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eduem@uem.br

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## Sensibility and resistance profiles to antibiotics of pathogens isolated in a hospital unit of food and nutrition

Eliza Marques Di Primio<sup>1</sup>, Bianca de Oliveira Schumacher<sup>2</sup>, Edcarlos Preuss<sup>2</sup>, Dulcinéa Blum-Menezes<sup>3</sup>, Eliezer Ávila Gandra<sup>4\*</sup> and Elizabete Helbig<sup>1</sup>

<sup>1</sup>Programa de Pós-graduação em Nutrição e Alimentos, Faculdade de Nutrição, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil. <sup>2</sup>Faculdade de Nutrição, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil. <sup>3</sup>Laboratório Genética de Microrganismos, Departamento de Microbiologia e Parasitologia, Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil. <sup>4</sup>Laboratório de Ciência dos Alimentos e Biologia Molecular, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Campus Capão do Leão, Prédio 4, Cx. Postal 354, 96010-900, Pelotas, Rio Grande do Sul, Brazil. \*Author for correspondence. E-mail: gandraea@hotmail.com

**ABSTRACT.** The objective of this study was to evaluate the sensibility and resistance profiles to antibiotics of pathogens isolated in a hospital unit of food and nutrition. It was evaluated as sample spots, with repetitions, environmental samples, utensils, equipment, manipulators' hands and usually diet that would be served to the hospitalized patients. In the evaluated samples, coagulase positive *Staphylococcus* (CPS) and Gram negative bacillus were enumerated, and also, it was possible to isolate *Klebsiella* spp and *Escherichia coli*. The antimicrobial of less efficiency for CPS was oxacillin and penicillin-G, and for *Klebsiella* spp ampicillin e cephalothin. It should be emphasized that it was found strains multiresistants of CPS, *Klebsiella* spp and *E. coli*. The hospital unit of food and nutrition was evaluated as microbiological inadequate in several points, and it was verified resistance profiles to antibiotics of pathogens isolated in this unit.

**Keywords:** *Staphylococcus*, *Klebsiella*, *Escherichia coli*, drug resistance, anti-bacterial agents.

## Perfil de sensibilidade e resistência a antimicrobianos de patógenos isolados em uma unidade de alimentação e nutrição hospitalar

**RESUMO.** O objetivo deste estudo foi avaliar os perfis de sensibilidade e resistência a antibióticos de patógenos isolados em uma unidade de alimentação e nutrição hospitalar. Foram analisados como pontos de amostragem, com repetições, de ambientes, de utensílios, de equipamentos, de mãos de manipuladores e de dieta normal a qual seria servida a pacientes hospitalizados. Nas amostras analisadas, *Estafilococos* coagulase positiva (ECP) e bacilos Gram negativos no ágar Mac Conkey foram enumerados, e *Klebsiella* spp e *Escherichia coli* foram isoladas. Os antimicrobianos de menor eficiência para ECP foram oxacilina e penicilina-G e para *Klebsiella* spp ampicilina e cefalotina. Cabe ressaltar que foram encontradas cepas multirresistentes de ECP, *Klebsiella* spp e *E. coli*. A unidade de alimentação e nutrição hospitalar foi avaliada como inadequada do ponto de vista microbiológico em diversos pontos e verificou-se a presença de perfis de resistência a antibióticos de patógenos isolados nesta unidade.

**Palavras-chave:** *Staphylococcus*, *Klebsiella*, *Escherichia coli*, farmacoresistência bacteriana, antibacterianos.

### Introduction

The procedures each time more invasive, the indiscriminated use of medication and the antimicrobial resistance are factors the point the hospital infections as a problem of public health (Starling, Fialho, Alves, Moura, & Couto, 2004). Its incidence is dependent of the sanitary conditions of health services and the presence of pathologic microorganism's vectors (Alves, Costa, Martins, Souza, & Pires, 2011).

Once that are different pathogenic mechanisms, one infection can be treated by many antibiotics, and

should be chosen that one the bacteria shows sensibility for. However, besides the big diversity of chemical structures and different action mechanisms of antibiotics, the infection treatment has been each time more difficult due to the appearance of multiresistant bacterial strains (Alves et al., 2011).

The nutrition service act as integrant of the epidemiologic chain of the infection vehicle by food, once that are many diseases by food contamination that can result in infection crusade or intoxication, resulting of wrong stock and manipulation of food. Being the hospital diet important for guarantee the supply of nutrients to the patient and thus, to

preserve and/or to recuperate its nutritional status, it is highlighted that the nutrition service requires special attention (Garcia, 2006).

In this context, the aim of this research was to evaluate the sensibility and resistance profiles to antibiotics of pathogens isolated in a hospital unit of food and nutrition.

## Methods

The research was conducted in a hospital, of Rio Grande city, Rio Grande do Sul State, Brazil, by previous authorization of the direction of the hospital and approbation by the Ethical Research Committee of the Medicine College of Federal University of Pelotas, in accord with the Resolution 196/96 of National Health Council (process OF. 17/11). The collect of samples from the manipulators' hands was made through signature of Free and Clarified Consent Term by the evaluated collaborators.

It was analyzed 13 sample spots of the production line. A total of 4 collections were made in different times, totaling 52 samples. For all the sample spots the collection was made in duplicate for each repetition.

The sample spots were chosen according with the food production line, and the microbiological risk spots were chosen and subdivided in: environment (sample by faucet and tables of kitchen), utensils (pan, table, vegetable knife, cut knife and food's bag), equipment (blender and transport car of meals), manipulators' hands (cooker and assistant), and the standard meal (usual diet, served meal to the patients without food restrictions).

The collection of samples of the environment, utensils, equipment and manipulators' hands was made by the technique of surface scrub (*swab technique*), the material collected was maintained in semi-solid Cary Blair transport medium in environmental temperature until the laboratorial process. In the standard food collection, it was take away one representative aliquot of approximately one quarter of the meal, ranging from 50 to 100g, and then placed in sterile plastic bags and conditioned in isothermal box with ice.

The samples were immediately sent to the Microorganism Genetic Laboratory of the Biology Institute, to the realization of the microbiological analyses concerning to the isolation of *Listeria monocytogenes*, and to the Food Analysis Laboratory of the Nutrition College to the remaining microbiological determinations, both in the Federal University of Pelotas (Brazil).

## Microbiological analysis

The microbiological determinations were made according with the recommendations of Downes and Ito (2001).

### Coagulase positive *Staphylococcus*

It was inoculated 0.1 mL of each serial dilution, by the surface sow in Baird Parker agar in duplicate, and then the plates were incubated to 37°C for 24 to 48 hours. The Colony-Forming Unit (CFU) was enumerated in a minimum 5 CFU with typical morphology, and 5 atypical morphologies were chosen to the realization of the free coagulase production test. The *strains* that showed positive reaction were stored in agar Brain Heart Infusion (BHI) to be evaluated for sensibility and resistance to the antibiotics.

### Enumeration of Gram negative bacillus and isolation of *E. coli* e *Klebsiella* spp

It was inoculated 1 mL of each serial dilution, by the deep plaque technique in Mac Conkey Agar, in duplicate. The plates were incubated in 37°C for 24 a 48 hours. After the incubation it was enumerated the CFU present in the plates, and the CFU that showed typical morphology of *E. coli* e *Klebsiella* spp (3 to 5 CFU by plate) were used to the realization of the proof of IMViC: indol production (I), Metila Red test (VM), Voges-Proskauer test (VP) and Citrate utilization test (C). For this proofs it was followed the method described by Mac Faddin (1976). After the discrimination of the cologne as *E. coli* or *Klebsiella* spp, as well as the case of CPS, these CFU were stored in BHI semi-solid to posterior tests with antibiotics.

### *Pseudomonas* spp

The quantification of *Pseudomonas* spp was made in Ágar *Pseudomonas*, by the surface sow technique, inoculating 0.1 mL of each serial dilution, in duplicate, and incubating the plates in 30°C for 48 hours. After the incubation, 3 to 5 typical CFU were submitted to confirmation tests for type, using the NF KIT (PROBAC of Brazil), that is made by the tests of oxidase, utilization of glucose in basis broth, decarboxylation of lysina and arginine (Moeller basis), liquid of gelatin, hydrolysis of urea, DNase, and sensibility to polymyxin.

### *Listeria monocytogenes*

By the *swabs* transported in Cary Blair Media and the samples of standard food, it was made enrichment in *Listeria* enrichment broth (LEB UVM-II) and incubation at 30°C for until 7 days.

The samples were observed day by day, during the period of enrichment, and when turbid, it was sowed in selective agars, Moxalactam, Oxford, Aloa and Hicrome. The plates with selective broths were incubated in 35°C for until 7 days. These plates also were observed day by day, during the 7 days of incubation, to be possible to obtain isolated CFU. After the isolation of suspect CFU, with typical morphology of *Listeria* spp, it was made coloration Gram tests, catalase production (hydrogen peroxide 3%), motility in 25°C and fermentation of dextrose, ramnose, xylose and mannitol, to confirmation of specie.

#### Resistance and sensibility to the antibiotic profile

The resistance/sensibility tests to antibiotics was made according with the protocol proposed by National Committee for Clinical Laboratory Standards (NCCLS, 2003) e Clinical and Laboratory Standards Institute (CLSI, 2011), using the disk diffusion technique.

It was utilized disks with 12 antibiotics of ordinary use, for the Gram positive bacteria: cefepime (COM, 30 µg), ciprofloxacin (CIP, 5 µg), clindamycin (CLI, 2 µg), chloramphenicol (CLO, 30 µg), erythromycin (ERI, 15 µg), gentamicin (GEN, 10 µg), oxacillin (OXA, 1 µg), penicillin G (PEN, 10 un), rifampicin (RIF, 5 µg), sulfazotrim (SUT, 23.75 + 1.25µg), tetracycline (TET 30 µg), vancomycin (VAN, 30 µg). For the Gram negative bacteria the antibiotics were: amikacin (AMI, 30 µg),

amoxicillin + clavulanate (AMC, 20+10 µg), ampicillin (AMP, 10 µg), cephalothin (CFL, 30 µg), cefepime (COM, 30 µg), cefoxitin (CFO, 30 µg), ceftazidime (CAZ, 30 µg), cefuroxime (CRX, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GEN, 10 µg), meropenem (MER, 10 µg), sulfazotrim (SUT, 23.75 + 1.25 µg).

#### Statistic treatment

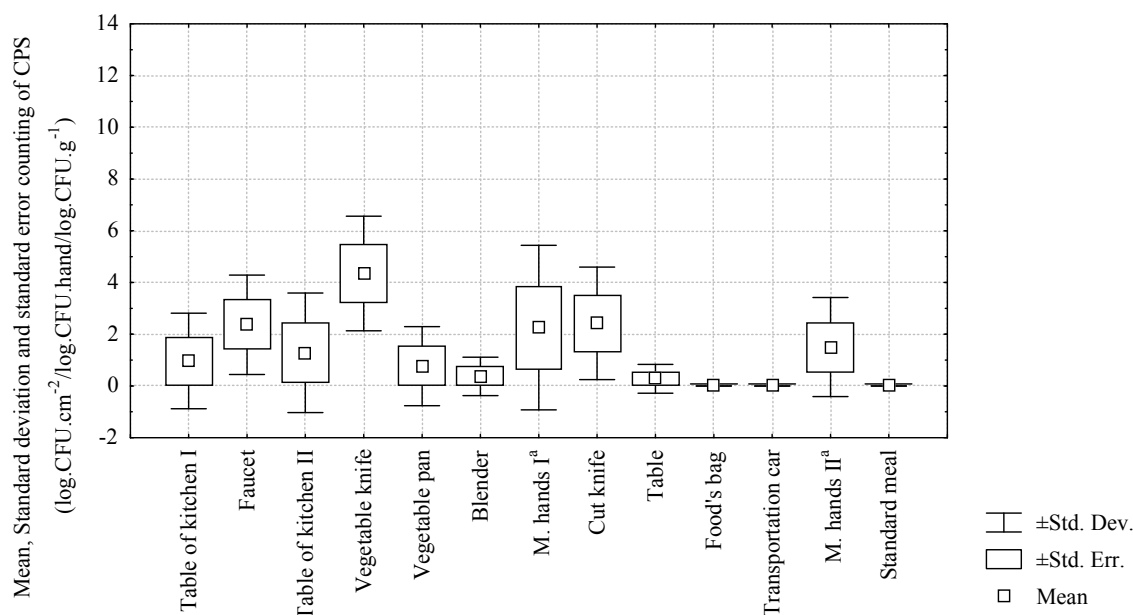
The results of the microbiological counting were transformed to log values and submitted to analysis of variance (ANOVA) followed by Tukey test 5%.

#### Results

##### Enumeration and isolation of pathogens in hospital unit of food and nutrition.

In the 52 samples analyzed between environment, utensils, equipment, manipulators hands and standard meal, it was enumerated CPS in 59.6% (31), with values ranging from 0.05 CFU to 980.4 CFU (being CFU cm<sup>-2</sup> for the environmental surfaces, utensils and equipment, CFU.hand<sup>-1</sup> for the manipulators' hand surface and CFU g<sup>-1</sup> for food samples).

The mean values, standard deviation and standard error counting of coagulase positive *Staphylococcus*, in 4 collections by sample spot in a production line of a food and nutrition hospital unit are show in log in the Figure 1.



**Figure 1.** Mean values, standard deviation and standard error counting of coagulase positive *Staphylococcus*, in 4 collections by sample spot in a production line of a food and nutrition hospital unit, Rio Grande, Rio Grande do Sul, Brazil, 2012.

<sup>a</sup> M. hands = Manipulator's hands

The sample spots of food bag, transportation car and standard food didn't present counting of CPS, and differed significantly of the vegetable knife ( $p < 0.05$ ). The high counting of CPS in the vegetable knife can be explained by cursed contamination, which the knife could be received of contaminated surfaces like manipulators' hands and tables, due the absence of periodic cleaning practices or due to the inadequate cleaning. It can be emphasized the proper hygiene of the previous items and the transportation car, that didn't show evidence of contamination in the standard meal.

The vegetable knife had average counting of CPS  $4.35 \log \text{CFU cm}^{-2}$ , that is a value above the maximum limit recommended for this kind of microorganism by the criteria adopted by Silva Jr. (2002) or Brazilian's surface of food contact, who considers 'satisfactory' the count of  $3.91 \log \text{CFU}^{-1} \text{cm}^{-2}$  for CPS in equipment and utensils. When analyzed the individual values for each collection, for this same sample spot, 2 (50%) samples showed counting above the maximum recommended, in the samples 2 and 4, it was found values of  $6.89 \log \text{CFU}^{-1} \text{cm}^{-2}$  and of  $5.28 \log \text{CFU}^{-1} \text{cm}^{-2}$ , respectively.

The average counting of the others sample spots, utensils, environment and equipment was below  $3.91 \log \text{CFU}^{-1} \text{cm}^{-2}$ , and can be considered 'adequate' or 'satisfactory' when used the same standards adopted by Silva Jr. (2002).

Although the sample spots of manipulators' hands I, table of kitchen II, faucet e cut knife (Figure 1), showed average values below the limit stipulated by Silva Jr. (2002), in one collection it was found superior values when compared with other collections, and these values were responsible by the increase of the standard deviation in these sample spots.

In the same sample spots searched for CPS, it was enumerated Gram negative bacillus in 47 (90.4%) of the analyzed samples. The mean values, standard deviation and standard error, by sample spot in the 4 collections, are represented in logarithm in the Figure 2.

According with the data shown in the Figure 2, the counting obtained in the sample spot table of kitchen II  $5.94 \log \text{CFU}^{-1} \text{cm}^{-2}$  differed significantly ( $p < 0.05$ ) from the obtained in the food's bag and the transportation car, that did not show counting in Mac Conkey agar.

The counting in the sample spot manipulator hand I differed significantly ( $p < 0.05$ ) from the obtained in the spots: vegetables pan ( $2.06 \log \text{CFU}^{-1} \text{cm}^{-2}$ ), manipulator hand ( $1.06 \log \text{CFU}^{-1}$

$\text{cm}^{-2}$ ), blender ( $0.98 \log \text{CFU}^{-1} \text{cm}^{-2}$ ), standard food ( $0.58 \log \text{CFU}^{-1} \text{g}^{-1}$ ), and spots of food's bag and transportation car that had no counting.

It was detected the presence of *Klebsiella* spp and *E. coli* in 76.9% (40) and 13.5% (7) of samples, respectively. For *Listeriamonocytogenes* and *Pseudomonas* spp it was not verified the presence in the evaluated samples.

From the sampling spots already described, it was isolated 49 CPS strains, 99 *Klebsiella* spp and 8 *E. coli*, that were submitted to the tests for determination of antimicrobial sensibility and resistance profile. The antimicrobial sensibility and resistance profile of these 156 isolated strains are shown in the Table 1.

In the Table 1 it can be observed that for CPS, 49% of isolated strains have shown resistance to the antibiotics oxacillin and penicillin-G, 22% to the vancomycin, and to the antibiotics cefepime, ciprofloxacin and chloramphenicol it was not observed resistant strains.

For *Klebsiella* spp 47% of isolated strains have shown resistance to the antibiotic ampicillin and 37% to the cephalothin. To the antibiotics amikacin, ciprofloxacin, gentamicin, sulfazotrim it was not obtained resistant strains.

However, for *E. coli*, 13% of the isolated strains have shown resistance to the antibiotics amoxicillin with clavulanate, ampicillin, cephalothin, cefepime, cefoxitin, ceftazidime, cefuroxime and meropenem. For the antibiotics amikacin, ciprofloxacin, gentamicin and sulfazotrim, the resistant strains did not occurred.

Of the 156 isolated and evaluated strains for the resistance and sensibility to the antibiotics, 61 (39.1%) showed to be multiresistant for at least 2 antibiotics, 49 (31.4%) showed resistance for 1 of the evaluated antibiotics, and 46 (29.5%) showed sensibility for all of the evaluated antibiotics. The multiresistant strains found were distributed in 12 sample spots, and only the manipulator hand sample spot didn't have this evidence. These data are in the Table 2.

## Discussion

The present research makes a contrast with the found of Souza and Campos (2003), which analyzed the hygienic-sanitary conditions of a hospital unit of food and nutrition and didn't observe the presence of *S. aureus* in equipment, utensils and manipulators' hands.

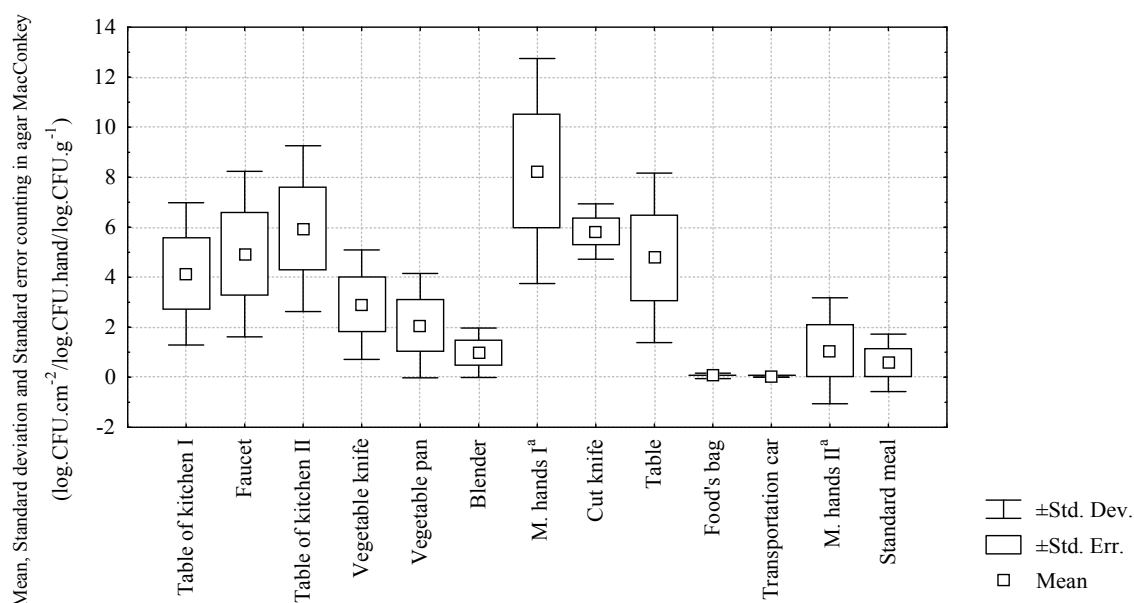
According with the exposed, the results of the CPS contamination verified in vegetables knives, cut

knives, table of kitchen and faucet, can be arising of deficient practices in the hygienization, and also can be arising of cursed contamination because the same utensils and environments were used by other workers of the unit.

According with Castro and Iaria (1984), one of the most important sources of contamination of *S. aureus* in Brazilian's hospital unit of food and nutrition are the manipulators' nose pit. These authors analyzed 78 food manipulators in hospital kitchens and observed that 42.3% of them were nasal bearers of this bacterium, however, in our

research, the CPS counting in manipulators' hands was low, when compared to this values.

The high counting of Gram negative bacillus in the Table 2 and in manipulator's hand can be attributed to possible inadequacies in the hygienization techniques, and it is explained also by the elevate nutrients available and humidity found in tables of production units during the food preparation, that turn possible the microbial grow. It was possible to watch suitable hygiene for the food's bag and the transportation car.



**Figure 2.** Average, standard deviation, standard error counting of bacillus Gram negatives in agar MacConkey, in 4 collections by sample spot in a production line of a hospital food and nutrition unit, Rio Grande, Rio Grande do Sul, Brazil, 2012.

<sup>a</sup> M. hands = Manipulator's hands

**Table 1.** Microbial sensibility and resistance profile of isolated strains in a food and nutrition hospital unit, Rio Grande, Rio Grande do Sul, Brazil, 2012.

| Antibiotics                                  | CPS   |       |       | Klebsiellaspp |       |       | Escherichia coli |       |       |
|----------------------------------------------|-------|-------|-------|---------------|-------|-------|------------------|-------|-------|
|                                              | R (%) | I (%) | S (%) | R (%)         | I (%) | S (%) | R (%)            | I (%) | S (%) |
| Amikacin (AMI) <sup>b</sup>                  |       |       |       | 0             | 0     | 100   | 0                | 0     | 100   |
| Amoxicillin + clavulanate (AMC) <sup>b</sup> |       |       |       | 24            | 12    | 64    | 13               | 0     | 87    |
| Ampicillin (AMP) <sup>b</sup>                |       |       |       | 47            | 14    | 39    | 13               | 0     | 87    |
| Cephalothin (CFL) <sup>b</sup>               |       |       |       | 37            | 15    | 47    | 13               | 0     | 87    |
| Cefepime (CPM) <sup>a,b</sup>                | 0     | 0     | 100   | 1             | 0     | 99    | 13               | 0     | 87    |
| Cefoxitin (CFO) <sup>b</sup>                 |       |       |       | 25            | 5     | 70    | 13               | 0     | 87    |
| Ceftazidime (CAZ) <sup>b</sup>               |       |       |       | 4             | 0     | 96    | 13               | 0     | 87    |
| Cefuroxime (CRX) <sup>b</sup>                |       |       |       | 9             | 2     | 89    | 13               | 0     | 87    |
| Ciprofloxacin (CIP) <sup>a,b</sup>           | 0     | 0     | 100   | 0             | 0     | 100   | 0                | 0     | 100   |
| Clindamycin (CLI) <sup>a</sup>               | 4     | 20    | 76    |               |       |       |                  |       |       |
| Chloramphenicol (CLO) <sup>a</sup>           | 0     | 0     | 100   |               |       |       |                  |       |       |
| Erythromycin (ERI) <sup>a</sup>              | 10    | 6     | 84    |               |       |       |                  |       |       |
| Gentamicin (GEN) <sup>a,b</sup>              | 2     | 0     | 98    | 0             | 0     | 100   | 0                | 0     | 100   |
| Meropenem (MER) <sup>b</sup>                 |       |       |       | 5             | 40    | 55    | 13               | 25    | 62    |
| Oxacillin (OXA) <sup>a</sup>                 | 49    | 16    | 35    |               |       |       |                  |       |       |
| Penicillin-G (PEN) <sup>a</sup>              | 49    | 0     | 51    |               |       |       |                  |       |       |
| Rifampicin (RIF) <sup>a</sup>                | 4     | 0     | 96    |               |       |       |                  |       |       |
| Sulfazotrim (SUT) <sup>a,b</sup>             | 2     | 0     | 98    | 0             | 0     | 100   | 0                | 0     | 100   |
| Tetracycline (TET) <sup>a</sup>              | 4     | 2     | 94    |               |       |       |                  |       |       |
| Vancomycin (VAN) <sup>a</sup>                | 22    | 0     | 78    |               |       |       |                  |       |       |

CPS: coagulase positive *Staphylococcus*. (R) Resistant; (I) Intermediary; (S) Sensitive. <sup>a</sup>Antibiotic tested for CPS (Gram positive bacteria). <sup>b</sup>Antibiotics tested for *Klebsiellaspp* and *E.coli* (Gram negative bacteria).

**Table 2.** Multiresistance profile of isolated strains in a food and nutrition hospital unit, Rio Grande, Rio Grande do Sul, Brazil, 2012.

| Sample spot <sup>a</sup>                       | Pathogen              | Strain <sup>b</sup> | Multiresistance <sup>c</sup> |     |     |     |     |     |     |     |  |  |
|------------------------------------------------|-----------------------|---------------------|------------------------------|-----|-----|-----|-----|-----|-----|-----|--|--|
| Faucet                                         | CPS                   | C3 - 01             | OXA                          | PEN | VAN |     |     |     |     |     |  |  |
|                                                | CPS                   | C3 - 02             | OXA                          | PEN | VAN |     |     |     |     |     |  |  |
|                                                | CPS                   | C3 - 03             | OXA                          | PEN |     |     |     |     |     |     |  |  |
|                                                | CPS                   | C4 - 02             | ERI                          | OXA | PEN | VAN |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C2 - 2K             | CFL                          | AMP | CFO |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C3 - 1K             | AMC                          | CFL | AMP |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C4 - 1K             | AMC                          | CFL | AMP | CFO |     |     |     |     |  |  |
| Table 1<br>(Table of kitchen I)                | <i>Klebsiella</i> spp | C4 - 2K             | AMP                          | CFO |     |     |     |     |     |     |  |  |
|                                                | CPS                   | C3 - 01             | OXA                          | PEN | RIF | VAN |     |     |     |     |  |  |
|                                                | CPS                   | C3 - 02             | OXA                          | PEN | VAN |     |     |     |     |     |  |  |
|                                                | CPS                   | C4 - 01             | OXA                          | PEN | SUT | VAN |     |     |     |     |  |  |
|                                                | CPS                   | C4 - 02             | OXA                          | PEN | VAN |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C1 - 1K             | CFL                          | CFO |     |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C3 - 3K             | AMC                          | CFL | CFO |     |     |     |     |     |  |  |
| Table 2<br>(Table of kitchen II)               | <i>Klebsiella</i> spp | C4 - 1K             | AMC                          | CFL | AMP | CFO |     |     |     |     |  |  |
|                                                | CPS                   | C1 - 01             | ERI                          | PEN |     |     |     |     |     |     |  |  |
|                                                | CPS                   | C1 - 02             | OXA                          | PEN |     |     |     |     |     |     |  |  |
|                                                | CPS                   | C1 - 03             | OXA                          | PEN |     |     |     |     |     |     |  |  |
|                                                | CPS                   | C1 - 04             | PEN                          | TET |     |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C1 - 1K             | AMC                          | CRX | CFL | AMP |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C1 - 2K             | AMC                          | CFL | AMP | CFO |     |     |     |     |  |  |
| Vegetable knife                                | <i>Klebsiella</i> spp | C2 - 1K             | AMC                          | AMP | MER |     |     |     |     |     |  |  |
|                                                | <i>E. coli</i>        | C3 - 4E             | AMC                          | CRX | CFL | AMP | CFO |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C4 - 1K             | CFL                          | AMP |     |     |     |     |     |     |  |  |
|                                                | CPS                   | C1 - 02             | OXA                          | PEN |     |     |     |     |     |     |  |  |
|                                                | CPS                   | C3 - 02             | OXA                          | PEN |     |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C1 - 1K             | CRX                          | CFL |     |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C1 - 2K             | CRX                          | CFL |     |     |     |     |     |     |  |  |
| Vegetable pan                                  | <i>Klebsiella</i> spp | C2 - 1K             | AMC                          | CFL | AMP | MER | CAZ | CFO |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C2 - 2K             | AMC                          | CFL | AMP | CFO |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C2 - 3K             | AMC                          | CFL | AMP | CFO |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C3 - 1K             | AMC                          | CFO |     |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C3 - 3K             | CRX                          | CFL |     |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C4 - 1K             | AMC                          | CFL | CFO |     |     |     |     |     |  |  |
|                                                | CPS                   | C1 - 03             | OXA                          | PEN |     |     |     |     |     |     |  |  |
| Blender (Liquefier)                            | <i>Klebsiella</i> spp | C1 - 1K             | CRX                          | CFL | AMP |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C1 - 2K             | CRX                          | CFL | AMP | CAZ |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C1 - 3K             | AMC                          | CRX | CFL | AMP | MER | CAZ | CPM | CFO |  |  |
|                                                | <i>Klebsiella</i> spp | C1 - 4K             | CRX                          | CFL | AMP |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C2 - 1K             | CFL                          | AMP |     |     |     |     |     |     |  |  |
| Manipulator's hands I                          | <i>Klebsiella</i> spp | C3 - 1K             | AMC                          | CFO |     |     |     |     |     |     |  |  |
| Cut knife                                      | CPS                   | C2 - 02             | PEN                          | VAN |     |     |     |     |     |     |  |  |
|                                                | CPS                   | C3 - 01             | OXA                          | PEN | VAN |     |     |     |     |     |  |  |
|                                                | CPS                   | C3 - 02             | CLI                          | ERI | OXA | PEN | RIF | VAN |     |     |  |  |
|                                                | <i>E. coli</i>        | C1 - 2E             | CAZ                          | CPM |     |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C2 - 1K             | AMC                          | CRX | CFO |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C2 - 2K             | AMC                          | CFO |     |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C3 - 3K             | CFL                          | CFO |     |     |     |     |     |     |  |  |
| Table                                          | <i>Klebsiella</i> spp | C4 - 1K             | AMP                          | CFO |     |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C4 - 2K             | AMC                          | AMP | CFO |     |     |     |     |     |  |  |
|                                                | CPS                   | C3 - 01             | CLI                          | OXA | PEN | VAN |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C1 - 1K             | AMC                          | AMP | CFO |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C1 - 3K             | CFL                          | AMP |     |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C3 - 1K             | AMC                          | CFL | AMP | CFO |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C4 - 1K             | CFL                          | AMP | CFO |     |     |     |     |     |  |  |
| Food's bag                                     | <i>Klebsiella</i> spp | C4 - 5K             | AMC                          | CFL | AMP |     |     |     |     |     |  |  |
|                                                | <i>Klebsiella</i> spp | C3 - 2K             | CFL                          | MER |     |     |     |     |     |     |  |  |
| Transportation car<br>(car transport of meals) | CPS                   | C4 - 03             | PEN                          | TET |     |     |     |     |     |     |  |  |
| Standard meal                                  | <i>Klebsiella</i> spp | C1 - 1K             | AMP                          | MER |     |     |     |     |     |     |  |  |

CPS: coagulase positive *Staphylococcus*. <sup>a</sup> The sample spot manipulators' hands didn't have multiresistant strains, and for this reason it isn't in this table. <sup>b</sup> The strains are presented by the analyzes conditions. <sup>c</sup> Analyzed antibiotics: amikacin (AMK); amoxicillin + clavulanate (AMC); ampicillin (AMP); cephalothin (CFL); cefepime (CPM); cefoxitin (CFO); ceftazidime (CAZ); cefuroxime (CRX); ciprofloxacin (CIP); clindamycin (CLI); chloramphenicol (CLO); erythromycin (ERI); gentamicin (GEN); meropenem (MER); oxacillin (OXA); penicillin-G (PEN); rifampicin (RIF); sulfazotrim (SUT); tetracycline (TET); vancomycin (VAN).

The contamination variation was also observed in a research about isolation of cockroach (*Periplaneta americana*) enterobacterial, categorized in 15 species, between them, *K. pneumonia* 17%, *K. oxytoca* 4%, *K. ozaenae* 3%, *E. coli* 2%,

*K. rhinoscleromatis* 2%, besides other microorganisms (Prado et al., 2002).

It doesn't exist any establish standard for the counting of Gram positive bacillus in environment, utensils, equipment, manipulators' hands and food,

but, considering the direct relationship between the quantity of this microorganisms and the isolation of *Klebsiella* spp and *E. coli*, it is noteworthy the existence of hygienic mistakes in the hospital unit of food and nutrition, especially in the sampled spots.

The appearance of bacteria resistant to the antibiotics can be considered as a natural manifestation by the evolutionary beginning of genetic adaptation of organisms to changes in its environment. As the time of bacteria duplication can be only 20 minutes in favorable conditions, there is the possibility of being produced many generations in only few hours, having, thus, many opportunities of evolve to adaptation (Silveira, Nome, Gesser, & Sá, 2006). Tavares (2000) affirms that the antibiotic resistance can be explained not only by the presence of resistance genes, but also by this genes expression, that is controlled by the environment.

Maldaner, Cavalli, Rossi, Scapin and Sardigla (2011), in a research that evaluated the resistance to antibiotics profile of isolated *Escherichia coli* strains of people with suspect urinary tract infection, the antibiotics: ampicillin, cephalothin and amikacin were the ones that showed a bigger resistance indices, especially in the isolated of patients take into the hospital when compared to the isolated of communitarian patients.

The isolation of resistant strains was observed by Carneiro et al. (2008), which showed the *E. coli* specie as the most frequent, followed by *Staphylococcus* sp., *Enterococcus* and after *Klebsiella* and *Aeromonas* sp. *Escherichia coli* was prevalent in all the studied spots, stressing that, during the research, it was found contaminated boots in all hospital environment researched.

The penicillin was the first antibiotic described in the literature in the 1940 decade and still has its role in the modern medicine, the use of this medication had a increase since its description and nowadays it continues being the indication of choice for some diseases (Grumach & Ferraroni, 2006).

The penicillins pass to represent a therapeutic option in the treatment and prevention of different infection process and its complications. It is a high efficiency antibiotic when correctly used and has a low price, being a right option to syphilis, prophylaxis primary and secondary of rheumatic fever, glomerulonephritis post-streptococcal, abscess cerebral, actinomycosis, diphtheria, endocarditic enterococcal bacterial, gangrene gases, infections of flat tissues (erysipelas and impetigo), infections of upper respiratory tract (tonsillitis and pharyngitis), otitis and meningitis by bacteria (Grumach & Ferraroni, 2006).

The main resistance mechanism of bacteria to penicillin is based on its own production of enzymes, the penicillinases, which degrade the penicillin before it can have effect (Daum, 2007).

The oxacillin is an antibiotic of the penicillin group, and it is resistant to the staphylococcal penicillinase. Its main indication are the infections provoked by these microorganisms in many places, usually abscesses, septicemias, pneumonias (Souza, 2011). In our research, it was verified that the oxacillin had action in 51% of the CPS strains tested.

In the last decades, in the hospital ambit, one option is the glycopeptides, mainly the vancomycin, but, in 1996 was identified, in Japan, the first isolated of *S. aureus* with reduced fragility to the vancomycin.

Currently, the penicillin has no more place as therapeutic drug for the cases of grave infection by *S. aureus*. For such grave cases, with the suspect of *S. aureus* be the etiological agent, is safer to use the vancomycin due to the high indices of resistance of the microorganism to the oxacillin, but, always when the diagnostic of the microorganism was obtained and confirmed that isn't resistant, is advisable to use oxacillin or other sensitive antibiotic with good penetration in the infection site, suggested as a way to retard the resistance to the vancomycin (Souza, 2011).

Ampicillin and cephalothin are antibiotics indicated on meningitis, paratyphoid fever, bacterial pharyngitis, gonorrhea, bacterial pneumonia, bacterial septicemia, skin infections and flat tissues, infections of tract genitourinary, sepsis, gastric-intestinal infections, and bones and joint infections.

Considering the exposed above, in regard of the antibiotics with relevant clinical importance, the values found in the present research must be emphasized, since 47 and 13% of the *Klebsiella* spp. and *E. coli* isolated types, respectively, showed resistance to the ampicillin antibiotic, and the cephalothin antibiotic had 37% of the *Klebsiella* spp. strain resistant.

Nowadays, there are many resistant strains of *E. coli* and *S. aureus*, for example, Bai et al. (2016) worked in a study to characterize multidrug-resistant Shiga toxin-producing *Escherichia coli* (STEC) harbouring the *mcr-1* gene on plasmids cultured from pigs in China and Marino, Blanco, Ginestra, Nostro and Bisignano (2016) characterized the antibacterial activity of gemifloxacin against methicillin-resistant *Staphylococcus aureus* (MRSA) ocular isolates in vitro and in a modified *ex vivo* rabbit keratitis model.

In the present research, it was found multiresistant strains that change the resistance

between 2 and 8 antibiotics of ordinary use, a delicate situation once that the researched environment it's a food and nutrition hospital unit that attend sick and weak people, in which the immunological system can be compromised and more susceptible to the infections, a fact that emphasize the importance of good practices in make food harmless to the patient's healthy.

All of these facts added to the results found in this research, show the existence of a care situation about the resistance to the antibiotics observed in this strains. 'Antibiotic resistant' microorganisms are not inhibited by systemic concentrations of microbial agents usually reachable in the normal therapeutic regime (NCCLS, 2003), and depending on the asepsis conditions, it can reach the patients creating difficult treatment infections.

In this context, it is necessary to point out that this research contributed for the knowledge of the rate of local resistance, and this is one of the basic steps for establishment of particular strategies related to the rational use of antibiotics. In the end, the results found make clear the necessity of control and make the hygienic practices more suitable, as also the necessity of implantation of a more effective system in the microbiologic control for the evaluated hospital.

It was possible to enumerate coagulase positive *Staphylococcus* and Gram negative bacillus thus as the isolation of *E. coli* e *Klebsiella* spp in 13 sample spots in a hospital unit of food and nutrition of the Rio Grande city, Rio Grande do Sul State, Brazil. The presence of pathogens verified in the food and nutrition unit researched alerts for the risk of food crusade contamination that can bring these pathogens to the patients. In addition, it was verified the presence of CPS, *E. coli* and *Klebsiella* spp. strains resistant to the antibiotics of ordinary use, totaling 39.1% of total microorganisms evaluated that presented multiresistance. The results of the antibiotic resistance indicate the necessity of strategy to suit the use of antibiotics in the evaluated hospital.

## Conclusion

The hospital unit of food and nutrition was evaluated as microbiological inadequate in several points, and it was verified resistance profiles to antibiotics of pathogens isolated in this unit.

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