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Microbiological Quality Assessment of Sashimi and Sushi Sold in the Federal District, Brazil

Karolina Oliveira Gomes, Flávia Bianca Amaral Alves, Ana Carolina Almeida de Oliveira Ferreira, Calliandra Maria de Souza Silva, Izabel Cristina Rodrigues da Silva, Daniela Castilho Orsi*

University of Brasilia (UnB/FCE), Laboratory of Food Control, Centro Metropolitano, Conjunto A, lote 01, Ceilandia, CEP: 72220-900, Brasilia, DF, Brazil
*Corresponding author: danielacastilhoorsi@gmail.com

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Abstract This study aimed to evaluate the microbiological quality of sashimi and sushi sold in the Federal District, Brazil. A total of 54 samples (27 sashimi and 27 sushi) were collected in 25 commercial establishments. Samples were tested for counts of mesophilic and psychrotrophic bacteria, determination of total and thermotolerant coliforms, *Staphylococcus aureus* counts, and presence of *Salmonella*. The results showed that 40.7% of sashimi (11/27) and 25.9% of sushi (7/27) samples were unfit for consumption according to Brazilian legislation. *Salmonella* (genetically confirmed by the invA gene's presence) was present in 25.9% of the sashimi samples (7/27). Thermotolerant coliforms were positive in 92.5% of sashimi samples (25/27) and 74.0% of sushi samples (20/27), and one sashimi sample was unfit for consumption ($\geq$ 2.0 log MPN g^{-1}). *S. aureus* bacteria exceeded the acceptable limit of 3.0 log CFU g^{-1} in 14.9% of sashimi samples (4/27) and 25.9% of sushi samples (7/27). A high count of *S. aureus* bacteria in some sushi and sashimi samples indicates improper personal hygiene while processing these ready-to-eat foods. *Salmonella* spp. can occur by improper handling of these foods or by the contact of fish with waters contaminated with sewage. These results can pose a health risk to consumers and indicated the need to improve good hygienic practices in establishments selling sashimi and sushi in Federal District, Brazil.

Keywords: *foodborne pathogens, food hygiene, Salmonella, S. aureus, ready-to-eat foods, sashimi, sushi*

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1. Introduction

In recent years, especially in large Brazilian cities, a change in the population dietary profile as to include the habit of consuming oriental dishes containing raw fish, such as sushi and sashimi, has become increasingly common. This change is probably due to the increased search for a healthy diet which tends to favor the consumption of Japanese food for it is colorful and careful preparations, with a wide variety of vegetables and little to no cooking, which helps to preserve the foods nutritional value [1,2,3].

The two best-known foods of Japanese cuisine are sushi and sashimi. Sushi usually is made from vinegar-acid cooked Japanese rice containing topping or stuffing of raw fish and vegetables. The most common type of sushi presentation is a hand-shaped rice ball, wrapped externally with nori algae and containing fish, vegetables, fruits, or eggs as a filling [4,5,6]. The word sashimi means "sliced meat." Sashimi consists of fresh, raw, thinly sliced fish or seafood [6,7].

Considering the rise in sushi and sashimi consumption, there has been increased concern about the microbiological hazards inherent in these ready-to-eat foods in foodborne disease transmission [2,6,7,8]. This concern with these foods' safety is prominent as they are prepared by manual handling and consumed raw, without thermal processing, requiring adequate hygienic-sanitary conditions for their preparation [3,5,9,10].

The high-quality raw material is essential for these raw fish-based foods' safety for the fish's microbiota reflects the water where it lives. Disposal of untreated sewage in rivers, seas, and lakes contaminate fish with pathogenic bacteria such as *Salmonella* and *E. coli*. Another worrying factor is the use of raw vegetables in sushi fillings. These vegetables increase the chances of contamination, and lack of hygiene or handling errors can carry pathogenic microorganisms [5,7,9,10]. Therefore, this study aimed to investigate the microbiological quality of sushi and sashimi sold in the Federal District, Brazil.

2. Materials and Methods

2.1. Samples, Microbiological and Molecular Analyses

A total of 54 samples (27 samples of sashimi and 27 samples of sushi) were collected in 25 different commercial establishments (restaurants, supermarkets, and

bakeries) in the Federal District between 2018 and 2019. The sashimi samples collected in the restaurants were prepared with salmon, tuna, or tilapia, and the sashimi samples collected in supermarkets and bakeries were prepared with salmon. Sushi samples were made with rice and nori seaweed and various fillings such as salmon, tuna or Kani-kama, cream cheese, cucumber, carrot, sesame, or poppy seeds. Samples were kept in their original packaging and transported in 1 h to the laboratory in portable coolers, and microbiological analysis was performed within 2 h.

Samples were analyzed for the following bacteriological determinations: total counts of mesophilic and psychrotrophic bacteria, total and thermotolerant coliforms, *Salmonella* spp. and *Staphylococcus aureus*. All samples were analyzed in triplicate. An amount of 25 g from each sample was diluted in 225 mL of 0.1% peptone water. Samples were homogenized, and an initial 10^{-1} dilution was obtained. Then, the homogenates' serial dilutions were prepared in 0.1% peptone water (up to 10^{-5}).

For total mesophilic and psychrotrophic bacteria counts, serial dilutions of the samples were surface plated in Plate Count Agar (PCA) (HiMedia, USA), following incubation at 37°C for 24 h for mesophilic bacteria and 8-10°C for 7 days for psychrotrophic bacteria. The results were expressed by colony-forming unit per gram (CFU g^{-1}). For total and thermotolerant coliforms detection, one mL of each dilution was transferred to three-tube series containing Lauryl Sulfate Tryptose (LST) (HiMedia, USA) with Durhan tubes in its interior. Total coliforms were enumerated in Brilliant Green Bile Broth 2% (HiMedia, USA), incubated at 37°C for 24 h, and thermotolerant coliforms were determined in *E. coli* broth (EC) (Acumedia, USA) incubated at 45°C for 24 h. The results were expressed by the most probable number per gram (MPN g^{-1}).

For total *Staphylococcus aureus* counts, the samples' serial dilutions were surface plated in Mannitol Salt Agar (HiMedia, USA), following incubation at 37°C for 48 h. The colonies were counted and sub-cultured in Mannitol Salt Agar tubes. The characteristic colonies of *Staphylococcus aureus* (yellow colonies with yellow zones, mannitol-fermenting) were stained by Gram's Method to confirm Gram-positive cocci. The *S. aureus* colonies were further confirmed through molecular analyses.

For the detection of *Salmonella*, the samples were inoculated in 0.1% peptone water (w/v) at 37°C for 24 h, and aliquots of this broth were transferred to the selective broth tetrathionate and iodine solution (Acumedia, USA), following incubation at 37°C for 24 h. From the selective broth, the strains of *Salmonella* were isolated in the differential media of Salmonella-Shigella Agar (SS) (HiMedia, USA) and Xylose Lysine Deoxycholate Agar (XLD) (HiMedia, USA). The plates were incubated at 37°C for 24 h in order to isolate characteristic colonies of *Salmonella* spp. Triple sugar iron (TSI) (HiMedia, USA), Lysine Iron Agar (LIA) (HiMedia, USA), and Phenylalanine Agar (HiMedia, USA) were used for presumptive confirmation of colonies that were further confirmed through molecular analyses.

The bacteria *S. aureus* and *Salmonella* were identified using the technique of polymerase chain reaction (PCR). Table 1 presents the primers specific for the termonuclease Nuc gene of *S. aureus* and the primers specific for the invasion A (InvA) gene of *Salmonella*. For DNA extraction, the NucleoSpin Food® kit (Macherey-Nagel, Düren, Germany) was used, following the manufacturer's instructions. Extracted DNA was stored at -20°C. PCR was performed in a reaction mixture of 25 µl final volume containing 2.5 µl of PCR buffer; 0.7 µl of $MgCl_2$; 1.5 µL of dNTP (2,5 mM); 0.5 µl of Taq DNA polymerase; 1.5 µL of each primer forward and reverse and 18.3 µl of Milli-Q water.

PCR amplification was performed with an initial denaturing step at 95°C for 1 min, followed by a 35-cycle reaction (95°C for 1 min and 60°C for 1 min). A final extension step was undertaken at 72°C for 1 min. All thermal cycling reactions were performed with Techne TC-512 thermal cycler (Bibby Scientific Inc., USA). Both negative and reagent controls were included in each PCR run. The reagent control consisted of all PCR components except for the template DNA. The amplified DNA was separated by electrophoresis at 100 V for 50 min in 1.5% (w/v) agarose gel and stained with ethidium bromide. Gels were visualized under UV light. A 100 bp DNA ladder was used as a molecular weight marker.

Table 1. Primer sequence used to identify Nuc and invA genes

Primer	Sequence 5´- 3´	Amplified product	Bacteria
Nuc foward	TGTTTGTGATGCATTTGCTG	105 bp	*S. aureus*
Nuc reverse	AAAGGGCAATACGCAAAGAG		
invA foward	GCTGATGCCGGTGAAATTAT	445 bp	*Salmonella* spp.
invA reverse	CGACAAGACCATCACCAATG		

Table 2. Microbiological limits for ready-to-eat food such as sashimi and sushi

Parameters (log CFU g^{-1} or log MPN g^{-1})	Acceptable or Satisfactory	Borderline or Unsatisfactory	Potentially Hazardous
Total Bacteria[a]	< 7.0	> 7.0	-
Total coliforms[b]	< 3.0	> 3.0	-
Thermotolerant coliforms[c]	< 1.0	> 1.0 - < 2.0	> 2.0
S. aureus[c]	< 2.0	> 2.0 - < 3.0	> 3.0
Salmonella[c]	Presence in 25 g	-	Absence in 25 g

[a] Mesophilic and Psychrotrophic Bacteria values recommended by ICMSF [13]; [b] values retrieved from Santos et al. [11] and INSA [12]; [c] values retrieved from the Brazilian legislation [14]

2.2. Classification of Microbiological Quality Levels

Samples, according to Santos et al. [11] and INSA [12], were classified into three microbiological quality levels: Acceptable or Satisfactory - results indicate a good microbiological quality; Borderline or Unsatisfactory - results indicate that the product does not meet one or more of the established values; Potentially Hazardous or Unacceptable - results indicate the presence of pathogenic microorganisms and toxins that may pose a health risk. The Brazilian legislation (14) defines the microbiological potentially hazardous limits for ready-to-eat foods, such as sushi and sashimi, as displayed in Table 2.

3. Results and Discussion

High counts of mesophilic and psychrotrophic bacteria (>7 log CFU g^{-1}) may indicate inappropriate contamination of raw material or contamination during food preparation as well as the appropriateness of storage conditions [5]. In sashimi samples, mesophilic bacteria counts ranged from 2.39 to 6.71, and psychrotrophic bacteria counts ranged from 3.13 a 7.70 log CFU g^{-1} (Table 3).

The highest percentage of sashimi samples for mesophilic bacteria counts (33.3%) were from 3 to 4 log CFU g^{-1} and for psychrotrophic bacteria counts (44.4%) were from 5 to 6 log CFU g^{-1} (Table 3). One sample of sashimi (3.7%) presented a psychrotrophic bacteria count of 7.70 log CFU g^{-1} and was considered unsatisfactory according to the microbiological limits stated in Table 2.

In sushi samples, mesophilic bacteria counts ranged from 2.10 to 5.75, and psychrotrophic bacteria counts ranged from 2.76 a 6.25 log CFU g^{-1}. The highest percentage of sushi samples for mesophilic bacteria counts (40.7%) were from 3 to 4 log CFU g^{-1} and for psychrotrophic bacteria counts (40.7%) were from 4 to 5 log CFU g^{-1} (Table 4). Sushi samples showed lower mesophilic and psychrotrophic bacteria counts compared to sashimi samples. This lower microbial load is probably due to the lower pH in acidified rice that may constitute a not suitable substrate for microbiological growth [15].

Total coliforms are a known hygiene indicator used to evaluate if acceptable hygiene practices are being implemented in the food production chain and as evidence of improper environment [7]. The total coliforms showed a range from 0.48 to 3.04 log CFU g^{-1} in sashimi samples and ND to 2.85 log CFU g^{-1} in sushi samples (Table 3). Five sashimi samples (18.5%) presented enumeration of total coliforms above the recommended limit (>3.0 log MPN g^{-1}) and were considered unsatisfactory, and all sushi samples presented enumeration of total coliforms within acceptable limits (< 3.0 log MPN g^{-1}) (Table 4).

Table 3. Microbiological analysis of sashimi and sushi samples

[a] Count interval	Mesophilic Bacteria n (%)	Psychrotrophic bacteria n (%)	Total coliforms n (%)	Thermotolerant coliforms n (%)	*S. aureus* n (%)
Sashimi samples					
ND	-	-	-	2 (7.4)	11 (40.7)
1-2	-	-	16 (59.3)	24 (88.9)	2 (7.4)
2-3	1 (3.7)	-	6 (22.2)	-	10 (37.0)
3-4	9 (33.3)	-	5 (18.5)	1 (3.7)	4 (14.9)
4-5	7 (25.9)	12 (44.4)	-	-	-
5-6	7 (25.9)	8 (29.6)	-	-	-
6-7	3 (11.2)	6 (22.2)	-	-	-
7-8	-	1 (3.7)	-	-	-
Sushi samples					
ND	-	-	6 (22.2)	11 (40.7)	11 (40.7)
1-2	-	-	16 (59.3)	16 (59.3)	-
2-3	5 (18.5)	3 (11.1)	5 (18.5)	-	9 (33.4)
3-4	11 (40.7)	8 (29.6)	-	-	7 (25.9)
4-5	5 (18.5)	11 (40.7)	-	-	-
5-6	6 (22.2)	5 (18.5)	-	-	-

[a] Count interval expressed in log CFU g^{-1} or log MPN g^{-1}; n (%) = number and percentage of positive samples; ND = not detected.

Table 4. Microbiological quality levels of sashimi and sushi samples

Microbiological Parameter	Sashimi samples (n = 27)			Sushi samples (n = 27)		
	Acceptable n (%)	Borderline n (%)	Potentially Hazardous n (%)	Acceptable n (%)	Borderline n (%)	Potentially Hazardous n (%)
MB	27 (100.0)	-	-	27 (100.0)	-	-
PB	26 (96.3)	1 (3.7)	-	27 (100.0)	-	-
TC	22 (81.5)	5 (18.5)	-	27 (100.0)	-	-
TT	19 (70.4)	7 (25.9)	1 (3.7)	20 (74.1)	7 (25.9)	-
S. aureus	13 (48.1)	10 (37.0)	4 (14.9)	11 (40.8)	9 (33.4)	7 (25.9)
Salmonella	20 (74.1)	-	7 (25.9)	27 (100.0)	-	-

n (%) = number and percentage of samples; MB = Mesophilic Bacteria; PB = Psychrotrophic bacteria; TC = Total Coliforms; TT = Thermotolerant Coliforms.

According to Miguéis et al. [10], the high number of *Enterobacteriaceae* in sashimi samples collected from restaurants in Portugal could be explained by cross-contamination of surfaces or utensils that have been in contact with vegetables, in which these microorganisms are commensal, and used in the preparation of sushi or other types of foods, indicating cross-contamination in the preparation of sashimi.

Thermotolerant coliforms indicate direct or indirect fecal contamination and expose the possible presence of enteropathogens [5]. The sashimi samples (92.6%) were more contaminated with thermotolerant coliforms than the sushi samples (59.3%). One sashimi sample (3.7%) presented a thermotolerant coliform enumeration of 3.0 log MPN g^{-1} and was unfit for consumption because it exceeded the acceptable limit (maximum value of 2 log MPN g^{-1}) (Table 3). Most sushi (74.1%) and sashimi (70.4%) samples were classified as acceptable with thermotolerant coliforms enumeration less than 1.0 log MPN g^{-1}. Although, 25.9% of sashimi and sushi samples were classified as borderline with thermotolerant coliforms counts between 1.0 and 2.0 log MPN g^{-1} (Table 4).

Liang et al. [5], in a Hong Kong study, reported similar results. Among the 120 sushi samples analyzed, 97.5% were classified as acceptable, 0.8% borderline, and 1.7% were considered potentially hazardous with *E. coli* content higher than 2 log CFU g^{-1}. Studies done in different Brazilian cities (Ji-Paraná, Porto Alegre, and Aracaju) reported high counts of thermotolerant coliforms in sashimi or sushi samples, with 25 to 50% of the samples unfit for consumption as their thermotolerant coliforms counts were higher than 2.0 log MPN g^{-1} [16,17,18].

S. aureus is part of the human microbiota; therefore, its presence in sushi/sashimi indicates poor personal hygiene, as these products are manually handled, the *S. aureus* found probably originated from the workers' hands and were transferred to these cuisines [19]. In this study, *S. aureus* bacteria were detected in 16 sashimi samples (59.3%) and 16 sushi samples (59.3%). Additionally, 7 sushi samples (25.9%) and 4 sashimi samples (14.9%) exceeded the acceptable limit for *S. aureus* of 3.0 log CFU g^{-1} and were classified as potentially hazardous (Table 3). Finally, 37.0% of sashimi samples and 33.4% of sushi samples were classified as borderline with *S. aureus* counts between 2.0 and 3.0 log CFU g^{-1} and 48.1% of sashimi samples and 40.8% of sushi samples were classified as acceptable with *S. aureus* counts less than 2.0 log CFU g^{-1} (Table 4).

Miguéis et al. [10] reported similar microbiological quality results analyzing 114 sashimi samples collected from 23 restaurants in Portugal. The most prevalent pathogen detected by the study was *S. aureus*, found in 40 samples (35.1%), and of which 6 samples (5.3%) were classified as potentially hazardous. Muscolino et al. [6] carried out a study on 38 samples of sushi collected from restaurants and take-away outlets from Messina and Catania, Southern Italy, and noted that 16 of the samples (42.1%) presented positive results for *S. aureus* with values from 2.0 to 3.6 log CFU g^{-1}.

Strains of *S. aureus* isolated from the sashimi and sushi samples were confirmed genetically through the amplification of the Nuc gene's amplification. The Nuc gene encodes a termonuclease unique to the bacterium *S. aureus* and vital for its pathogenesis [20,21].

A disquieting result from this study was the *Salmonella* isolation in 25.9% of the sashimi samples (7/27) analyzed, which automatically classified them as potentially hazardous. Conversely, none of the sushi samples (0/27) were positive for *Salmonella* bacteria (Table 4). *Salmonella* spp. is not part of the natural microbiota of fish and is responsible for many foodborne infection outbreaks, and its presence in sashimi samples can be explained by contamination of a water source and poor hygiene during the capture, handling, and transportation of fish. The feeding practice of fish with inappropriate and contaminated feed can also contribute to its prevalence in aquaculture fish [22,23].

Some studies in Brazil have reported *Salmonella* in samples of sashimi and sushi. Braghini et al. [2] analyzed 15 samples of sashimi collected from five restaurants in Maringá city and revealed that 3 samples (20%) had positive results for *Salmonella*. Malavota et al. [24] detected *Salmonella* in 12.5% of the sashimi samples (8/64) collected from 2 restaurants in Rio de Janeiro. Furthermore, Souza et al. [8] studied the microbiological quality of 15 sushi samples collected from 5 restaurants in João Pessoa and reported *Salmonella* in 2 samples (13.3%).

In these sashimi samples, *Salmonella* strains were confirmed genetically through the presence of the invA gene. Amplification of the invA gene has been recognized as an international standard for detecting *Salmonella* genus. The invA gene contains sequences unique to the *Salmonella* species. This gene encodes a protein in the inner invasion of the host's epithelial cells [25].

Table 5 shows that, of the 54 samples of sashimi and sushi analyzed in this study, 35.2% of samples (19/54) were acceptable for consumption, 31.5% (17/54) were borderline yet still acceptable for consumption, and 33.3% (18/54) were classified as potentially hazardous.

Table 5. Overall microbiological quality of sashimi and sushi samples

Samples	Acceptable n (%)	Borderline n (%)	Potentially Hazardous n (%)
Sashimi Total samples (n = 27)	8 (29.6)	8 (29.6)	11 (40.7)
Sushi Total samples (n = 27)	11 (40.7)	9 (33.3)	7 (25.9)
Total samples (n = 54)	19 (35.2)	17 (31.5)	18 (33.3)

n (%) = number and percentage of samples.

For the sashimi samples, 11 of the 27 samples (40.7%) were classified as potentially hazardous due to 3 samples collected in restaurants exceeded the acceptable limit for *S. aureus*, 1 sample collected in restaurant exceeded the acceptable limit for thermotolerant coliforms, and *Salmonella* was present in 7 sashimi samples collected in supermarkets and bakeries. Any of the sashimi samples collected in restaurants presented *Salmonella*. Miguéis et al. [10] revealed a difference in the quality of sashimi samples collected from typical Japanese restaurants compared with non-typical restaurants in Portugal. According to their study, 6 of the samples (5.3%) analyzed were classified as potentially hazardous, and most of the cases were from non-typical restaurants (83.33%).

For sushi samples, 40.7% of samples (11/27) were acceptable for consumption, 33.3% of samples (9/27) were borderline, and 7 of the 27 samples (25.9%) were classified as potentially hazardous because exceeded the acceptable limit for *S. aureus* (5 samples sold in restaurants and 2 samples sold in supermarkets and bakeries). Liang et al. [5] reported better microbiological quality results for sushi sold in Hong Kong. Among the 120 samples analyzed, 63.3% were acceptable for consumption, 35.0% were borderline, and only 1.7% (one sample) was classified as potentially hazardous for containing more than 2 log CFU g^{-1} of *E. coli*.

4. Conclusions

Although most of the sushi and sashimi samples sold in the Federal District were acceptable or borderline for consumption (66.7%), the results revealed that 33.3% of the samples were potentially hazardous. The occurrence of high counts of *S. aureus* indicates improper personal hygiene during food processing as it is part of the human microbiota. *Salmonella* spp., on the other hand, is not part of the natural microbiota of fish nor humans, and its presence in sashimi samples might be justified by improper handling during the production or by the fish's contact with fecal-contaminated waters. These results indicated the need to improve the reliability of quality-control practices in the preparation of sashimi. Sashimi and sushi preparation requires an exceptionally high standard of hygiene, and the quality of its raw materials must also meet these high standards. Consumers should be aware of the risks associated with these fresh ready-to-eat foods, which must be consumed immediately or kept adequately refrigerated until consumption.

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Conflicts of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, or publication of this article.

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