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

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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 detect, and Listeria monocytogenes occurred in four (0.83%) samples. Salmonella were not detect. 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 micro biological standards for foods in Brazil.

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

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 no socomial infections in the United States, United Kingdom, France, Canada, Germany, the Netherlands and Spain were report 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 handler, 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 isothemic plastic boxes containing recyclable ice and transported to the laboratory where it was analyze on the same day as the collection.

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 selective media, 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 isothemic plastic boxes containing recyclable ice and transported to the laboratory where it was analyze on the same day as the collection.

Complementary analysis

Testing for enterotoxin-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 Illustra 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\textsubscript{2}, 200 nMol of dNTP (Invitrogen®), 2.5 µL of PCR buffer (10x) (Invitrogen®), 3 µL of DNA sample, and the required quantity of water free of nucleases (USB®) to give a volume of 25 µL.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence</th>
<th>Base pairs</th>
<th>Annealing Temperature</th>
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<td>SEE-2</td>
<td>gcaggtactctataagtgcc</td>
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</table>

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 (sec), ATCC 19095 (see), FRI 361 (sed), and ATCC 27664 (sec) 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® Green (Invitrogen®), 3 µL of DNA sample, and the required quantity of water free of nucleases (USB®) to give a volume of 25 µL.

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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.

Figure 1: Measure the area of the hands of food handlers (cm²).
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 1.0×10⁰ to 8.8×10⁰ CFU/ml or cm². 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 1.3×10⁵ CFU/ml of total coliforms. At General Kitchen 19.83% of the samples showed 1.0×10¹ to 1.1×10² CFU/g or cm², and from the Special Diet Kitchen 18.97% of the samples showed 7.0×10⁰ to 7.0×10² CFU/g or cm². At HB Milk Dispensary 8.62% of the samples were contaminated with 2.0×10⁰ to 2.0×10¹ CFU/ml or cm² from General Kitchen, 11.21% showed <1.0×10¹ to 2.3×10¹ CFU/g or cm², from the Special Diet Kitchen, 7.76% of the samples showed 1.0×10¹ to 9.2×10¹ CFU/g, and from the utensils and equipment used both in the General and Special Diet Kitchens, 19.83% showed 1.0×10¹ to 1.7×10⁴ CFU/cm².

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 <1.0×10⁰ to 4.0×10⁰ CFU/g, the Special Diet Kitchen 22.22% with 1.1×10¹ to 2.0×10¹ CFU/g, one of that, a hot meal was inappropriate for consumption, since it was contaminated with 2.0×10¹ 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 2.0×10⁰ to 2.5×10¹ CFU/cm².

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 contaminant 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 (1.0×10¹ to 3.0×10⁰ CFU/cm²), 18.75% from meals and hands of food handlers at the General Kitchen (1.0×10⁰ to 1.0×10² CFU/g or cm²) and 18.75% from the Special Diet Kitchen (9.5×10⁶ to 1.1×10⁻² CFU/g or cm²).

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 1.0×10⁷ CFU/cm³), 12.5% from the General Kitchen (<1.0×10⁰ to 1.0×10² CFU/g or cm²), 18.75% from the Special Diet Kitchen (4.3×10¹ to 2.0×10² CFU/g or cm²) and 6.25% from the utensils and equipment used in the General and Special Diet Kitchens (an equipment with 4.3×10⁷ CFU/cm³).

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 (sea 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 10⁵ 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 1.0×10⁰ to 1.0×10⁴ CFU/g. 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 3.1×10⁰ to 1.3×10⁴ CFU/cm². The General Kitchen had 18.9% of the contaminated samples (1.8×10⁰ to 1.6×10⁴ CFU/g or cm²), and the Special Diet Kitchen 18.11% of the samples (5.1×10⁶ to 6.8×10⁴ CFU/g or cm²), totaling 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 1.1×10⁶ to 1.4×10⁴ CFU/cm². From the General Kitchen we were 11.02% of the samples (<1.0×10⁶ to 4.1×10⁴ CFU/g or cm²), 11.81% from the Special Diet Kitchen (3.1×10⁴ to 5.0×10⁴ CFU/g or cm²), and 11.02% from the utensils and equipment used in the General and Special Diet Kitchens (3.3×10⁴ to 1.4×10⁵ CFU/g or cm²).

The high counts of coagulase-negative Staphylococcus should not be disregarded, since these microorganisms are potential

\[\text{Table 2: Sanitary microbiological standards for foods [11].}\]

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Recommended Tolerance for Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Coliforms</td>
</tr>
<tr>
<td>Ready-to-serve or instant products which will be consumed by children over one year of age after the addition of liquids</td>
<td>20</td>
</tr>
<tr>
<td>Ready-to-serve or instant products which will be consumed by babies under one year of age after the addition of liquids</td>
<td>10</td>
</tr>
<tr>
<td>Infant formulas for premature babies</td>
<td>10</td>
</tr>
<tr>
<td>Bottled water for the preparation of feeding bottles</td>
<td>Absent</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td>-</td>
</tr>
<tr>
<td>Fresh unprocessed vegetables prepared for consumption</td>
<td>-</td>
</tr>
<tr>
<td>Ready-to-serve meals (ready-to-serve foods of kitchens, restaurants, etc.) based on cooked meat, fish, eggs and so on.</td>
<td>-</td>
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</tbody>
</table>
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 been 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 addiction 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 contaminate by *L. monocytogenes*, and for one sample, the count was 1.2×10³ 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.

**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**


