Nutrición Hospitalaria

Nutr Hosp. 2015;32(5):2280-2285
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original/*Otros*

Microbiological assessment of lettuce salads and antimicrobial resistance of *Staphylococcus* spp

Josi Guimarães César[1], Andriele Madruga Peres[2], Caroline Pereira das Neves[2], Érica Tupiniquim Freitas de Abreu[2], Jozi Fagundes de Mello[2], Ângela Nunes Moreira[1,2,3] and Kelly Lameiro Rodrigues[1,2]

[1]Programa de Posgrado en Nutrición y Alimentos, Universidad Federal de Pelotas, Facultad de Nutrición, Campus Porto, Pelotas, RS. [2]Departamento de Nutrición, Universidad Federal de Pelotas, Facultad de Nutrición, Campus Porto, Pelotas, RS. [3]Programa de Posgrado en Biotecnología, Centro de Desarrollo Tecnológico, Universidad Federal de Pelotas, Campus Capão do Leão, Pelotas, RS, Brasil.

Abstract

Introduction: **self-service restaurants in which food is served ready to be consumed are liable to have some products contaminated by pathogenic microorganisms causing food-transmitted diseases.**

Aim: **evaluates the microbiological quality of lettuce salads in restaurants in Pelotas RS Brazil by counts of thermo-tolerant coliforms, *E. coli*, *Staphylococcus* spp. and detection of *Salmonella* spp. Antimicrobial resistance of *Staphylococcus* spp. isolates are also assessed.**

Methods: **thirty-six samples of lettuce salads were collected from nine restaurants and thermotolerant coliforms, *Escherichia coli* and *Staphylococcus* spp. were quantified, coupled to a research on *Salmonella* spp., following methodology by the Bacteriological Analytical Manual. *Staphylococcus* spp. isolates underwent antimicrobial resistance test by the disc-diffusion method.**

Results and discussion: **results showed that 61.1% of the salad samples contained more thermotolerant coliforms than allowed by Brazilian legislation and *E. coli* was confirmed in 5.6% of the samples. Positive and negative coagulase *Staphylococcus* occurred respectively in 5.6% and 77.8% of isolates, but no sample had *Salmonella* spp. Further, 56.7% of the thirty isolates of *Staphylococcus* spp. tested were resistant to penicillin; 46.7% to oxacillin; 26.7% to erythromycin and 23.3% were multi-resistant.**

Conclusion: **inadequate quality of the salad was due to pathogenic microorganisms, while *Staphylococcus* spp. isolates had a high percentage of antimicrobial resistance.**

(*Nutr Hosp*. 2015;32:2280-2285)

DOI:10.3305/nh.2015.32.5.9632

Key words: *Microorganism markers*. *Restaurants*. *Antibiotics*.

Correspondence: Josi Guimarães César.
Universidad Federal de Pelotas.
Calle Pinto Martins, 652 A apto. 31. Barrio Centro.
Pelotas, RS, Brasil. CEP 96020-350.
E-mail: josigcesar@gmail.com

Recibido: 10-VII-2015.
Aceptado: 14-VIII-2015.

EVALUCIÓN MICROBIOLÓGICA DE ENSALADAS DE LECHUGA Y PERFIL DE RESISTENCIA ANTIMICROBIANA DE *STAPHYLOCOCCUS* SPP

Resumen

Introducción: **la procura por estabelecimientos que ofrecen alimentos prontos para consumo ha aumentado, sin embrago, los alimentos disponibles en estos locales pueden estar contaminados con microorganismos patogénicos, pudiendo causar enfermedades transmitidas por alimentos.**

Objetivos: **evaluar la calidad microbiológica de las ensaladas de lechuga en los restaurantes de Pelotas RS Brasil, a través de los recuentos de coliformes termotolerantes, *Escherichia coli*, *Staphylococcus* spp. y la detección de *Salmonella* spp. Resistencia a los antimicrobianos de *Staphylococcus* spp. también se evalúan.**

Métodos: **fueron colectadas 36 muestras de ensaladas de lechuga en nueve restaurantes y realizada la cuantificación de coliformes termotolerantes, *Escherichia coli* y *Staphylococcus* spp. e investigación de *Salmonella* spp., siguiendo la metodología del *Bacteriological Analytical Manual*. Los aislados de *Staphylococcus* spp. fueron sometidos al examen de resistencia a antimicrobianos por el método de difusión con discos.**

Resultados y discusión: **de las 36 muestras de ensalada de lechuga, 61,1% presentaron cuantificación de coliformes termotolerantes superiores a lo permitido por la legislación brasileña, y hubo confirmación de *E. coli* en 5,6% de las muestras. La cuantificación de *Staphylococcus* coagulasa positiva representó 5,6% de los aislados y *Staphylococcus* coagulasa negativa representó 77,8%. Todas las muestras presentaron ausencia de *Salmonella* spp. De los 30 aislados de *Staphylococcus* spp. examinados, 56,7% fueron resistentes a penicilina, 46,7% a oxacilina, 26,7% a eritromicina y 23,3% fueron multirresistentes.**

Conclusión: **la calidad microbiológica de las ensaladas de lechuga se mostró inadecuada debido a la presencia de microorganismos patogénicos, y los aislados de *Staphylococcus* spp. presentaron elevado porcentaje de resistencia antimicrobiana.**

(*Nutr Hosp*. 2015;32:2280-2285)

DOI:10.3305/nh.2015.32.5.9632

Palabras clave: *Microorganismos indicadores*. *Restaurantes*. *Antibióticos*.

Abbreviations

FD: foodborne disease.
MPN: Most Probable Number.
CFU: Colony-Forming Units.
PCS: positive coagulase *Staphylococcus*.
NCS: negative coagulase *Staphylococcus*.

Introduction

Self-service restaurants in which food is served ready to be consumed are liable to have some products contaminated by pathogenic microorganisms causing foodborne diseases (FD)[1,2,3]. Thermotolerant coliforms and *Escherichia coli* are microorganism indicators of fecal contamination; *Staphylococcus* spp. indicates poor hygiene conditions during food processing; and *Salmonella* spp. is a pathogenic microorganism which may cause infections by food[4]. Epidemiological data in Brazil show that *Salmonella* spp., positive coagulase *Staphylococcus* and *Escherichia coli* are the main agents in FD outbreaks[5].

Lettuce (*Lactuca sativa*) is consumed worldwide and has an important role on the Brazilian market. In fact, this leafy vegetable has beneficent qualities for health due to its fiber rates and antioxidant properties[2,6,7,8]. Since the lettuce is consumed raw, adequate hygiene process should be undertaken to eliminate pathogen microorganisms[9,10,11]. In fact, the lettuce has been associated with contamination by certain pathogenic microorganisms such as *Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes*[12,13].

Staphylococcus spp., especially positive coagulase *Staphylococcus*, may occur in food intoxication outbreaks[5,10,11]. Further, since it acquires antimicrobial resistance and bacterial multiresistance, it becomes the main problem in the treatment of infections[14].

Antimicrobial resistance is actually a growing health issue in animal breeding and public health. Several bacteria in the environment are resistant and may contaminate water and food with serious consequence to health[15,16]. Due to their capacity in acquiring antimicrobial resistance, *Staphylococcus* infections are on the increase, with a greater number of multi-resistant strains and rising difficulties in their treatment[17,18,19,20].

Current analysis evaluates the microbiological quality of lettuce salads in restaurants in Pelotas, RS, Brazil by counts of thermo-tolerant coliforms, *E. coli*, *Staphylococcus* spp. and detection of *Salmonella* spp. Antimicrobial resistance of *Staphylococcus* spp. isolates are also assessed.

Methods

Experimental design and sampling

A transversal analysis was conducted in self-service restaurants in Pelotas, RS, Brazil. Information on the number and place of the restaurants was retrieved from the sanitary and food vigilance sector of the Municipal Health Office for the experimental design. Sampled population comprised 10% of the number (91) of self-service restaurants in the town, with the random selection of 9 restaurants.

Collection of samples

A sample of lettuce salad was retrieved from each restaurant (n=9), once a week, during four weeks, with a total of 36 samples. Samples were retrieved as if one were buying lettuces; collection occurred at the distribution section and the lettuces were packed in disposable thermal packages available at the restaurant. The package was closed, identified and taken immediately to the laboratory for analysis.

Microbiological analyses

Thermo-tolerant coliforms, *Escherichia coli* and *Staphylococcus* spp were counted and search for *Salmonella* spp. was undertaken, following requirements by Brazilian legislation[21]. Microbiological analyses followed methodology by the Bacteriological Analytical Manual[22]. Counts of thermo-tolerant coliforms followed the Most Probable Number (MPN) method in a Lauril Sodium Sulfate broth test (LST, Merck®) and a confirmation test in *Escherichia coli* broth (EC, Merck®). Confirmation analysis for *E. coli* was undertaken where the confirmed culture was streaked by Eosin-Methylene Blue Agar Plate Protocol (L-BEM, Merck®), incubated at 36 °C for 24 h. Typical colonies were transferred to Count Standard Agar plates (PCA, Merck®) and incubated at 36 °C for 24 h. Colonies were Gram stained and pure cultures underwent biochemical tests.

For the analysis for *Salmonella* spp., pre-heating was done in Buffered Peptone Water (Merck®), at 37 ºC for 24 h. After incubation, 1 mL was transferred to Tetrathionate Broth (Merck®) to which 0.1mL of brilliant green and 0.2 mL iodine were added; 0.1 mL was transferred to Rappaport-Vassiliadis broth (Merck®) and incubated at 42 ºC for 24 h. Seeding in Hektoen Agar (HE, Merck®) and agar Xylose-lysine-deoxycholate (XLD, Merck®) was performed after the above selective enrichment; it was then incubated at 37 ºC for 24 h. Characteristic colonies underwent biochemical and serum tests.

Three decimal dilutions were performed for the isolation of *Staphylococcus* spp. and 1 mL of each was divided into three Agar Baird Parker plates (BP, Merck®) enriched with egg yolk emulsion and potassium tellurite 1% by surface spreading, and incubated at 37 ºC for 48 h. Further, typical and atypical presumptive colonies were counted and given in Colony-Forming Units per gram of food (CFU/g). Isolated colonies were Gram-stained and the characteristic and pure ones were

selected. A loopful of each selected colony was inoculated in Brain Heart Infusion broth (BHI, Merck®) and incubated at 37 ºC for 24 h. The coagulase test was performed and colonies with coagulates were considered positive; otherwise they were considered negative.

Antimicrobial resistance

Antimicrobial resistance by disc diffusion technique was verified from isolates of positive coagulase *Staphylococcus* (PCS) and negative coagulase *Staphylococcus* (NCS), according to the Clinical and Laboratory Standards Institute (CLSI)[23]. Isolates were first cultivated in BHI broth (Merck®) at 36 ºC and inoculated in a saline solution 0.85 % (Merck®) till Mac Farland scale 0.5. The culture was then spread on a plate containing Agar Muller Hinton (MH, Merck®) and the antimicrobial discs (Invitrogen®); they were similar spread and incubated at 37 ºC for 24 h for Gram positive bacteria. Halo was measured in centimeters from one extremity to the other and result was obtained by comparing tables for CLSI resistance standard[23]. The 12 antimicrobial agents tested composed the antimicrobial disc: ampicillin (10 μg), penicillin (10 units), oxacillin (1 μg), clindamycin (2 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), chloramphenicol (30 μg), erythromycin (15 μg), gentamicin (10 μg), tetracycline (30 μg), vancomycin (30 μg), ciprofloxacin (5 μg), cefepime (30 μg), rifampicin (5 μg) (CLSI, 2007).

Statistical analysis

Statistical analyses were performed with SPSS® (Chicago, v. 17.0, 2008) for antimicrobial resistance test.

Results and discussion

Twenty-two (61.1%) of the 36 lettuce salad samples analyzed contained thermotolerant coliforms above the limit allowed by Brazilian legislation, or rather, up to 10^2 NMP/g[21] (Table I).

Table I
Amount of thermotolerant coliforms in samples of lettuce salads. Pelotas, 2015. (n=36)

	Samples	
	n (%)	*Counts (MPN/g)*
Thermotolerant coliforms	15 (41.7)	$1.1x10^3$ a > $1.1x10^3$
	7 (19.4)	$1.6x10^2$ to $4.6x10^2$
	10 (27.8)	3.6 to 93
	4 (11.1)	< 3

MPN/g – Most Probable Number per gram of food.

Brandão et al.[24] evaluated 30 samples of lettuce salad in self-service restaurants in Rio de Janeiro, RJ, Brazil and detected 22 samples (73.3%) contaminated by thermotolerant coliforms, with 6 samples (19.9%) above the limit allowed by Brazilian legislation. Another study evaluated the microbiological quality of lettuce salads in 10 self-service restaurants in Gurupi, TO, Brazil and reported 12 samples (60%) contaminated by thermotolerant coliforms above the minimum rate[25].

Moreover, two samples (5.6%) out of the 36 samples in current analysis were confirmed for *E. coli*, with 3.6 MPN/g in each sample. Several other studies register *E. coli* in lettuces ready for consumption, with percentages ranging between 3.5 and 30% contamination[24,26,27,28].

The amount of thermotolerant coliforms at percentages higher than those permitted by the legislation in ready foods, and the confirmation of *E. coli* indicate either improper prime matter conditions, or unsatisfactory food processing conditions or inadequate hygiene-sanitary conditions[21,24,29,30,31]. Contamination in current analysis may be related to the fact that lettuce salads were served raw and either there were inadequate hygiene procedures during food handling or contamination occurred in the post-processing period[4,24,27,28].

No *Salmonella* spp. was reported in any of the analyzed samples (25 g of food) and thus Brazilian legislation was complied with (BRASIL, 2001). Results were similar to other studies that assessed *Salmonella* spp. in lettuce salad[24,26,27,28,32].

Two (5.6%) out of the 36 samples were reported with PCS, whereas 28 (77.8%) were reported with NCS (Table II).

Whereas a study in São Bernardo do Campo, SP, Brazil evaluated 30 samples of raw green vegetables in self-service restaurants and detected one sample (3.3%) with PCS[33]. Another study in Porto Alegre, RS,

Table II
Quantity of Staphylococcus spp. in lettuce salad samples. Pelotas, 2015. (n=36)

	Samples	
	n (%)	*Counts (CFU/g)*
Positive coagulase *Staphylococcus*	1 (2.8)	$2x10^5$
	1 (2.8)	$2x10^3$ (est.)
	34 (94.4)	< 10
Negative coagulase *Staphylococcus*	13 (36.1)	$1x10^5$ a $5x10^5$
	9 (25.0)	$1x10^4$ a $7.0x10^4$
	5 (13.9)	$3x10^3$ a $9x10^3$
	1 (2.8)	$9x10^2$
	8 (22.2)	< 10

CFU/g – Colony-Forming Unit per gram of food; (est.) - estimated count

Brazil evaluated 26 samples of different types of food, including raw and boiled salads, and reported 15 samples (57.7%) contaminated with NCS. The same study assessed the hands of 21 food handlers and discovered 9 people with *Staphylococcus* spp.[34].

Although staphylococcus contamination occurs by inadequate hygiene of hands, the production of toxins occurs in food with *Staphylococcus* spp. counts over 10^5 CFU/g[5,18,30,31,35,36,37].

Moreover, *Staphylococcus* spp. is resistant to several antimicrobial classes and may cause illness and even death owing to infections[38,39,40,41]. The microbial resistance test (n=30) was undertaken from *Staphylococcus* spp. isolates. Results revealed that isolates had a higher resistance percentage to penicillin (56.7%), oxacillin (46.7%) and erythromycin (26.7%). Only one isolate (3.3%) had an intermediate resistance to the antimicrobial agent erythromycin. All *Staphylococcus* spp. isolates under analysis were sensitive to the antimicrobial agent gentamycin (Table III).

Other studies that evaluated the antimicrobial resistance of *S. aureus*, a PCS isolated from food and humans, detected 31 (58.5%) of samples resistant to penicillin[40]; 133 (47.6%) resistant to oxacillin[42]; 29 (22.7%) resistant to erythromycin[43] and 8 (100%) samples sensitive to gentamicin[44].

Penicillin and oxacillin belong to the antimicrobial β-lactam class and antimicrobial resistance occurs through the interference in the synthesis and remodeling of bacterial peptidoglycans. However, erythromycin belongs to the macrolide class and its antimicrobial resistance occurs by the inhibition of protein synthesis of the susceptible bacterial cells in the 50S ribosomal subunit. β-lactam resistance in positive Gram bacteria mainly occurs on the alteration of the target protein, with enzyme degradation[39,45,46].

Seven (23.3%) among the 30 *Staphylococcus* spp. isolates evaluated for antimicrobial resistance were multi-resistant, of which one isolate was resistant to six antimicrobial agents (clindamycin, vancomycin, erythromycin, rifampicin, oxacillin and penicillin). Similar studies on antimicrobial resistance of *Staphylococcus* isolates also reported multi-resistance to the same anti-microbial agents[44,47,48].

Several mechanisms may trigger bacterial multi-resistance: either by intercellular decrease of the antimicrobial agent by an alteration in the permeability of the external membrane and decrease transport through the internal membrane, or by active efflux through enzyme mutation or modification and the deviation of the drug to the target[49]. Multi-resistant isolates may increase the number of highly persistent infections, limit the use of available antimicrobial agents and, consequently, make difficult clinical treatment, increase the dissemination of infections, hospital costs and mortality rates[14,50].

Conclusion

The microbiological quality of lettuce salads is very poor. Although *Salmonella* spp. was not detected, more than half the samples contained thermotolerant coliforms above the limit permitted by legislation. Important pathogens in food, such as *E. coli* and *Staphylococcus* spp. were reported, coupled to a high percentage of antimicrobial resistance of isolated microorganisms.

Acknowledgement

The researchers would like to thank the Coordination of Superior Level Staff Improvement (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES).

Table III
Antimicrobial resistance test of Staphylococcus spp. isolates in lettuce salad. Pelotas, 2015. (n=30)

Antimicrobial agents	*Sensitive (%)*	*Intermediate (%)*	*Resistant (%)*
Vancomycin	96.7	0	3.3
Erythromycin	70	3.3	26.7
Chloramphenicol	93.3	0	6.7
Rifampicin	96.7	0	3.3
Cefepime	93.3	0	6.7
Oxacillin	53.3	0	46.7
Penicillin	43.3	0	56.7
Ciprofloxacin	90	0	10
Gentamicin	100	0	0
Tetracycline	96.7	0	3.3
Sulfamethoxazole/trimethoprim	96.7	0	3.3

References

1. Welker CAD, Both JMC, Longaray SM, Haas S, Soeiro MLT, Ramos RC. Análise microbiológica dos alimentos envolvidos em surtos de doenças transmitidas por alimentos (DTA) ocorridos no estado do Rio Grande do Sul, Brasil. *Rev Bras Bioc* 2010; 8: 44-48.
2. Pereira EL, Rodrigues A, Ramalhosa E. Influence of working conditions and practices on fresh-cut lettuce salads quality. *Food Control* 2013; 33: 406–412. doi: 10.1016/j.foodcont.2013.03.021.
3. Leong D, Alvarez-Ordóñez A, Jordan K. Monitoring occurrence and persistence of Listeria monocytogenes in foods and food processing environments in the Republic of Ireland. *Front. Microbiol* 2014; 5: 4361-4368. doi: 10.3389/fmicb.2014.00436.
4. Franco BDGM, Landgraf M. *Microbiologia dos alimentos*. Vol. I. Artmed, Porto Alegre; 2008.
5. Brasil. 2014. Ministério da Saúde. Secretaria de Vigilância e Saúde. *Vigilância Epidemiológica das Doenças de Transmissão de Alimentos Hídrica e Alimentar (VE-DTA). Dados epidemiológicos de 200 a 2014*. [Internet]. 2001 [acesso 2015 abr 12]. Disponível em: <http:// www.saude.gov.br/
6. Abreu IMO, Junqueira AMR, Peixoto JR, Oliveira AS. Qualidade microbiológica e produtividade de alface sob adubação química e orgânica. *Ciênc Tecnol Aliment* 2010; 30: 108-118. doi: 10.1590/S0101-20612010000500018.
7. Zhou T, Harrison AD, Mckellar R, Young JC, Odumeru J, Piyasena P. Determination of acceptability and shelf life of ready-to-use lettuce by digital image analysis. *Food Res Int* 2004; 37: 875-881. doi: 10.1016/j.foodres.2004.05.005.
8. Mogharbel ADI, Masson ML. Perigos associados ao consumo da alface, (*Lactuca sativa*), *in natura*. *Alim Nutr Araraquara* 2005; 16: 83-88.
9. Moreira IS, Souza FC, Santos FM, Feitosa MKSB, Marques LF. Eficiência de soluções antimicrobiana na desinfecção de alface tipo crespa comercializada em feira livre. *Rev Verde* 2013; 8: 171-177. doi: 10.18378/rvads.v8i2.2196.
10. Bennett SD, Walsh KA, Gould LH. Foodborne disease outbreaks caused by Bacillus cereus, Clostridium perfringens, and *Staphylococcus aureus* - United States, 1998-2008. *Clin Infect Dis* 2013; 57: 425-433. doi: 10.1093/cid/cit244.
11. Ho J, Boost M, O'Donoghue M. Sustainable reduction of nasal colonization and hand contamination with *Staphylococcus aureus* in foodhandlers, 2002-2011. *Epidemiol Infect* 2014; 13: 1-10. doi: 10.1017/S0950268814002362.
12. Jablasone J, Warriner K, Griffiths M. Interactions of *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* plants cultivated in a gnotobiotic system. *Int J Food Microbiol* 2005; 99: 7-18.
13. Jeddi MZ, Yunesian M, Gorji ME, Noori N, Pourmand MR, Khanik GRJ. Microbial Evaluation of Fresh Minimally-processed Vegetables and Bagged Sprouts from Chain Supermarkets. *J Health Popul Nutr* 2014; 32: 391-399.
14. Deleo FRE, Chambers HF. Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. *J Clin Invest* 2009; 119: 2464-2474. doi: 10.1172/JCI38226.
15. Normanno G, La Salandra G, Dambrosio A, Quaglia NC, Corrente M, Parisi A, Santagada G, Firinu A, Crisetti E, Celano GV. Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *Int J Food Microbiol* 2007; 115: 290-296.
16. Resch M, Nagel V, Hertel C. Antibiotic resistance of coagulase-negative staphylococci associated with food and used in starter cultures. *Int J Food Microbiol* 2008; 127: 99-104. doi: 10.1016/j.ijfoodmicro.2008.06.013.
17. Gorwitz RJ, Kruszon-Moran D, Mcallister SK, MCquillan G, Mcdougal LK, Fosheim GE, Jensen BJ, Killgore G, Tenover FC, Kuehnert MJ. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. *J Infect Dis* 2008; 197: 1226-1234. doi: 10.1086/533494.
18. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat Rev Microbiol* 2009; 7: 629-641. doi: 10.1038/nrmicro2200.
19. Montoya IC, Mira MO, Álvarez IA, Cofre JG, Cohen JV, Donoso GW, Torres JPT. Resistencia inducible a clindamicina em *Staphylococcus aureus* meticilino resistente. *Rev Chil Pediatr* 2009; 80: 48-53. doi: 10.4067/S0370-41062009000100006.
20. Rinsky JL, Nadimpalli M, Wing S, Hall D, Baron D, Price LB, Larsen J, Stegger M, Stewart J, Heaney CD. Livestock-Associated Methicillin and Multidrug Resistant Sta*phylococcus aureus* Is Present among Industrial, Not Antibiotic-Free Livestock Operation Workers in North Carolina. *PLoS One* 2013; 8: e67641. DOI: 10.1371/journal.pone.0067641.
21. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. *Regulamento Técnico sobre padrões microbiológicos para alimentos*. Resolução Resolução nº. 12 de 02 de janeiro de 2001. [Internet]. 2001 [acesso 2015 abr 12]. Disponível em: <http://www.anvisa.gov.br/
22. Food and Drug Administration (FDA). *Bacteriological Analytical Manual*. AOAC International, Gaithersburg; 2001.
23. Clinical and Laboratory Standards Institute (CLSI). *Performance standard for antimicrobial susceptibility testing*. EUA; 2007.
24. Brandão MLL, Almeida DO, Bispo FCP, Bricio SML, Marin VA, Miagostovich MP. Assessment of Microbiological Contamination of Fresh, Minimally Processed, and Ready-to-Eat Lettuces (*Lactuca sativa*), Rio de Janeiro State, Brazil. *J Food Sci* 2014; 79: 961-966. doi: 10.1111/1750-3841.12459.
25. Junior JP, Gontijo EEL, Silva MG. Perfil parasitológico e microbiológico de alfaces comercializadas em restaurantes *self-service* de Gurupi-TO. *Rev Cient do ITPAC Araguaína*. 2012; 5: Pub.2.
26. Loncarevic S, Johannessen GS, Rorvik LM. Bacteriological quality of organically grown leaf lettuce in Norway. *Lett Appl Microbiol* 2005; 41: 186-189.
27. Oliveira M, Usall J, Viñas I, Anguer AM, Gatius F, Badias M. Microbiological quality of fresh lettuce from organic and conventional production. *Food Microbiol* 2010; 27: 679-684. doi: 10.1016/j.fm.2010.03.008.
28. Althaus D, Hofer E, Corti S, Julmi A, Stephan R. Bacteriological Survey of Ready-to-Eat Lettuce, Fresh-Cut Fruit, and Sprouts Collected from the Swiss Market. *J Food Prot* 2012; 75: 1338-1341. doi: 10.4315/0362-028X.JFP-12-022.
29. Silva ND, Junqueira VCA, Silveira NFA, Taniwaki MH, Santos RFS, Gomes RAR. *Manual de métodos de análise microbiológica de alimentos*. Vol. IV. Livraria Varela, São Paulo; 2010.
30. Silva Júnior EA.. *Manual de controle higiênico-sanitário em serviços de alimentação*. Vol. VI. Livraria Varela, São Paulo; 2012.
31. Oliveira ABAC, Silveira R, Trindade JT, Cardoso EC, Itapema MR. Avaliação da presença de microrganismos indicadores higiênico-sanitários em alimentos servidos em escolas públicas de Porto Alegre, Brasil. *Ciên & Saúde Coleti* 2013; 18: 955-962.
32. Maistro LC, Miyaa NTN, Sant'anab AS, Pereira JL. Microbiological quality and safety of minimally processed vegetables marketed in Campinas, SP – Brazil, as assessed by traditional and alternative methods. *Food Control* 2012; 28: 258-264. doi:10.1016/j.foodcont.2012.05.021.
33. Calil BEM, Ferreira FLA, Brazão CS, Sovenhi CC. Qualidade microbiológica de saladas ofrecidas em restaurantes tipo *self-service*. *ASA* 2013: 1, 36-42.
34. Mello JF, Rocha LB, Lopes ES, Frazzon J, COSTA MSanitary quality, occurrence and identification of *Staphylococcus* sp. in food services. *Braz J Microbiol* 2014; 45: 1031-1037. doi: 10.1590/S1517-83822014000300036.
35. Rizek CF, Matté MH, Dropa M, Mamizuka EM, DE Almeida LM, Lincopan N, Matté, GR, Germano, PM. Identification of Staphylococcus aureus carrying the mecA gene in ready-to-eat food products sold in Brazil. *Foodborne Pathog Dis* 2011; 8: 561-563. doi: 10.1089/fpd.2010.0706.
36. Gücükoğlu A, Çadirci Ö, Terzi G, Kevenk TO, Alişarli M. Determination of enterotoxigenic and Methicillin Resistant Staphylococcus aureus in ice cream. *J Food Sci* 2013; 78: 738-741. doi: 10.1111/1750-3841.12093.
37. Bhunia A. *Foodborne microbial phatogens – mechanisms and pathogenesis*. Springer, New York; 2008.

38. Erkan M, Vural A, Oezekinci T. Investigating the presence of *Staphylococcus aureus* and coagulase negative Staphylococci (CNS) in some leafy green vegetables. *Res Biol Sci* 2008; 3: 930-933.
39. Spanu V, Scarano C, Cossu F, Pala C, Spanu C, De Santis EP. Antibiotic Resistance Traits and Molecular Subtyping of Staphylococcus aureus Isolated from Raw Sheep Milk Cheese. *J Food Sci* 2014; 79: 2066-2071. doi: 10.1111/1750-3841.12590.
40. Wang X, Wang Y, Guo G, Usman T, Hao D, Tang X, Zhang Y, Yu Y. Antimicrobial resistance and toxin gene profiles of Staphylococcus aureus strains from Holstein milk. *Lett Appl Microbiol* 2014; 58: 527-534. doi: 10.1111/lam.12221.
41. Souza M, Silva N, Igrejas G, Silva F, Sargo R, Alegria N, Benito D, Gómez P, Lozano C, Gómez-Sanz E, Torres C, Caniça M, Poeta P. Antimicrobial resistance determinants in Staphylococcus spp. recovered from birds of prey in Portugal. *Vet Microbiol* 2014; 171: 436-440. doi: 10.1016/j.vetmic.2014.02.034.
42. Pournajaf A, Ardebili A, Goudarzi L, Khodabandeh M, Narimani T, Abbaszadeh H. PCR-based identification of methicillin-resistant Staphylococcus aureus strains and their antibiotic resistance profiles. *Asian Pac J Trop Biomed* 2014; 4: 293-297. doi: 10.12980/APJTB.4.2014C423.
43. Kumar R, Yadav BR., Singh RS. Genetic determinants of antibiotic resistance in *Staphylococcus aureus* isolates from milk of mastitic crossbred cattle. *Curr Microbiol* 2010; 60: 379-386. doi: 10.1007/s00284-009-9553-1.
44. Hassan AS, Altalhi AD, Gherbawy YA, El-Deeb BA. Bacterial Load of Fresh Vegetables and Their Resistance to the Currently Used Antibiotics in Saudi Arabia. *Foodborne Pathog Dis* 2011; 8: 1011-1018. doi: 10.1089/fpd.2010.0805.
45. Xu J, Shi C, Song M, Xu X, Yang P, Paoli G, Shi X. Phenotypic and Genotypic Antimicrobial Resistance Traits of Foodborne Staphylococcus aureus Isolates from Shanghai. *J Food Sci* 2014; 79: 635-642. DOI: 10.1111/1750-3841.12405.
46. Weisblum B. Erythromycin Resistance by Ribosome Modification. *Antimicrob Agents Chemother* 1995; 39: 577-585.
47. Hammad AM, Watanabe W, Fujii T, Shimamoto, T. Occurrence and characteristics of methicillin-resistant and-susceptible *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci from Japanese retail ready-to-eat raw fish. *Int J Food Microbiol* 2012; 156: 286-289. doi: 10.1016/j.ijfoodmicro.2012.03.022.
48. Ye Y, Jiang Q, Wu Q, Zhang J, Lu J, Lin L. The Characterization and Comparison of Staphylococcus aureus by Antibiotic Susceptibility Testing, Enterobacterial Repetitive Intergenic Consensus–Polymerase Chain Reaction, and Random Amplified Polymorphic DNA–Polymerase Chain Reaction. *Foodborne Pathog Dis* 2012; 9: 168-171. doi: 10.1089/fpd.2011.0927.
49. Depardieu F, Podglajen I, Leclercq R, Collatz E, Courvalin P. Modes and modulations of antibiotic resistance gene expression. *Clin Microbiol Rev* 2007; 20: 79-114. doi: 10.1128/CMR.00015-06.
50. Wang X, Li G, Xia X, Yang B, Xi M, Meng J. Antimicrobial susceptibility and molecular typing of methicillin-resistant S*taphylococcus aureus* in retail foods in Shaanxi, China. *Foodborne Pathog Dis*. 2014; 11: 28128-28136. doi: 10.1089/fpd.2013.1643.