

## Research Article

# Meals, Preparation Environment and Hands of Food Handlers - Microbiological Status in Hospital Kitchens

Julia Arantes Galvão<sup>1\*</sup>, Loredana d'Ovidio<sup>2</sup>, Kate Aparecida Buzi<sup>3</sup>, Ricardo Seiti Yamatogi<sup>3</sup>, Vera Lúcia Mores Rall<sup>4</sup> and José Paes de Almeida Nogueira Pinto<sup>3</sup>

<sup>1</sup>Department of Veterinary Medicine, Federal University of Paraná, Curitiba, Brazil

<sup>2</sup>Department of Animal and Food Production, University of the State of Santa Catarina, Lages, Brazil

<sup>3</sup>Department of Veterinary Hygiene and Public Health, Paulista State University, Botucatu, Brazil

<sup>4</sup>Department of Microbiology and Immunology, Botucatu Institute of Biosciences, Paulista State University, Botucatu, Brazil

## Abstract

The aim of this study was to assess the microbiological conditions of ready-to-serve meals, the preparation environment and the hands of food handlers at two public hospital allocated in São Paulo State, Brazil. Were evaluated 480 samples of food, equipment, utensils, drains and hands of the staff from three kitchen sectors of two public hospitals in Brazil. Total coliforms were found in 116 (24.17%) samples, *Escherichia coli* in nine (2.08%) samples and coagulase-positive *Staphylococcus* in 17 (3.54%) samples, two of these strains showed gene encoders for classic enterotoxins production. Coagulase-Negative *Staphylococcus* (CNS) occurred in 98 (20.41%) samples, in which 19 gene encoders for classic enterotoxins production were detected, and *Listeria monocytogenes* occurred in four (0.83%) samples. *Salmonella* were not detected. The microbiological quality of most samples evaluated was considered satisfactory; however, the presence of *L. monocytogenes* and other microorganisms, even at low frequency and with low counts, represents a risk of cross contamination of the food items, which can transmit pathogens to the patients, as well as forming bio film. The great concern is that *Listeria* and CNS were not included at sanitary microbiological standards for foods in Brazil.

**Keywords:** *Escherichia coli*; *Listeria monocytogenes*; Total coliforms; *Staphylococcus*; *Salmonella*

\*Corresponding author: Julia Arantes Galvão, Department of Veterinary Medicine, Federal University of Paraná, Curitiba, Brazil, Tel: +55 4192255697; E-mail: juliaarantesgalvao@gmail.com

**Citation:** Galvão JA, d'Ovidio L, Buzi KA, Yamatogi RS, Rall VLM, et al. (2017) Meals, Preparation Environment and Hands of Food Handlers - Microbiological Status in Hospital Kitchens. J Food Sci Nutr 3: 018.

**Received:** January 31, 2017; **Accepted:** April 28, 2017; **Published:** May 12, 2017

## Introduction

Enteric pathogens are harmless to most of the population, never the less they may cause illness and even death in susceptible individuals, particularly those immune compromised. A total of 1022 outbreaks of nosocomial infections in the United States, United Kingdom, France, Canada, Germany, the Netherlands and Spain were reported from 1966 to 2002 and only 3.32% of these were identified as food borne diseases [1].

However, the data in the literature do not reveal the true incidence of diseases originated from food in hospital units since most cases are not reported. The main factors that contribute to the occurrence of food borne diseases are poor personal hygiene habits of the food handlers, the cooking and storage of food at inappropriate temperatures, the acquisition of raw materials from unreliable sources and the use of poorly sanitized equipment. Since patients have a greater risk of becoming ill when exposed to potential food borne pathogens and given that the food services need to provide a wide variety of foods, it is essential that appropriate food handling practices are maintained [2].

The aim of this study was to evaluate the hygiene-sanitary quality of food prepared in two kitchens at public hospitals in São Paulo State, Brazil, as well as to study the dynamics of the contamination of ready-to-serve meals from utensils, equipment, the environment and the food handlers involved in the process.

## Material and Methods

### Hospital kitchens

Sample collections were carried out at two public hospitals. The Hospital A (HA) showed 467 beds and served approximately 2000 meals/day, the Hospital B (HB) showed 318 beds and served around 1000 meals/day. At each hospital, three specific areas of food preparation were assessed:

- Milk dispensary: an isolated area, with restricted access to staff using special clothing and hand sanitation. Utensils and equipment were restricted to this environment;
- General kitchen: the patients without dietary restrictions, staff and students had the meals prepared here. At HA the equipment and utensils were of exclusive use at this area, while at HB it has shared with the Special Diet Kitchen. The salads served at HA are acquired ready-to-serve, only being divided into portions in this environment, while at HB, the salads are sanitized, prepared and divided into portions in this environment; and
- Special Diet Kitchen, where the meals were prepared for patients with some dietary restriction - diabetic, hypertensive, etc.

### Study protocol

A prospective study was carried out over 10 months, with a monthly collection at each hospital, totalizing 240 samples/hospital, amounting 480 samples.

Around 100 ml/g of each food product (meat, rice, soup, beans, chicken meat, potato, and other cooked food available for patients)

were collected. For the equipment and utensils a smear of the surface delimited by molds, carried out with swabs, which were transfer to tubes containing 10 ml of Lethen broth. It was collected smears from the drains using sterile sponges soaked in 10 ml of Lethen broth and transferred to Whirl-Pak® bags containing 100 ml of Lethen broth.

The staff hands area was measured as shown in figure 1, so rinsed for around 1 min. in Whirl-Pak® bags containing 100 ml of Lethen broth. At each visit at the Milk Dispensary, was collected a sample of milk or substitute at feeding bottle, two swabs of utensils, two swabs of equipment and two rinses of the hands of the staff. At the General Kitchen a hot meal, a cold meal, two swabs of utensils, two swabs of equipment, two rinses of the hands of the staff and a swab of a drain was collected; and in the Special Diet Kitchen the same protocol was designed, except for the cold meal (that was the same). At HB the General and the Special Diet Kitchens shared the utensils and equipment, so the total swabs were sampled from the same place. The samples were stored in isothermal plastic boxes containing recyclable ice and transported to the laboratory where it was analyze on the same day as the collection.

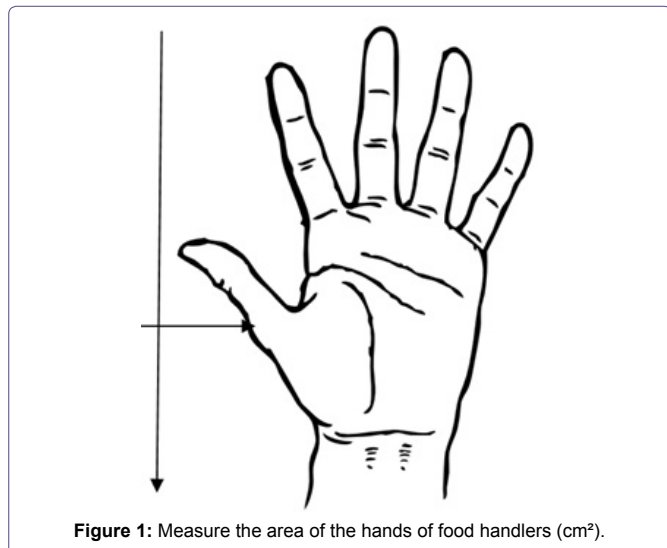


Figure 1: Measure the area of the hands of food handlers (cm²).

### Microbiological analysis

*Salmonella* spp. [3] and *Listeria monocytogenes* [4] were assessed in select samples of food, utensils, equipment, drains and hands of the food handlers. These samples (with the exception of the drains) were either tested for coagulase-positive and coagulase-negative *Staphylococcus* [5,6], total coliforms and *Escherichia coli* by Petrifilm™ EC (3M®).

To test for *Salmonella* spp. variable volumes of 1% buffered peptone water was added to test bags or tubes, observing a ratio of 1:9 (sample/diluent). The same procedure was used to test for *L. monocytogenes*, in this case, employing *Listeria* broth enrichment, and for the enumeration of coagulase-positive and negative *Staphylococcus*, total coliforms and *E. coli* tenfold dilution were carried out in 0.85% saline solution.

All culture media were of the brand Difco®, with the exception of Petrifilm™ EC (3M®) and the agar Cromo Cen *Listeria* (base Agar *Listeria* Ottaviani Agosti; Biocen®).

### Complementary analysis

Testing for entero toxin-encoding genes of *Staphylococcus* A, B, C, D and E were carried out through the Polymerase Chain Reaction (PCR). For the extraction and purification of the genetic material was used Ilustra blood genomic Prep Mini Spin Kit (GE Healthcare®) and the PCR reactions were performed with 500 nMol of each primer (Table 1), one unit of Taq DNA polymerase (Invitrogen®), 2 µMol of MgCl<sub>2</sub>, 200 nMol of dNTP (Invitrogen®), 2.5 µL of PCR buffer (10×) (Invitrogen®), 3 µL of DNA sample, and the required quantity of water free of nucleases (USB®) to give a volume of 25 µL.

Gene	Primer	Sequence	Base pairs	Annealing Temperature
<i>sea</i> <sup>(7)</sup>	SEA-1	ttggaacgggttaaaccgaa	120	50°C
	SEA-2	gaaccttcccatcaaaaaaca		
<i>seb</i> <sup>(7)</sup>	SEB-1	tcgcatcaaacgacaaacg	478	
	SEB-2	gcaggctactctataagtgcc		
<i>sec</i> <sup>(7)</sup>	SEC-1	gacataaaagctaggaattt	257	
	SEC-2	aaatcggattaacattatcc		
<i>sed</i> <sup>(7)</sup>	SED-1	ctagttggtaatatctcct	137	
	SED-2	taatgctatatcttataggg		
<i>see</i> <sup>(8)</sup>	SEE-1	aggttttttcacaggatcatcc	209	
	SEE-2	ctttttttctcgggcaatc		

Table 1: Oligonucleotides and their properties used in the detection of coagulase-positive and coagulase-negative *Staphylococcus* genes, producers of toxins A, B, C, D and E.

Oligonucleotides and their properties used in the detection of coagulase-positive and coagulase-negative *Staphylococcus* genes, producers of toxins A, B, C, D and E.

For the amplification, the Gene Amp PCR System 9700 (Applied Bio systems®) was used, with the following program: 94°C/7 min (initial denaturation), followed by 30 cycles of 94°C/30s, 50°C/30s and 72°C/30s, with a reduction of 1°C per cycle in the annealing phase until reaching 45°C. For the final period, 72°C had applied for 5 min. In all of the reactions the strains ATCC 13565 (*sea*), ATCC 14458 (*seb*), ATCC 19095 (*sec*), FRI 361 (*sed*), and ATCC 27664 (*see*) were used as positive controls and ultrapure water free of nucleases was used as the negative control. Universal primers originating from 16S rRNA forming a product of 371bp has used as the internal control [9].

The products of the PCR reactions were submitted to electrophoresis (Electrophoresis Power Supply Model EPD 600 - Amersham-Pharmacia Biotech Inc.) in 1.5% agarose gel (Prodinasa®) in Tris-boric acid-EDTA 1X (TBE 1X) buffer and developed with 1 µL of SYBR® safe (Invitrogen®)/10 ml of agarose gel. Comparative analysis were carried out with a label of 50 bp (LGC Biotecnologia®), and photographs of the DNA fragments has taken with an image analyzer (AlphaImager - AlphaEase FC Software - AlphaInotech Corporation®). The amplification of the internal control verified the good performance of the PCR and the absence of inhibitory agents in the reaction and extraction.

The *L. monocytogenes* strains were tested in API *Listeria*® (Biomérieux®) and serotyping [10], through pulsed-field gel electrophoresis (PulseNet protocol) at the Pharmaceutical Sciences Department of University of São Paulo (Faculdade de Ciências Farmacêuticas da Universidade de São Paulo).

All the results were interpreted according to the limits established by Brazilian legislation (Table 2). The foods contaminated by *L. monocytogenes* were classified as unsafe products.

Food Group	Recommended Tolerance for Sample			
	Total Coliforms	Coliforms 45°C	Coagulase-Positive Staphylococcus	Salmonella
Ready-to-serve or instant products which will be consumed by children over one year of age after the addition of liquids	20	1	50	Absent
Ready-to-serve or instant products which will be consumed by babies under one year of age after the addition of liquids	10	Absent	Absent	Absent
Infant formulas for premature babies	10	Absent	Absent	Absent
Bottled water for the preparation of feeding bottles	Absent	-	-	-
Pasteurized milk	-	4	-	Absent
Fresh unprocessed vegetables prepared for consumption	-	100	-	Absent
Ready-to-serve meals (ready-to-serve foods of kitchens, restaurants, etc.) based on cooked meat, fish, eggs and so on.	-	20	1000	Absent

Table 2: Sanitary microbiological standards for foods [11].

### Ethical aspects

Considering that the hands of the food handlers were rinsed with culture broth, before collecting the samples the subjects read the “free and informed terms of consent” which highlighted the test conditions and the physical risks to which they would be subjected on participating in the project. After the reading and the clarification of any queries, if the handler agreed to participate in the study, the terms were signed by the food handler and by the researcher. The Ethical Committee in the University approved this procedure.

### Results and Discussion

Total coliforms were found in 116 (24.17%) samples. At HA 52.58% of the samples were contaminated, the Milk Dispensary showed 13.79% of the samples at the range  $1.0 \times 10^0$  to  $8.8 \times 10^3$  CFU/ml or  $\text{cm}^2$ . A feeding bottle was inappropriate [11] for consumption by premature babies or by children both under and over one year of age, since it represents an infant food, which contains  $1.3 \times 10^2$  CFU/ml of total coliforms. At General Kitchen 19.83% of the samples showed  $1.0 \times 10^1$  to  $1.1 \times 10^4$  CFU/g or  $\text{cm}^2$ , and from the Special Diet Kitchen 18.97% of the samples showed  $7.0 \times 10^0$  to  $7.0 \times 10^3$  CFU/g or  $\text{cm}^2$ . At HB Milk Dispensary 8.62% of the samples were contaminated with  $2.0 \times 10^0$  to  $2.0 \times 10^3$  CFU/ml or  $\text{cm}^2$ , from General Kitchen, 11.21% showed  $<1.0 \times 10^1$  to  $2.3 \times 10^3$  CFU/g or  $\text{cm}^2$ , from the Special Diet Kitchen, 7.76% of the samples showed  $1.0 \times 10^1$  to  $9.2 \times 10^3$  CFU/g, and from the utensils and equipment used both in the General and Special Diet Kitchens, 19.83% showed  $1.0 \times 10^0$  to  $1.7 \times 10^4$  CFU/ $\text{cm}^2$ .

Nine (2.08%) samples were contaminated by *E. coli*, and none of these was originated from HA or from the Milk Dispensary of HB. At HA, the general kitchen had 33.33% of the samples with counts by  $<1.0 \times 10^1$  to  $4.0 \times 10^1$  CFU/g, the Special Diet Kitchen 22.22% with  $1.1 \times 10^1$  to  $2.0 \times 10^1$  CFU/g, one of that, a hot meal was inappropriate for consumption, since it was contaminated with  $2.0 \times 10^1$  CFU/g of *E. coli* [11]. Furthermore, 44.44% from the utensils and equipment used in the General and Special Diet Kitchens at HB had  $2.0 \times 10^0$  to  $2.5 \times 10^1$  CFU/ $\text{cm}^2$ .

The results obtained for the total coliforms and *E. coli* counts of the samples collected from the hands of the food handlers reveal that good hygiene practices were adopting in relation to the hands at both units. In another study, of 180 samples analyzed, 8% was contaminate with *E. coli* [12].

Coagulase-positive *Staphylococcus* occurred in 17 (3.54%) samples, two of these strains showed gene encoders for classic enterotoxins production, 12.5% of these samples came from hands of food handlers

at the Milk Dispensary ( $1.0 \times 10^1$  to  $3.0 \times 10^2$  CFU/ $\text{cm}^2$ ), 18.75% from meals and hands of food handlers at the General Kitchen ( $1.0 \times 10^1$  to  $1.0 \times 10^2$  CFU/g or  $\text{cm}^2$ ) and 18.75% from the Special Diet Kitchen ( $9.5 \times 10^1$  to  $1.1 \times 10^2$  CFU/g or  $\text{cm}^2$ ).

At HB the samples contaminated by coagulase-positive *Staphylococcus* was 12.5% originated from the Milk Dispensary (it was a hand of food handler with  $1.0 \times 10^2$  CFU/ $\text{cm}^2$ ), 12.5% from the General Kitchen ( $<1.0 \times 10^2$  to  $1.0 \times 10^2$  CFU/g or  $\text{cm}^2$ ), 18.75% from the Special Diet Kitchen ( $4.3 \times 10^1$  to  $2.0 \times 10^2$  CFU/g or  $\text{cm}^2$ ) and 6.25% from the utensils and equipment used in the General and Special Diet Kitchens (an equipment with  $4.3 \times 10^3$  CFU/ $\text{cm}^2$ ).

The strains with gene encoders for production of classic enterotoxins has detected at a blender (*sea e sec*) from General Kitchen of HA and, at the hands of a food handler (*seb e sec*) from General Kitchen of HB.

Regarding the presence of coagulase-positive *Staphylococcus*, a low frequency of this microorganism was found and when it was present, it did not reach a significant count, considering national legislation [11] and the number of viable cells required for the production of toxins, which is over  $10^5$  CFU/g of food [13].

Other authors, who evaluated 70 samples of salads to be served to hospitalized individuals in Turkey, have obtain results of greater concern, eight (11%) of the samples being contaminated by coagulase-positive *Staphylococcus*, with counts ranging from  $1.0 \times 10^3$  to  $1.0 \times 10^4$  CFU/g<sup>12</sup>. Coagulase-Negative *Staphylococcus* (CNS) occurred in 98 (20.41%) samples, in which 19 gene encoders for classic enterotoxins production were detect (Table 3).

At HA 14.17% of the contaminated samples with CNS came from the Milk Dispensary, none of that was by feeding bottles and the count was by  $3.1 \times 10^1$  to  $1.3 \times 10^4$  CFU/ $\text{cm}^2$ . The General Kitchen had 18.9% of the contaminated samples ( $1.8 \times 10^1$  to  $1.6 \times 10^5$  CFU/g or  $\text{cm}^2$ ), and the Special Diet Kitchen 18.11% of the samples ( $5.1 \times 10^1$  to  $6.8 \times 10^4$  CFU/g or  $\text{cm}^2$ ), totalizing 51.18% of the samples contaminated by coagulase-negative *Staphylococcus*. At HB 14.96% of the contaminated samples had been originated from the Milk Dispensary, in the same way observed in HA none of that was by feeding bottles, and the counts were by  $1.1 \times 10^1$  to  $1.4 \times 10^4$  CFU/ $\text{cm}^2$ . From the General Kitchen were 11.02% of the samples ( $<1.0 \times 10^2$  to  $4.1 \times 10^4$  CFU/g or  $\text{cm}^2$ ), 11.81% from the Special Diet Kitchen ( $3.1 \times 10^1$  to  $5.0 \times 10^4$  CFU/g or  $\text{cm}^2$ ), and 11.02% from the utensils and equipment used in the General and Special Diet Kitchens ( $3.3 \times 10^1$  to  $1.4 \times 10^4$  CFU/g or  $\text{cm}^2$ ).

The high counts of coagulase-negative *Staphylococcus* should not been disregarded, since these micro organisms are potential

Hospital	Toxin gen	Milk Dispensary		General Kitchen		Special Diet Kitchen	
		Sample	Strain	Sample	Strain	Sample	Strain
Hospital A	sea	-	-	-	-	-	-
	seb	-	-	-	-	-	-
	sec	-	-	1 Hand	<i>S. warneri</i>	-	-
	sed	-	-	-	-	-	-
	see	1 Hand	<i>S. warneri</i>	1 Hand	<i>S. warneri</i>	1 Food handler / 2 Hands	<i>S. epidermidis</i>
		-	-	3 Food handler / 4 Hands	<i>S. saprophyticus</i>	3 Food handler / 3 Hands	<i>S. warneri</i>
-		-	1 Tomato sauce	<i>S. saprophyticus</i>	-	-	
Hospital B	sea	-	-	-	-	-	-
	seb	-	-	1 Spoon	<i>S. xylosus</i>	-	-
		-	-	1 Fork	<i>S. xylosus</i>	-	-
		-	-	1 Food handler / 1 Hand	<i>S. saprophyticus</i>	-	-
	sec	1 Food handler / 1 Hand	<i>S. lugdunensis</i>	-	-	1 Food handler / 1 Hand	<i>S. warneri</i>
		-	-	-	-	1 Fork	<i>S. xylosus</i>
	sed	-	-	-	-	-	-
	see	-	-	1 Food handler / 1 Hand	<i>S. caprae</i>	-	-

**Table 3:** Coagulase-negative *Staphylococcus* strains from Hospitals A and B with gene encoders for production of classic enterotoxins.

producers of SE. It has created a great concern, especially because this pathogen was not included at national legislation [11] for the hospital food, hands of staff or kitchens environment, which hinders the implementation of corrective/preventative actions. However, the production of toxins has not tested in this study.

*Listeria monocytogenes* occurred in four (0.83%) samples. It was detected at a drain from HA (serotype 1/2b, 3b, 7), and at two samples of drains (serotype 4a, 4c and 1/2b, 3b, 7) and at an equipment (a blender-serotype not identified) at HB. In addition an equipment (a vase) from HA and a drain from HB were contaminated with *L. innocua*.

There were none ready-to-serve meal contaminated by *L. monocytogenes*. In another study, 29 of 950 sandwiches prepared in a hospital in the United Kingdom were contaminated by *L. monocytogenes*, and for one sample, the count was  $1.2 \times 10^3$  CFU/g [14].

The detection of *Listeria* at the equipment and environment created a great concern, especially because the absence of this pathogen was not included at national legislation (Table 2) for the hospital kitchens environment. An improvement in relation to the sanitation and disinfection of the equipment and drains was poignantly recommend, since the presence of *L. monocytogenes* and yours marker (*L. innocua*) is unacceptable particularly at an environment that prepares meals for hospitalized individuals.

There was none sample contaminated by *Salmonella* spp. The absence of pathogens in the final meals, as well as the low counts for *E. coli*, detected only in the cold meals and within the standards established by legislation [11], lead to the conclusion that the units present good hygiene-sanitary control in relation to their operations.

So, the microbiological quality of most samples evaluated has considered satisfactory; however, the presence of *L. monocytogenes* and other microorganisms, even at low frequency and with low counts, represents a risk of cross contamination of the food items, which can transmit pathogens to the patients, as well as the possible formation of a biofilm.

The detection of *Listeria* at the equipment and environment created a great concern, especially because this pathogen was not included at national legislation (Table 2) for the hospital kitchens environment.

An improvement in relation to the sanitation and disinfection of the equipment and drains was poignantly recommend, since the presence of *L. monocytogenes* and yours marker (*L. innocua*) is unacceptable particularly at an environment that prepares meals for hospitalized individuals.

## Acknowledgment

We thank for Maria Teresa Destro (Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Departamento de Alimentos e Nutrição Experimental) for confirmation and serotyping of *Listeria* strains. We also thank the nutritionists and the hospital management for permit this study.

## References

- Gastmeier P, Stamm-Balderjahn S, Hansen S, Nitzschke-Tiemann F, Zschneid I, et al. (2005) How outbreaks can contribute to prevention of nosocomial infection: Analysis of 1022 outbreaks. Infect Control Hosp Epidemiol 26: 357-361.
- Réglier-Poupert H, Paraina C, Beauvais R, Descamps P, Gillet H, et al. (2005) Evaluation of the quality of hospital food from the kitchen to the patient. J Hosp Infect 59: 131-137.
- Andrews WH, June GA, Sherrod PS, Hammack TS, Amaguana, et al. (1998) Food and Drug Administration. Bacteriological Analytical Manual, Gaithersburg: AOAC International, Maryland, USA.
- Pagotto F, Daley E, Farber JM (2011) Enumeration of *Listeria monocytogenes* in food. Compendium of analytical methods, MFLP-74. Health Canada, Ontario, Canada.
- Ministry of Agriculture, Livestock and Supply (2003) Normative Instruction N° 62. Methods for microbiological analysis of food of animal origin and water. Brasília, Brazil.
- Cunha ML, Sinzato YK, Silveira LV (2004) Comparison of Methods for the Identification of Coagulase negative *Staphylococci*. MemInst Oswaldo Cruz 99: 855-860.

7. Johnson WM, Tyler SD, Ewan EP, Ashton FE, Pollard DR, et al. (1991) Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin1 in *Staphylococcus aureus* by the polymerase chain reaction. *J Clin Microbiol* 29: 426-430.
8. Mehrotra M, Wang G, Johnson WM (2000) Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J Clin Microbiol* 38: 1032-1035.
9. Greisen K, Loeffelholz M, Purohit A, Leong D (1994) PCR primers and probes for the 16S rRNA gene of most species of pathogenic bacteria, including bacteria found in cerebrospinal fluid. *J Clin Microbiol* 32: 335-351.
10. Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P (2004) Differentiation of the Major *Listeria monocytogenes* Serovars by Multiplex PCR. *J Clin Microbiol* 42: 3819-3822.
11. Ministry of Health Resolution (2001) RDC N° 12. Technical Regulation on microbiological standards for foods, Brazil.
12. Ayçiçek H, Sarimehmetoglu B, Çakiroglu S (2004) Assessment of microbiological quality of meals sampled at the meal serving units of a military hospital in Ankara, Turkey. *Food Control* 15: 379-384.
13. Tranter HS (1990) Foodborne *Staphylococcal* illness. *Lancet* 336: 1044-1046.
14. Meldrum RJ, Smith RM (2007) Occurrence of *Listeria monocytogenes* in sandwiches available to hospital patients in Wales, United Kingdom. *J Food Protect* 70: 1958-1960.