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Original Article

Phenotypic and Molecular Aspects of *Staphylococcus* spp. Isolated from Hospitalized Patients and Beef in the Brazilian Amazon

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Abstract

The aim of this study was to characterize and compare *Staphylococcus* spp. isolated from hospitalized patients and beef marketed in the city of Porto Velho-RO, Brazil. The isolates were subjected to antibiogram tests, adherence capacity tests, detection of the *mecA* gene, and epidemiological investigation by the random amplified polymorphic DNA (RAPD) technique, using the primers M13 and H12. Among the 123 *Staphylococcus* spp. isolates, 50 were identified as *S. aureus* and 73 as coagulase-negative *Staphylococcus*; among the latter, 7 species were identified. It was observed that the coagulase-negative *Staphylococcus* isolates showed greater adhesion ability than *S. aureus*. The profile of antimicrobial susceptibility was different among isolates, all of which were susceptible to vancomycin and linezolid, and had high penicillin resistance rates, varying according to the bacterial class and the source. In this study, all strains were negative for *mecA* gene detection; however, 36% of *S. aureus* and 17% of coagulase-negative *Staphylococcus* were resistant to oxacillin. The genetic relationship of these bacteria, analyzed by RAPD, was able to discriminate the species of coagulase-negative *Staphylococcus* strains of *S. aureus* along its origin. It was concluded that the isolates of *Staphylococcus* spp. derived from beef and human infections differ genetically. Thus, it is suggested that isolates from beef, which were grouped within hospital isolates, were probably carried via contact with beef in hospital professionals or patients.

Introduction

Among bacteria of the genus *Staphylococcus*, *S. aureus* is considered, in Brazil, to be the second most common pathogen related to food poisoning, with lower incidence only compared to *Salmonella* spp. (Brasil, 2007). The incubation period and the severity of the symptoms depend on the amount of enterotoxin and the individual's susceptibility. Foods most often involved are those with a high water content and high concentration of protein, such as cheese, meat, and meat products (Gottardi *et al.*, 2006).

S. aureus is widespread in nature and is part of the autochthonous microbiota of the skin and mucosa of mammals and birds; the nasal cavity is the main habitat in humans (Tortora *et al*., 2012). It is transmitted to food by handlers and it produces a large number of enzymes and toxins that contribute to hampering phagocytosis by neutrophils. These characteristics are responsible for many hospital infections, exacerbated by resistance to antimicrobials (Schaechter *et al*., 2002). Infections often result from the introduction of these bacteria after trauma, abrasions of the skin and mucous membranes, or during surgical procedures (Manegotto and Picoli, 2007). Such infections culminate in need for antimicrobial treatment, increasing the risk of the emergence of resistant microorganisms (Ueno, 2001). The main way of transmitting the bacteria through hospitals is via the transiently colonized hands of health professionals. These microorganisms survive on inanimate surfaces, such as bedding, personal clothing, and fomites, which can participate in the epidemiological chain in isolated cases and outbreaks (John *et al*., 1996). Survival is associated with the adhesion capacity of these bacteria (Królasik *et al*., 2010).

Most species of coagulase-negative *Staphylococcus* are not recognized as an important cause of disease, but it is known that some may also produce enterotoxins (Baird-Parker, 1990; Santana *et al.*, 2010) and may be isolated in foods as both humans and animals are common carriers of these strains (Pereira *et al*., 2000). These microorganisms are primarily opportunistic infections but can produce serious infections and have been recognized as etiologic agents of some

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infectious processes (Kloos *et al*., 1994). A critical factor in the transmission of the microorganism from one person to the environment and to other people is the ability of these bacteria to survive on environmental surfaces (Neely *et al*., 2000).

The random amplified polymorphic DNA (RAPD) test has been used to distinguish different isolates of *S. aureus* (Reinoso *et al*., 2004). It is a simple and fast technique, capable of identifying genetic variation between different organisms, in particular within the same group. This assay can be performed with small amounts of DNA and short synthetic oligonucleotide primers. These primers allow for amplification without the need for any information about the genome or nucleotide sequence (Idil and Bilkay, 2014). Polymorphisms are recognized by the presence or absence of a specific amplified DNA fragment (Williams *et al*., 1990).

A comparative study of *Staphylococcus* spp. isolated from fresh beef to the phenotypic and molecular level with those isolated from hospitalized patients can be done to assess whether there is a genetic correlation between these microorganisms. The aim of this study was to perform a phenotypic and genotypic evaluation of *Staphylococcus* spp. isolated from humans with nosocomial infections and fresh beef in the city of Porto Velho, RO, Brazil.

Materials and Methods

We evaluated 123 isolates of *Staphylococcus* spp.: 43 obtained from different sources in patients with a nosocomial infection hospitalized in the Cemetron Hospital, Porto Velho, RO, Brazil, and 80 isolates obtained from 48 beef samples collected in two sampling times from two butchers and two supermarkets in the same city. All isolates were characterized presumptively through classical biochemical tests such as the mannitol fermentation, coagulase test, DNAse, and thermonuclease tests (Koneman *et al*., 2001).

S. aureus identification was performed using the Pastorex Staphy Kit Plus (Bio Rad) following the manufacturer's instructions, based on the simultaneous detection of antigenic affinity to fibrinogen, protein A, and capsular polysaccharides of *S. aureus*. Coagulase-negative *Staphylococcus* were identified with the use of biochemical tests (Microgen Staph-ID; Microgen), following the manufacturer's instructions. The results were interpreted using the Microgen-ID system software (Microgen).

Adhesion capacity was evaluated according Pfaller *et al*. (1988) using 96-well microtiter plates. Into each well, 20 μL of 10^5 colony-forming units/mL of the bacterial suspension and 180 μL of tryptic soy broth were added. After 18 h of aerobic incubation at 35°C, the wells were aspirated, washed three times with phosphate-buffered saline (pH 7.2), and dried for 1 h at 60°C. The adhered cells were fixed with methanol for 20 min and then the wells were stained with crystal violet for 1 min. Crystal violet was removed and the wells were washed again. The plates were read in a spectrophotometer at 490 nm absorbance, after the addition of 200 μL of ethanol. Sterile tryptic soy broth was used as a negative control and *Staphylococcus epidermidis* strain ATCC 35984 was used as a positive control. The optical density values (OD) were interpreted according to the ability to produce a biofilm: OD <0.500 (negative); OD between 0.500 and 1.500 (moderate), and OD >1500 (positive). The test was performed in triplicate.

The extraction of genomic DNA was assessed by the phenol chloroform method. The bacteria were grown in 3 mL of tryptic soy broth at 37°C under stirring for 18 h. The cells were collected by centrifugation at 12,000 × *g* for 10 min and the pellet was resuspended in 300 μL of TEN buffer (0.05 M Tris-HCl, 0.05 M EDTA, 0.1 M NaCl) and 30 μL of lysozyme (20 mg/mL) and incubated for 30 min at room temperature. Next, 50 μL Triton X-100 (10%) and 20 μL 3 M NaCl were added and heated to 60°C for 5 min, then immediately cooled to room temperature. After this period, 10 μL of RNAse A (10 mg/mL) was added and incubated for 30 min at 37°C. Then, 50 μL of 10% sodium dodecyl sulfate and 3 μL of proteinase K (10 mg/mL) were added and homogenized, then incubated for 15 min at 37°C. The samples were stirred with 450 μL of phenol for 5 min and centrifuged at 12,000 × *g* for 10 min. The aqueous phase was recovered and washed with 450 μL of chloroform, then stirred for 5 min. The aqueous phase was recovered by centrifugation at 12,000 × *g* for 10 min and the DNA was precipitated by adding 40 μL of 3 M NaCl and adding slowly 1 ml of 100% ethanol at −20°C. The DNA was centrifuged at 12,000 × *g* for 10 min and hydrated with 70% ethanol. The pellet was dried and resuspended in 200 μL TE buffer (0.01 M Tris-HCl, 0.01 M EDTA, pH 8.0) and stored at −20°C.

Kirby-Bauer disc diffusion testing was performed as recommended by Clinical and Laboratory Standards Institute (CLSI, 2013) for the antibiotics shown in Table 1 to establish antimicrobial susceptibility of isolates. The bacteria were incubated in Müller-Hinton agar with antimicrobial discs for 24 h at 37°C. The results were obtained by performing halo measurements for each antimicrobial as recommended by the CLSI (2013). The results were evaluated by the chi-square test of independence with a significance level of 0.05 using the software GraphPad InStat version 3.6 (GraphPad Software Inc., La Jolla, CA).

Table 1. Susceptibility to Different Antimicrobials in *Staphylococcus aureus* (MO 1) and Coagulase-Negative *Staphylococcus* (MO 2) Isolated from Hospitalized Patients and Beef Marketed for Retail Sale from the City of Porto Velho, RO, Brazil

MO	*source*	*GEN*	*CIP*	*CLO*	*OXA*	*CLI*	*VAN*	*DOX*	*LZD*	*AZI*	*PEN*
1	Beef	62.9	68.6	91.5	85.7	34.3	100	62.8	100	51.5	34.3
1	Hospital	26.7	33.4	46.7	60.0	33.4	100	80.0	100	20.0	00.0
2	Beef	97.8	97.8	100	100	86.7	100	97.8	100	93.4	75.6
2	Hospital	67.9	71.5	67.86	71.5	64.3	100	71.5	100	78.6	46.5

GEN, gentamicin; CIP, ciprofloxacin; CLO, chloramphenicol; OXA, oxacillin; CLI, clindamycin; VAN, vancomycin; DOX, doxycycline; LZD, linezolid; AZI, azithromycin; PEN, penicillin.

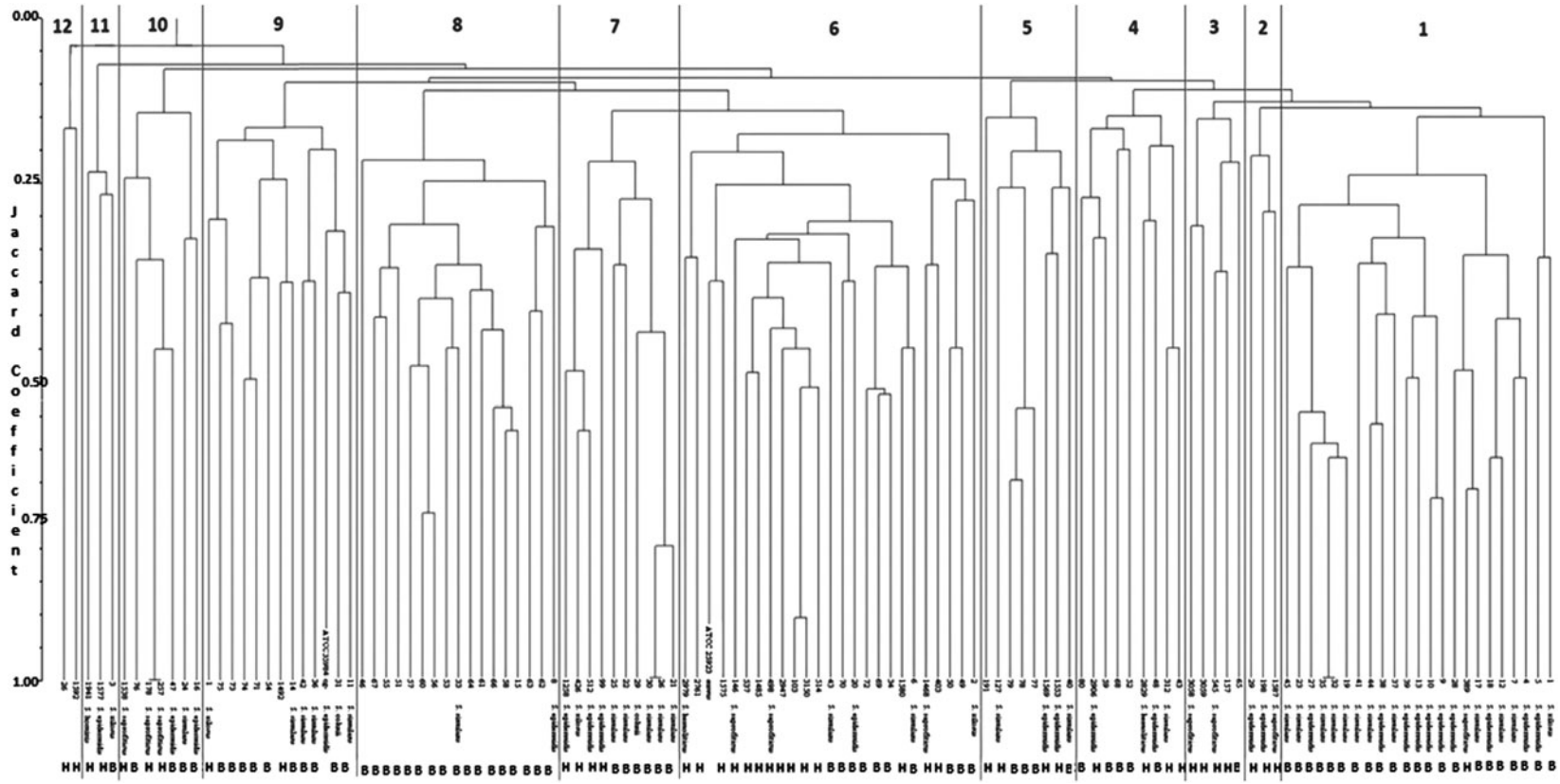

FIG. 1. Neighbor-joining tree generated by the UPGMA method (pair-group method using the unweighted arithmetic average), with the genetic grouping of 123 isolates of *Staphylococcus* spp. obtained from beef samples (B) and from patients with nosocomial infections (H) in Porto Velho-RO, Brazil, after samples were submitted to random amplified polymorphic DNA (RAPD). All isolates without a species name are *S. aureus* and other species are coagulase-negative *Staphylococcus*.

TABLE 2. DISTRIBUTION OF 123 ISOLATES OF *STAPHYLOCOCCUS* GENUS OBTAINED FROM BEEF ($N=80$) AND HOSPITALIZED PATIENTS ($N=43$) IN PORTO VELHO, BRAZIL

			Human samples isolates									
Species/source	*Beef isolates*	% (n = 80)	*Vaginal discharge*	*Urethral discharge*	*Nasal discharge*	*Lip discharge*	*Ear discharge*	*Tracheal discharge*	*Catheter*	*Blood*	*Sputum*	*Urine*	% (n = 43)
S. aureus	35	43.75	0	1	0	1	1	3	1	3	0	5	34.88
S. epidermidis	15	18.75	1	0	0	0	0	3	0	3	0	2	20.93
S. xilosus	3	3.75	0	0	0	0	0	1	1	0	0	0	4.65
S. simulans	25	31.25	0	0	0	0	0	1	0	0	0	1	4.65
S. cohnii	2	2.50	0	0	0	0	0	0	0	0	0	0	0.00
S. saprofiticus	0	0.00	1	1	0	0	0	0	0	0	1	8	25.58
S. hemoliticus	0	0.00	0	1	1	0	0	0	0	0	0	0	4.65
S. hominis	0	0.00	0	0	0	0	0	0	0	0	2	0	4.65

The detection of the *mecA* gene by polymerase chain reaction (PCR) was performed following Alcaraz *et al*. (2003), using the following oligonucleotide sequences: *mecA* 1–5′AAA ATC GAT GGT AAA GGT TGG C 3′ and *mecA* 2—5′AGT TCT GCA GTA CCG GAT TTG C 3′, which produced a amplicon of 533 bp. PCR was performed in a final volume of 25 μL, containing 2 μL of chromosomal DNA, 1x amplification buffer (BioLabs), 1 μM of each oligonucleotide, 200 μM of each deoxynucleotide triphosphate (dATP, dTTP, dCTP, and dGTP), 1.5 mM $MgCl_2$,and 2 U of *Taq* polymerase (BioLabs). The amplification protocol was performed in a BioRad thermocycler (My cycler thermal cycler model) programmed for a pre-denaturing cycle at 94°C for 5 min followed by 35 cycles of 94°C for 2 min, 57°C for 2 min, and 72°C for 1 min, and a final extension step of 72°C for 7 min. Amplified products were separated by agarose gel electrophoresis on a 1.5% 1x Tris-Borate-Ethylenediamine tetraacetic acid (TBE) for 30 min at 100 V, and stained with ethidium bromide (0.5 mg/mL). In each run, we used a 1 Kbplus DNA ladder (Invitrogen) molecular weight marker and *S. aureus* ATCC 25923 was used as the positive control.

RAPD reactions were conducted based on the protocol developed by Williams *et al*. (1990). The two oligonucleotides used were: *M13*: 5′ – GAG GGT GGC GG TTC T – 3′ (Hsueh *et al.,* 1999; Sloos *et al.,* 2000; Lee *et al.,* 2004) and *H12:* 5′ – ACG CGC ATG T – 3′ (Lee *et al.,* 2004; Hsueh *et al.,* 1999). Amplification reactions were performed in 200-μL tubes in a final volume of 25 μL containing 1x amplification buffer (BioLabs), 25 ng of chromosomal DNA, 1.6 pmol of oligonucleotides, 0.25 mM of each deoxynucleotide triphosphate (dATP, dTTP, dCTP, and dGTP), 2.5 mM $MgCl_2$, and 2.5 U of Taq polymerase (BioLabs). The reaction was performed on an Eppendorf thermocycler (Mastercycler gradient model). The amplification protocol was programmed for a pre-denaturation cycle at 94°C for 3 min followed by 40 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 2 min, and final extension step at 72°C for 10 min. The amplified fragments were separated by agarose gel electrophoresis on a 1.5% 1x TBE for 3 h at 70 V and stained with ethidium bromide (0.5 mg/mL). In each run, the 1 Kbplus DNA ladder (Invitrogen) molecular weight marker was used and *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 35984 were used as positive controls. The generated fragments were photographed and analyzed using a KODAK 1D digital camera and the Kodak 1D scientific imaging system (Scientific Imaging Systems), version 3.5.2.

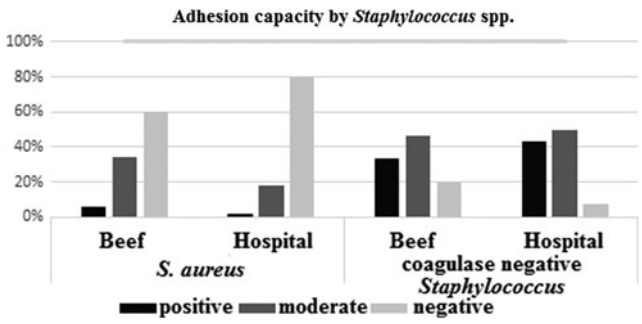

FIG. 2. Adhesion capacity as the percentage (%) of isolates of *Staphylococcus* spp. isolated from beef and hospitalized patient samples.

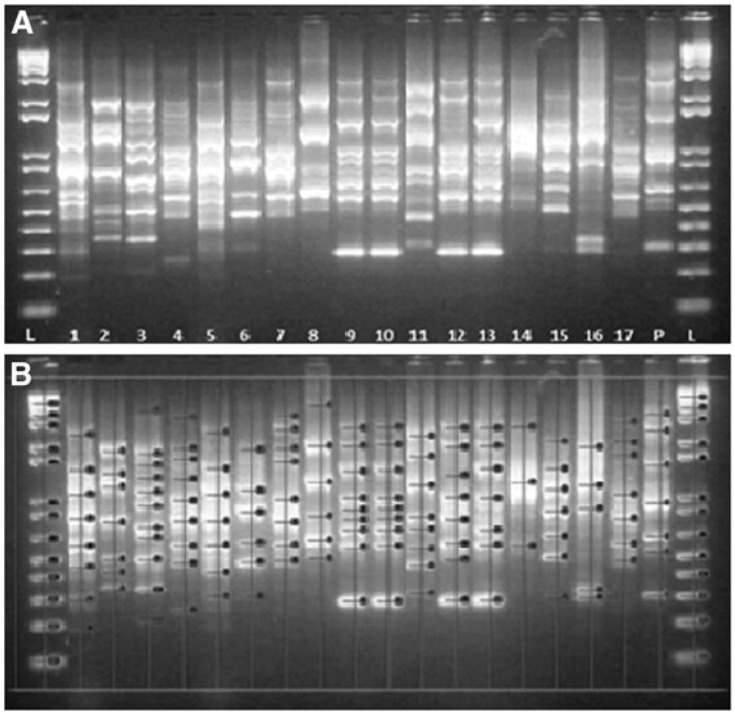

FIG. 3. Random amplified polymorphic DNA profiles obtained by the amplification of the genomic DNA of coagulase-negative *Staphylococcus* using the primer M13; (L) molecular weight marker 1 kb plus; (1–17) strains isolated from beef; (P) strain *S. aureus* ATCC 25923 (A) gel obtained after separation of the bands by electrophoresis; (B) gel with polymorphisms marked by the Kodak 1D scientific imaging system.

RAPD polymorphisms were used to construct the binary matrix of presence and absence of strips, which was set as 0 for no and 1 for the presence of a band. The similarity between samples was estimated by using the Jaccard coefficient done through the NTSYS-pc software (numerical taxonomy and multivariate analysis system), version 1.7 (Exeter Software). Using the similarity matrix, it was possible to determine the groups by the UPGMA method (unweighted pair-group method using the arithmetic average).

Results and Discussion

In this study, we identified eight species of *Staphylococcus* (Fig. 1 and Table 2) isolated from fresh beef and patients hospitalized in Cemetron Hospital in Porto Velho.

The presence of coagulase-negative *Staphylococcus* was significant in beef isolates (Table 2). According Türkyilmaz and Kaya (2006), species in this group of bacteria isolated from several types of samples of animal origin possess virulence factors of great importance in the pathogenesis of infections, which may be as harmful as *S. aureus*. This is because these microorganisms have the capacity to adhere to inert surfaces such as fomites and surgical instruments, which increases the risk of infections in patients when hospitalized.

Figure 2 shows the adhesion ability of *Staphylococcus* spp. isolates from beef and hospital samples. The *S. aureus* isolates from both sources were negative for adhesion (i.e., 60.00% [$n=21$] and 80.00% [$n=12$]) from beef and hospital samples, respectively. The species of coagulase-negative *Staphylococcus* had moderate adhesiveness in 46.67% ($n=21$) and 50.00% ($n=14$) of isolates from hospital and beef, respectively; positive adhesiveness was found in 42.86% ($n=12$) of the hospital isolates and 33.33% ($n=15$) of isolates from beef. There were significant differences ($p<0.001$) between the isolates of *S. aureus* and coagulase-negative *Staphylococcus* regarding adhesion ability; however, this ability did not differ between the two sources ($p<0.498$). When only isolates of *Staphylococcus* spp. positive for adhesion ability (Fig. 2) were analyzed, it was observed that the species of coagulase-negative *Staphylococcus* had greater adhesion ability compared with *S. aureus* from both isolation sources.

According to Christensen *et al*. (1994), the number of medical appliances contaminated with coagulase-negative *Staphylococcus* is greater than those contaminated with *S. aureus*, suggesting the adhesiveness thereof and coinciding with the adhesion results of this study. A similarity was observed in coagulase-negative *Staphylococcus* adhesion ability from both sources, which is similar to the results found by Alcaraz *et al*. (2003), who observed a similar frequency in the adhesion ability of coagulase-negative *Staphylococcus* isolates from clinical and environmental origins.

Results of the antimicrobial susceptibility tests are presented in Table 1. All isolates were sensitive to the antibiomicrobials vancomycin and linezolid. *S. aureus* isolates arising from the hospital were not sensitive to penicillin. Regarding penicillin, as it is the oldest antimicrobial originating in the 1940s, many studies in the literature have confirmed the resistance of many bacterial isolates to this antimicrobial agent, whether obtained from hospitals, the environment, or healthy animals; this also agrees with the findings of this study. Catão *et al*. (2013) found similar results, with 100% of *S. aureus* isolates from hospitals displaying penicillin resistance. Regarding *S. aureus* from beef, we observed high sensitivity to 8 antimicrobials of the 10 tested. Only penicillin and clindamycin showed less effectiveness, where 65.71% of the isolates were resistant (Table 1). Comparing the susceptibility to these antimicrobials in *S. aureus* isolates collected from beef and hospitalized patients, there was no significant difference ($p=0.2031$).

Coagulase-negative *Staphylococcus* isolated from beef showed high sensitivity to all antimicrobials tested. For the penicillin results in these bacteria, it was observed that more than 45% were resistant; however, it is relevant to highlight that susceptibility was greater for beef isolates, showing that in the hospital environment, these bacteria suffer from selective pressure and thus present higher numbers of resistant strains.

Despite the increase in antimicrobial resistance presented by bacteria in biofilms showed by Costa *et al*. (2014), our results did not present statistical correlation between the ability to adhere to surfaces and higher antimicrobial resistance by our isolates.

There was no detection of the *mecA* gene in any of the 123 *Staphylococcus* spp. isolates analyzed by PCR; this gene was only found in the positive control strain, *S. aureus* ATCC 25923. This gene, when expressed, confers methicillin resistance, and also resistance to other drugs of the same class, such as oxacillin used in this study. However, 36% of *S. aureus* and 17% of coagulase-negative *Staphylococcus* in this study were resistant to this antimicrobial on the disk diffusion test. According to Alcaraz *et al*. (2003), this difference was also found in environmental and clinical isolates of *Staphylococcus* spp. Thus, as found in this study, these results suggest that another mechanism of resistance is present in resistant isolates.

RAPD assays generated 96 polymorphic bands (Fig. 3), resulting in 12 separate clusters within the dendrogram (Fig. 1). Most species of coagulase-negative *Staphylococcus* formed distinct clusters compared with isolates of *S. aureus*, as shown in many fields of the dendrogram, indicating that the species of coagulase-negative *Staphylococcus* have similar molecular profiles among them, unlike the species of *S. aureus*. It is likely that the variations in the polymorphisms refer to the gene responsible for the production of the enzyme coagulase, as this is the main different feature between these two groups. Kwok *et al*. (2003), in a phylogenetic study of the *Staphylococcus* genus, showed that species of coagulase-negative *Staphylococcus* are contiguously linked among them.

It was observed that many isolates were grouped according to their source; for example, clusters 1 and 8 were of beef origin and 2 and 12 were of human origin. Genetic differences between species isolated from different sources can occur due to the fact that these bacteria belong to a specific host. A study by Reinoso *et al*. (2004), also using RAPD with *S. aureus* isolated from bovine and human hosts, was able to determine the specialization of a particular host, suggesting that strains isolated from different hosts differ from one another, which is corroborated by the clusters determined in this study.

However, despite this, it is relevant to highlight that some clusters presented in Figure 1 (e.g., clusters 3–7) contain isolates obtained from both sources, which could occur via cross-contamination from beef prepared in the hospital kitchen for patients and from food preparation workers in this environment. The *Staphylococcus* spp. derived from beef could come from contact between food preparation workers and beef, through manipulation, thus presenting a genetic similarity in common. Several factors lead to the contamination of food, such as constant handling by employees and consumers, contaminated utensils and equipment, and commercial refrigerators with fluctuating temperature conditions, which could allow for the proliferation of microorganisms. Evangelista-Barreto and Vieira (2003) isolated *S. aureus* in 60% of food handlers, with the largest numbers occurring in the oropharyngeal cavity, saliva, nasal cavity, and hands, confirming the high incidence of this microorganism in these persons. The cleanliness and quality of equipment in food industries and trades are essential in hygienic sanitary control, and is an important issue for the safety of the final product and, consequently, consumer health (Oliveira *et al*., 2008).

Conclusions

Coagulase-negative *Staphylococcus* isolates had greater adhesion ability than *S. aureus* isolates. For both groups, in the antimicrobial susceptibility test, there was no difference in resistance among isolates from beef and from patients with hospital infections. Despite the absence of the *mecA* gene, 36% of *S. aureus* isolates and 17% of coagulase-negative *Staphylococcus* isolates were resistant to oxacillin, which suggests the use of another mechanism of resistance. The genetic relationship between these bacteria, analyzed by RAPD, was used to differentiate isolates of coagulase-negative *Staphylococcus* and *S. aureus* along with their origins. *Staphylococcus* spp. from different hosts usually differed due to the specialization for each host. However, in this work, we present several isolates from different sources—beef and hospitalized patients—grouped in the same genetic cluster, suggesting the existence of cross-contamination between food handlers in the hospital environment and the beef processed there. This is of great concern to public health in the region, which should seek preventive measures to avoid this cross-contamination in hospitals.

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Disclosure Statement

No competing financial interests exist.

References

Alcaraz LE, Satorres SA, Lucero RM, Centorbi ONP. Identification, slime production and oxacillin susceptibility in coagulase-negative staphylococci isolated from nosocomial specimens. Braz J Microbiol 2003;34:45–51.

Baird-Parker AC. The staphylococci: An introduction. J Appl Bacteriol 1990;19:1S–8S.

Brasil. Vigilância epidemiológica das doenças transmitidas por alimentos no Brasil. 2007. Available at: http://portal.saude.gov.br/portal/arquivos/pdf/apresentacao_dta.pdf, accessed February 6, 2012.

Catão RMR, Silva PMF, Feitosa RJP, Pimentel MC, Pereira HS. Prevalência de infecções hospitalares por *Staphylococcus*

aureus e perfil suscetibilidade a antimicrobianos. J Nurs 2013; 6:57–64.

Christensen GD, Baldassari L, Simpson WA. Colonization of medical devices by coagulase-negative staphylococci. In: Bisno AL, Waldvogel FA. *Infection Associated With Indwelling Medical Devices*. 2nd ed. Washington: ASM Press, 1994, pp. 45–78,.

[CLSI] Clinical and Laboratory Standards Institute. *Suggested Grouping of US-FDA Approved Antimicrobial Agents That Should Be Considered for Routine Testing and Reporting on Nonfastidious Organisms by Clinical Laboratories*. CLSI Publication M100-S23. Wayne, PA: CLSI, 2013.

Costa JCM, Espeschit IF, Pieri FA, Carvalho IA, Moreira MAS. Sensitivity profile of planktonic and sessile cells of *Escherichia coli* to antimicrobial used in the treatment of bovine mastitis. Arq Bras Med Vet Zoo 2014;66:129–136.

Evangelista-Barreto NS, Vieira RHSF. Investigação sobre possíveis portadores de *Staphylococcus aureus* em duas indústrias de pesca. Higiene Alimentar 2003;17:49–57.

Gottardi CPT, Souza CAS, Schmidt V. *Surtos de Toxinfecção Alimentar no Município de Porto Alegre/RS, no Período de 1995 a 2002*. Higiene Alimentar 2006;20:50–55.

Hsueh P, Teng L, Yang P, Pan H, Chen Y, Wang L, Ho S, Luh K. Dissemination of two methicillin-resistant *Staphylococcus aureus* clones exhibiting negative staphylase reactions in intensive care units. J Clin Microbiol 1999;37:504–509.

Idil N, Bilkay IS. Application of RAPD-PCR for determining the clonality of methicillin resistant *Staphylococcus aureus* isolated from different hospitals. Braz Arch Biology Tech 2014;57:548–553.

John JF, Barg NL. *Staphylococcus aureus*. In: Mayhall CG (ed.). *Hospital Epidemiology and Infection Control.* Baltimore: Williams and Wilkins, 1996, pp. 275–276.

Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. Clin Microbiol Rev 1994; 7:117–140.

Koneman EW, Allen SD, Janda WM. *Diagnóstico Microbiológico—Texto e Atlas Colorido*, 5th ed. Rio de Janeiro: MEDSI Editora Médica e Científica Ltda., 2001.

Królasik J, Zakowska Z, Krepska M, Klimek L. Resistance of bacterial biofilms formed on stainless steel surface to disinfecting agent. Polish J Microbiol 2010;59:281–287.

Kwok AYC, Chow AW. Phylogenetic study of *Staphylococcus* and *Macrococcus* species based on partial hsp60 gene sequences. Int J Systemat Evolut Microbiol 2003;53:87–92.

Lee JH, Jeong JM, Park YH. Evaluation of the methicillin-resistant *Staphylococcus aureus* (MRSA)—Screen latex agglutination test for detection of MRSA of animal origin. J Clin Microbiol 2004;42:2780–2782.

Manegotto FR, Picoli SU. *Staphylococcus aureus* oxacilina resistant (MRSA): Incidência de cepas adquiridas na comunidade (CA-MRSA) e importância da pesquisa e descolonização em hospital. RBAC 2007;39:147–150.

Neely NA, Maley MP. Survival of enterococci and staphylococci on hospital fabricas and plastic. J Clin Microbiol 2000; 38:724–726.

Oliveira MMM, Brugnera DF, Mendonça AT, Piccoli RH. Condições higiênico-sanitárias de máquinas de moer carne, mãos de manipuladores e qualidade microbiológica da carne moída. Higiene Alimentar 2008;32:1893–1898.

Pereira MS, Praso MA, Sousa JT, Tipple AFV, Souza ACS. Controle de infecção hospitalar em unidades de terapia intensiva: Desafios e perspectivas. Rev Eletronica Enfermagem 2000;2:1.

Pfaller MA, Davenport D, Bale M. Development of the cuantitative micro-test for slime production by coagulase negative staphylococci. Eur J Clin Microbiol Infect Dis 1988;7:30–33.

Reinoso E, Susana B, Cecilia F. RAPD-PCR analysis of *Staphylococcus aureus* strains isolated from bovine and human host, Microbiologic Res 2004;159:245–255.

Santana EHW, Beloti V, Aragon-Alegro LC, Mendonça MBO. Estafilococos em alimentos: Artigo de revisão. Arq Inst Biol 2010;77:545–554.

Schaechter M, Eisenstein BI. Estabelecimento de doenças infecciosas. In: Schaechter M, Engleberg NC, Eisenstein BI, Medoff G. *Microbiologia Mecanismo das Doenças Infecciosas*. 3rd ed. Rio de Janeiro: Guanabara-Koogan, 2002, pp. 3–9,.

Sloos JH, Dijkshoorn L, Vogel L, van Boven CPA. Performance of phenotypic and genotypic methods to determine the clinical relevance of serial blood isolated of *Staphylococcus epidermidis* in patients with septicemia. J Clin Microbiol 2000;38:2488–2493.

Tortora GJ, Funke BR, Case CL. *Microbiologia*. 10th ed. Porto Alegre: Artmed, 2012.

Turkyilmaz S, Kaya O. Determination of some virulence factors in *Staphylococcus* sp. isolated from various clinical samples. Turk J Vet Anim Sci 2006;30:127–132.

Ueno M. Caracterização de *Staphylococcus aureus* resistentes a meticilina envolvidos em infecções nosocomiais, por meio de técnicas fenotípicas e análise de perfil plasmidial. Rev Biociência 2001;7:15–22.

Williams JGK, Kubelik ARK, Livak JL. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 1990;18:6531–6535.

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