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Microbiological quality of hamburgers sold in the streets of Cuiabá - MT, Brazil and vendor hygiene-awareness

Qualidade microbiológica de sanduíche comercializado nas ruas de Cuiabá - MT, Brasil e a percepção dos manipuladores

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Abstract

This study evaluated the microbiological quality of hamburgers and the microbe community on the hands of vendors in Cuiabá, Mato Grosso, Brazil, in relation to vendors' awareness as to what constitute acceptable food-handling practices as part of a broad-spectrum research programme on street foods in Brazil. Sale of the hamburger known as the 'baguncinha' is common and widespread in urban Cuiabá, Mato Grosso, Brazil. Food inspectors encounter various difficulties in carrying out inspections. One hundred and five hamburgers samples were evaluated using conventional methods including tests for facultative aerobic and/or anaerobic mesophytic bacteria, coliform counts at 45 °C, the coagulase test for *Staphylococcus*, Gram-staining for the presence of *Bacillus cereus*, *Clostridium* sulphite reductase and *Salmonella* spp. The hamburgers were categorized as unsuitable for human consumption in 31.4% of samples, with those testing positive for coliforms and *Staphylococcus* at unacceptably high levels by Brazilian standards. High levels of microbiological contamination were detected on the hands of the food handlers and mesophytic bacterial counts reached 1.8×10^4 CFU/hand. Interviews were carried out by means of questionnaires to evaluate levels of awareness as to acceptable food handling practices and it was found that 80,1% of vendors had never participated in any kind of training.

Keywords: food quality; food safety; education; evaluation.

Resumo

Este estudo avaliou a qualidade microbiológica de sanduíches e a microbiota nas mãos dos vendedores em Cuiabá - Mato Grosso, Brasil, relacionando com o conhecimento dos comerciantes sobre boas práticas de manipulação, como parte de um amplo programa de pesquisa sobre alimentos de rua no Brasil. O comércio de sanduíche denominado baguncinha é típico na região metropolitana de Cuiabá - Mato Grosso, Brasil. A vigilância sanitária tem problemas estruturais e financeiros para a fiscalização. Cento e cinco amostras de sanduíches foram avaliadas utilizando-se métodos convencionais de determinação de bactérias mesófilas aeróbias e/ou anaeróbias facultativas, contagem de coliformes a 45 °C, estafilococos coagulase positiva, *Bacillus cereus*, clostrídio sulfito-redutor e presença de *Salmonella* spp. Os sanduíches foram classificados como impróprios para o consumo em 31,4% das amostras, com níveis de coliformes e de estafilococos coagulase positiva superiores aos padrões brasileiros. Altos níveis de contaminação microbiológica foram detectados nas mãos dos manipuladores do baguncinha e a contagem de bactérias mesófilas atingiu valores de $1,8 \times 10^4$ UFC/mão. Foram realizadas entrevistas por meio de questionários para avaliar a percepção relacionada ao conhecimento sobre manipulação adequada de alimentos e 80,1% dos vendedores nunca participaram de um treinamento.

Palavra-Chave: qualidade dos alimentos; segurança alimentar; educação; avaliação.

1 Introduction

Data from the bureau Family Budget Research estimate that 25.7% of the average of family outgoings is spent on eating out (Instituto Brasileiro de Geografia e Estatística, 2002). Contaminated food results in economic losses and poses a risk to human health (MUDGIL; AGGARWAL; GANGULI, 2003). Epidemiological data show that the prime cause of intestinal infections is eating from roadside food (MONSUPYE; HOLY, 2000). Department of Health food inspectors encounter logistical and financial difficulties in carrying out inspections of such places (DALLARI et al., 2000). The "Baguncinha" is a typical sandwich of the metropolitan region of the city of Cuiabá - MT, Brazil. It is

an adaptation of the traditional version of the "X-Tudo" (bread, mayonnaise, hamburger, cheese, ham, Frankfurter sausage, egg, lettuce, and tomato) with fewer ingredients. The low price makes the sandwich popular among poor people, particularly adolescents. Even though some ingredients reach a temperature that is ideal to ensure the food is cooked thoroughly, cross-contamination during preparation has been traced back to the use of uncooked vegetables and unhygienic handling (FRANCO, 1996; SOTO et al., 1996; GERMANO M. I. S.; GERMANO P. M. L., 2003; REIJ; AANTREKKER, 2004). Hence, a study was carried out with Brazilian street vendors to verify whether any previous

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food-preparation training have resulted in significant changes in incidences of contamination of food prepared by them (BEZERRA, 2007, 2008). Ineffective educational intervention has worried international health organizations which have encouraged the training of those food handlers (WORLD HEALTH ORGANIZATION, 2006). This study evaluated the microbiology of the “baguncinha” sandwich and the cleanliness of the sandwich makers’ hands in Cuiabá in relation to their knowledge of Good Manufacture Practice (GMP) as part of a wider programme of hygienic and sanitary conditions involved in the preparation of street food in Brazil.

2 Materials and methods

Thirty five snack bars were evaluated in the period from March to July 2005. The sample comprised snack bars chosen at random from a selection situated in districts of urban Cuiabá that had been previously mapped out. The microorganisms researched included those that posed an indirect risk to the consumer’s health, such as fecal coliforms; a direct risk, such as *Salmonella* and *Staphylococcus* (INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATIONS FOR FOODS, 2006), and other indicators of contamination such as *Clostridium* sulphite reductase and *Bacillus cereus* (LANDGRAF, 1996). Three sandwiches with an average weight of 221 g were collected from each point of sale totalizing 105 samples analyzed. The samples were collected under aseptic conditions, wrapped in sterile plastic bags, sealed, appropriately labeled, and kept in ice boxes containing recycled ice (AMERICAN PUBLIC HEALTH ASSOCIATION, 2001).

The 35 vendors were told to wash both their right and left hands in saline solution contained in a sterile plastic bag, and this dirty water was then taken for bacteriological analysis (DESTRO, 1995; KARAM; MIGLIORANZA; OLIVEIRA, 1998).

Vendor awareness regarding to the appropriate hygiene for sandwich preparation was studied via responses to 13 questions (BEZERRA, 2007, 2008) included in the questionnaire (MINAYO, 2000; AZANZA; GATCHALIAN; ORTEGA, 2000; GERMANO, 2003).

The researchers’ presence at the point of sale enabled systematic observation of sandwich handling practices and frequency of hand washing (BEZERRA, 2007, 2008).

Ethical aspects conformed to the Brazilian code of practice (Resolução CNS n.196/1996 (BRASIL, 1996)).

2.1 Sandwich microbiological analysis

The microbiological analyses carried out included isolation and identification of pathogens, following standard procedures, to presumptively quantify *Clostridium* sulfite reducers, *Bacillus cereus*, *Staphylococcus* coagulase positive, the Most Probable Number (MPN) of coliforms at 45 °C, and the presence of *Salmonella* spp. (AMERICAN PUBLIC HEALTH ASSOCIATION, 2001; SILVA; JUNQUEIRA; SILVEIRA, 1997). Twenty-five grams of a sample sandwich were weighed, put in 225 mL of saline peptone solution (SPS) 0.1%, and homogenized for the following procedures.

Coliform counts at 45 °C

The MPN of coliforms was determined using three series of dilutions (10^{-1} to 10^{-3}). Using a sterile pipette, 1 mL of each dilution was transferred into a series of three tubes containing lauryl sulphate tryptose (LST) with Durham tubes and incubated at 36 °C for 48 hours for the presumptive test. All tubes that had become turbid and in which gas had been produced were selected and transferred into tubes containing *E. coli* culture. They were then incubated in a bain-marie (or double-boiler) at 45 °C for 48 hours. The tubes of *E. coli* medium that showed turbidity and gas production were quantified and the MPN of coliforms per gram was determined according to the MPN table for the three tubes (AMERICAN PUBLIC HEALTH ASSOCIATION, 2001; SILVA; JUNQUEIRA; SILVEIRA, 1997).

Differentiation of *Staphylococcus* spp. using the coagulase test

A 0.1 mL extract was drawn off from each of the series of dilutions (10^{-2} to 10^{-4}) and poured into duplicate Petri-dishes containing Baird Parker Agar (BPA), spread with a Drigalsky handle, and incubated at 36 °C for 48 hours. The dishes containing between 20 and 200 colonies were selected and expressed as Colony-Forming Units per gram of sandwich (CFU.g⁻¹). Typical and atypical colonies were tested using Gram staining, catalase, and coagulase tests (AMERICAN PUBLIC HEALTH ASSOCIATION, 2001; SILVA; JUNQUEIRA; SILVEIRA, 1997).

Counts of *Clostridium* sulphite reductase

For the *Clostridium* counts, 0.1 mL of the dilutions 10^{-2} to 10^{-4} were spread on duplicate Petri-dishes containing tryptose sulphite cycloserine agar (TSC) using a Drigalsky handle. After drying completely, the Petri dishes were covered with a layer of TSC and incubated at 35 °C for 24 hours in anaerobic conditions. After incubation, the Petri dishes containing between 20 and 200 of the black colonies that typify *Clostridium* cultures were selected. The result was expressed in Colony-Forming Units per gram of sandwich (CFU.g⁻¹) (AMERICAN PUBLIC HEALTH ASSOCIATION, 2001; SILVA; JUNQUEIRA; SILVEIRA, 1997).

Bacillus cereus counts

An extract of 0.1 mL was taken from the 10^{-2} to 10^{-4} dilutions and spread on duplicate Petri dishes containing mannitol-egg yolk- polymyxin agar (MYP) using a Drigalsky handle. After the surface of the agar had dried completely, the Petri dishes were incubated at 30 °C for 24 hours. Following incubation, dishes containing from 10 to 100 typical *B. cereus* colonies were selected, and at least five were taken for confirmatory testing (APHA, 2001; SILVA; JUNQUEIRA; SILVEIRA, 1997). The result was expressed in Colony-Forming Units per gram of sandwich (CFU.g⁻¹).

Detection of *Salmonella* spp.

Prior to enrichment, 25 g of the sample were homogenized in 225 mL of lactose nutrient broth (LNB) and incubated at 36 °C for 24 hours. Then, 1.0 and 0.1mL of this suspension

were then added to test tubes containing 10 mL of tetrathionate broth (TT) and Rappaport-Vassiliadis (RV) nutrient broth and incubated at 35 and 42 °C for 24 hours, respectively, in baird-parker. Prior to that, a drop of each nutrient broth was spread on Petri dishes of Hoekten Enteric (HE) and Rambach (RAM) agar, previously incubated at 36 °C for 24 hours. Presumptive colonies of *Salmonella* were placed into tubes containing Nutrient Agar (ANI) and incubated at 35 °C for 24 hours. Typical *Salmonella* spp. colonies were subjected to biochemical tests in Triple Sugar Iron (TSI), Lysine Iron (LIA), and Simmons' citrate agar. The TSI and LIA agars were incubated at 35 °C for 24 hours and that of Simmons' citrate at 35 °C for 96 hours. Colonies manifesting biochemical behavior patterns similar to those of *Salmonella* were subjected to agglutination testing using polyvalent somatic anti-*Salmonella* serum (PROBAC) (AMERICAN PUBLIC HEALTH ASSOCIATION, 2001; SILVA; JUNQUEIRA; SILVEIRA, 1997).

2.2 Bacteriological analysis of water vendors' hands were washed in (handwashing water)

Microbiological analyses included standard counts in Petri dishes and quantification of *Staphylococcus* spp. that had been differentiated by testing positive in the coagulase test. For the standard counts in Petri dishes serial dilutions (10^0 to 10^{-2}) of hand washing water (water that vendors had washed both hands in) were made up with 0.1% saline peptone solution. Then, 0.1 mL extracts were spread on duplicate Petri dishes of Plate Count Agar (PCA) using a Drigalsky handle. The Petri dishes were incubated at 35 °C for 48 hours. For the counts, Petri dishes with between 25 and 250 colonies were selected and the results were expressed in Colony-Forming Units per pair of Hands (CFU/H) (AMERICAN PUBLIC HEALTH ASSOCIATION, 2001; SILVA; JUNQUEIRA; SILVEIRA, 1997).

2.3 Interpretation of results

The interpretation of the microbiological results followed standards of the regulations in RDC n.12, which approves the technical regulation of food microbiological standards (ANVISA, 2001). Considering the lack of standards or official specifications concerning the interpretation of data on dirty water from hand washing, the quantification of pathogens from food-handlers' hands was established by adapting recommendations postulated by other authors (ALMEIDA et al. 1995; ANDRADE; SILVA; BRABES, 2003). The results for the levels of awareness regarding good practices in food preparation by sandwich vendors and the systematic observation of the practices employed when handling sandwiches were analyzed (AZANZA; GATCHALIAN; ORTEGA, 2000; BEZERRA, 2007; 2008) by means of the absolute and relative frequencies obtained using the programme SPSS 10.0.

3 Results

Among the burger bars evaluated, eleven (31.4%) were found lacking with respect to standards of hygiene, as shown in Table 1. They showed levels of fecal coliforms and *Staphylococcus* spp. above those allowed by specific Brazilian legislations (BRASIL, 2001).

The presence of *Salmonella* spp. was not detected in any of the sandwich samples analyzed. *Bacillus cereus* and *Clostridium* sulphite reductase were present but met hygiene standards allowed by the Brazilian law (BRASIL, 2001) (Table 1).

The hands of the sandwich makers' showed high levels of microbiological contamination in 80% of samples. The standard counts from the Petri dish cultures varied from $<10^2$ to 1.8×10^4 CFU/hand and the *Staphylococci* counts varied from 1.6×10^2 to 4.4×10^4 CFU/hand.

Questionnaire findings showed that among the vendors of 'baguncinha' sandwiches in Cuiabá, 27 (77.1%) believed they possessed adequate knowledge of what constituted (GMP) good manufacture practice (Table 2). Only 19.9% of the vendors said they had acquired this knowledge through training. The rest mentioned other vendors, television, radio, newspapers, or magazines as sources of information about what constituted good standards of hygiene for the handling of foodstuffs.

According to the results of this study, 11.4% of sandwich samples of burger bars fell short of safe levels for fecal coliforms permitted by Brazilian laws (Table 3).

Only three (8.7%) of the samples of burger bars were contaminated with *Staphylococcus aureus*; however the number of individuals was above the limit set by the RDC n.12 guidelines for sandwiches served hot (BRASIL, 2001), which is 10^3 CFU.g⁻¹ (Table 4).

The hygiene level of contamination on the hands of the sandwich handlers researched was considered unsatisfactory in 80% of the handlers (Table 5).

Table 1. Microbiological profile of 'baguncinha' sandwiches compared with accepted Brazilian standards of hygiene.

Microorganism taxon	Brazilian standard*	"Baguncinha" sandwich data
Coliforms at 45 °C (MPN.g ⁻¹)	10^2	<3 a 887
<i>Staphylococcus</i> spp. (UFC.g ⁻¹)	10^3	10^3 a 1.7×10^6
<i>Bacillus cereus</i> (UFC.g ⁻¹)	10^3	< 10^3
<i>Clostridium</i> sulphite reductase (UFC.g ⁻¹)	10^3	< 10^3
<i>Salmonella</i> spp./25 g	Absent	Absent

*BRASIL/ANVISA/RDC n. 12, 2001.

Table 2. Knowledge level about GMP perceived by sandwiches vendors. Cuiabá - MT (BRAZIL, 2005).

	Frequency	Valid (%)	Cumulative(%)
Valid			
Total	35	32.9	62.9
Partially	5	14.3	77.1
None	8	22.9	100.0
Total	35	100.0	

Table 3. Safe level for coliforms at 45 °C per burger bars in Cuiabá - MT, (BRAZIL, 2005*).

	Frequency	Valid (%)	Cumulative (%)
Valid			
No	4	11.4	11.4
Yes	31	88.6	100.0
Total	35	100.0	

*Permitted under Brazilian law.

Table 4. Number of individuals of *Staphylococcus aureus* (CFU.g⁻¹) of samples per burger bars in Cuiabá - MT (BRAZIL, 2005^a).

		Frequency	Valid (%)	Cumulative (%)
Valid	100.00	32	91.4	91.4
	47000.00	1	2.9	94.3
	52000.00	1	2.9	97.1
	1700000.00	1	2.9	100.0
	Total	35	100.0	

^aPermitted under Brazilian law.**Table 5.** Level of contamination on the hands of the handlers researched in Cuiabá - MT (Brazil, 2005).

		Frequency	Valid (%)	Cumulative (%)
Valid	Acceptable	7	20.0	20.0
	Unacceptable	28	80.0	100.0
	Total	35	100.0	

The maximum time for interviewing and collecting samples of food and hand washing water at the “baguncinha” hamburger bars was six hours per vendor, which corresponded to approximately 75% of the working day of the vendors researched. Although the majority of the food handlers (94.3%) stated that they washed their hands frequently, this behavior was rarely (11.4%) observed.

4 Discussion

Due to the informal nature of street food vending, little has been studied and published about this theme, thus making it relevant to engage in a detailed investigation from the standpoint of hygienic and sanitary quality (CARDOSO; SANTANA; GUIMARÃES, 2007).

Similar studies on the microbiological quality of sandwiches sold on the streets of São Paulo and Rio Grande do Sul showed high levels of contamination by fecal Coliforms (BROD, 2002; LOPES, 2005). These results probably reflect the dirtiness of the water used for hand washing by the sandwich makers, insufficient hand washing, insufficiently-heated food, and unsuitable conditions of ingredients storage. Therefore, the likelihood of contamination by fecal Coliforms in the sandwiches sold on Brazilian roadsides is similar to that which occurs in other countries in Latin America (GARIN et al., 2002).

The data found on the hands of the handlers researched in this study suggest that physical contact between the maker's hands and the sandwich could have been one of the possible causes of contamination by *Staphylococcus aureus*.

The high percentage of handlers with dirty hands was similar to that found by the analysis of hand and fingernail cleanliness of snack vendors in Presidente Prudente - SP (LOPES, 2005). *Staphylococcus aureus* is of great economic importance because apart from causing diseases, it can give rise to asymptomatic infections due to its ability to colonize different regions of an organism (OKURA et al., 2005).

The high rate of contamination of roadside foodstuff vendors' hands also stimulated a study in Jakarta, Indonesia.

Researchers took statements from vendors who claimed (50%) that they always washed their hands before handling food. However, since these authors did not observe actual food handling at the snack bars concerned, they were unable to compare the stated practices (opinion) with observed practices (van KAMPEN et al., 1998).

The disparity between stated practices and those observed concerning the hand washing habits of the “baguncinha” sandwich vendors suggests that the high level of hand contamination might be explained by neglect of this essential step in good practice in the handling of foodstuffs. Similar results were obtained for studies of hot dog vendors in the city of São Paulo, whose authors concluded that the lack of hygiene was due to lack of knowledge (LUCCA; TORRES, 2002, 2006). Other authors have affirmed that the best way to reduce the risk of contamination is to train the people involved in food handling (OLIVEIRA et al., 2006).

It is opportune to emphasize that the likelihood of some consumers prefer badly processed hamburger associated to the precarious knowledge of vendors about GMP and the low microbiological profile of ‘baguncinha’ sandwiches suggests a potential risk of food poisoning.

However, due to the fact that the majority of ‘baguncinha’ sandwich makers believed they had adequate knowledge of good practices, it may be at least partially understood why education has not changed inappropriate handling practices (BEZERRA, 2007, 2008). This fact has important implications for the evaluation of the efficiency of educational intervention which seeks to change unsuitable foodstuff handling practices at the roadside.

The findings from this study point out a need of further investigations about educational programmes for street food handlers, mainly for the “baguncinha” vendors. Furthermore, educational interventions should extrapolate the field of information by including the consideration of values, customs, models, and social symbols that lead to specific forms of behavior and practices. The scientific vision should therefore be set aside and a significant advance be made in understanding the complexity of education and health.

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