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Microbiological study of vinaigrette salad sold at pasty stalls in street markets in the City of São Paulo, Brazil

Estudo microbiológico de vinagretes comercializados em barracas de pastéis em feiras livres na Cidade de São Paulo, Brasil

Estudio microbiológico de vinagretas vendidas en puestos de pastelería en mercados abiertos de la Ciudad de São Paulo, Brasil

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Abstract

Foodborne diseases (FBD) present high prevalence worldwide and more than 250 types have been reported. The main agents are *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* and certain parasites. The habit of consuming foods sold in street markets in the city of São Paulo exposes consumers to the risk of acquiring FBD. 25 samples of vinaigrette salad were collected in the central, northern, southern, eastern and western regions of the city of São Paulo, Brazil. The samples were inoculated in selective, enriched culture media and biochemical analyses were performed. Bacterial and fungal growth occurred in all samples. The microorganisms detected were *E. coli* (64%), *Enterobacter* (60%), *S. aureus* (52%), *Klebsiella* (40%), *Proteus sp*. (32%), *Shigella sp*. (28%), *Citrobacter sp*. (16%), *Edwardsiella sp*. (12%), *Alcaligenes sp.* (8%), *Serratia sp.* (8%), *Salmonella sp*. (4%), *Pseudomonas sp*. (4%) and; 72% were positive for aflatoxins. Twelve species were identified, 50% of which can cause FBD. *S. aureus* and fecal coliforms can cause FBD, while *Alcaligenes sp.* can cause nosocomial infections and *Edwardsiella sp*. can cause hepatic abscess, meningitis and septicemia. Aflatoxins may cause mycotoxicoses and liver cancer. Therefore, inspections, and prevention and awareness measures should be reinforced to minimize the risks of contracting FBD from foods sold in street markets in the city of São Paulo.

Keywords: Foodborne disease; Food microbiology; Salad; *Escherichia coli*; Aflatoxins; *Staphylococcus aureus*.

Resumo

As doenças transmitidas por alimentos (DTA) apresentam alta prevalência em todo o mundo e mais de 250 tipos foram relatadas. Os principais agentes são *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* e alguns parasitas. O hábito de consumir alimentos vendidos em feiras livres da cidade de São Paulo expõe o consumidor ao risco de adquirir o DTA. 25 amostras de salada de vinagrete foram coletadas nas regiões centro, norte, sul, leste e oeste da cidade de São Paulo, Brasil. As amostras foram inoculadas em meios de cultura enriquecidos seletivos e análises bioquímicas foram realizadas. O crescimento bacteriano e fúngico ocorreu em todas as amostras. Os microrganismos detectados foram *E. coli* (64%), *Enterobacter* (60%), *S. aureus* (52%), *Klebsiella* (40%), *Proteus sp*. (32%), *Shigella sp.* (28%), *Citrobacter sp*. (16%), *Edwardsiella sp.* (12%), *Alcaligenes sp*. (8%), *Serratia sp*. (8%), *Salmonella sp*. (4%), *Pseudomonas sp.* (4%) e 72% foram positivos para aflatoxinas. Doze espécies foram identificadas, 50% das quais podem causar DTA. *S. aureus* e coliformes fecais podem causar DTA, enquanto *Alcaligenes sp.* podem causar infecções nosocomiais e *Edwardsiella sp.* pode causar abscesso hepático, meningite e septicemia. As aflatoxinas podem causar micotoxicoses e câncer de fígado. Portanto, inspeções e medidas de prevenção e conscientização devem ser reforçadas para minimizar os riscos de contrair DTA em alimentos comercializados em feiras livres da cidade de São Paulo.

Palavras-chave: Doenças transmitidas por alimentos, Microbiologia de Alimentos; Salada; *Escherichia coli*; Aflatoxinas; *Staphylococcus aureus*.

Resumen

Las enfermedades transmitidas por alimentos (ETA) tienen una alta prevalencia en todo el mundo y se han reportado más de 250 tipos. Los principales agentes son *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* y algunos parásitos. El hábito de consumir alimentos vendidos en mercados abiertos de la ciudad de São Paulo expone a los consumidores al riesgo de adquirir ETA. Se recolectaron 25 muestras de ensalada de vinagreta en las regiones central, norte, sur, este y oeste de São Paulo, Brasil. Las muestras se inocularon en medios de cultivo enriquecidos selectivos y se realizaron análisis bioquímicos. Se produjo crecimiento de bacterias y hongos en todas las muestras. Los microorganismos detectados fueron *E. coli* (64%), *Enterobacter* (60%), *S. aureus* (52%), *Klebsiella* (40%), *Proteus sp.* (32%), *Shigella sp.* (28%), *Citrobacter sp*. (16%), *Edwardsiella sp*. (12%), *Alcaligenes sp*. (8%), *Serratia sp.* (8%), *Salmonella sp.* (4%), *Pseudomonas sp.* (4%) y 72% fueron positivos para aflatoxinas. Se identificaron doce especies, el 50% de las cuales pueden causar ETA. *S. aureus* y coliformes fecales pueden causar ETA, mientras que *Alcaligenes sp.* puede causar infecciones nosocomiales y *Edwardsiella sp.* puede causar absceso hepático, meningitis y septicemia. Las aflatoxinas pueden causar micotoxicosis y cáncer de hígado. Por lo tanto, se deben reforzar las inspecciones y las medidas de prevención y sensibilización para minimizar los riesgos de contratar ETA en los alimentos vendidos en los mercados abiertos de la ciudad de São Paulo.

Palabras clave: Enfermedades transmitidas por los alimentos; Microbiología de los alimentos; Ensalada; *Escherichia coli*; Aflatoxinas; *Staphylococcus aureus*.

1. Introduction

Foodborne Diseases (FBD) is caused by the ingestion of water or food contaminated with microorganisms and/or their toxins. There are over 250 types of FBDs, and the main causative agents include *Staphylococcus aureus*, *Escherichia coli*, *Salmonella sp.* and *Shigella sp*. Vegetables, fruits, milk and dairy products, eggs and meat are the vehicles most involved in outbreaks (Secretaria de Vigilância em Saúde, 2018). The symptoms of gastrointestinal FBD are colic, diarrhea, vomiting and fever, which may vary in intensity and characteristics according to the etiological agent, microbial load and health status of the individual affected (Secretaria de Vigilância em Saúde, 2018; Herrera et al., 2006; Riddle et al., 2016; Silva et al., 2008). Is outbreak considered when two or more people show events, signs and symptoms similar after ingesting food or water from the same source within the same time frame, or when cases of serious diseases occur, such as cholera and botulism (Secretaria de Vigilância em Saúde, 2018). The investigation is conducted to identify the etiological agent through laboratory, bromatological and clinical exams and/or epidemiological norms (Secretaria de Vigilância em Saúde, 2018; Ganho et al., 2011).

The consumption of food purchased in street markets is a common practice in the city of São Paulo, especially a type of deep-fried pasty (a pastel) that is served with a salad made of onion, tomato, pepper and cabbage with a vinaigrette dressing, known as vinaigrette salad. It is packaged in a disposable plastic bag or cup (Silva et al., 2008; Ganho et al., 2011). It is a fresh food that remains exposed on stalls where it is handled with minimal criteria concerning the prevention of contamination. The

presence of pathogenic microorganisms in this food may contribute to an increase in the number of outbreaks and the development of FBD. Thus, the purpose of this research was to investigate the presence of pathogenic microorganisms in vinaigrette salad offered as an accompaniment to the pasties sold in street markets in the city of São Paulo (Silva et al., 2008).

2. Methodology

***Sample**s*

Twenty-five samples of vinaigrette salad sold in street markets in São Paulo were collected, six from the central region, five from the northern region, four from the southern region, six from the eastern region and four from the western region, Table 1.

Table 1. Vinaigrette salad Collection Areas in São Paulo.

Region	Address	Sample
Central (1)	Charles Miller Square, Pacaembu	1.1, 1.2, 1.3, 1.4
Central (1)	Street Antônio Coruja, Bom Retiro	1.5
Central (1)	Street Herculano de Freitas, Bela Vista	1.6
Northern (2)	Street Dr. Gabriel Piza, Santana	2.7, 2.8, 2.9, 2.10
Northern (2)	Street Manuel Gaya, Vila Mazzei	2.11
Southern (3)	Street Eça de Queiroz, Vila Mariana	3.12, 3.13, 3.14, 3.15
Eastern (4)	Street Pires de Campos, Mooca	4.16, 4.17, 4.18, 4.19
Eastern (4)	Street Pitagoras, Jardim Coimbra	4.20
Eastern (4)	Street Sebastião Barbosa, Tatuapé	4.21
Western (5)	Street Cayowa, Perdizes	5.22, 5.23
Western (5)	Street Tavares Bastos, Pompéia	5.24, 5.25

Source: Own authorship.

Culture mediums

The culture media used were blood agar, MacConkey agar, cystine lactose electrolyte-deficient (CLED) agar, *Salmonella-Shigella* (SS) agar and Sabouraud agar (Microbac®, Jacarepaguá, RJ, Brazil), each chosen for their selective and differential characteristics. Each culture medium was prepared according to the manufacturer's recommendations. Following inoculation of the medium, the plates were incubated at 35±2°C for 18-24h (Blood, MacConkey, CLED and SS agars) or at 25°C for seven days (Sabouraud agar).

Enzymatic and biochemical tests

The following biochemical tests were performed: the Rugai method (Cecon® Vila Sonia, SP, Brazil) and the mannitol, DNAse and TSI tests (Microbac®, Jacarepaguá, RJ, Brazil), each according to the manufacturer's recommendations.

Aflatoxin test

A fluorescence detector was used in qualitative assays to verify the presence of aflatoxins. Sabouraud culture medium plates containing aflatoxins were exposed to a 366 nm ultraviolet lamp, such that aflatoxin molecules absorb the photons and emit a blue light that indicates the presence of aflatoxins B and green light when detect aflatoxins G (Rasooly et al., 2016).

3. Results

All the samples inoculated on blood, MacConkey CLED, SS and Sabouraud media (n=25) showed colonies of bacterial growth, except one (1.5), which showed no bacterial growth on MacConkey and SS media.

Colonies that grew on blood agar and presented characteristics compatible with those of the genus *Staphylococcus* were inoculated on mannitol and DNAse media to identify and confirm the presence of *S. aureus*. Colonies grown on MacConkey, CLED and SS media were inoculated on Rugai medium to identify Gram negative bacteria. The following bacteria were identified: *S. aureus*, *E. coli*, *Klebsiella*, *Enterobacter*, *Shigella*, *Proteus*, *Citrobacter*, *Edwardsiella sp.*, *Alcaligenes*, *Serratia*, *Pseudomonas* and *Salmonella*, Table 2.

Table 2. Bacterial identification in biochemical test samples.

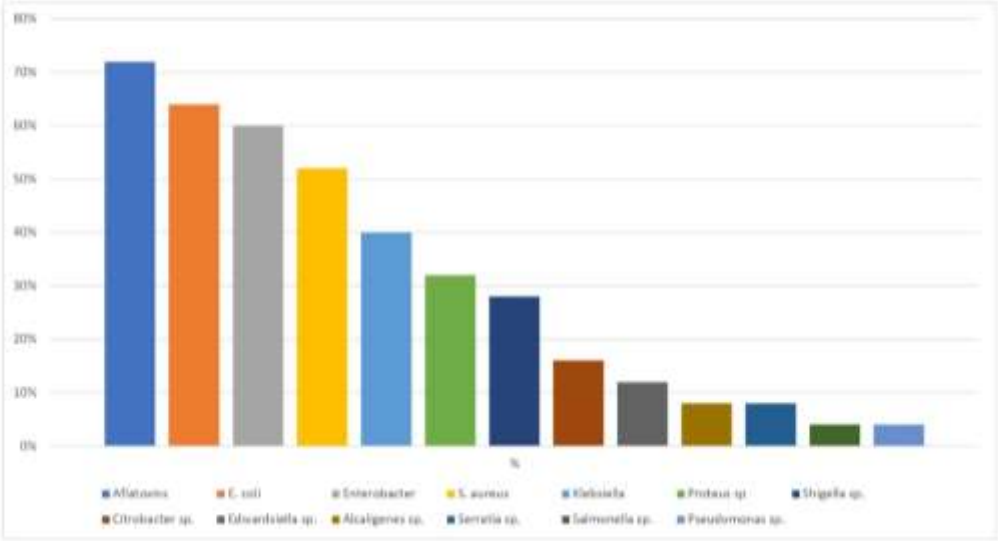
Sample	Microorganisms
1.1	*E. coli, Pseudomonas sp., Citrobacter, Enterobacter, Klebsiella*
1.2	*S. aureus, E. coli, Enterobacter, Salmonella sp., Proteus sp.*
1.3	*S. aureus, E. coli, Enterobacter, Klebsiella*
1.4	*S. aureus, Klebsiella, Enterobacter*
1.5	*S. aureus*
1.6	*S. aureus, Enterobacter, Klebsiella, Shigella, Alcaligenes sp.*
2.7	*S. aureus, Edwardsiella sp., Proteus*
2.8	*S. aureus, E. coli, Enterobacter*
2.9	*S. aureus, E. coli, Klebsiella*
2.10	*E. coli, Klebsiella, Shigella, Proteus*
2.11	*E. coli, Klebsiella, Alcaligenes sp.*
3.12	*E. coli, Edwardsiella sp., Proteus*
3.13	*E. coli, S. aureus*
3.14	*E. coli, Citrobacter*
3.15	*E. coli, Enterobacter, Shigella*
4.16	*E. coli, Enterobacter*
4.17	*E. coli, Enterobacter*
4.18	*S. aureus, E. coli, Enterobacter*
4.19	*E. coli, Klebsiella, Shigella*
4.20	*Klebsiella, Enterobacter, Shigella, Proteus, Citrobacter*
4.21	*S. aureus, Enterobacter*
5.22	*Proteus, Serratia*
5.23	*S. aureus, Klebsiella, Enterobacter, Shigella, Proteus*
5.24	*E. coli, Edwardsiella sp, Serratia sp.*
5.25	*S. aureus, Klebsiella, Enterobacter, Shigella, Proteus, Citrobacter*

Source: Own authorship.

All the Sabouraud medium plates (n=25) showed fungal growth. Of these, the following 18 (72%) samples were positive for aflatoxins: 1.4, 1.6, 2.7, 2.9, 2.11, 3.12, 3.13, 3.14, 3.15, 4.16, 4.17, 4.18, 4.19, 4.20, 4.21, 5.22, 5.23, 5.25.

All the inoculated samples (n=25) showed bacterial and fungal growth. The microorganisms identified were *S. aureus* (52%), *E. coli* (64%), *Klebsiella* (40%), *Enterobacter* (60%), *Salmonella sp.* (4%), *Shigella sp.* (28%), *Citrobacter sp.* (16%), *Pseudomonas sp.* (4%), *Proteus* (32%), *Edwardsiella sp.* (12%), *Alcaligenes sp.* (8%) and *Serratia sp.* (8%). Aflatoxins were present in 72% of the samples analyzed, Figure 1.

Figure 1. Distribution of microorganisms in São Paulo.



Source: Own authorship.

In the central region (n=6), the microorganisms identified were: *S. aureus* (83%), *Enterobacter* (83%), *Klebsiella* (67%), *E. coli* (50%), *Salmonella sp.* (17%), *Alcaligenes* (17%), *Pseudomonas sp.* (17%), *Citrobacter* (17%), *Proteus* (17%), *Shigella* (17%) and aflatoxins (33%), Figure 2.

In the northern region (n=5), the microorganisms identified were: *E. coli* (80%), *S. aureus* (60%), *Klebsiella* (60%), *Proteus* (40%), *Shigella* (20%), *Alcaligenes* (20%), *Edwardsiella* (20%), *Enterobacter* (20%) and aflatoxins (60%), Figure 2.

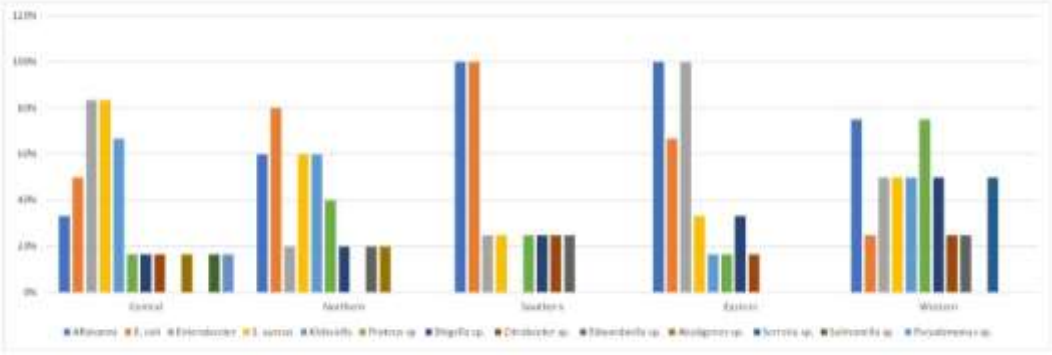
In the southern region (n=4), the microorganisms identified were: *E coli* (100%), *Enterobacter* (25%), *Shigella* (25%), *Edwardsiella* (25%), *Citrobacter* (25%), *Proteus* (25%) and *S. aureus* (25%) and aflatoxins (100%), Figure 2.

In the eastern region (n=6), the microorganisms identified were: *Enterobacter* (100%), *Klebsiella* (17%), *E. coli* (67%), *S. aureus* (33%), *Shigella* (33%), *Citrobacter* (17%), *Proteus* (17%) and aflatoxins (100%), Figure 2.

In the western region (n=4), the microorganisms identified were: *S. aureus* (50%), *Proteus* (75%), *Enterobacter* (50%), *Klebsiella* (50%), *Shigella* (50%), *Serratia* (50%), *E. coli* (25%), *Edwardsiella* (25%), *Citrobacter* (25%) and aflatoxins (75%), Figure 2.

The distribution of microorganisms in São Paulo identified in this study was *E. coli*, *Enterobacter*, *Klebsiella*, *S. aureus*, *Shigella*, *Proteus* and aflatoxins present in samples from all five regions; *Citrobacter* present in three regions (southern, eastern and western); *Edwardsiella* in three regions (northern, southern and western); *Alcaligenes* in two regions (central and northern), *Salmonella* in one region (central) and *Serratia* in one region (western), Figure 2.

Figure 2. Distribution of microorganisms in Areas of São Paulo.



Source: Own authorship.

4. Discussion

The Centers for Disease Control and Prevention (CDC), through its FOODNET (Network for active surveillance of foodborne diseases) portal, has received reports of 25,866 cases of infection, 6,164 hospitalizations and 122 deaths in 2019. Most are infections caused by bacteria and their toxins, fungi, viruses and others. Numerous outbreaks are related to the consumption of food that does not present organoleptic changes, since the microbial load necessary to cause food infection is lower than that to decompose the food (Tack et al., 2020).

In 2018, the Brazilian Ministry of Health registered 503 outbreaks of FBD. The vehicles were mixed foods (8.6%), water (6.2%), eggs and egg products (3.7%), milk and dairy products (2.8%), meat and cold cuts (2.1%), sweets and desserts (2.0%), cereals, flour and derivatives (1.9%), other types of food (6.3%) (Secretaria de Vigilância em Saúde, 2018). However, the majority of these (66.4%) were ignored or were inconclusive regarding the suspected food. The main etiological agents identified are *E. coli*, *Salmonella*, *S. aureus* and coliforms (Secretaria de Vigilância em Saúde, 2018; Centro de Vigilância Epidemiológica, 2008). In São Paulo, from 2007 to August 2020, the Epidemiological Surveillance Bulletin on Water and Foodborne Illness (Boletim de Vigilância Epidemiológica de Doenças Transmitidas por Água e Alimentos) linked to the Health Surveillance Coordination reported 2592 outbreaks involving 24832 patients caused by contaminated food, and the main etiological agents cited were *E. coli* and *Shigella sp.* (Coordenadoria de Vigilância em Saúde, 2020; Souza et al., 2015).

The trade in street food has grown as a practical and economical alternative the population (Silva et al., 2016; Santos et al., 2016). In São Paulo, street markets form part of the city's culture, they are distributed throughout the five regions and operate Tuesday through Sunday. The consumption of deep-fried pasties accompanied by a vinaigrette salad is a common habit that could be associated with the acquisition of FBD (Silva et al., 2016). Food poisoning caused by *S. aureus* occurs due to the presence of toxins that lead to symptoms like vomiting, diarrhea, nausea and colic (Secretaria de Vigilância em Saúde, 2018). These toxins can trigger other infections, such as pneumonia and septicemia, important diseases in the hospital environment that can contribute to the development and permanence of methicillin resistant strains (MRSA) (Sciezyńska at al., 2012). *S. aureus* is part of the human microbiota, as well as being found in numerous environments, so food handling must be performed rigorously, because the handler can be a vehicle of contamination. In the United States of America (USA), 27.8% of raw meat samples analyzed were positive for *S. aureus* (Sciezyńska at al., 2012). In this study, we observed that 60% of the samples were contaminated with *S. aureus*.

Thermotolerant coliforms (*E. coli*, *Klebsiella* and *Enterobacter*) can contaminate water and food through untreated sewage disposal. They are useful indicators for assessing water and food contamination (Martin et al., 2016). Some species of

E. coli stand out as producers of Shiga toxin, which leads to severe conditions like hemorrhagic colitis and uremic hemolytic syndrome that can lead to renal failure. The most severe cases can be fatal among children and the elderly with poor health (Martin et al., 2016). In India, *E. coli* was detected in 66% of samples of salads, eggs, chicken, sheep meat and unpasteurized buffalo milk, which corroborates the findings of our study, where 64% of samples were contaminated with *E. coli* (Rasheed et al., 2014). Besides being associated with FBD outbreaks, *Enterobacter* and *Klebsiella* are also involved in other diseases, such as pneumonia, and wound and urinary infections (Martin et al., 2016). In Ghana, 65% of pasta and plantain samples were contaminated with *Enterobacter* (Saba & Gonzalez-Zorn, 2012). In Barcelona, microbiological analysis performed on hospital food detected 8.8% of *Klebsiella* in the samples analyzed (Calbo et al., 2011). Among our samples, 60% were positive for *Enterobacter* and 40% for *Klebsiella*.

Salmonella sp. and *Shigella sp.* can cause typhoid, salmonellosis and enteric fever. Salmonellosis is one of the leading causes of human bacterial gastroenteritis and the second most reported in the European Union (Mughini-Gras et al., 2014). In Salvador, 11.25% of fresh eggs, and in Recife 12.7% of curd cheeses presented *Salmonella* (Evêncio-Luz et al., 2012). In Mexico, the presence of *Salmonella* was detected in 2% of cooked vegetable salad samples (Bautista-De Leon et al., 2013). In Ethiopia, 7.4% of beef samples were positive for *Shigella* (Garedew et al., 2016). In this study, 4% of the samples presented *Salmonella* and 28% presented *Shigella sp*.

Citrobacter sp., *Pseudomonas sp.*, *Proteus sp.*, *Alcaligenes sp.* and *Serratia sp.* are microorganisms resident in the human gut, and contaminated water and soil. They can cause various numerous diseases, including urinary and respiratory infections, meningitis, bacteremia and septicemia, and more severe cases can result in death (Jarvis et al., 2010). In China, 35.7% of fresh vegetable samples were positive for *Citrobacter freudi* (Liu et al., 2018). Among our samples, 16% were contaminated with *Citrobacter sp*. In Switzerland, a study of fish and seafood detected *P. aeruginosa* in 27% of the samples (Boss et al., 2016). In France, an analysis of cheese detected the presence of *Proteus* in 68% of samples (Deetae et al., 2009). Here, we detected *Pseudomonas* in 4% of the samples studied and *Proteus* in 32%. In Dhaka, Bangladesh, 17.5% of frozen animal foods were contaminated with *Alcaligenes* (Sultana et al., 2014). In Germany, 26% of *Serratia* contamination was detected in fresh pork meat (Schill et al., 2017). In our research, 8% of the samples showed the growth of both *Alcaligenes sp*. and *Serratia sp*.

Edwardsiella sp. is found in the aquatic environments and is responsible for enteric infection, endocarditis, liver abscess, and more severe cases of meningitis and septicemia. Mortality can affect 61.1% in patients with soft tissue infection (Ota et al., 2011; Takeuchi et al., 2009). In Spain, the presence of *Edwardsiella* was detected in 8% of the grouper fillets studied (Herrera et al., 2006). In this study, we detected *Edwardsiella sp*. in 12% of the samples.

Aflatoxins are products of the secondary metabolism of *Aspergillus spp.* and other fungi. Isoform B1 causes acute toxicity in mammals, fish, birds and humans. They are found in agricultural products like corn and peanuts (Rasooly et al., 2016). Aflatoxin contamination leads to suppression of the immune system and to liver failure, which can progress to cancer and in more severe cases, to death. Yearly estimates suggest that in South Africa and the Sahara, 26,000 deaths are associated with chronic aflatoxin exposure. This contamination is a serious food security problem worldwide (Rasooly et al., 2016; Shekhar et al., 2017). In India, *Aspergillus flavus* was detected on 83.33% of corn kernels (Shekhar et al., 2017), while herein, 72% of the samples were positive for aflatoxins.

In São Paulo, SP, Brazil, 45% of the fish sold in street markets was unfit for consumption due to the levels of thermotolerant coliforms (*E. coli*) and non Vibrio-cholerae. This indicates that the inadequate food handling and hygiene observed in street markets in São Paulo could be endangering public health and facilitating FBD (Silva et al., 2008).

Food sold on the street is a known vehicle for microorganisms that cause FBD. The source of bacterial contamination can be present throughout the production chain, from the use of contaminated water, poor hygiene with regard to the food, the

utensils and even cross contamination from prepping surfaces, when handling fresh meat and fish, for example, in the case of *Edwardsiella sp.* (Santos et al., 2016; Schill et al., 2017).

During sample collection, we observed that vinaigrette salad is almost always exposed, with minimal protection or refrigeration. In all the stalls, the trash cans were close to where the product was stored. The sellers frequently manipulated money concomitant with serving vinaigrette salad. The sum of these factors makes it impossible to determine at what point product contamination occurs during the transaction.

5. Final Considerations

This study detected the presence of pathogenic microorganisms in vinaigrette salad offered as an accompaniment to deep fried pasties sold in street markets in the city of São Paulo. The Gram-positive bacteria *S. aureus*, Gram-negative bacteria *E. coli*, *Klebsiella*, *Enterobacter*, *Proteus*, *Shigella*, *Citrobacter*, *Edwardsiella sp.*, *Alcaligenes sp.*, *Serratia sp.*, *Pseudomonas sp.* and *Salmonella sp.*, and aflatoxins were detected in the samples studied. Microorganisms were detected in all five regions (central, northern, southern, eastern and western) of São Paulo. The ingestion of the vinaigrette salad commonly sold in the city of São Paulo can lead to an imminent risk of contamination and development of enteric and other types of infection, since several of the bacteria identified are responsible for more than one type of disease, such as *E. coli.* and *Salmonella typhi*. Other species may present multiresistance to effective treatment, such as *S. aureus* (MRSA) and *Klebsiella* (KPC). The presence of aflatoxins increases the risk of consumers developing mycotoxicosis and liver cancer. These findings reinforce recommendations to promote training for sellers, suppliers and handlers concerning food management and food safety practices and to intensify inspections as the main actions to safeguard the population from possible infections. Further studies are required to provide a clearer understanding of the foodborne illness outbreaks in the city of São Paulo and throughout Brazil.

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