Microbiological quality of finger food and snacks

Qualidade microbiológica de lanches e salgados

RIALA6/1570

Sarah Hwa In LEE^{1*}, Carlos Henrique CAMARGO^{1,2}, Elisângela de Souza MIRANDA¹, Ary FERNANDES JUNIOR¹, Vera Lúcia Mores RALL¹

*Endereço para correspondência: ¹Departamento de Microbiologia e Imunologia, Instituto de Biociências de Botucatu da UNESP - Campus de Botucatu, Distrito de Rubião Júnior, S/N. CEP: 18618-970, Botucatu/SP, Brasil. E-mail: sarah.hwa.in.lee@gmail.com ²Centro de Bacteriologia, Instituto Adolfo Lutz, São Paulo, SP, Brasil. Recebido: 13.03.2013 - Aceito para publicação: 06.08.2013

ABSTRACT

Although finger food is convenient, it may be easily contaminated from the stage of preparation to the moment of consumption. This study aimed at evaluating the microbiological quality of finger food and sandwiches sold in Botucatu – SP, Brazil, by following the standards established by the Brazilian Health Surveillance Agency, ANVISA. The analysis was conducted according to APHA. A hundred and twenty-two samples of meat, chicken, shrimp, cheese, and vegetable finger food and sandwiches were tested from August 2008 to March 2009. Seventeen (13.9 %) samples of meat, cheese, vegetables and chicken were in disagreement with the ANVISA standards — some of them in more than one parameter. High counting of thermotolerant coliforms and coagulase-positive staphylococci were found in ten (8.2 %) and eight (6.5 %) samples, respectively. *Salmonella* spp. was detected in two samples (1.6 %). No *Bacillus cereus* and sulfite-reducing clostridia were isolated. Although only 10 samples (8.2 %) showed pathogenic bacteria contamination, these results are relevant, since they indicate that the population is generally exposed to risks of acquiring foodborne diseases. Thus, the sanitary authorities might implement actions for supervising the quality of the food sold in Botucatu, and to strengthen the food sellers to improve the hygienic conditions and be aware of the risks of food contamination.

Keywords. food quality, finger food, CPS, pathogenic bacteria, ready-to-eat food, snacks

RESUMO

Neste trabalho foi avaliada a qualidade microbiológica de salgados e sanduíches comercializados em Botucatu-SP, seguindo-se a legislação em vigor. As análises foram realizadas de acordo com APHA. Foram analisadas 122 amostras de salgados de carne, frango, camarão, queijo e vegetais, e sanduíches no período de agosto/2008 a março/2009. Dezessete (13,9%) amostras de carne, queijo, vegetais e frango, e em algumas em mais de um parâmetro, estavam em desacordo com a legislação em vigor. Foram detectadas elevadas contagens de coliformes termotolerantes e estafilococos coagulase-positiva, respectivamente, em dez (8,2%) e oito (6,5%) amostras. *Salmonella* spp. foi isolada em duas amostras (1,6%). As contagens de *Bacillus cereus* e de Clostrídio Sulfito Redutor não ultrapassaram os padrões da legislação. A maioria dos salgados mostrou resultados dentro dos padrões estabelecidos pela legislação. A presença de bactérias patogênicas como *S. aureus* e *Salmonella* spp. foi demonstrada em 10 amostras (8,2%); e este resultado é relevante, pois indica que a população está exposta a riscos de doenças veiculadas por alimentos. Torna-se necessário colocar em prática a vigilância dos alimentos comercializados em Botucatu, incentivar a melhoria de condições de higiene pelos comerciantes, e ter ciência dos riscos e das implicações da contaminação microbiológica dos alimentos.

Palavras-chave. qualidade de alimentos, lanches, ECP, bactérias patogênicas, alimentos prontos para consumo, salgados.

INTRODUCTION

The choice for quick meals out of the house was stimulated by the insertion of women into labor market and by the lack of time either to go home to eat or to prepare a meal. This situation resulted in an increased number of people who eat snacks or fast food commercialized near their employment place.

Microbiological contamination may come from the raw food or occur at preparation, storage or the moment of consumption. Eggs, chicken and meat are the most commonly contaminated foods¹. During preparation, cross-contamination can occur either due to the use of poorly sanitized kitchenware or kitchen equipment, or due to negligence or ignorance of the food handler regarding the use of gloves or mob-caps, hand asepsis, and good handling practices. Transmission of enteric pathogens and other microbial hazards to ready-to-eat foods through air, contaminated utensils and food contact surfaces has also been widely reported, so attention should be paid to food hygiene and compliance with the Hazard Analysis and Critical Control Points (HACCP) system in order to prevent foodborne illness outbreaks².

Contamination with microbes or their toxins is a particularly important problem, causing at least 48 million illnesses annually in the United States and, likely, hundreds of millions globally³. It is known that often the causative agent of foodborne diseases is not identified⁴. In addition, it is known that outbreak cases are underestimated, because most cases are not notified to the Brazilian health authorities. Some outbreaks, however, were sporadically reported in Brazil^{5,6}.

Besides the natives, Botucatu receives about 550 new students every year, due to the presence of a university. Almost all of these students live in Fraternity or Sorority houses and do not cook due to the lack of time. Thus, they usually eat ready to eat foods or fast meals that are cheap and tasty⁷.

The aim of this study was to evaluate the microbiological safety of finger foods and sandwiches commercialized in Botucatu snack bars, street vendors, groceries and convenience stores, according to the Brazilian food sanitation standards established by ANVISA⁸.

MATERIAL AND METHODS

Sample collection

Sixty-one groceries, cafes, food trailers, snack bars, bakeries and street vendors located in

important areas in Botucatu, SP, were analyzed two times, between August 2008 and March 2009. Samples of meat, chicken, shrimp, cheese, vegetable finger food and sandwiches were collected. All temperature conditions for food storage were adequate except for 2 snack bars. Samples were transported in sterile plastic bags (114 x 229 mm - Inlab), under refrigeration, in a cooler box until processing at the lab on the same day.

Microbiological Analysis

According to APHA⁹ protocols, 25 g of food were homogenized in 225 mL of peptone water in plastic bags at Stomacher (Lab Blender 400) for 30 seconds. Other dilutions were made from this 10⁻¹ dilution.

All culture media were obtained from Difco, Becton Dickinson (Sparks, MD). Determination of most probable number (MPN) of thermotolerant coliform (TC) was performed using technique of multiples tubes⁹.

Coagulase-positive staphylococci (CPS) were detected and identified according to Lancette and Bennett⁹ by spread plating an appropriate dilution (0.1 mL) onto Baird Parker agar plate, incubating at 35 °C/48 h. Black colonies with 2 halos were presumptive of CPS. Such presumptive strains were confirmed by catalase, coagulase, TNase and the "Dry Spot Staphytect Test" (Oxoid). Acetoin production (Voges-Proskauer) and β -galactosidase tests were performed in order to separate *S. aureus* from *S. intermedius*.

The detection protocol employed for *Salmonella* spp. was described by APHA⁹. Thus,25 g of food were homogenized in a Stomacher (Lab-Blender 400) with 225 mL buffered peptone water. After incubation at 35 °C/24 h, 1 mL was inoculated in 10 mL of tetrathionate broth (35 °C/24 h) and 0.1 mL in 10 mL Rappaport Vassiliadis (42 °C/24 h). After incubation, a loopful of each suspension was plated onto xylose-lysine-desoxycholate agar and *Salmonella-Shigella* agar. After incubation at 35 °C/24 h, five typical colonies from each agar plate were biochemically tested, using triple sugar iron agar and the API 20E test kit (bioMérieux). The colonies were also submitted to serological tests, using polyvalent somatic and flagellar antisera (Probac).

Enumeration of sulfite-reducing clostridia (SRC): using the spread plate method, 0.1 mL serial dilutions was plated onto Sulfite Polymyxin Sulfadiazine (SPS) agar plates, incubated under anaerobic atmosphere in a jar with atmosphere generation system (Anaerogen/ Oxoid), at 46 °C/48 h. Plates showing between 25 and 250 black and small colonies were chosen for enumeration⁹.

For *Bacillus cereus* group detection, spread method was also used in Mannitol Egg Yolk Polymyxin Agar at 35 °C/24 h. Typical colonies, rough and dry with bright pink background surrounded by an egg yolk precipitate, were transferred to Tryptic Soy Agar (TSA) and further confirmed by biochemical characterization².

Microbiological Parameters

ANVISA⁸ legislation RDC no. 12 determines the following parameters to cakes and similar food, either sweet or salty, with or without filling, and finger food in general: maximum score of 10³ CFU/g for coagulase positive staphylococci, *B. cereus* and sulfite-reducing clostridia, 10² MPN/g for thermotolerant coliforms and absence of *Salmonella* in 25 g.

RESULTS AND DISCUSSION

Overall, according to ANVISA⁸ RDC no. 12, 13.9 % of the samples were in disagreement with those established standards. Most of the samples in disagreement were chicken, meat, cheese and vegetable, from the highest value to the lowest. It is possible to notice in Table 1 that the total number is higher, but there were some samples with more than one parameter in disagreement. The values can be observed in Table 1.

It is important to notice that in 3 samples the values of two of the parameters were exceeded. Two samples of chicken finger food exceeded in thermotolerant coliform values and had *Salmonella* spp. at the same time. One sample of cheese finger food exceeded in thermotolerant coliform and Coagulase-positive staphylococci values.

sandwiches and Finger food generally hold potential health risks associated with initial contamination of raw food by pathogenic bacteria, as well as contamination by handler during the preparation and through post-cooking handling, besides crosscontamination. Microbiological load in snacks in the developing world is highly dependent on the raw ingredients, traditional methods of processing and packaging, and maintaining temperature, which can determine the rate of deterioration of the ready-toeat food products and snack foods¹⁰. Also, many of the facilities were not clean, which can reflect in food quality. The risk of contamination is much higher when vendors wear dirty uniforms.

We are aware that foodborne illness caused by microorganisms is a major international health problem associated with food safety in developing countries. Contamination of street food has been attributed to exposure to polluted environment, poor sanitation and poor hygienic practices by the vendors.

Even though the São Paulo state health surveillance authority (Centro de Vigilância Sanitária da Secretaria de Estado de Saúde de São Paulo¹¹) requires that the center of fried finger food reaches at least 74 °C during preparation, one seller said that, in order to be crispy, the temperature inside does not reach that values; thus, potential pathogens are not eliminated. Chicken finger food is a good example, because poultry is manipulated after it has been cooked, when it is wrapped in dough and fried later on. *Salmonella* spp. is thermosensitive, being eliminated at 65 °C, after 15 minutes. We found *Salmonella* spp. in a fried

Table 1. Microbiological resu	lts of 122 finger food and s	andwiches retailed in Botucatu, SP
0	0	

Sample of finger food or sandwich	N*	Thermotolerant coliform	Coagulase-positive staphylococci	B. cereus	Sulfite-reducing clostridia	Salmonella spp.
		n (%)**	n (%)**	n (%)**	n (%)**	n (%)***
Sandwich	5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chicken	44	3 (6.8)	5 (11.3)	0 (0)	0 (0)	1 (2.3)
Vegetable	5	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)
Cheese	34	2 (5.9)	2 (5.9)	0 (0)	0 (0)	1 (2.9)
Meat	33	4 (12.1)	1 (3.0)	0 (0)	0 (0)	0 (0)
Shrimp	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	122	10 (8.2)	8	0	0 (0)	2

*number of samples analyzed; **number and percentage of samples above the limit; ***number and percentage of positive samples

finger food with cheese and chicken filling. There is a positive correlation between long holding times at room temperature and high bacterial counts¹². It was observed that in 2 snack bars, the heater was turned off in order to save energy, and we isolated *Salmonella* spp. from the snacks sold in those places. This type of food has to be kept at temperatures above 65 °C, to avoid bacterial growth or toxin production. Reheating previously cooked foods increased the risk of *Salmonella* spp. contamination. Snacks and sandwiches are generally prepared in the early morning; they are stored at room temperature and frequently are not separated from raw chicken and vegetables⁴. Therefore, it is crucial to avoid any recontamination.

In similar studies^{5,13,14}, this bacteria was not found, which indicates a low prevalence of *Salmonella* spp. in this kind of food, but when it is present, it represents a risk for the consumer. Salmonellosis is usually acquired by ingestion of contaminated water or food, and poultry products are a major source of *Salmonella* spp. in many developing countries.

Brazilian legislation provides regulation of microbiological standards for different kinds of food⁸. Above these limits, the food is considered improper to consume. Thermotolerant coliform indicates the hygiene and sanitization conditions the product underwent and shows possible fecal contamination and presence of pathogens.

We found 8.2 % of samples with thermotolerant coliform beyond the limit considered safe by ANVISA. Almeida et al¹ reported that the frequency of fecal contamination of street foods in other Latin American cities ranged from 9.4 % to 56.7 % above the standard, being considered of unsatisfactory hygienic condition. Furlaneto et al¹⁴ found 70 % of samples presenting thermotolerant coliform beyond the limit; Hanashiro et al⁷, in a study conducted in São Paulo, found 30 % of the samples containing thermotolerant coliform beyond the limit and Curi¹³ did not find any improper food in another study in Brazil. The presence of thermotolerant coliforms in the samples indicates a high risk that other pathogenic organisms have also contaminated the food⁷.

Coagulase-positive staphylococci enumeration beyond the established limit was found in 8 (6.5 %) samples. Furlaneto et al¹⁴ found similar value, with 10 % of contamination. Higher numbers were observed by Rodrigues et al⁶ and Curi¹³, of 37 % and 34 %, respectively. The presence of high quantity of CPS indicates lack of hygiene on the preparation, bad manipulation, improper cooking and maintenance of the food.

None of *Bacillus cereus* or sulfite-reducing clostridia count from our samples exceeded the limit established by ANVISA. Curi¹³ also did not find any samples with *B. cereus* and SRC counting exceeding the recommended limit. SRC is commonly isolated from pasture soil and from the digestive tract of healthy animals. Hanashiro et al⁷ isolated *B. cereus* in 12.5 % of the samples, which may be considered low comparing to studies around the world.

Although this study brings up some important information regarding the ready-to-eat food commercialized as snacks and sandwiches, it has some limitation. Nevertheless, this work showed that consumers should be aware and look for quality.

CONCLUSION

The results show that most of the samples were in accordance with the microbiological standards established by ANVISA. However, 8.2 % of them had pathogens such as *S. aureus* and *Salmonella* spp., exposing population to risks. Considering the data reported in this study, we present some suggestions for improving the microbiological quality of ready-to-eat food sold in Botucatu: educational and training programs for vendors; improvement of vendors' equipment for preparation and storage; adequate sanitation and refuse disposal facilities; and also adoption of the HACCP system.

ACKNOWLEDGEMENTS

The authors wish to thank São Paulo Research Foundation (FAPESP) for the research fellowship support provided to the student Sarah Hwa In Lee.

REFERENCES

- 1. Almeida CF, Araújo ES, Soares YC, Diniz RFC, Fook SML, Vieira KVM. Epidemiological profile of food intoxication reported by the Poisoning Information Center of Campina Grande, Paraíba. Rev Bras Epidemiol. 2008;11:139-46.
- 2. Lund BM, O'Brien SJ. Microbiological safety of food in hospitals and other healthcare settings. J Hosp Infect. 2009;73:109-20.
- 3. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M, Roy SL, et al. Foodborne Illness Acquired in the United States — Major Pathogens. Emerg Infect Dis. 2011;17(1):7-15.
- 4. Cardinale TE, Gros-Claude PJD, Tall F, Guèye EF, Salvat G. Risk factors for contamination of ready-to-eat street-vended

poultry dishes in Dakar, Senegal. Int J Food Microbiol. 2005;103:157-65.

- Caetano VC, Saltini DA, Pasternak J. Surto de salmonelose por Salmonella enterica em profissionais de saúde, causado por alimentos consumidos em uma festa de ano novo realizada dentro da Unidade de Terapia Intensiva. Einstein. 2004;2:33-5.
- Rodrigues KL, Moreira NA, Almeida ATS, Chiochetta D, Rodrigues MJ, Brod CS, et al. Intoxicação estafilocócica em restaurante institucional. Cienc Rural. 2004;34(1):297-9.
- Hanashiro A, Morita M, Matté GR, Matté MH, Torres EAFS. Microbiological quality of selected street foods from a restricted area of São Paulo city, Brazil. Food Control. 2004;16:439-44.
- Brasil. Ministério da Saúde. Resolução RDC nº 12, de 02 de janeiro de 2001. Aprova o Regulamento Técnico sobre Padrões Microbiológicos para Alimentos. Diário Oficial da União. 10 jan 2001. Seção 1:45-53.
- 9. APHA. Standard Methods for the Examination of Water and Wastewater. 21st ed. Washington (DC): American Public Health Association; 2001.

- Ezekiel CN, Anokwuru CP, Fari A, Olorunfemi MF, Fadairo O, Ekeh HA, et al. Microbiological quality and proximate composition of peanut cake (*Kulikuli*) in Nigerian markets. Acad Arena. 2011;3(4):103–11.
- Centro de Vigilância Sanitária. Portaria CVS-6/99, de 10 de março de 1999. Aprova Regulamento Técnico, que estabelece os Parâmetros e Critérios para o Controle Higiênico-Sanitário em Estabelecimentos de Alimentos. Diário Oficial do Estado de São Paulo. 10 mar 1999.
- 12. Bryan FL. Risks of practices, procedures and processes that lead to outbreaks of foodborne diseases. J Food Prot. 1988;5:663-73.
- Curi JDP. Condições microbiológicas de lanches (cachorro quente) adquiridos de vendedores ambulantes, localizados na parte central da cidade de Limeira, SP [dissertação de mestrado]. Piracicaba (SP): Universidade de São Paulo; 2006.
- 14. Furlaneto L, Kataoka AF. Análise microbiológica de lanches comercializados em carrinhos de ambulantes. Lecta. 2004;22:49-52.