

Enterotoxin and slime gene detection in *Staphylococcus* coagulase negative isolated from ground beef

Ana Claudia Lemes Pavan,¹ Giovana Hashimoto Nakadomari,² Vanessa Kelly Capoa Vignoto,¹ Ricardo Antonio Pilegi Sfaciotte,³ Fernanda Danielle Melo,³ Sandra Maria Ferraz,³ Patrícia Marques Munhoz,¹ Sheila Rezler Wosiacki¹

¹ Department of Veterinary Medicine, Maringá State University, Brazil

² Department of Preventive Veterinary Medicine, Londrina State University, Brazil

³ Department of Veterinary Medicine, Santa Catarina State University, Brazil

Correspondence: Sheila Rezler Wosiacki, Department of Veterinary Medicine, Umuarama Regional Campus, Maringá State University, Estrada da Paca s/n, Bairro São Cristóvão, Umuarama-Paraná, CEP 87507-190, Brasil. +55(44)3621-9456, Email: srwosiacki@gmail.com

Artigo enviado em: 16/10/2018, aceito para publicação em 05/12/2018

DOI: <http://dx.doi.org/10.4025/revcivet.v6i1.44965>

ABSTRACT

Among the meat products, ground beef presents a greater risk of contamination, due to excessive manipulation and greater surface contact. *Staphylococcus* indicates inadequate hygienic-sanitary conditions of food. Coagulase-negative *Staphylococci* (CoNS) cover most of the existing staphylococci species and among the virulence factors presented by this group, biofilm production and staphylococcal enterotoxins (SE) are the most prominent. The objective of this study was to detect the presence of enterotoxin and biofilm genes in coagulase-negative *Staphylococci* isolates obtained from ground beef samples. Twenty four strains of CoNS isolated from fresh bovine meat, phenotypically characterized as biofilm producers, were used. The samples were collected in twenty four commercial establishments (butchers and supermarkets) in Umuarama City from Paraná State, Brazil. The CoNS count found was between 1.0×10^3 and 3.8×10^5 CFU/g of food. All 24 samples showed genes belonging to the *ica* operon, 21 (87.50%) carrying the *icaA*, *icaC* and *icaD* genes and 3 (12.50%) of the *icaA* and *icaD* genes. 95.83% (23/24) of the strains were carriers of the *sea* enterotoxin gene, being 9 (37.5%) only *sea*, one (4.2%) *sea* and *seb*, 7 (29.1%) *sea* and *see* and 6 (25.0%) *sea*, *seb* and *see*. One (4.2%) strain did not show any of the enterotoxin genes. The detection of enterotoxin and biofilm genes in CoNS demonstrate the pathogenic potential of this microorganism. The lack of epidemiological data neglects the actual fault of the CoNS in foodborne diseases.

Keywords: food poisoning, handling, contamination, PIA, resistance

RESUMO

Dentre os produtos cárneos, a carne moída apresenta maior risco de contaminação, por sofrer manipulação excessiva e possuir maior superfície de contato. Os *Staphylococcus* indicam condições higiênicas-sanitárias inadequadas dos alimentos. Os estafilococos coagulase negativo (ECN) abrangem a maioria das espécies de estafilococos existentes e entre os fatores de virulência apresentados por esse grupo, destacam-se a produção de biofilme e as enterotoxinas estafilocócicas (ES). Este estudo teve por objetivo detectar a presença de genes produtores de enterotoxina e biofilme em isolados de estafilococos coagulase negativo, obtidos de amostras de carne moída *in natura*. Foram utilizadas 24 cepas de ECN isoladas de carne moída bovina *in natura*, caracterizadas fenotipicamente como produtoras de biofilme. As amostras foram coletadas em vinte e quatro estabelecimentos comerciais (açougues e supermercados) no município de Umuarama – PR. A contagem de ECN encontrada foi entre $1,0 \times 10^3$ a $3,8 \times 10^5$ UFC/g de alimento. Todas as 24 amostras apresentaram genes pertencentes ao operon *ica*, sendo 21 (87,5%) portadoras dos genes *icaA*, *icaC* e *icaD* e 3 (12,5%) dos genes *icaA* e *icaD*. 95,83% (23/24) das cepas foram portadoras do gene da enterotoxina *sea*, sendo 9 (37,5%) somente

sea, 1 (4,2%) *sea* e *seb*, 7 (29,1%) *sea* e *see* e 6 (25,0%) *sea*, *seb* e *see*. Uma (4,2%) cepa não apresentou nenhum dos genes de enterotoxina. A detecção de genes de enterotoxina e biofilme em ECN demonstram o potencial patogênico desse microrganismo. A falta de dados epidemiológicos negligência a real culpa dos ECN nas doenças transmitidas por alimentos.

Palavras-chave: Intoxicação Alimentar, manipulação, contaminação, PIA, resistência.

INTRODUCTION

Due to its nutritional composition, high humidity and pH close to neutrality, the meat constitutes an excellent culture medium, favoring the installation and multiplication of pathogenic microorganisms. Among the meat products, ground beef presents a greater risk of contamination, because it is subjected to excessive manipulation and has a larger contact surface (FERREIRA and SIMM, 2012).

When found in food, bacteria of the *Staphylococcus* genus may indicate inadequate hygienic-sanitary conditions, as they colonize the skin and mucous membranes of humans (SILVA; BERGAMINI; OLIVEIRA, 2010). These bacteria are Gram-positive cocci, facultative anaerobic, catalase positive, classified into two main groups: coagulase-negative *Staphylococci* (CoNS) and coagulase-positive *Staphylococci* (CoPS) (FREITAS, 2005; PODKOWIK *et al.*, 2013).

CoNS cover most of the existing species, with *Staphylococcus epidermidis* being the most isolated in humans

(OLIVEIRA and CUNHA, 2010). Among the virulence factors presented by this group, biofilm production stands out, giving them protection both in the environment and in the host (O'GARA and HUMPHREYS, 2001).

The presence of biofilms in food processing areas can generate economic losses, since microorganisms become resistant to sanitization processes, representing an original source of contamination (OLIVEIRA *et al.*, 2010).

The composition of the biofilm is given by the grouping of cells in layers, through an extracellular polysaccharide matrix. In *Staphylococci*, the main molecule responsible for intercellular aggregation is the polysaccharide intercellular adhesin (PIA), composed by 130 residues of N-acetylglucosamine in β -1,6 linkage (MACK *et al.*, 1999).

The *ica operon* is responsible for the synthesis of PIA and is composed of *icaA*, *icaD*, *icaB* and *icaC* genes, in addition to the *icaR* regulatory gene (MORALES *et al.*, 2004). The *icaA* gene is responsible for the coding of the N-acetylglucosaminyl transferase, which

synthesizes N-acetylglucosamine, whereas the *icaA* gene increases the activity of N-acetylglucosaminyl transferase (GOTZ, 2002). Expression of the *icaC* gene together with the *icaA* and *icaD* genes causes the synthesis of N-acetylglucosamine chains longer than those synthesized by the expression of the *icaA* and *icaD* gene pair. The function of the *icaB* gene is not well established (GERKE *et al.*, 1998).

Staphylococcal enterotoxins (SE) are another important pathogenicity factor observed in CoNS. They are superantigen proteins that stimulate the proliferation of T cells, releasing cytokines in an uncontrolled way (MCCORMICK *et al.*, 2001).

The classical forms of SE are SEA, SEB, SEC, SED and SEE, encoded by the genes *sea*, *seb*, *sec*, *sed* and *see*, respectively (FREITAS, 2005). Only toxins whose emetic effect has been proven by oral administration in primates may be called enterotoxins (SILVA *et al.*, 2017).

These proteins are resistant to trypsin, chymotrypsin, renin, papain and pepsin, which enables their action in the gastrointestinal tract. The enterotoxins are also thermoresistant, which increases their importance in the food industry since most

foods are heat treated (PODKOWIK *et al.*, 2013).

The constant multiplication of *Staphylococcus* in foods originates to the production of enterotoxins, which when ingested promotes food poisoning. Doses less than 1 µg can cause symptoms, this amount is reached when the population of these microorganisms reaches values above 10⁶ CFU/g of food (SILVA *et al.*, 2017).

They may represent a potential risk to public health, being associated with the form of gastroenteritis clinically manifested with vomit, and may or may not be associated with diarrhea (DINGES *et al.*, 2013). The classic symptoms appear between one and seven hours after ingestion, with a predominance of upper gastrointestinal symptoms, involving outbreaks with a short interval between ingestion and the onset of symptoms (SILVA *et al.*, 2017).

The objective of this study was to detect the presence of classical enterotoxin-producing genes and *ica* operon genes, which participate in the synthesis of PIA for biofilm production in coagulase-negative *Staphylococcal* isolates obtained from ground beef samples, given importance of this microorganism to consumers and food industries, which

could lead to public health problems as well as economic losses.

MATERIAL AND METHODS

Samples

Twenty four strains of Coagulase-Negative *Staphylococci* (CoNS) phenotypical producers of biofilm isolated from fresh bovine meat were used in this study. The samples were collected in twenty four commercial establishments (butchers and supermarkets) in Umuarama City, Paraná State, Brazil, during the months of March and April of 2016.

The methodology contained in Normative Instruction No. 62 was used for the isolation of *Staphylococci* (BRASIL, 2011). In an aseptic environment, 25 grams of the sample were weighed and diluted with 225 mL of 0.1% peptone water, at where serial dilution was performed to 10^{-4} . 0.1 ml of inoculum were spread over the entire surface of the Baird Parker agar with the Drigalsky loop that was incubating at 36 ° C for 48 hours.

After the incubation time, the plates containing 20 to 200 colonies were selected and the counting was performed. Typical colonies (convex, black and two halos) and atypical (black, gray or brownish and with one or no halo) were counted separately. Then 3 colonies of each were separated and submitted to the

coagulase test. The isolated colonies were inoculated in BHI broth (Brain Heart Infusion) and incubated at 36 ° C for 24 hours. An aliquot of 0.3 ml of the broth was transferred to a tube containing 0.3 ml of equine plasma and incubated at 36 ° for 6 hours. *Staphylococcus* colonies, confirmed negative for the coagulase test, were submitted morpho-tinctorial and biochemical characteristics.

All the atypical colonies selected from each plate were confirmed as negative coagulase, so the result was equal to the initial count, taking into account the dilution used. The results were expressed in Colony Forming Units (CFU) per gram of feed.

The CoNS were sown in Congo Red Agar according to Greco et al., (2008) modified, for phenotypic characterization of biofilm production. Samples that had black colonies were considered biofilm producers.

All isolates were stocked in TSB broth (20% glycerol) at -20 ° C.

DNA Extraction

The bacterial DNA extraction was performed by the chloroform/isoamyl alcohol technique. Initially 200 µl of the bacterial inoculum (10^8 CFU) was added in eppendorf type microtube, followed by the addition of 500 µl of chloroform/isoamyl

alcohol (24:1). The contents were homogenized in vortex and heated in water bath for 30 minutes at 56°C, after homogenization and centrifugation for 10 minutes at 12,000 x G.

The supernatant was transferred to another microtube, to which was added 600 µl of ice cold 70% alcohol. The microtubes were homogenized and centrifuged at 13,500 x G for 20 minutes. The supernatant was discarded by inversion. The microtube with the DNA was oven-dried at 56 ° C for one hour for drying.

After drying, the material was suspended in 200 µl of sterile Milli-Q water and stored in a freezer at -20 ° C until use.

Polymerase Chain Reaction (PCR) for detection of *icaA*, *icaD* and *icaC* genes

The primers described by Arciola et al. (2005) (table 1) were used for the detection of biofilm producer genes. As internal control of the reaction the 16S primer was used (ASFOUR and DARWINSH, 2011).

Table 1 Nucleotide sequence and PCR amplified product for the primers used in the detection of biofilm

Gene	Primer	Primer sequence	Amplified product (bp)
<i>IcaA</i>	ICAA-F	5'- ACA GTC GCT ACG AAA AGA AA - 3'	103
	ICAA-R	5'- GGA AAT GCC ATA ATG ACA AC - 3'	
<i>IcaD</i>	ICAD-F	5'- ATG GTC AAG CCC AGA CAG AG - 3'	198
	ICAD-R	5'- CGT GTT TTC AAC ATT TAA TGC AA - 3'	
<i>IcaC</i>	ICAC-F	5'- TAA CTT TAG GCG CAT ATG TTT T - 3'	400
	ICAC-R	5'-TTC CAG TTA GGC TGG TAT TG - 3'	
16S	16S-F	5' - AGGTGGCAAGCGTTATCC - 3'	228
	16S-R	5' - CGCACATCAGCGTCAG - 3'	

There were made 2 reactions, the first was a multiplex PCR used to detect *icaA*, *icaC* and 16S genes and the second was a simplex PCR for *icaD* gene detect. Reactions contained the following reagent concentrations: 0.4 pmol of each primer (Invitrogen®), 0.4 mM of each deoxyribonucleotide (dNTP) (Invitrogen®), 2 mM MgCl₂, 1x PCR Buffer, 1.25 units of

Platinum® Taq DNA polymerase (Invitrogen®) and 10% of the DNA extracted. The final volume of the reaction was adjusted to 25 µL with ultrapure water.

DNA amplification was performed on a thermocycler (Veriti Applied Biosystems®), and consisted on the following steps: the first step of 5 minutes

at 94°C for initial denaturation; the second step of 50 cycles of 30 seconds at 94°C for denaturation, 30 seconds at 54°C for annealing and 1 minute at 72°C for extension; and the third step of a final extension of 2 minutes at 72°C (ARCIOLA et al., 2005).

PCR for detection of *sea*, *seb*, *sec*, *sed* and *see* genes

Primers used in the detection of classical enterotoxin encoding genes have been described by Freitas (2005) (table 2). In this reaction the 16S primer was also used as internal reaction control (ASFOUR and DARWINSH, 2011).

Table 2 Nucleotide sequence and PCR amplified product for the primers used in the detection of classical enterotoxins

Gene	Primer	Primer sequence	Amplified product (bp)
<i>sea</i>	IBR 1)F	5' - GGT TAT CAA TGT GCG GGT GG - 3'	102
	IBR 1)R	5' - CGG CAC TTT TTT CTC TTC GG - 3'	
<i>seb</i>	IBR 2)F	5' - GTA TGG TGG TGT AAC TGA GC - 3'	164
	IBR 2)R	5' - CCA AAT AGT GAC GAG TTA GG - 3'	
<i>sec</i>	IBR 3)F	5' - AGA TGA AGT AGT TGA TGT GTA TGG - 3'	451
	IBR 3)R	5' - CAC ACT TTT AGA ATC AAC CG - 3'	
<i>sed</i>	IBR 4)F	5' - CCA ATA ATA GGA GAA AAT AAA AG - 3'	278
	IBR 4)R	5' - ATT GGT ATT TTT TTT CGT TC - 3'	
<i>see</i>	IBR 5)F	5' - AGG TTT TTT CAC AGG TCA TCC - 3'	209
	IBR 5)R	5' - CTT TTT TTT CTT CGG TCA ATC - 3'	

Two multiplex reactions were performed: one containing the primers of the *sea*, *sec* and *see* genes, and the other of *seb*, *sed* and 16S primers. Reactions contained the following reagent concentrations: 0.4 pmol of each primer (Invitrogen®), 0.4 mM of each deoxyribonucleotide (dNTP) (Invitrogen®), 2 mM MgCl₂, 1x PCR Buffer, 1.25 units of Platinum® Taq DNA polymerase (Invitrogen®) and 10% of template DNA. The final volume of the reaction was adjusted to 25 µL with ultrapure water.

For DNA amplification the same thermocycler as mentioned above was used. Cycles consisted of the first step of 3 minutes at 94°C for initial denaturation; the second step of 35 cycles of 30 seconds at 94°C for denaturation, 30 seconds at 57°C for annealing and 30 seconds at 72°C for extension; and the third step with an extension of 10 minutes at 72°C (ÂNGELO, 2010).

Detection of Amplified Products

The amplified products were submitted to 1.5% Agarose gel electrophoresis, stained with SYBR[®] Safe (Invitrogen[®]). The gel was observed in transilluminator under Ultra Violet (UV) light and the images documented through photography.

Statistical Analysis

All the results obtained were subjected to descriptive analysis by comparing the relative and absolute frequencies.

RESULTS AND DISCUSSION

Despite the excessive manipulation suffered by ground beef, there is no standard established by the Brazilian legislation regarding the presence of *Staphylococcus* spp. Resolution RDC No. 12 of 2001 (BRAZIL, 2001) establishes as a parameter of microbiological quality only the absence of *Salmonella* spp. in 25g of this food.

The Coagulase-negative *Staphylococcal* count ranged from 1.0×10^3 CFU/g to 3.8×10^5 CFU/g of food. Inadequate storage conditions could increase this count significantly, representing public health risk, since the production of enterotoxins occurs when

Staphylococcus reaches values above 10^6 CFU/g of food (SILVA *et al.*, 2017).

All 24 samples phenotypically characterized as biofilm producers presented genes belonging to the *ica operon*. This result demonstrates the importance of adequate hygiene in the food processing areas, since biofilm can make the microorganisms an original source of contamination due to the resistance to sanitizers (OLIVEIRA; BRUGNERA; PICCOLI, 2010). In relation to the genes found, 21 (87.5%) CoNS were carriers of the 3 genes tested (*icaA*, *icaC* and *icaD*) and 3 (12.5%) were carried only the *icaA* and *icaD* genes. According to Oliveira and Cunha (2010), the *icaA* and *icaD* genes are the main responsible for the formation of PIA, but the presence of the *icaC* gene may further favor biofilm formation. The research confirms the importance of the *ica operon* in the formation of biofilm in CoNS.

The detection of classical enterotoxin genes was performed in 95.83% (23/24) of the samples studied. According to Franco and Landgraf (2005), meat and meat products have already been incriminated in outbreaks of staphylococcal intoxication. Although poorly diagnosed, the Coagulase-negative *Staphylococci* can also be a cause of food poisoning.

Of these 24 strains, 23 (95,8%) were carriers of the *sea* gene, where 9 (37.5%) were carriers of only the *sea* gene, 1 (4.2%) were *sea* and *seb* genes carriers, 7 (29.1%) were *sea* and *see* genes carriers, 6 (25.0 %) were *sea*, *seb* and *see* genes carriers and 1 (4.2%) strain showed only

the 16S gene used as internal control, and no enterotoxin gene was found (table 3). Freitas (2005) and Ângelo (2010), claim that enterotoxin A is the most common detected in outbreaks of food poisoning, such an assertion agrees with this study.

Table 3 Distribution of classical enterotoxin genes amplified by PCR in Coagulase-negative *Staphylococcus* isolated from ground beef

Enterotoxin genes	Number of samples	Percentage
<i>Sea</i>	9	37.5
<i>sea</i> e <i>seb</i>	1	4.2
<i>sea</i> e <i>see</i>	7	29.1
<i>sea</i> , <i>seb</i> e <i>see</i>	6	25.0
ausente	1	4.2
Total	24	100

In relation to the enterotoxin and biofilm genes: all 9 strains carrying the *sea* gene were also carriers of the *icaA*, *icaD* and *icaC* genes; in the carrier strain of the *sea* and *seb* genes only the *icaA* and *icaD* genes were detected; of the 7 strains carrying the genes *sea* and *see*, 6 had the 3 biofilm producing genes and 1 only *icaA* and *icaD* genes; of the 6 strains carrying the genes *sea*, *seb*, and *see*, 5 had the 3 biofilm producing genes and 1 only *icaA*

and *icaD* genes (Table 4). The association between biofilm and enterotoxin genes in a microorganism, since many outbreaks of foodborne diseases have been found to be associated with biofilm. It is well documented that biofilm has become a problem in the food industry because it makes its inhabitants resistant to antimicrobial agents and cleaning (SREY; JAHID; HÁ, 2013).

Table 4 Relationship between enterotoxin and biofilm genes detected by PCR in Coagulase-negative *Staphylococcus* isolated from ground beef

Enterotoxin genes	Biofilm genes		
	<i>icaA</i>	<i>icaD</i>	<i>icaC</i>
<i>sea</i>	9	9	9
<i>sea e seb</i>	1	1	0
<i>sea e see</i>	7	7	6
<i>sea, seb e see</i>	6	6	5
Total	23	23	20

CONCLUSION

The detection of enterotoxin and biofilm genes in Coagulase-negative *Staphylococcus* demonstrates the pathogenic potential of these microorganisms.

The lack of epidemiological data neglects the actual participation of CoNS in foodborne diseases. Further research is needed on the risks offered to the consumer and the food industry.

Acknowledgements

Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná and Programa Institucional de Bolsas de Iniciação Científica nas Ações Afirmativas (PIBIC-AF-IC-CNPq/FA/UEM) of Universidade Estadual de Maringá.

Conflicts of Interest

Author declares there is no conflict of interest.

REFERENCES

- ÂNGELO, F. F. **Detecção de genes de exotoxinas em *Staphylococcus* spp. isolados de leite cru refrigerado e de leite de vacas com mastite.** 2010. 70 p. Tese (Doutorado) em ciência e tecnologia de alimentos, Universidade Federal de Viçosa - UFV, Viçosa, 2010.
- ARCIOLA, C. R.; GAMBERINI, S.; CAMPOCCIA, D.; VISAI, L.; SPEZIALE, P.; BALDASSARRI, L.; MONTANARO, L. A multiplex PCR method for the detection of all five individual genes of *ica* locus in *Staphylococcus epidermidis*. A survey on 400 clinical isolates from prosthesis-associated infections. **Journal of Biomedical Materials Research Part A**. v. 75, n. 2, p. 408-13, 2005.
- ASFOUR, H.A.E.; DARWISH, S.F. Phenotypic and genotypic detection of both *mecA* and *blaZ* genes mediated β -lactâm resistance in *Staphylococcus* strains isolated from bovine mastitis. **Global Veterinária**, v. 6, n. 1, p. 39-50, 2011.

- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 62, de 26 de agosto de 2003. **Oficializa os métodos analíticos oficiais para análises microbiológicas para controle de produtos de origem animal e água.** Diário Oficial [da] República Federativa do Brasil, Brasília, 18 de setembro de 2003. Seção 1, p. 14.
- BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução RDC Nº 12, de 02 de janeiro de 2001. Regulamento Técnico sobre padrões microbiológicos de alimentos. **Diário Oficial da União**, Brasília, 2001.
- DINGES, M. M.; ORWIN, P. M.; SCHLEIVERT, P. M. Exotoxins of *Staphylococcus aureus*. **Clinical Microbiology Reviews**. v.13, n. 1, p. 16-34, 2000.
- FERREIRA, R.; SIMM, E. M. Análise microbiológica da carne moída de um açougue da região central do município de Pará de Minas/MG. **SynThesis Revista Digital FAPAM**, Pará de Minas, n.3, p. 37 - 61, 2012.
- FRANCO, B. D. G. M.; LANDGRAF, M. **Microbiologia dos Alimentos**. São Paulo: Editora: Atheneu, 2005.
- FREITAS, E. I. **Deteção de genes de enterotoxinas de *Staphylococcus* sp. isolados de queijo Minas Frescal**. 2005. 106 p. Dissertação (Mestrado) em Vigilância Sanitária – Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro, 2005.
- GERKE, C.; KRAFT, A.; SUBMUTH, R.; SCHWEITZER, O.; GOTZ, F. Involved in the Biosynthesis of the *Staphylococcus epidermidis* Polysaccharide Intercellular Adhesin. **Journal of Biological**. v. 273, n. 29, p. 18586–18593, 1998.
- GOTZ, F. *Staphylococcus* and biofilms. **Molecular Microbiology**, v. 43, n. 6, p. 1367–1378, 2002.
- GRECO, C.; MASTRONARDI, C.; PAGOTTO, F.; MACK, D.; RAMIREZ-ARCOS, S. Assessment of biofilm-forming ability of coagulase-negative staphylococci isolated from contaminated platelet preparations in Canada. **Transfusion**. v. 48, p. 969-977, 2008.
- MACK, D.; RIEDEWALD, J.; ROHDE, H.; MAGNUS, T.; FEUCHT, H. H.; ELSNER H-A.; LAUFS, R.; RUPP M. E. Essential functional role of the polysaccharide intercellular adhesin of *Staphylococcus epidermidis* in hemagglutination. **Infection and Immunity**. v. 67, n. 2, p. 1004-1008, 1999.
- MCCORMICK, J. K.; YARWOOD, J. M.; SCHLIEVERT, P. M. Toxic shock syndrome and bacterial superantigens: an

- update. **Annual Review of Microbiology**, v. 55, p. 77–104, 2001.
- MORALES, M.; MENDEZ-ALVAREZ, S.; MARTIN-LOPES J-V.; MARREIRO, C.; FREYTES, C. O. Biofilm: the microbial “bunker” for intravascular catheter-related infection. **Support Care Cancer**, v. 12, p. 701-707, 2004.
- O’GARA, J. P.; HUMPHREYS, H. *Staphylococcus epidermidis* biofilms: importance and implications. **J. Med. Microbiol.** v. 50, p. 582-587, 2001.
- OLIVEIRA, A.; CUNHA, M. L. RS. Comparison of methods for the detection of biofilm production in coagulase-negative staphylococci. **BMC Research Notes**, v. 3, p. 1-8.
- OLIVEIRA, M. M. M.; BRUGNERA, D. F.; PICCOLI, R. H. Biofilmes microbianos na industria de alimentos: uma revisão. **Revista do Instituto Adolfo Lutz**, São Paulo, v. 69, n. 3, p. 277-284, 2010.
- PODKOWIK, M.; PARK, J. Y.; SEO, K. S.; BYSTRONÍ, J.; BANIA, J. Enterotoxigenic potential of coagulase-negative staphylococci. **International journal of Food Microbiology**, v. 163, p. 34-40, 2013.
- SILVA, E. P.; BERGAMINI, A. M. M.; OLIVEIRA, M. A. Alimentos e agentes etiológicos envolvidos em toxinfecções na região de Ribeirão Preto, SP, Brasil: 2005 a 2008. **Boletim Epidemiológico Paulista**, São Paulo, v. 7, n. 77, p. 4-10, maio 2010.
- SILVA, N.; JUNQUEIRA, V. C. A.; SILVEIRA, N. F. A.; T, M. H.; G, R. A. R.; OKAZAKI, M. M. **Manual de Métodos de Análise Microbiológica de Alimentos e Água**. 5ª ed. São Paulo. Editora: Blucher, 2017.
- SREY, S.; JAHID, I. K.; HÁ, S. Biofilm formation in food industries: A food safety concern. **Food Control**, v. 31, p. 572-585, 2013.