Original Article

Microbiological analysis of the hands of food handlers

Análise microbiológica das mãos de manipuladores de alimentos

Análisis microbiológico de las manos de los manipuladores de alimentos

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ABSTRACT

Background and Objectives: The convenience of eating meals outside home poses a risk to consumers' health, especially due to the exposure to possible disease-causing agents present in food. This is a result of generally poor hygienic-sanitary conditions, which increases the likelihood of microbial contamination of these foods. Thus, the aim of this study is to investigate the presence of total and thermotolerant coliforms and *Staphylococcus* spp. in samples taken from the hands of street food handlers. **Methods:** Two approaches were used in this investigation: a) the collection of microorganisms present in the hands of street vendors, which is directly associated with foodborne diseases, and b) the conduction of a questionnaire to gather information on the hygiene conditions of the handlers. **Results:** The microbiological analyses identified the presence of total coliforms and *Escherichia coli*, as well as *Staphylococcus* coagulase-positive (*S. aureus*) and coagulase-negative. **Conclusion:** The hygiene conditions of the handlers were considered unsatisfactory according to the results presented in the analysis and the questionnaire applied to the subjects.
KEYWORDS: Microbiological analysis. *Staphylococcus aureus.* Snacks.

RESUMO

Justificativa e Objetivos: A praticidade de fazer refeições fora de casa representa um risco à saúde do consumidor, especialmente pela exposição a possíveis agentes causadores de doenças presentes nos alimentos. Isto ocorre devido às condições higiênico-sanitárias, geralmente precárias, o que aumenta a probabilidade de contaminação microbiana desses alimentos. Assim, o presente estudo possui como objetivo verificar a presença de coliformes totais e termotolerantes e de *Staphylococcus* spp. em amostras das mãos de manipuladores de lanches de rua. **Métodos:** A investigação de microrganismos foi realizada de duas formas: a) coleta dos microrganismos presentes nas mãos de vendedores ambulantes, o que está diretamente associado a doenças transmitidas por alimentos, e b) aplicação de um questionário com a finalidade de verificar informações sobre as condições de higiene dos manipuladores. **Resultados:** Através das análises microbiológicas, foram identificadas a presença de coliformes totais e de *Escherichia coli*, bem como a presença de *Staphylococcus* coagulase positiva (*S. aureus*) e coagulase negativa. **Conclusão:** As condições de higiene dos

manipuladores se mostraram insatisfatórias conforme os resultados apresentados na análise e no questionário aplicado para esses indivíduos.
PALAVRAS-CHAVE: Análise microbiológica. *Staphylococcus aureus*. Lanches.

RESUMEN
Justificación y Objetivos: La practicidad de comer fuera de casa presenta un riesgo para la salud del consumidor, especialmente por la exposición a posibles agentes causantes de enfermedades alimentarias. Esto se debe a las condiciones higiénico-sanitarias, generalmente precarias, que aumenta la probabilidad de contaminación microbiana de esos alimentos. El presente estudio tuvo como objetivo verificar la presencia de coliformes totales y termotolerantes de *Staphylococcus* spp. en muestras de las manos de manipuladores de alimentos en la calle. **Methods:** Se realizó el análisis de microorganismos de dos maneras: a) se recolectó la presencia del microorganismo presente en las manos de los vendedores ambulantes, lo que está directamente asociado a las enfermedades transmitidas por alimentos; y b) se aplicó un cuestionario con el fin de recoger informaciones sobre las enfermedades y condiciones de higiene de los manipuladores. **Results:** Se identificó la presencia de coliformes totales y de *Escherichia coli*, así como la presencia de *Staphylococcus* coagulase positivo (*S. aureus*) y coagulase negativo. **Conclusión:** Las condiciones de higiene de los manipuladores se mostraron insatisfactorias conforme los resultados presentados en el análisis y en el cuestionario aplicado a esos individuos.
PALABRAS CLAVE: Análisis microbiológico. *Staphylococcus aureus.* Bocadillos.

INTRODUCTION

Having meals and snacks on street food vendors is a practical and easy way of eating, being common in several countries. In Brazilian culture, the diversity of food preparation and urbanization are directly reflected on the habits of the population.[1,2] Worldwide, 2.5 billion people eat street food every day, which represents a cultural, social and economic phenomenon that is closely related to urbanization.[3]

Street food is part of the daily intake of many consumers and, as a result, several studies have revealed increases on the number of vendors of this type of food.[2] Thus, street trade is crucial in creating jobs and providing low-cost food in easily accessible places. Moreover, street food may reflect the economic and social condition of the country.[2,4]

However, the convenience of having meals outside home poses a risk to consumers' health, since the likelihood of the microbial contamination of these foods is high, especially due to hygienic-sanitary conditions. Consequently, infections and food poisoning are the most common complications caused by the lack of satisfactory hygiene conditions. *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and *Yersinia enterocolitica*, among others, are some of the most relevant agents, with *Yersinia enterocolitica* being the main responsible for foodborne diseases and high hospitalization rates.[5-7]

Many studies have shown that street foods are often kept at inadequate temperatures, over handled by their vendors and exposed to inadequate hygienic conditions. These

circumstances can make food prone to contamination.[8] Moreover, most vendors have little or no formal education[8], which can indicate little knowledge about the proper methods of food manipulation and contribute to the transmission of microorganisms.[2] Thus, the microbiological safety of street foods is an important factor to assess their problems, so that the concerned organizations can take proper actions to improve safety and sanitation in this sector.[8]

Given that the hands of food handlers may be a potential source of pathogens in food services, the aim of this study was to investigate the presence of total and thermotolerant coliforms and *Staphylococcus* spp. in samples of the hands of street food handlers.

METHODS

This is an experimental, cross-sectional, descriptive study conducted in the municipality of Caruaru, in the countryside of Pernambuco. Sample collection was carried out on the city's public streets, where food handlers of the informal snack trade were randomly chosen. They were located at different points of sale near schools, universities, hospitals, bus stations and formal businesses.

This study was approved by the Research Ethics Committee of the Centro Universitário do Vale do Ipojuca (CEP - UniFavip DeVry), under the number 2,149.339, CAEE 67688117.9.0000.5666.

Study population

The study population consisted of handlers of street food, of both sexes. Inclusion criteria included: handlers who sell any type of snack (typical Brazilian snacks such as coxinha, risole, esfiha, enroladinho, pie, pastel, hot dog, skewers, churros, popcorn, tapioca, acarajé, hamburger, among others), and commercialization could be made from specific carts for snacks, adapted carts and tents. Individuals younger than 18 years old were excluded.

Sample collection and preparation

In total, 30 vending locations of snacks were visited, and 30 handlers participated in the survey, one from each vending place. Every participant signed an Informed Consent Form and then, with the help of sterile swab moistened in NaCl at 0.9 %, a sample of material of each subject's dominant hand was collected.[9]

Soon after the collection, the material was deposited in a test-tube with peptone water. The tubes were then sealed and labeled, and maintained in styrofoam until being taken to the UniFavip/DeVry microbiology laboratory. In addition, a questionnaire composed of 20

questions was applied to the vendors to investigate their hygiene practices. The questions included: name, age, gender, schooling, having taken a training course on good food handling practices, for how long and how many days they work at the vending location, how many foods are produced and sold, hand washing, wearing gloves, bouffant cap, uniform and adornments, and good self-presentation such as clean hands with short nails and no nail polish.

Microbiological analysis

The samples collected from the handlers' hands were analyzed in coliform count plates – total, thermotolerant and *S. aureus*. The collected material was cultivated in Petri dishes containing about 20 mL of solidified nutrient agar. In the laboratory, the dishes were inverted and incubated at 35°C ± 2°C for 24 hours for the growth of microorganisms.[6,7,9] The number of isolates per sample varied in accordance with the heterogeneity on the growth observed in the nutrient agar medium.

To analyze total coliforms, once diluted, samples of cultures in duplicate were used. A suspected colony was cultivated in nutrient agar and separately isolated in test-tubes containing brilliant green bile broth (BGB) 2% with inverted Durham tubes, incubated at 35°C ± 2°C for 24 to 48 hours. To analyze thermotolerant coliforms, positive BGB were cultivated in tubes containing *E. coli* broth with inverted Durham tubes and incubated at 45°C for 24 to 48 hours. Tubes that developed a clouding of the medium, as well as presence of gas inside of the Durham tube, were considered positive.[7,9,10]

To phenotypically identify *S. aureus*, the colonies were analyzed according to their growth characteristics when in base agar plus 8% defibriated sheep blood, incubated at 35°C for 24 hours, and their production of hemolysis and pigment.[11]

Data Processing and Analysis

The data obtained with the microbiological analysis and the questionnaire were properly stored and tabulated in Microsoft Excel 2016. The bacteria were identified, and the results were presented in the form of percentage and compared with studies from the literature.

RESULTS

Among the 30 handlers that were interviewed, 50% were aged between 35 and 55 years, and 53.33% were male. Regarding schooling, 53.33% graduated from high school, and 80% stated that they had not taken any food hygiene courses. Regarding the working time in the vending place, 73.33% of the subjects reported working in this area for over 10 months, and

most work from 4 to 6 days a week. The studied population sells around 75 snacks a day; 3 subjects refused to provide this information (Table 1).

Table 1 - Data for the samples from the hands of food handlers regarding their age, schooling, having taken a training course on food hygiene and working time separated by gender

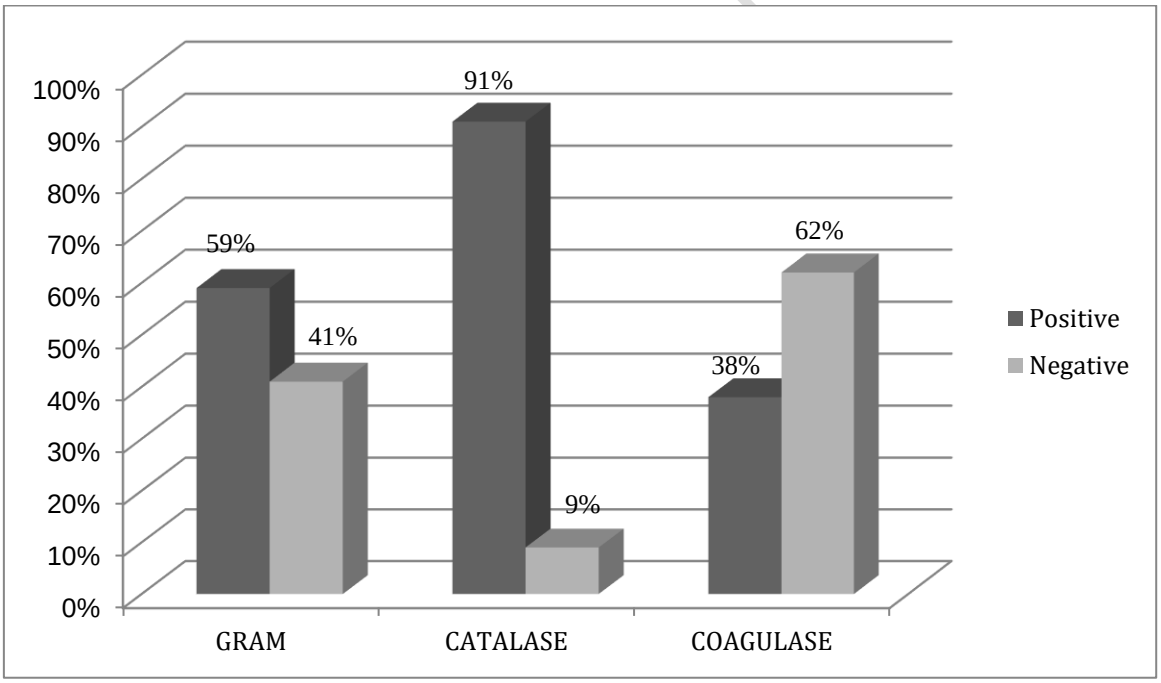
Variables	**Male n (%)**	**Female n (%)**	**Total n (%)**
Age (years)			
18-25	1 (3.33)	1 (3.33)	2 (6.66)
26-35	7 (23.33)	2 (6.66)	9 (29.99)
36-55	9 (30)	6 (20)	15 (50)
> 55	2 (6.66)	2 (6.66)	4 (13.32)
Schooling			
Incomplete Elementary School	4 (13.33)	2 (6.66)	6 (19.99)
Elementary School	5 (16.66)	3 (10)	8 (26.66)
High School	7 (23.33)	9 (30)	16 (53.33)
Food Hygiene Course			
Yes	4 (13.33)	2 (6.66)	6 (19.99)
No	12 (40)	12 (40)	24 (80)
Working time			
< 1 month	1 (3.33)	0 (0)	1 (3.33)
1-5 months	1 (3.33)	1 (3.33)	2 (6.66)
5-10 months	2 (6.66)	3 (10)	5 (16.66)
> 10 Months	12 (40)	10 (33.33)	22 (53.33)

With regard to hand hygiene when handling the food and dealing with the payment, 43.33% of the interviewees reported not washing their hands; among these, 77% stated that they lack a place for the procedure. Among the 56.67% that reported washing their hands, 47.06% do this sanitizing procedure using water and detergent, 23.53% only water, 17.65% water and soap, 5.88% Veja limpeza®, and 5.88% water with detergent and hand sanitizer.

Regarding the use of disposable gloves when handling the food, 63.33% reported not wearing gloves and 36.67% to wear it, and the average number of pairs of gloves wore was 2 per day. Regarding food storage, 36.67% of the handlers reported using plastic canisters. In addition, it was also possible to observe that 66.67% do not wear closed uniform (coat), 76.67% use adornments and do not keep their hair protected with bouffant caps, and 63.33% do not denote good personal presentation.

Through the microbiological analysis of the 30 samples collected from the hands of the handlers, it was possible to isolate 78 microorganisms in blood agar. The Gram staining technique showed that 58.97% of the samples were Gram-positive and 41.03% Gram-negative. To characterize the microorganisms present in the sample, catalase and coagulase tests were conducted, and 91.3% of the samples were considered catalase-positive and 38.10% coagulase-positive, suggesting the presence of *S. aureus* (Figure 1).

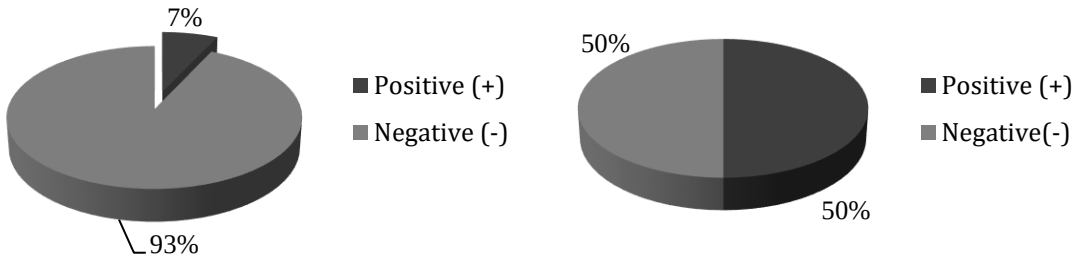
Figure 1. Results of Gram analyses and coagulase and catalase tests.



Regarding total coliforms, 4 isolates presented clouding of the medium and gas production; whereas for thermotolerant coliforms, 2 isolates had positive test results (Graph 2).

Figure 2. Results of total and thermotolerant coliform analyses.

Coliformes Totais **Thermotolerant coliforms**



DISCUSSION

The application of the questionnaire allowed us to identify that the scarce knowledge about good hygiene practices by handlers is very common. Moreover, food is prone to contamination due to the lack of a proper physical structure since the handlers' vending place is set on the streets and does not offer proper conditions for suitable personal hygiene.

Factors such as the precarious infrastructure, the characteristics of the products and the insufficient action of health surveillance services are responsible for the increasing concern about the reliability of street food. These factors, when associated with mishandling, disregard for storage and preservation practices, as well as the ineffective protection of food against insects and dust, increase the hazards on the transmission of foodborne microbial diseases.[10]

Annually, hundreds of thousands of people are believed to be victims of foodborne diseases worldwide. According to the Brazilian Ministry of Health, the country faced 673 foodborne diseases outbreaks only in 2015; among these, 90.5% of the microorganisms involved were bacteria, and the main etiological agents were *Salmonella*, *E. coli* and *S. aureus*.[12,13]

The World Health Organization states that diseases transmitted by street foods are a public health concern, leading countries to spend many resources on public health services when diseases could be minimized simply by implementing good handling practices.[2,10,12]

The presence of total and thermotolerant coliforms in the samples of the handlers' hands attests the inadequate practices of hygiene and manipulation, thus serving as an important vehicle for microorganisms such as *E. Coli*, which indicates fecal contamination, and *S. aureus*, which indicates the presence of nasal material, both present in the results.[4,11,14,15]

The family *Enterobacteriace* is the main agent of gastrointestinal infections. Among them, the diarrheagenic categories of *E. coli* have a prominent role. Studies show that several

serotypes of *E. coli* are responsible for diarrheal diseases, constituting a serious public health problem worldwide, with over two million deaths reported each year.[10,15]

The tests performed to identify *Staphylococcus* spp. were positive. This genus of bacteria is catalase- and thermonuclease-positive, and, depending on the species, coagulase--positive or negative. Only the species *S. aureus*, *S. delphini*, *S. medimedius*, *S. schleiferi coagulans* and some strains of *S. hyicus* are coagulase-positive. Most *Staphylococcus* strains are coagulase-negative.[11,16-19]

The main hosts of this pathogen are humans and animals. Thus, it can be found in external environments, skinfolds, armpits, vagina, intestine, human skin, nasal, oral and auricular mucosa, etc., being the nasal cavity its primary habitat on humans. Consequently, food handlers carrying the *S. aureus* are eminent sources of food contamination.[15,20-22]

One simple and important control measure when handling food is hand hygiene. Hand washing is crucial to protect food from possible microbiological contamination, since the pathogens transmitted via the hands are mostly originated from fecal contamination due to the handlers' inefficient hygiene habits.[1,6]

Food hygiene is fundamental to ensure the safety of the food from its production to its consumption. However, the quality of consumed food is assured by the Brazilian National Food and Nutrition Policy.[12,13] Many studies address the importance of food safety intervention, aiming to ensure a safe consumption of food. Thus, as food handlers are considered a key source of contamination, it is important to highlight their hygienic-sanitary conditions. Moreover, to modify inadequate food handling practices, people must be provided with proper training.[2,12]

Food intake depends not only on personal and cultural factors, but also on the surrounding food environment. The food environment is one of the main areas in which policies can intervene to improve the availability, accessibility and acceptability of healthier foods. By improving nutritional labeling, offering healthier foods, setting standards in public institutions, using cost-effective tools to address food accessibility, restricting food advertising, improving the quality of food supply and establishing incentives and rules to create a healthy retail network, the food environment can better support consumers to make healthier choices.[23]

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Authors' contribution:

Luciclecia Edjanira da Silva and Willyane da Silva Ferreira dos Santos also participated in all stages of the article preparation. Márcia Gabrielle Silva Viana worked in the study orientation.