other foods cannot be ruled out.⁽²²⁾ Among the 50 artisanal fresh pork sausages samples, 12% (6/50) were contaminated with *Salmonella* spp., out of which 6% (3/50) were identified through PCR as *S. Typhimurium*, 2% (1/50) as *S. Choleraesuis* and 4% (2/50) as *Salmonella* spp. None of the samples were positive for *S. Enteritidis*. Among the 50 inspected pork sausages samples, some positive results were also obtained, totaling 6% (3/50), out of which 2% (1/50) were identified as *S. Typhimurium* and the other 4% (2/50) were identified as *Salmonella* spp. None of the inspected samples were positive for *S. Enteritidis* or *S. Choleraesuis*. Results showed that the samples did not meet the standards established through the legislation, which recommends that *Salmonella* spp. should be absent in 25 grams of food.⁽²³⁾ Most *Salmonella* isolates were identified as belonging to the serovar Typhimurium, corroborating with the results reported by other authors such as Costa,⁽²⁴⁾ who observed a total positivity of 14.5% (20/138) for *Salmonella* spp. in meat products and that serovar Typhimurium was the predominant type in meat products, accounting for 45% (9/20).

Regarding *Salmonella* spp. detection, Silva *et al.*⁽²⁵⁾ reported 5/32 (17.86%) of positive *frescal linguiça* samples, Sabioni *et al.*⁽²⁶⁾ found an occurrence in 17,9% of fresh *linguiça* samples and Souza *et al.*⁽²⁷⁾ reported 30% of positivity in artisanal *linguiças* – they identified the following: *S. enterica* subs. *enterica*, *S. Derby*, *S. Agona*, *S. Enteritidis*

and *S. Typhimurium*. In Brazil, *S. Typhimurium*, Angona, Infantis and Enteritidis have been implied as the cause of foodborne infection in human.⁽²⁸⁾

In the coagulase-positive *Staphylococcus* count of artisanal fresh pork samples, 58% (29/50) presented counts higher than the limit regulated by the Resolution RDC n° 12⁽²²⁾ (reference value: up to 5×10^3 CFU/g), while none of the inspected fresh pork sausage samples presented counts above the permitted levels. The positivity rate of CPS among the artisanal samples analyzed was quite significant, since the presence of this microorganism in food can cause food poisoning and thus represents a risk to consumers' health.

Mata⁽²⁹⁾ reported 72.5% samples contaminated by coagulase-positive *Staphylococcus*, and 20% of them with counts above the legislation limits. These results were similar to 25% of *frescal linguiça* samples with high counts of ECP described by Marques *et al.*⁽³⁰⁾ Almeida Filho and Sigarini⁽³¹⁾ showed 60% of the samples contaminated with *S. aureus*, all above the legislation limits.

Morot-Bizot *et al.*⁽³²⁾ stated that the various species of *Staphylococcus* found in sausage samples show correlations with raw materials and the conditions of the production scheme. *Staphylococcus* spp. can colonize poorly sanitized surfaces of equipment, as well as the skin and mucosa of warm-blooded animals and humans,⁽³³⁾ which makes people who handle these foods an important source of contamination.

Most probable number of total and thermotolerant coliforms quantification revealed that out of the 50 samples of artisanal sausages analyzed, 76% (38/50) presented counts above the permitted levels, considering the established reference value⁽²³⁻¹⁴⁾ therefore the studied samples were considered not acceptable for consumption. Among the 50 inspected sausages samples, 24% (12/50) showed results above the acceptable limit for consumption. Santos *et al.*⁽³⁴⁾ found 8.9% (4/45) of their samples with thermotolerant coliform levels above the acceptable standard, among sausages subject to inspection by the municipal service in Goiás city. In the literature, some studies have also

presented very high rates both for the presence of thermotolerant coliforms and total coliforms and for the presence of *E. coli*. Merlini *et al.*⁽³⁵⁾ isolated this microorganism in 50% (20/40) of artisanal sausages samples analyzed. Lima *et al.*⁽³⁶⁾ found that 35% (7/20) of their samples were above the limit for total coliform count and 25% (5/20) were above the limit for thermotolerant coliforms.⁽²³⁾ Fecal coliforms and thermotolerants presence indicates an improper handling and no application of good manufacturing procedures, thus it may point a fecal contamination that is considered a risk for consumers health.⁽³⁷⁾

Another result from the present study was the detection of *eae* and *pfpA* genes from enteropathogenic *E. coli* (EPEC) in 2% (1/50) of the inspected products samples. The *astA* gene was detected in two samples from inspected sausages and in nine artisanal sausage samples, respectively (Table 1). To our knowledge, this is the first report with positive findings about the investigation of virulence factors for these bacteria in samples of pork sausages in Brazil, which has implications for one health control programs.

Regarding other *E. coli* serogroups, occurrences of Shigatoxigenic *E. coli* (STEC) in poultry and pigmeat products have been reported in studies conducted in other countries.⁽³⁸⁾ Lee *et al.*⁽³⁹⁾ detected STEC strains in 7.3% of chicken meat samples and in 2% of pork samples purchased from Korean retailers. Mayrhofer *et al.*⁽⁴⁰⁾ analyzed meat products purchased from Austrian retailers and observed that STEC prevalence in beef and pork were 5.2% and 1.7%, respectively. In Switzerland, Fantelli and Stephan⁽⁴¹⁾ analyzed 400 samples of beef and pork collected from butchers and detected STEC in 1.7% of these samples. They isolated five different serotypes, and none of them belonged to serotype O157:H7.

Campylobacter spp. was not detected in any samples by means of either microbiological analysis or PCR. In the literature, few studies have reported the presence of this microorganism in pork sausages. Costa⁽²⁴⁾ investigated several meat products for *Campylobacter* spp. and none pork

sausage sample was positive for this pathogen. Several studies on the presence of *Campylobacter* spp. in chickens and their derivatives have been conducted; however, in Brazil, we did not find any other studies on occurrences of *Campylobacter* spp. in pork sausages, or even in beef products.

During the processing stages for meat products, heat treatment, cooling and freezing can cause reduction of the counts of *Campylobacter* spp. in samples, or even its elimination,⁽⁴²⁾ which may explain the absence of this pathogen in the sausage samples studied.

Table 1 - Occurrence of *Salmonella* spp., *Campylobacter* spp., coagulase positive *Staphylococcus* and total and thermotolerant coliforms counts in fresh pork sausages in the state of São Paulo.

Bacteriological isolates / Methods	Artisanal fresh pork sausages	Inspected pork sausages	P - value
<i>Salmonella</i> spp. / Isolation	6/50 (12%)	3/50 (6%)	
<i>S.</i> spp. / PCR	2/50 (4%)	2/50 (4%)	
<i>S. Thyphimurium</i> / PCR	3/50 (6%)	1/50 (2%)	
<i>S. Choleraesuis</i> / PCR	1/50 (6%)	0	
<i>S. Enteritidis</i> / PCR	0	0	
Coagulase positive <i>Staphylococcus</i> count	29/50 (58%)	0	<0.0001
Most probable number of total and thermotolerant coliforms	38/50 (76%)	12/50 (24%)	<0.0001
<i>eae</i> and <i>pfpA</i> genes / PCR	0	1/50 (2%)	
<i>Campylobacter</i> spp. / Isolation	0	0	
<i>Campylobacter</i> spp. / PCR	0	0	

Source: the authors.

There was a significant difference between the artisanal and inspected samples, regarding the proportions of positive results for *Staphylococcus* coagulase counts and most likely numbers of total and thermotolerant coliforms (p - value: <0.0001). Regarding the proportions of positive results for *Salmonella* spp., there was no significant difference between the artisanal and inspected samples.

Based on the results from the present study, it can be said that the hygiene conditions relating to handlers, contamination from utensils and temperature on the point-of-sale counter for the products may have allowed multiplication of pathogens. Both artisanal and inspected sausage samples, presented high contamination levels that turned these products into health risks for consumers. Processed meat production involves a couple manipulation steps, allowing contamination by diverse microorganisms, compromising the microbiological quality of the final product.

Conclusion

The results obtained from the samples of artisanal and inspected sausages showed that their levels of contamination by bacterial pathogens were outside of the acceptable standards under the current legislation. Although both artisanal and inspected products presented contamination levels above legislation limits, we can affirm that the artisanal ones may represent a higher risk to public health according to the statistically significant difference (CPS and MPN) between the groups of the present study.

Acknowledgements

Funding: this study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), under finance code 001.

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Received on: Jan. 18, 2023

Accepted on: Apr. 8, 2023