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Microbiological profile of different types of salads from hospital kitchens

Perfil microbiológico de diferentes tipos de saladas provenientes de cozinhas hospitalares

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ABSTRACT: The objective of this work was to verify the microbiological profile of different types of salads from hospital kitchens. During the period from 2010 to 2014, the Public Food Guidance Service (SOAP) received 641 samples of salads from two public hospitals in the Central West region of the São Paulo state, where they were submitted to microbiological analysis in order to determine the most probable number (MPN) of coliforms at 35 and 45ºC, carry out *Salmonella* spp. study and coagulase-positive staphylococci count. The results showed that in 30.56% of samples the coliform count at 35ºC was above 1,100 MPN/g and 12.17% of samples presented coliforms at 45ºC above 100 MPN/g, which is the maximum limit established by Brazilian law. The prevalence of contaminated samples among those without heat treatment was at 97.44%, and for samples with heat treatment the prevalence was at 2.56% for both cooked and braised foods. All samples were negative for *Salmonella* spp. presence and showed coagulase-positive staphylococci count at $< 1.0 \times 10^2$ UFC/g. Although no pathogenic agents were found, the high count for indicator microorganisms in a large number of samples suggests that the practices of obtaining and manipulating these foods are inadequate, facilitating the risk of contamination with pathogens, including other agents not included in this research. Thus, food and nutrition units must pay attention to food preparation procedures, especially since these meals are served to indoor patients.

KEYWORDS: coliforms; *Staphylococci*; hospital; vegetables; *Salmonella* spp.

RESUMO: O objetivo deste trabalho foi verificar o perfil microbiológico de diferentes tipos de saladas provenientes de cozinhas hospitalares. No período de 2010 a 2014, o Serviço de Orientação à Alimentação Pública (SOAP) recebeu 641 amostras de saladas provenientes de dois hospitais públicos da região centro-oeste do estado de São Paulo, onde foram submetidas às análises microbiológicas para a determinação do número mais provável (NMP) de coliformes a 35 e 45ºC, pesquisa de *Salmonella* spp. e contagem de estafilococos coagulase positiva. Os resultados revelaram que em 30,56% das amostras a contagem de coliformes a 35ºC foi maior que 1.100 NMP/g, e 12,17% apresentaram coliformes a 45ºC acima de 100 NMP/g, limite máximo estabelecido pela legislação brasileira. A prevalência de amostras contaminadas sem tratamento térmico foi de 97,44% e de 2,56% para amostras com tratamento térmico, cozidas ou refogadas. Todas as amostras foram negativas para presença de *Salmonella* spp. e apresentaram contagem de estafilococos coagulase positiva $< 1{,}0 \times 10^2$ UFC/g. Embora não tenham sido encontrados agentes patogênicos, as altas contagens de micro-organismos indicadores em grande parte das amostras sugerem que as práticas de obtenção e manipulação desses alimentos estão inadequadas, possibilitando risco de contaminação por patógenos, inclusive outros agentes não contemplados nesta pesquisa. Portanto, as unidades de alimentação e nutrição hospitalares devem se atentar quanto aos procedimentos de preparo de alimentos, sobretudo porque essas refeições são servidas a indivíduos hospitalizados.

PALAVRAS-CHAVE: coliformes; estafilococos; hospital; vegetais; *Salmonella* spp.

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INTRODUCTION

Vegetables are herbaceous plants whose part(s) is (are) used in its natural form by humans as food (BRASIL, 1978). In accordance with the comestible part, these can be classified as tuberoses (roots, bulbs and tubers), fruits (vegetables and seeds) and greens (leaves, stems and flowers) (BEVILACQUA, 2014; FAO, 2014). These vegetables are indispensable in human diet, acting as adjuvants in the prevention and treatment of diseases (OYEBODE et al., 2014; WANG et al., 2014). However, there has been an outbreak of foodborne diseases associated with vegetable consumption (TABAN; HALKMAN, 2011; BENNETT, 2014).

The majority of foodborne diseases are caused by biological agents that can generate infections, poisoning and toxinfections (BRASIL, 2014). Estimates from Centers for Disease Control and Prevention (CDC) revealed that 48 millions of Americans get ill each year as a result of foodborne diseases, 128,000 are hospitalized and 3,000 die. From 2010 to 2014, 114 outbreaks of foodborne diseases occurred in Brazilian hospital units, while in the United States only 108 outbreaks were registered during the period from 1998 to 2012 (BRASIL, 2014; CDC, 2014).

KOZAK et al. (2013) verified the outbreaks associated with fresh vegetables and fruits in Canada during the period from 2001 to 2009, where 27 items of plant origin were associated with the occurrence of 1,549 outbreaks of foodborne diseases. Among the most commonly encountered bacteria were *Salmonella* spp. (50%), *Escherichia coli* (33%) and *Shigella* spp. (17%).

There is no specific syndrome associated with foodborne diseases. However, as they enter the body orally, the initial clinical symptoms are associated with the gastrointestinal tract, although extraintestinal symptoms are also possible, affecting several organs and systems (BRASIL, 2010; CDC, 2012).

The contamination of vegetables can occur during any production phase and can have its origin in the primary phase as a result of contamination of the equipment and the environment (soil, water and air), as well as during, before and after harvesting (CEUPPENS et al., 2014). At the same time, food manipulators, utensils and contact surfaces are all important sources of contamination during the preparation of meals, which may cause hazards of biological, chemical or physical nature in a salad ready to be consumed (BALZARETTI; MARZANO, 2013; BRANDÃO et al., 2014).

In order to ensure the safety of vegetables, as well as dishes prepared using these vegetables, the National Sanitary Surveillance Agency (ANVISA), by way of its Resolution No. 12/01, established microbiological standards, requiring complete absence of *Salmonella* spp. in 25 g of product, 10^2 NMP/g of coliforms at 45ºC and coagulate-positive staphylococci count at $< 1.0 \times 10^2$ UFC/g (BRASIL, 2001).

Considering the characteristics attributed to the studied food, like the presence of vegetables in the composition of dishes that make part of dietary habits of Brazilians, especially in hospital diets, as well as their relevance for health promotion and as adjuvants in the treatment of some diseases, when speaking about outbreaks of foodborne diseases, research is needed to obtain information about the microbiological profile of meals served in Hospital Food and Nutrition Units (UAN).

Thus, the objective of this study was to verify the microbiological profile of different types of salads from hospital kitchens of two public hospitals of the Central West region of São Paulo state, by determining the Most Probable Number (MPN) of coliforms at 35 and 45ºC, *Salmonella* spp. research and coagulate-positive staphylococci count.

MATERIAL AND METHODS

From January 2010 to July 2014, the Public Food Guidance Service (SOAP) of Botucatu *campus* of Faculty of Veterinarian Medicine and Animal Science (FMVZ) received 641 samples of salads from hospitals located in Botucatu and Bauru regions of São Paulo state. These were put in a sterile collector bag and sent in an exothermic box to microbiological laboratory to realize the following tests: determination of the MPN of coliforms at 35 and 45ºC, *Salmonella* spp. study and coagulate-positive staphylococci count.

Preparation and Dilution of Samples

The samples were weighed using analytical balance (Gehaka BK 3000, Gehaka, Brazil) under a laminar flow hood (Veco Bioseg 12, Veco, United States). Immediately after cleaning the packages with 70% alcohol, 25 g of homogenized samples were weighed aseptically and 225 mL of 0.1% Oxoid buffered peptone water was added, followed by homogenization realized in *Stomacher*. One mL of this 10^{-1} dilution was transferred to a threaded tube containing 9 mL of 0,9% saline solution, which allowed to obtain a 10^{-2} dilution, and after repeating the procedure — a 10^{-3} dilution.

Microbiological Analysis

The analytical methods for the determination of the MPN of coliforms at 35 and 45ºC and for coagulate-positive staphylococci count were carried out in accordance with the description given in Normative Instruction No. 62 of the Ministry of Agriculture, Livestock and Supply (MAPA) (BRASIL, 2003). The *Salmonella* spp. study was conducted according to the methodology recommended by the Bacteriological Analytical Manual (ANDREWS et al., 2011).

Data Collection

The results were collected through verification of reports generated by the Sample Management for Analysis Program

(PGAA) and transferred to an Excel 2010 spreadsheet for further statistical analysis.

Statistical Analysis

The distribution of MPN values for coliforms at 35ºC was verified through quantile and box plot charts, showing the absence of normality. Statistical analysis was performed using non-parametric Wilcoxon test for independent samples via PROC NPAR1WAY (SAS, 2011), since the logarithmic scale was not effective for normalization. At the same time, the results were depicted graphically using the logarithmic scale for a better visualization.

A logistic regression model was used via PROC GLIMMIX (SAS, 2011) in order to estimate the chances of finding samples considered to be positive for coliforms at 45ºC as an explanatory variable function: heat processing and vegetable category. The interaction term between heat processing and vegetable category was tested in this model. The results were presented in percentage. For all tests, the significance level was set at 5% ($p < 0.05$).

RESULTS

From January 2010 to July 2014, 641 samples of salads from hospital kitchens of two public hospitals from Central West region of São Paulo state were analyzed. Of these, 607 (94.70%) did not receive any type of heat treatment (SPT) and 34 (5.30%) did pass through a heat treatment process, which means cooking or braising (CPT).

According to the part of vegetables intended for human consumption, the samples were categorized as leaves, fruits and tuberoses. A fourth group called "mixed" was created to categorize samples composed of more than one ingredient (Table 1).

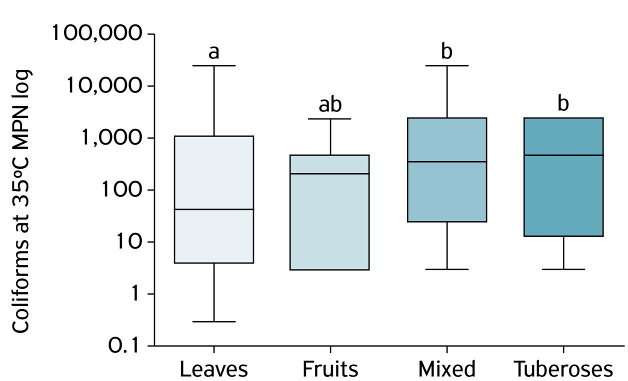
All 641 samples (100%) showed absence of *Salmonella* spp. in 25 g of product and the coagulate-positive staphylococci count was $< 1.0 \times 10^2$ UFC/g. Of the total amount of samples, 196 (30.60%) showed presence of coliforms at 35ºC above 1,100 MPN/g. The median value (min.–max.) was 93 (0.3–24,000) for SPT samples and 10 (3–2,400) for CPT samples ($p = 0.01$).

Figure 1 shows the MPN of coliforms at 35ºC in salads for respective category. Among the four analyzed categories, only the leaves showed statistical difference in comparison with mixed and tuberoses categories ($p < 0.05$). The total coliform-positive percentage above 100 MPN/g, which is a standard limit set by current legislation, for samples at 45ºC was 78 (12.17%), in which 76 (97.44%) were heat treatment (SPT) samples and 2 (2.56%) were cooking or brasing (CPT) samples. The chances of SPT samples to be positive were 6.98 times greater when compared to samples of CPT group, with a confidence interval of 95%CI 1.48–32.65, showing statistical difference ($p < 0.05$).

Table 1. A percentage of SPT and CPT samples, and total percentage of salads analyzed at SOAP during the 2010-2014 period.

Samples	SPT (%)	CPT (%)	Total (%)
Fruits	26 (89.65)	3 (10.34)	29 (4.52)
Pumpkin	1 (50)	1 (50)	2 (0.31)
Squash	1 (100)	0 (0)	1 (0.16)
Eggplant	6 (0.75)	2 (25)	8 (1.25)
Tomato	14 (100)	0 (0)	14 (2.18)
Cucumber	4 (100)	0 (0)	4 (0.62)
Leaves	521 (98.67)	7 (1.32)	528 (82.37)
Chard	34 (97.14)	1 (2.86)	35 (5.46)
Cress	48 (100)	0 (0)	48 (7.49)
Lettuce	155 (100)	0 (0)	155 (24.18)
Romaine lettuce	87 (100)	0 (0)	87 (13.57)
Chive	2 (100)	0 (0)	2 (0.31)
Coriander	9 (100)	0 (0)	9 (1,40)
Chicory	9 (100)	0 (0)	9 (1,40)
Kale	25 (83.33)	5 (16.67)	30 (4.68)
Endive/ Escarole	42 (100)	0 (0)	42 (6.55)
Spinach	2 (100)	0 (0)	2 (0.31)
Cabbage	37 (97.37)	1 (2.63)	38 (5.93)
Ruccola	69 (100)	0 (0)	69 (10.76)
Parsley	2 (100)	0 (0)	2 (0.31)
Tuberoses	26 (76.47)	8 (23.52)	34 (5.30)
Potato	0 (0)	2 (100)	2 (0.31)
Beet	11 (100)	0 (0)	11 (1.72)
Onions	1 (100)	0 (0)	1 (0.16)
Carrots	14 (70)	6 (30)	20 (3.12)
Mixed	34 (68)	16 (32)	50 (7.8)
Total	607 (94.70)	34 (5.30)	641 (100)

SPT: no heat processing; CPT: heat processed.



Different letters indicate statistical difference ($p < 0.05$).

Figure 1. Coliforms at 35ºC MPN log for samples distributed in categories.

In Table 2, the tuberose vegetables showed the largest percentage of positivity for the presence of coliforms at 45ºC above the limits established by current legislation, with 15 (44.12%) showing statistical difference in relation to other categories ($p < 0.05$). The tuberoses had 10.53 (4.73–23.55) more chances to be positive than leaves, 8.93 (2.17–37.03) in relation to fruits and 3.55 (1.31–10.00) when compared to mixed category. There was also statistical difference ($p < 0.05$) between mixed and leaves categories, in which the former one had 2.98 (1.32–6.71) times more chances to be positive than the latter one. There was no statistical difference between fruits and leaves neither between mixed and fruits ($p > 0.05$).

DISCUSSION

Although there is no standard in Brazilian legislation concerning the MPN of coliforms at 35ºC, an elevated number of these microorganisms in prepared meals ready to be consumed provides information about the hygiene practices before, during and after food processing (JAY, 2005; OLAIMAT; HOLLEY, 2012).

In addition to obtained results, other authors report the occurrence of coliforms at 35ºC with values above 1,100 MPN/g in this type of product (LEÓN et al., 2013; BRANDÃO et al., 2014), since some varieties of bacteria belonging to this group are found naturally in fresh vegetables and soil (FRANCO; LANDGRAF, 2002; JAY, 2005). As for the MPN of coliforms at 45º, the results are similar to those obtained by BRANDÃO et al. (2014) in Brazil, showing a 20% prevalence of values above the standard limits in salad samples from restaurants, although lower than the results obtained in other studies conducted in Brazil, which showed a prevalence of 70% in samples of minimally processed salads originating from supermarkets (FRÖDER et al., 2007) and 100% in salads originating from restaurants (ROCHA et al., 2014) with values above 100 MPN/g.

The lesser prevalence of coliforms at 45ºC in salads that were submitted to a heat treatment process is evidence of the importance of temperature in maintaining the products safe for consumption (ALMUALLA et al., 2010; FARIAS et al., 2011). The lesser contamination of cooked salads in comparison with the raw ones was also noted in restaurants of Mexico (LEÓN et al., 2013). In accordance with the Sanitary Vigilance Center Ordinance (CVS-5) dated 04/2013, the temperature of cold meals ready for consumption should be 10ºC with a validity term of four hours, or between 10 and 21ºC, with a validity term of two hours (SÃO PAULO, 2013).

Table 2. Number and percentage of samples above the 10^2 MPN/g limit for coliforms at 45ºC, established by Resolution No. 12/01, divided by category.

Class	N Total	N Pos	% Pos
Tuberoses	34	15	44.12^{a}
Mixed	50	9	18^{b}
Fruits	29	3	10.34^{bc}
Leaves	528	51	9.66
Total	641	78	12.17

N Total: Total number of samples in a category; N Pos: Number of positive samples in a category; % Pos: Percentage of positive samples in a category; Different letters $^{(a, b, c)}$ indicate statistical difference ($p < 0.05$).

Salads that had tubers in their composition showed greater occurrence of coliforms. This result may be associated with the preparation method, as the tuberose vegetables consumed raw are subjected to more manipulation, such as peeling, cutting and grating/shredding, which results in freshly cut vegetables exhibiting higher moisture content and greater nutrient availability, apart from increased contact surfaces, favoring the growth of microorganisms (JAY, 2005; OLAIMAT; HOLLEY, 2012). These vegetables are also more exposed to contaminants during the initial production phase, since the part intended for consumption is found beneath the surface of the soil (BEVILACQUA, 2014; FAO, 2014). Thus, if directly after the harvesting stage some effective hygienic measures are not adopted, the final product may end up containing contaminants originating from the cultivation phase, since the soil and fertilizers are sources of contamination during the primary production phase (OLAIMAT; HOLLEY, 2012; CEUPPENS et al., 2014).

CEUPPENS et al. (2014), after analyzing the materials used in the production of lettuce in Brazil, showed that the prevalence of *Salmonella* spp. in organic fertilizers (manure) was 5,6%, while in the soil it was 2,6%. They also showed a strong relationship between the elevated number of coliforms at 45ºC and the increase in the prevalence of pathogens.

Irrigation water is another important source of contamination for raw vegetables during the initial production phase (OLAIMAT; HOLLEY, 2012; CEUPPENS et al., 2014). In accordance with Resolution No. 357/05 of the National Council for Environment (CONAMA), the maximum limit of coliforms at 45ºC for water used in irrigation of vegetables intended for consumption is 200 MPN/100 mL (BRASIL, 2005). Research conducted in Brazil in the regions of Botucatu/SP (FRAVET; CRUZ, 2007) and Brasília/DF (ABREU et al., 2010) revealed that 100% of irrigation water used in plots did not meet the requirements of the legislation in respect of MPN of coliforms at 45ºC.

The presence of coliforms in vegetables does not necessarily mean that there was a fecal contamination, because some strains of the *Enterobacter* and *Klebsiella* genus possess the same characteristics as coliforms at 45ºC and can be found in other environments, like fresh vegetables themselves, as well as soil (FRANCO; LANDGRAF, 2002; JAY, 2005). At the same time, high levels may compromise sensorial and nutritional quality, apart from being an evidence of unsatisfactory hygienic practices during the stage of washing the vegetables for preparation of salads (BRANDÃO et al., 2014).

During the preparation of salads, the food manipulators and contact surfaces are both important sources of contamination (BALZARETTI; MARZANO, 2013; BRANDÃO et al., 2014). In Brazil, RIBEIRO et al. (2014) analyzed the sanitary conditions of home kitchens and found that 47.5 and 3.8% of the hands of the person who, among the residents, was most likely to handle the food were contaminated with coliforms at 35ºC and *Escherichia coli*, respectively. In Italy, BALZARETTI; MARZANO (2013) evaluated the hygienic conditions of 44 UAN of three airports and found that 8.4 and 3.5% of hand washing by food handlers showed unsatisfactory limits with regard to total bacterial coagulate-positive staphylococci count, respectively.

The washing of vegetables helps reduce the number of microorganisms (JAY, 2005). RODRIGUES et al. (2011) evaluated two techniques used in the cleaning of vegetables and fruits, one of them made use of water and soap and the other one a 1% sodium hypochlorite solution with a 100–250 ppm active chlorine content. Both cleaning procedures lasted for 15 minutes. Only the later technique proved to be efficient in the quantitative reduction of microorganisms.

Although *Salmonella* spp. is the most commonly biological agent associated with outbreaks of foodborne diseases in Brazil (BENNETT, 2014; BRASIL, 2014), this study showed a notable absence of it in 25 g, which is in line with the results obtained by BRANDÃO et al. (2014). At the same time, ARAÚJO et al. (2011) obtained a 100% prevalence of *Salmonella* spp. in raw salads of restaurants in Pombal, in Paraiba state. In other studies carried out in UAN by FRÖDER et al. (2007), DIAS et al. (2011) and MAISTRO et al. (2012), the prevalence of *Salmonella* spp. in salad samples was 3, 28.57 and 16.86%, respectively. The presence of *Salmonella* spp. was also observed in salads from restaurants in Mexico, where GÓMEZ-ALDAPA et al. (2013) found a prevalence of 6.8% in raw salads, while LEÓN et al. (2013) found a prevalence of 1% in cooked salads.

The meals served by hospital Food and Nutrition Units must be safe in all respects, especially with regard to hygiene. Although foodborne diseases affect any type of person, some population groups are more susceptible than others, which is the case of indoor patients with impaired immune systems, whose state of health may be aggravated by ingestion of contaminated food (KONECKA-MATYJEK et al., 2012; SARKER et al., 2014).

The results of previous research performed with salad samples from different types of establishments are comparable with those shown by present study, as all of these establishments are considered Food and Nutrition Units and must meet the same hygienic and sanitary parameters (PINTO et al., 2013).

CONCLUSION

The microbiological profile of salad samples from kitchens of two public hospitals of the Central West region of São Paulo state proved to be compatible with current legislation regarding *Salmonella* spp. and coagulate-positive staphylococci count. Although no pathogenic agents were found, the high count of indicator microorganisms in the majority of samples suggests that the practices of obtaining and manipulating these products are inadequate, allowing the risk of contamination by pathogens, including other agents not included in this research. Therefore, hospital Food and Nutrition Units must pay attention to food preparation procedures, especially since these meals are served to hospitalized individuals.

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