



## Molecular identification and microbiological evaluation of isolates from equipments and food contact surfaces in a hospital Food and Nutrition Unit

S. Pieniz<sup>a\*</sup>, D. F. Rodrigues<sup>a</sup>, R. M. Arndt<sup>a</sup>, J. F. Mello<sup>a</sup>, K. L. Rodrigues<sup>a</sup>, R. Andreazza<sup>b</sup>,  
F. A. O. Camargo<sup>c</sup> and A. Brandelli<sup>d</sup>

<sup>a</sup>Departamento de Nutrição, Faculdade de Nutrição, Programa de Pós-graduação em Ciências Ambientais, Universidade Federal de Pelotas – UFPEL, Rua Gomes Carneiro, 1, CEP 96010-610, Pelotas, RS, Brasil

<sup>b</sup>Centro de Engenharias, Curso de Engenharia Ambiental e Sanitária, Programa de Pós-graduação em Ciências Ambientais, Universidade Federal de Pelotas – UFPEL, Rua Benjamin Constant, 989, CEP 96010-020, Pelotas, RS, Brasil

<sup>c</sup>Departamento de Ciência do Solo, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul – UFRGS, Avenida Bento Gonçalves, 7712, CEP 91540-000, Porto Alegre, RS, Brasil

<sup>d</sup>Departamento de Ciência dos Alimentos, Instituto de Ciência e Tecnologia de Alimentos – ICTA, Universidade Federal do Rio Grande do Sul – UFRGS, Avenida Bento Gonçalves, 9500, Campus do Vale, CEP 91501-970, Porto Alegre, RS, Brasil

\* e-mail: [nutrisimone@yahoo.com.br](mailto:nutrisimone@yahoo.com.br)

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### Abstract

The hygienic and sanitary control in Food and Nutrition Units (FNU) is considered a standard procedure to produce adequate meals and reduce the risk of foodborne diseases and hospital infections. This study aimed to evaluate the isolation and identification of bacteria from equipment and food contact surfaces in a hospital FNU as well as to evaluate the sanitary condition. Likewise, it was analyzed the adhesion of the microorganisms on polyethylene cutting boards. The presence of aerobic mesophilic microorganisms, yeasts, molds, coagulase-positive staphylococci, coliform and fecal coliform, and *Escherichia coli* were analyzed on eating tables, countertop surfaces and cutting boards used for meat or vegetable handling, and equipment such as microwaves and refrigerators. The molecular identification it was done by 16S rRNA gene sequencing. The adhesion of the microorganisms (biofilm formation) on meat and vegetable cutting boards was also evaluated by scanning electron microscopy. The results showed high numbers of all microorganisms, except for *E. coli*, which was not observed in the samples. The molecular analysis identified species of the Enterobacteriaceae family and species of the Pseudomonadaceae family. Scanning electron microscopy analyses revealed bacterial adhesion on the cutting board surfaces. The results obtained in this study indicated that the hygienic conditions of surfaces like plastic cutting boards and equipment in this hospital FNU were inadequate. The achievement and application of standard operating procedures could positively help in the standardization of sanitary control, reducing the microbial contamination and providing a safe food to hospitalized patients.

**Keywords:** food safety, hygienic-sanitary control, hospital food, foodborne diseases, microbial biofilms.

### Avaliação microbiológica e identificação molecular de isolados de equipamentos e superfícies de contato com alimentos em um hospital Unidade de Alimentação e Nutrição

#### Resumo

O controle higiênico e sanitário nas Unidades de Alimentação e Nutrição (UAN) é considerado um procedimento padrão para produzir refeições adequadas e reduzir o risco de doenças transmitidas pelos alimentos e infecções hospitalares. Este estudo teve como objetivo isolar e identificar bactérias de equipamentos e superfícies de contato com alimentos em uma UAN hospitalar, bem como avaliar a condição sanitária. Do mesmo modo, analisou-se a adesão dos micro-organismos em tábuas de corte de polietileno. A presença de micro-organismos aeróbios mesófilos, leveduras, fungos, *Staphylococcus* coagulase-positivos, coliformes, coliformes fecais e *Escherichia coli* foi analisadas na superfície de mesas do refeitório, superfícies de bancada e tábuas de corte usadas para manuseio de carne ou vegetais e, em equipamentos como micro-ondas e refrigeradores. A identificação molecular foi feita pelo sequenciamento do gene 16S rRNA. A adesão dos micro-organismos (formação de biofilmes) em tábuas de corte de carne e de vegetais também foi avaliada por microscopia eletrônica de varredura. Os resultados mostraram elevada contagem para todos os micro-organismos analisados, exceto para *E. coli*, a qual não foi observada nas amostras. A análise molecular

identificou espécies da família Enterobacteriaceae e Pseudomonadaceae. A análise de microscopia eletrônica de varredura revelaram adesão bacteriana nas superfícies das placas de corte. Os resultados obtidos neste estudo indicaram que as condições higiênicas das superfícies e de equipamentos nesta UAN hospitalar estavam inadequadas. A aplicação de procedimentos operacionais padrão poderia auxiliar positivamente na padronização do controle higiênico-sanitário, reduzindo a contaminação microbiana e fornecendo um alimento seguro para pacientes hospitalizados.

*Palavras-chave:* segurança alimentar, controle higiênico-sanitário, alimentos hospitalares, doenças transmitidas por alimentos, biofilmes microbianos.

## 1. Introduction

Good personal hygiene and good practices on sanitary handling at work are an essential part of any prevention program for food safety. Also, it is important the handlers of food have the skills and knowledge to handle safely the food, because the human handling errors have been generally implicated in outbreaks of food poisoning (Gaungoo and Jeewon, 2013). *Staphylococcus aureus*, *Escherichia coli* and spore forming bacteria are the main bacteria causing foodborne illness in hospital (Steinbrecher et al., 2000; Jalalpour, 2013). *S. aureus* is the most common gram-positive bacterium causing nosocomial infections (NIs) (Steinbrecher et al., 2000), and *E. coli* is the most common gram-negative bacterium, causing mainly NIs (Jalalpour, 2013). Poor sanitation of food contact surfaces, equipments, and processing environments have been an important factor in foodborne outbreaks. Surfaces cleaned improperly promote food debris and, with the presence of water, it contributes to the development of bacterial biofilms, which may contain pathogenic microorganisms (Chmielewski and Frank, 2003).

Microbial adhesion and biofilm formation have great importance for the food industry and occur on a variety of food contact surfaces (Oliveira et al., 2010), such as stainless steel and polyurethane. Therefore, surfaces of household and workplace such as sinks, countertops, toilets, and cutting boards can act as storage and source of pathogenic bacteria (Rao et al., 2005). So, the purpose of this study was to evaluate the microbiological status on surfaces and equipments used in food handling and food preparation as well as identify molecularly the isolates and also assess the presence of bacterial biofilms in polyethylene cutting boards in a hospital Food and Nutrition Unit (FNU).

## 2. Materials and Methods

This study took place in a hospital FNU in Pelotas City, Southern Brazil. Microbiological analyses were performed at the Laboratory of Food Microbiology at the College of Nutrition, Federal University of Pelotas (UFPEL) and the scanning electron microscopy (SEM) was done at the Center of Electronic Microscopy at the Federal University of Rio Grande do Sul (UFRGS).

### 2.1. Samples and conditions

Triplicate samples were sampled in twice with an interval of fifteen days and were performed after the daily cleaning of FNUs. The areas selected were countertop of

meat handling area, countertop of vegetable handling area, microwave, refrigerator, eating table and polyethylene cutting boards. Microorganisms were removed from surfaces considered cleaned and sanitized with sodium hypochlorite (100 ppm) by the swab technique. A sterile swab (0.5 cm diameter/2 cm long) was moistened in sterile saline solution (0.85%) and was rubbed back-and-forth three times forming an angle of 30° with the surface in a 50 cm<sup>2</sup> area to collect microorganisms. Swabs were transferred to 10 mL sterile saline solution (0.85% NaCl) and transported under refrigeration to the laboratory where they were immediately processed. Tubes were vortexed at high speed for 2 min in 10 s bursts and decimal dilutions in sterile saline solution (0.85% NaCl) (10<sup>-1</sup> to 10<sup>-3</sup>) were performed.

### 2.2. Determination of aerobic mesophilic microorganisms and coagulase-positive staphylococcus

For enumeration of aerobic mesophilic microorganisms, 1 mL of each dilution (10<sup>-1</sup> to 10<sup>-3</sup>) was added to petri dishes sterile and 20 mL of Plate Count Agar (PCA, Merck, Darmstadt, Germany). For isolation of coagulase-positive staphylococci, 0.1 mL of each dilution was surface plated onto Baird-Parker Agar (BP, Merck). Plates were incubated at 35 ± 2 °C for 48 h. The number of colony forming units (CFU) per cm<sup>2</sup> was obtained by multiplying the number of colonies by the dilution factor and divided by the product area. Confirmation as *Staphylococcus* isolates was performed by the coagulase test in accordance to Carpentier et al. (2012).

### 2.3. Determination of yeasts and molds

Each dilution was inoculated (0.1 mL) onto the surface of Potato Dextrose Agar (PDA, Merck) containing 10% (w/v) tartaric acid. Thereafter the plates were inverted and incubated at 25 ± 0.5 °C for 120 h. Following the incubation, colonies were counted and results expressed as CFU/cm<sup>2</sup>.

### 2.4. Determination of the Most Probable Number (MPN/cm<sup>2</sup>) of coliform

For determination of coliforms and fecal coliforms, 1 mL of each dilution was inoculated into three sets of three tubes containing Lauryl Sulfate Tryptose (LST, Merck) broth. After incubation at 35 ± 0.5 °C for 48 h, tubes showing gas production were considered positive. The positive results observed in LST broth were inoculated into Brilliant Green broth (Merck) for analysis of coliforms, which were incubated at 35 ± 0.5 °C for 48 h; and likewise,

the positive LST tubes were inoculated into *E. coli* (EC) broth for analysis of fecal coliforms, which were incubated at 44.5 °C for 24 h. For confirmation of *E. coli*, EC positive tubes were inoculated on plates containing Eosin Methylene Blue agar (EMB, Merck), and then incubated at 35 °C for 24 h. Subsequently, typical colonies were inoculated on PCA plates and incubated at 35 °C for 24 h. After the incubation period, the biochemical identification of typical colonies was performed in Koser Citrate broth (Difco, Sparks, MD), Methyl Red (MR, Difco) and Voges-Proskauer (VP, Merck) broth and 1% Tryptone broth. Results were expressed as MPN of coliforms, fecal coliform and *E. coli* /cm<sup>2</sup> (Feng et al., 2002).

### 2.5. Analysis of bacterial adherence in plastic cutting boards

This analysis was adapted from Di Bonaventura et al. (2008). Two polyethylene boards used for cutting meats and vegetables were analyzed in this study. Each polyethylene board was cut in the central part into four pieces of 1.2 cm<sup>2</sup>. Two pieces of each board were stored in sterile bags and kept under refrigeration, and two pieces were added in 50 mL of Brain Heart Infusion (BHI, Oxoid, Germany) broth and incubated for 48 h at 35 °C to induce biofilm formation. After the incubation, non adherent cells were removed by dipping each sample three times in sterile phosphate-buffered saline (PBS; pH 7.3). All pieces were further utilized for SEM analysis.

### 2.6. Scanning Electron Microscopy (SEM)

The SEM was performed to evaluate bacterial adhesion to the plastic cutting boards and the formation of a conditioning film above mentioned on the surface of the material. Samples were immersed in a fixing solution containing 25% (w/v) of glutaraldehyde, 0.2 M of phosphate buffer and distilled water. After 7 days, the samples were washed 3 times with 0.2 M of phosphate buffer and distilled water (1:1) by immersion for 30 min in each wash process. Then, the samples were dried (Tondo et al., 2010). The drying was performed on samples with increasing concentrations of acetone (30% for 10 min, 50% for 10 min, 70% for 10 min, 90% for 10 min, 90% for 20 min, 100% for 10 min and 100% for 20 min). The samples were then submitted to critical point drying with the aid of liquid CO<sub>2</sub> (Critical Point Dryer - Blazers CPD030®), and coated with gold in the Sputter Coater machine (Blazers SCD050®). After gold metallization, the samples were observed in a scanning electron microscope JEOL® JSM-6060 (Jeol, Tokyo, Japan).

### 2.7. DNA amplification by Polymerase Chain Reaction (PCR)

The 16S rRNA gene of the isolates were amplified using universal primers 27F (5'-AGATTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTACGACTT-3') (Lane, 1991). The reactions were prepared containing 17 µL of special mix for PCR (GoTaq- Promega, Madison, WI USA), 1 µL of each primers, 1 µL of 1 µL sample and ultra pure water (milli-Q), totaling 20 µL final volume. The 16S rRNA genes were amplified using the following thermal protocol:

initial denaturation 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 0.5 min, annealing at 50 °C for 1 min and extension at 72 °C for 1 min; and final extension at 72 °C 5 min. After, the amplified PCR products were analyzed on 1% agarose gel electrophoresis. After electrophoresis DNA the samples was staining with Syber Safe (Invitrogen®) and visualized by ultraviolet transilluminator. Millipore Montage PCR filter units (Millipore, Billerica, MA) were used to remove primers salts, and unincorporated dNTPs according to the manufacturer's instructions except that an additional 400 µL of sterile nuclease free water was added to wash off residual PCR ingredients.

### 2.8. DNA sequencing and phylogenetic analysis

DNA polymerase-mediated amplification of templates in the presence of mixtures of dNTPs, fluorescently-labeled dideoxynucleotide triphosphates and primer 519r (5'-GWATTACCGCGGCKGCTG-3') using Sanger (BigDye) terminator kit (Applied Biosystems). Extension products were fractionated by capillary electrophoresis using Applied Biosystems 3730-XL DNA sequencing machine following the manufacturers' instructions.

The 16S sequences obtained were submitted to the BLAST search program of the National Center for Biotechnology Information website (NCBI, <http://www.ncbi.nlm.nih.gov>) to search for homologous sequences. BioEdit (Hall, 1999) was used to edit sequences and a 16S-based phylogenetic tree was inferred from Clustal X alignments using the Neighbor-joining method (Saitou and Nei, 1987) in MEGA version 5.0 (Tamura et al., 2011). Genetic distance was calculated based on Kimura two-parameter model of nucleotide evolution (Kimura, 1980). The support of nodes was assessed with 1.000 bootstrap replications (Felsenstein, 1985).

## 3. Results

It was selected isolates from vegetable and meat countertop, and vegetable and meat cutting board for molecular identification as shown in Table 1. The molecular analysis identified species of the Enterobacteriaceae family as *Enterobacter aerogenes*, *Raoultella ornithinolytica*, *Klebsiella pneumoniae* and *Escherichia coli* and species of the Pseudomonadaceae family as *Pseudomonas aeruginosa* (Figure 1), showing 99% and 100% similar to sequences in the Genbank. Nucleotide sequence of isolates were submitted to the GenBank under accession number describe in Table 1.

The microbiological quality of food handling surfaces and equipment was investigated based on the parameters established by APHA (2001) for aerobic mesophilic microorganisms, mold and yeast, and coagulase-positive *Staphylococcus* (Table 2). For all surfaces (0.2 x 10<sup>1</sup> CFU/cm<sup>2</sup>) and equipments (1 x 10<sup>2</sup> CFU/cm<sup>2</sup>) studied, the CFU values of mesophilic aerobic microorganisms were higher than that required by the APHA (2001). This represents an unsatisfactory hygienic condition on food handling surfaces and equipments. Among the evaluated samples, plastic cutting boards used for meat or vegetable handling surprised by

**Table 1.** Identification of isolates by sequencing 16S rRNA.

Isolated	Source	Species	Family	Similarity	bp*	**Access#
B1	Vegetable countertop	<i>Enterobacter aerogenes</i>	Enterobacteriaceae	100%	446	KY497462
B2	Meat countertop	<i>Enterobacter aerogenes</i>	Enterobacteriaceae	99%	444	KY497463
B3	Meat countertop	<i>Raoultellaornithinolytica</i>	Enterobacteriaceae	99%	437	KY497464
B4	Vegetable cutting boards	<i>Escherichia coli</i>	Enterobacteriaceae	99%	419	KY497465
B5	Vegetable countertop	<i>Klebsiella pneumoniae</i>	Enterobacteriaceae	99%	450	KY497466
B6	Meat cutting boards	<i>Klebsiella pneumoniae</i>	Enterobacteriaceae	99%	447	KY497467
B7	Vegetable cutting boards	<i>Pseudomonas aeruginosa</i>	Pseudomonadaceae	99%	456	KY497468

\*bp: base pairs.\*\*Access#: GenBank accession number.

**Table 2.** Analysis of indicator microorganisms on food handling surfaces and equipment in a hospital food and nutrition unit at the first (1<sup>st</sup>) and second (2<sup>nd</sup>) sampling.

Sampling	Mesophilic		Yeasts and molds		Coagulase-positive <i>Staphylococcus</i>	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
	----- CFU/cm <sup>2</sup> -----					
Meat countertop	5.7 x 10 <sup>3</sup> ± 410	1.4 x 10 <sup>4</sup> ± 240	8.1 x 10 <sup>1</sup> ± 47	9.2 x 10 <sup>1</sup> ± 16	*	9.4 x 10 <sup>2</sup> ± 131
Vegetable countertop	6.3 x 10 <sup>3</sup> ± 94	7.1 x 10 <sup>3</sup> ± 183	2.0 x 10 <sup>2</sup> ± 10	1.3 x 10 <sup>3</sup> ± 12	*	8.0 x 10 <sup>2</sup> ± 75
Refrigerator	1.2 x 10 <sup>4</sup> ± 205	5.8 x 10 <sup>2</sup> ± 113	7.4 x 10 <sup>1</sup> ± 17	7.8 x 10 <sup>1</sup> ± 15	*	6.4 x 10 <sup>2</sup> ± 122
Microwave	3.3 x 10 <sup>2</sup> ± 64	2.4 x 10 <sup>2</sup> ± 40	3.2 x 10 <sup>1</sup> ± 03	5.4 x 10 <sup>1</sup> ± 10	*	*
Eating table	3.8 x 10 <sup>3</sup> ± 250	3.5 x 10 <sup>3</sup> ± 185	2.3 x 10 <sup>2</sup> ± 25	4.0 x 10 <sup>1</sup> ± 06	*	*
Meat cutting board	4.8 x 10 <sup>4</sup> ± 162	1.3 x 10 <sup>4</sup> ± 140	3.1 x 10 <sup>1</sup> ± 09	1.5 x 10 <sup>3</sup> ± 28	*	5.5 x 10 <sup>2</sup> ± 11
Vegetable cutting board	1.2 x 10 <sup>4</sup> ± 273	6.1 x 10 <sup>4</sup> ± 205	1.3 x 10 <sup>2</sup> ± 66	1.5 x 10 <sup>2</sup> ± 11	*	1.1 x 10 <sup>3</sup> ± 94

\* CFU values under the specified by APHA (2001), below the limit of 0.2 x 10<sup>1</sup> CFU/cm<sup>2</sup> for countertops surface or 1 x 10<sup>2</sup> CFU/cm<sup>2</sup> for equipment. The samples was collected in triplicate in the 1<sup>st</sup> and 2<sup>st</sup> analysis and the results were expressed in media CFU/cm<sup>2</sup> ± standard deviations. n=3.

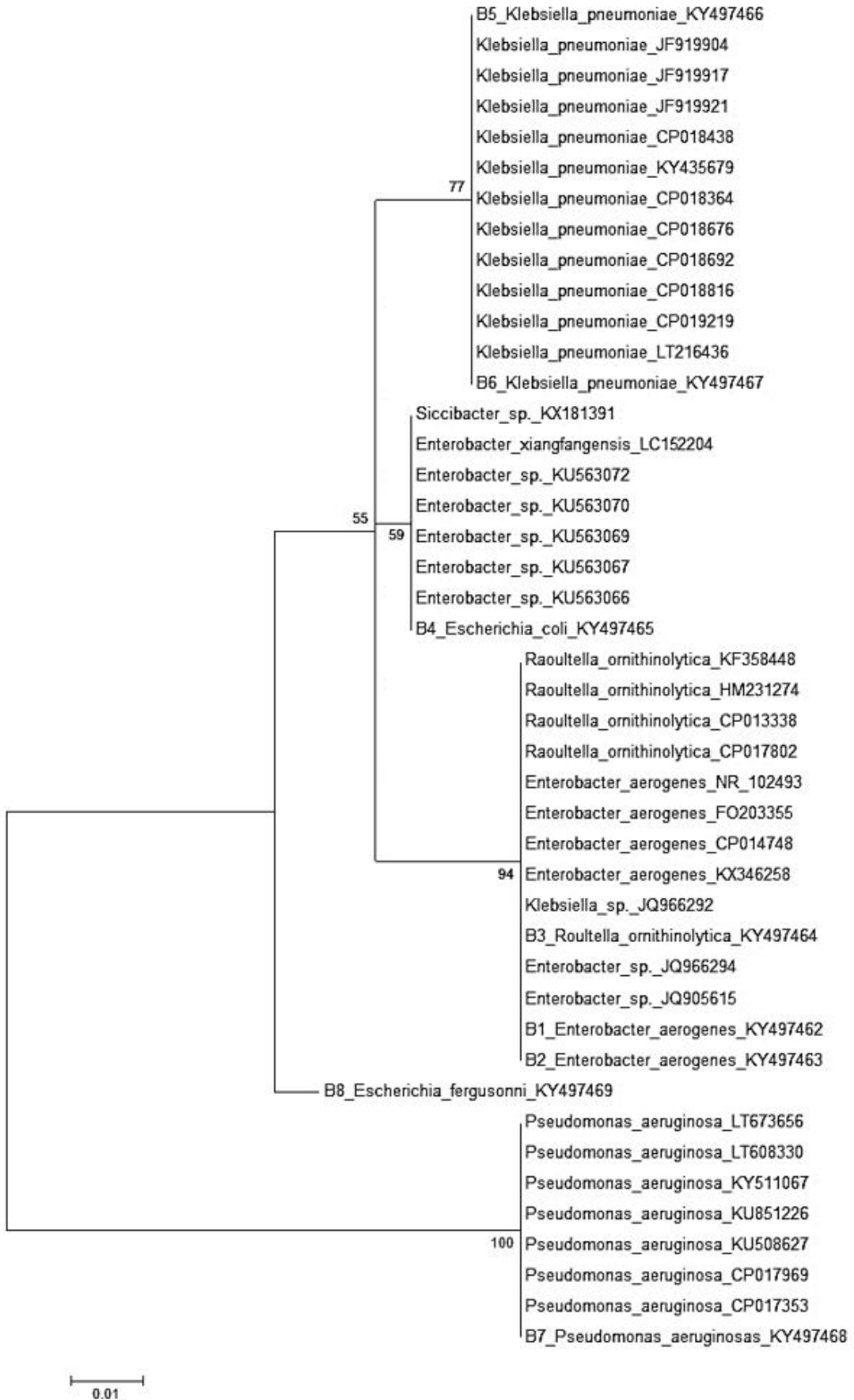
the high CFU values, reaching 6.1 x 10<sup>4</sup> ± 205 CFU/cm<sup>2</sup> in the second sampling for vegetable cutting board, and a similar value in the first sampling for vegetable cutting board (4.8 x 10<sup>4</sup> ± 162 CFU/cm<sup>2</sup>).

Yeasts and molds exhibited high CFU values for samples from countertops and plastic boards used for meat and vegetable handling, and refrigerator (Table 2). A CFU reduction from the first to the second sampling was exhibited in the meat handling countertop (from 8.1 x 10<sup>2</sup> ± 47 to 9.2 x 10<sup>1</sup> ± 16 CFU/cm<sup>2</sup>), in the refrigerator (from the 7.4 x 10<sup>1</sup> ± 17 to the 7.8 x 10<sup>1</sup> ± 15 CFU/cm<sup>2</sup>), in the eating table (from the 2.3 x 10<sup>2</sup> ± 25 to 4.0 x 10<sup>1</sup> ± 06 CFU/cm<sup>2</sup>) and in the vegetable cutting board (from 1.3 x 10<sup>3</sup> ± 66 to 1.5 x 10<sup>2</sup> ± 11 CFU/cm<sup>2</sup>). On contrast, increased CFU values were observed in the vegetable handling countertop (from 2.0 x 10<sup>2</sup> ± 10 to 3 x 10<sup>3</sup> ± 12 CFU/cm<sup>2</sup>) and in the meat cutting board (from 3.1 x 10<sup>1</sup> ± 09 to 1.5 x 10<sup>3</sup> ± 28 CFU/cm<sup>2</sup>).

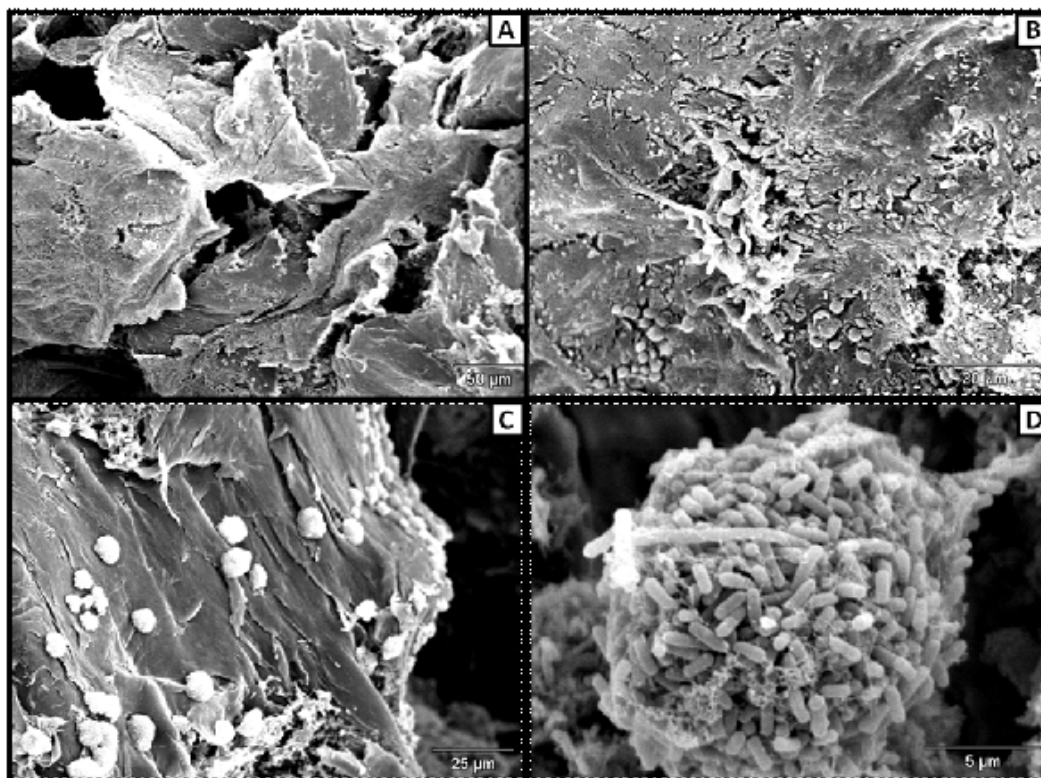
The analysis of coagulase-positive *Staphylococcus* showed that the microwave and the eating table were efficiently sanitized through the cleaning practices in both samples (Table 2). This result is important for the specific pathogenic characteristics of this microorganism as a source of NIs. However, the presence of coagulase-positive *Staphylococcus* was showed in the other samples with values achieving 1.1 x 10<sup>3</sup> ± 94 CFU/cm<sup>2</sup> on the vegetable cutting board.

Among the samples analyzed for coliforms and *E. coli*, only those from refrigerator and eating table had MPN values under the range values recommended by the APHA (Table 3). The countertop and cutting boards used for handling meat or vegetables showed unsatisfactory results for coliform analysis (at both coliforms and fecal coliforms). In general, the analysis of *E. coli* content showed low values to undetectable MPN (Table 3).

In the present study, the surface of polyethylene cutting boards used for vegetable or meat handling, with and without inducing the formation of biofilms in BHI medium, were analyzed by SEM. The cutting boards were sampled after the daily cleaning, it is cleaned with water and liquid detergent and sanitized with sodium hypochlorite solution (100 ppm). As expected, the cutting boards presented an irregular surface (Figure 2A), which can be favorable for adhesion and colonization by microorganisms. Even without growth inducing conditions, a noticeable occurrence of microorganisms adhered to the vegetable cutting board was verified (Figure 2B). When these polyethylene boards were incubated under favorable growth conditions, the accumulation of microorganisms on the surface was exhibited (Figure 2C and 2D). The presence of different microbial morphologies can be clearly visualized, showing heterogeneity of microorganisms growing on this surface.



**Figure 1.** Phylogenetic tree showing evolutionary distance between isolates from hospital based on 16s rRNA gene sequence (500 pb). The scale represents the evolutionary distance value. B1: *Enterobacter aerogenes*; B2: *Enterobacter aerogenes*; B3: *Raoultella ornithinolytica*; B4: *Escherichia coli*; B5: *Klebsiella pneumoniae*; B6: *Klebsiella pneumoniae*; B7: *Pseudomonas aeruginosa*.



**Figure 2.** Scanning electron microscopy of plastic cutting board used for vegetable handling after daily cleaning without the induction of biofilm formation (on typical surface topography of plastic cutting board (A); presence of microorganisms adhered on cutting board surface (B); the induction of biofilm formation (presence of colonies on the surface of cutting board (C); the presence of bacterial agglomerates adhered on cutting board surface (D).

**Table 3.** Most probable number of coliforms, fecal coliforms and *Escherichia coli* on food handling surfaces and equipment in a hospital food and nutrition unit at the first (1<sup>st</sup>) and second (2<sup>nd</sup>) sampling.

Sampling	Coliform		Fecal coliform		<i>E. coli</i>	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
	----- MPN/cm <sup>2</sup> -----					
Meat countertop	>22*	>22	> 22	>22	<3	<3
Vegetable countertop	>22	>22	> 22	>22	<3	<3
Refrigerator	<3	<3	<3	<3	<3	<3
Microwave	<3	>22	<3	>22	<3	<3
Eating table	<3	<3	<3	<3	<3	<3
Meat cutting board	>22	>22	>22	>22	<3	<3
Vegetable cutting board	>22	>22	>22	>22	<3	<3

\*Values of MPN >3 are considered contaminated.

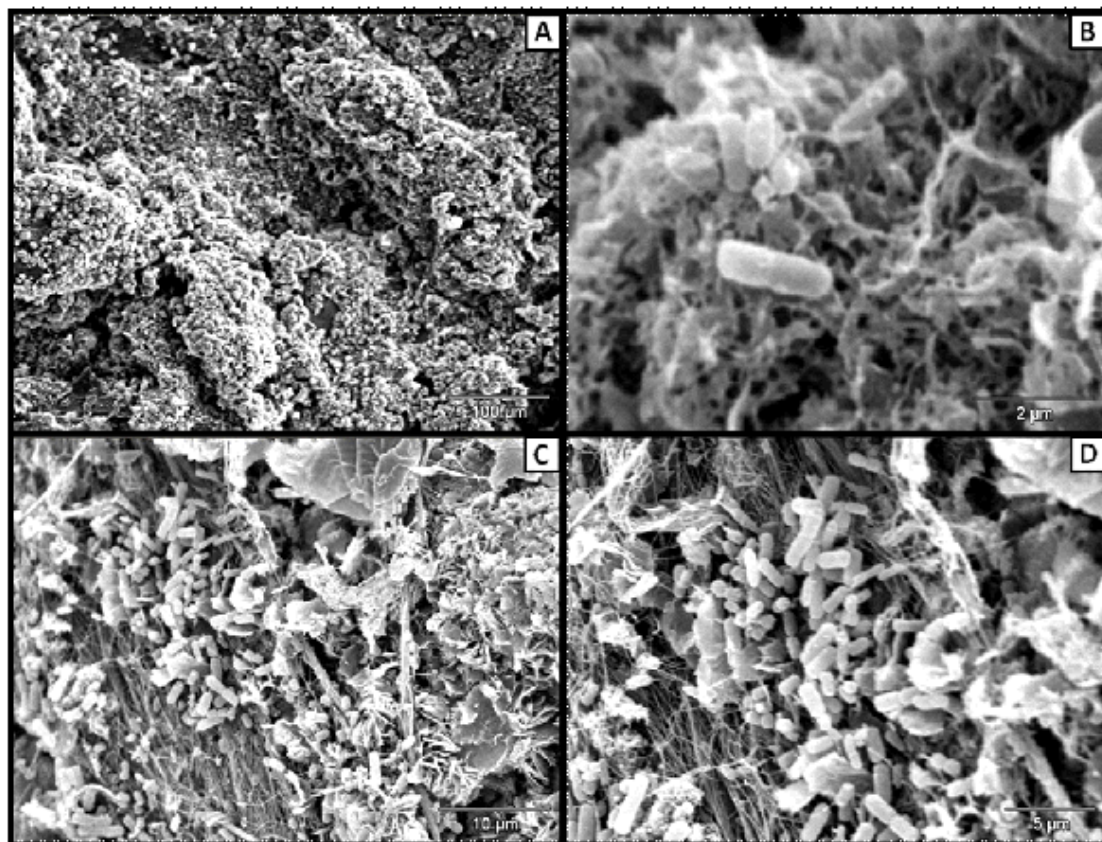
When the meat cutting boards were analyzed, a few amount of microorganisms on the surface were showed (Figure 3A), and bacteria cells could be only found at higher magnifications (Figure 3B). However, when the cutting

boards were subjected to biofilm-inducing conditions, an important increase in bacterial colonization was showed (Figure 3C and 3D). The presence of miscellaneous microbial morphologies adhered to the surface is noteworthy.

#### 4. Discussion

Bacteria are the cause of agents of foodborne illness in 60% of cases requiring hospitalization (Mead et al., 1999). Equipments and surfaces can accommodate a diverse microbiota. In the food services this can be influenced by contact with food (raw or processed), food handlers and by the processes of cleaning. Equipment and poorly sanitized environments may contain organic matter which combined with convenient extrinsic factors, can provide favorable conditions for the growth of microorganisms (Rode et al., 2007). Also, the contaminated equipment could be the source of microorganisms for food prepared with them (Silva Meira et al., 2012).

In this study, it was identified by molecular analyzes five species of the Enterobacteriaceae family and two bacteria identified as the Pseudomonadaceae family. According to Kramer et al. (2006) highly virulent microorganisms, particularly those known to cause nosocomial infections in admitted patients, such as *Escherichia coli*, *Klebsiella* species and *Pseudomonas aeruginosa* are capable of



**Figure 3.** Scanning electron microscopy of plastic cutting board used for meat handling after daily cleaning without the induction of biofilm formation (on typical surface topography of the plastic cutting board at different magnifications (A); the presence of bacteria on the surface of plastic cutting board (B); the induction of biofilm formation (extensive colonization of the surface of plastic cutting board) (C); the presence of different bacterial morphologies adhered on the surface of cutting board (D).

surviving for several days on hospital surfaces and are characteristically resistant to antibiotics and sanitizers (Sánchez-Gómez et al., 2015). This results represented a significant found, so the survival and persistence of pathogenic bacteria in natural environments associated the foodborne illness be considered serious public health problem (Medeiros et al., 2014).

Konecka-Matyjek et al. (2012) reported that food service areas are considered critical to health and, therefore, the microbiological quality of food handling surfaces in hospital kitchens must be assessed. Adequate nutrition and maintenance of cleanliness in hospital kitchens are conditions of medical treatment and contribute to recovery of hospitalized patients. These conditions directly affect the likelihood of complications and the length of a hospital stay. Thus, it is extremely important to identify potential sources of hazards during both the production and distribution of meals to ensure adequate hygienic conditions are being used in hospital kitchens. Sampling of the production and processing environments can be a useful tool to identify and control the presence of pathogenic microorganisms in foodstuffs (Konecka-Matyjek et al., 2012).

In addition, the present study exhibited that the counts of mesophilic microorganisms, yeasts and molds, and coagulase-positive *Staphylococcus* were above to that recommended by APHA (2001), and the results showed that the equipments and surfaces under study were classified as “unsatisfactory”, except for the analysis of coagulase-positive *Staphylococcus* at microwave and eating table. In accordance to the APHA recommendations, a count of  $< 0.2 \times 10^1$  CFU/cm<sup>2</sup> for countertop surfaces is recommended and for the food handling surfaces or equipments is  $1 \times 10^2$  CFU/cm<sup>2</sup> (Evancho et al., 2001). Similarly, if the results are compared with the value established by the Pan American Health Organization (PAHO), it can be showed that the results for all surfaces and equipment was in the inadequate category ( $> 1 \times 10^2$  CFU/cm<sup>2</sup>) (Silva et al., 2010). The values obtained in this study were compared with international standards since there is a gap in Brazilian law regarding microbiological standards for equipment and food handling surfaces. However, many authors consider the international standards too rigorous for Brazil due to climatic conditions. So, corroborating with

this, Silva Júnior (2005) recommended  $5 \times 10^1$  CFU/cm<sup>2</sup> for aerobic mesophilic bacteria.

Microorganisms can remain viable on food contact surfaces for significant periods, increasing the risk of cross-contamination events between food handlers, food products, and food contact surfaces (De Cesare et al., 2003). Epidemiological studies have revealed that a significant number of consumers were submitted to unsafe and risky practices during meal preparation (Redmond and Griffith, 2003) and they, do not implement proper hygienic procedures to prevent cross-contamination events (Fischer et al., 2007).

In this study, it was evaluated the bacterial adhesion in meat polyethylene cutting boards. The popularity of plastic cutting board in kitchen is inevitable, even if there is a significant risk of cross-contamination (DeVere and Purchase, 2007). The introduction of the cutting board of plastic in the 1970s replaced the traditional cutting board of wood, which is more susceptible to cross-contamination, especially from raw meat and poultry juices remaining on the surface (Oliveira et al., 2010). It was demonstrated in this study that meat cutting boards presented high microbial counts, which could be derived from cross-contamination. Klontz et al. (1995) described that 25% of food workers reported to reuse cutting boards without cleaning after cutting raw chicken. Furthermore, Gorman et al. (2002) showed that *Campylobacter*, *E. coli*, *Salmonella*, and *Staphylococcus aureus* originating from chicken were found on dishcloths, refrigerator handles, oven handles, countertops, and draining boards.

Elevated microbial counts and extensive bacterial adhesion were also observed in vegetable cutting boards. The contamination of these surfaces is more worrying because vegetables are often consumed raw, and it is known that in many times the cleaning is not properly done. Many studies have been conducted with regard to food outbreaks associated with contamination of fruits and vegetables. Raw and unheated foods can spread microorganisms associated with food poisoning and intestinal infections (Berger et al., 2010). According to Ferens and Hovde (2011), fresh vegetables have been identified as significant vehicles of relevant pathogens in public health, including the *E. coli* O157: H7.

It is important to emphasize that, even if a cutting board appears to be clean to naked eye, it does not mean that it is properly cleaned because if the food debris remains, together with proper environmental conditions as humidity and temperature, the surface can favor the adhesion for microorganisms, and consequently, favoring the formation of bacterial biofilms. Currently, microbial biofilms are attracting interest in many areas including food safety. Biofilm formation by foodborne pathogens can compromise the sanitation of food contact surface and cause cross-contamination to fresh produce (Oliveira et al., 2010).

In this study, the cutting boards used for handling vegetable and meat were analyzed by SEM, and bacterial adhesion was showed on the surfaces. The cutting boards are tools widely used in hospital kitchens. Because a large

number of patients are daily admitted in the hospital, the food service is continuous, and sometimes the cleaning can be inadequate promoting the adhesion of bacteria. Janssens et al. (2008) reported that approximately 80% of persistent bacterial infections in the United States were found associated with biofilms and, therefore, biofilms have become problematic in hospitals (Konecka-Matyjek et al., 2012) and in food industries (Srey et al., 2013). The induction of biofilm formation is important because when the surface is poorly cleaned and exposed to certain environmental conditions, such as adequate temperature, humidity and food debris, it can allow the development of microbial communities and biofilms.

In summary, the results obtained in this study indicate that the hygienic conditions of surfaces like plastic cutting boards and equipment in this hospital FNU were inadequate. Both cutting boards used for handling meat and vegetable showed high contamination, indicating that special attention should be given to more efficient strategies for disinfection. The achievement and application of standard operating procedures could positively help in the standardization of sanitary control at a FNU hospital, reducing the microbial contamination and providing a safe food to hospitalized patients. Other studies are being conducted with these isolated as susceptibility to antibiotics, in vitro biofilms formation, analysis of the presence of biofilm-forming genes, plasmid profile, among other evaluations.

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