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GENOTYPIC CHARACTERIZATION OF COAGULASE NEGATIVE *STAPHYLOCOCCUS* SPECIES FROM BOVINEMILK FOUND ON PROPERTIES AND MUNICIPALITIES OF NORTHERN MINAS GERAIS

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ABSTRACT

One hundred eleven Gram positive cocci from six dairy farms at north of Minas Gerais were identified by the proteomic technique. *Staphylococcus* coagulase negative (SCN) species were submitted to the disc diffusion test with conventional beta-lactam antibiotics. Strains resistant to meropenem were screened for the bla_{OXA-23} and bla_{KPC} genes by PCR. Staphylococcus coagulase-negative species were evaluated by Chi-square test for resistance and multidrug resistance index. For MALDI-TOF MS the most common genus with 56.8% was *Staphylococcus* spp, the SCN group had a frequency of 27%. The species *S. epidermidis* and *S. chromogenes* had a higher prevalence in the herds with 35.8%, respectively. In Janaúba Nova Prima property, the mean value of the multiresistance index found was 0.6, the other properties and municipalities were 0.5. Cefoxitin (100%), oxacillin (100%), meropenem (100%) and vancomycin (100%) were the antimicrobial resistance in the herds of dairy cattle. The bla_{OXA-23} and bla_{KPC} resistance genes weren't screened in the species *S. epidermidis*, *S. chromogenes*, *S. auriculares* and *S. haemolyticus*. Other mechanisms of resistance are present in the strains causing inefficiency in the antimicrobial treatment in the herds, compromising the well-being of the animals and public health.

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INTRODUCTION

The isolation frequency of the coagulase-negative *Staphylococcus* group in the late 1980s was considered an opportunistic agent of lesser impact, but it has become common in several countries as a cause of mastitis in dairy cattle, especially in young cows. This group includes a variety of species, which makes identification by conventional microbiological methods difficult (Bier *et al*, 2017) and interferes in epidemiological and pathogenic studies of mastitis caused by coagulase-negative *Staphylococcus*. Associated with the participation of coagulase-negative *Staphylococcus* in the pathogenesis of mastitis, in which there is a high prevalence of the identification of multidrug-resistant strains capable of transmitting genes to other bacteria, including *S. aureus*, there was a need for greater knowledge about the real participation of this group. in human and animal health (Isaac *et al*., 2017). Microorganisms have the bla_{KPC} gene, often have several genes responsible for resistance to other antimicrobial agents, such as aminoglycosides, quinolones, triemetropiclins (Chenamidas *et al*., 2014; Nowakhowska, 2016). Considering that the presence of coagulase-negative *Staphylococcus* in milk from cows with subclinical mastitis represents a public health problem, and may be recurrent in Brazilian dairy cattle, the objective of this work was to identify prevalent species, using proteomic analyzes as well as the presence of resistance-associated genes, $bla_{OXA-23}$and bla_{KPC}.

MATERIAL AND METHODS

This work was carried out according to criteria approved by the Chamber of Ethics and Animal Experimentation (CEUA) with protocol nº 145/2013 of the Federal University of Minas Gerais.

Bacterial isolates: One hundred and eleven strains identified as Gram-positive cocci from the laboratory of Animal Health Laboratory- CPCA- UFMG were used for this research. These came from milk from cows with subclinical mastitis in six properties in the North of Minas Gerais, as described in studies reported by Xavier *et al*. (2017).The strains were frozen in BHI broth (Brain Heart Infusion) (Prodimol Biotechnology) added with 20% glycerol and stored in a freezer at -20ºC (Teixeira *et al*., 2014). The microorganisms were activated by three consecutive repetitions in BHI broth and incubated for 24 h at 37ºC. To confirm the purity of the cultures, the colonies were microbiologically analyzed for morphology on Blood Agar (Oxoid) supplemented with 5% defibrinated sheep blood and Gram stain, according to Quinn *et al*., (2005); Koneman; Allen; Janda, (2001). Colonies that showed microscopic morphology of Gram-positive cocci in pure cultures were selected and striated on TSA (Tryptic Soy Agar) agar (Oxoid) for further identification through MALDI-TOF MS analysis.

Proteomic analysis by MALDI-TOF mass spectrometry: One hundred and eleven strains, presumptively identified as Gram positive cocci, were selected and duly forwarded for identification through proteomic analysis at the AQUACEN/REN QUA laboratory of the Veterinary School of the Federal University of Minas Gerais.MALDI TOF MS analysis was performed in accordance with Assis *et al*., (2017) using the Bruker Daltonics Microflex TM MALDI TOF MS instrument. A single fresh colony was spread with a sterile wooden stick and placed on a 96-well stainless steel plate. For each strain, 1µL of formic acid (70%) and 1µL of MALDI TOF MS matrix, constituted by a saturated solution of α-cyano-4-hydroxycinnamic (HCCA) Bruker Daltonics, Bremen, Germany, were applied in place and left to dry. room temperature. Prior to measurements, calibration was performed by a bacterial test standard (*E. coli* DH5 alpha; Bruker Daltonics). Real-time identification score criteria were those recommended by the manufacturer: score ≥ 2.000 indicates species-level identification, score ≥ 1.700 and ≤ 2000 indicates genus-level identification, and ≤ 1700 indicates unreliable identification.

Antimicrobial sensitivity profile: The strains identified as coagulase-negative *Staphylococcus* were analyzed for resistance to beta lactams, using the disk diffusion technique according to Wayne (2016), with the antibiotics: cefoxitin Laborclin (30µg), oxacillin Cecon (1µg), vancomycin Laborclin (30µg), meropenem Cecon (10µg), imepenem Cecon (10µg) and Laborclin ampicillin sulbactam (10µg). Soon after, the multiple antimicrobial resistance index (MAR) of the microorganisms was determined by the ratio between the number of antimicrobials that the sample is resistant to and the total number of antimicrobials tested. MAR index above 0.2 was characterized as multidrug resistance (Krumperman, 1983).

DNA extraction: The cryopreserved isolates of coagulase negative *Staphylococcus* species were reactivated by seeding in BHI and incubated at 37ºC for 24 hours. Bacterial cultures were sent to the Biotechnology laboratory of the Federal University of Minas Gerais, where they were subjected to DNA extraction by the proteinase K digestion method, followed by phenol chloroform (Barea *et al*., 2004) with modifications. A volume of 1.5 ml of each coagulase negative *Staphylococcus* culture was centrifuged at 5000 rpm for 15 minutes. The supernatant was discarded and the bacterial cell pellet ressuspended in 40µL of digestion buffer (0.9% NaCl, 0.2M EDTA and 20mg/mL proteinase K). 40µL of 20% SDS was added and the samples were incubated in a water bath at 60ºC for 10 minutes and then cooled to room temperature. The integrity and quantification of the extracted DNA's were verified by electrophoresis in a 1% agarose gel. This material was used in the PCR reactions.

Universal gene detection for 16S-rDNA bacteria: To ensure that the extracted DNA referred to bacteria, PCR was performed using the primers DG74 (5'-AGGAGGTGATCCAACCGCA-3') and RW01 (5'-2 AACTGGAGGAAGGTGGGGAT-3') generating an amplicon of 370 bp, under the conditions described by Xavier *et al* (2017). Reactions were conducted using a mmix containing 2XGoTaq® Green Master Mix (Promega Corporation, USA), MgCl2 (2.5 mm), 10 µM of each primer, and 50 ng bacterial DNA, in a final total reaction volume. of 50 µL. The conditions were as follows: an initial cycle of denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 45 s and final extension. of 10 min. The amplicons were visualized on 1.5% agarose gel stained with ethidium bromide and photographed and documented. As a positive PCR control, a nosocomial strain of *Klebsiella pneumoniae* identified by coding for the universal bacterial 16S rDNA gene was used.

PCR analysis for detection of resistence genes *bla* OXA23 and *bla* KPC: All primers used in this work were synthesized by Integrated DNA Technology USA and are presented in the following table:

The reactions for the presence of the bla_{OXA-23}gene were carried out in a mixture containing 1X Taq buffer from the Kappa PCR kit, 2.5 mM MgCl2, 1 µM deoxynucleotides, 0.5 U Taq Polymerase Taq, 1.25 µM of each primer, and 1 µL (50 ng / µL) of bacterial DNA, for a final reaction volume of 25 µL. The amplification conditions were as follows: an initial denaturation cycle at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and a final extension of 5min. As a positive control for PCR, we used an *Acinetobacter baumannii* strain isolated from the hospital and genetically identified by a Brazilian reference laboratory (Fundação Ezequiel Dias) as *A. baumannii* encoding the bla_{OXA-23}gene. As a negative control, we used a strain of *E. coli* ATCC 25922.

To search for the bla_{KPC}gene, amplifications were performed in a PCR containing 2x Go Taq Green Master Mix® (Promega, USA 2.5m MgCl2, 10µM each primer and 50ng of bacterial DNA in a final volume of 50µL. The amplification conditions were of 5 minutes at 95ºC, followed by 35 cycles of denaturation at 95ºC for 1 minute, annealing at 58ºC for 30 seconds and extension at 72ºC for 1.5 minutes. The amplification obtained a final extension at 72ºC for 10 minutes. As a positive control for PCR, use the *K. pneumoniae* strain and water as a negative control, Yigit *et al*., (2001). All amplicons of the genes described were visualized in 1.5% agarose gel stained with ethidium bromide and photodocumented. To assess the presence of resistance genes, amplification profiles were visually analyzed by two observers according to the presence or absence of bands compared to controls.

Statistical analysis: The results were submitted to descriptive statistics through the distribution of frequencies relative to the microbiological findings. The chi-square test at a significance level of 5% was used to assess the differences between the frequency of coagulase-negative *Staphylococcus* (SCN) in properties and municipalities. To verify if there was an association between antimicrobial resistance in the properties and in the municipalities, the same statistical test described above was performed. The analyzes were performed using the R version 3.5.0 program.

RESULTS AND DISCUSSION

Species of *Staphylococcus* spp. Identified by MALDI-TOF MS: Using the MALDI - TOF MS technique, the most common genus with 56.8% (n= 63/111) was *Staphylococcus* spp. Among these, *S. aureus* corresponded to 70.0% (n=19 44/63) and the coagulase-negative *Staphylococcus* group presented a frequency of 27.0% (n=22 17/63). Table 2 presents the results of localization of the strains identified as SCN, as well as the results of staining by the Gram method, coagulase test and proteomic analysis.*S. epidermidis* and *S. chromogenes* were identified at the same frequency, corresponding to

Table 1. Resistance and profile of the coagulase-negative Staphylococcus group species against beta lactams isolated from bovine milk in the authorities of properties at the North of Minas multi-resistance Gerais

Oligonucleotides	Sequence	Target Gene	Amplicon	Reference
OXA23F	GATGTGTCATAGTATTCGTCG	*bla*OXA-23	1057 pb	Fonseca*et al.*, (2013)
OXA23R	TCACAACAACTAAAAGCACTG-3			
*Bla*KPC F	TGTCAC TGTATC GCC GTC	*bla*KPC	876 pb	Yigit*etal*.,(2001).
*Bla*KPCR	CTCAGTGCT CTACAGAAA ACC			

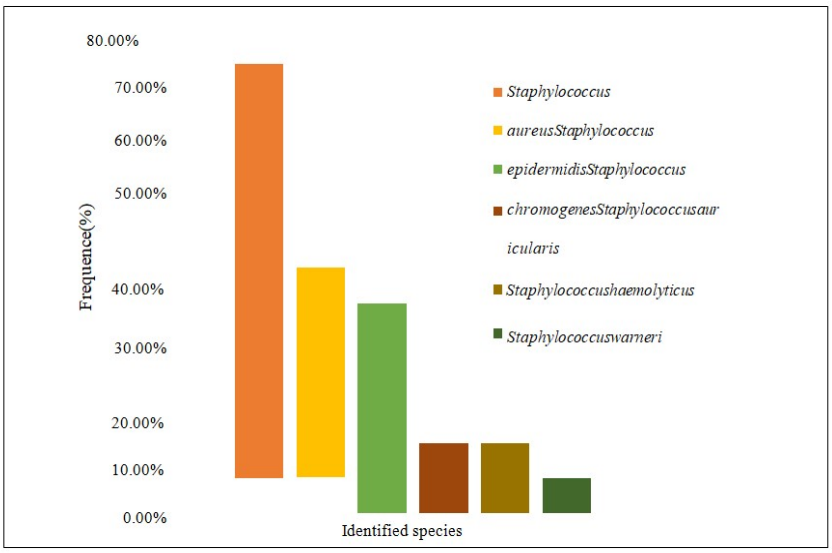
Species of SCN	Municipilaties	Antimicrobial resistence among municipalities (%)						MAR between the unicipalities	Farms	Antimicrobial resistance among farms (%)						MAR between the farms
		CFO	OXA	VAN	MER	SBA	IMP			CFO	OXA	VAN	MER	SBA	IMP	
	Janaúba	100	100	33,3	33,3	0	0	0.6	NovaPrima	100	100	33,3	33,3	0	0	0.6
S.epidermidis	Porteirinha	50	50	50	0	0	0	0.5	Muganga	50	50	50	0	0	0	0.5
	Icaraí deMinas	100	100	0	0	0	0	0.3	GU	100	100	0	0	0	0	0.3
S.chromogenes	Bocaíuva	100	100	0	100	0	0	0.5	Triunfo	100	100	0	100	0	0	0.5
	Icaraí deMinas	100	100	100	0	0	0	0.5	GU	100	100	100	0	0	0	0.5
S.haemolyticus	Janaúba	100	100	0	0	0	0	0.3	NovaPrima	100	100	0	0	0	0	0.3
	Montes Claros	100	100	0	100	0	0	0.5	FEHAN	100	100	0	100	0	0	0.5
S.auricularis	Montes Claros	50	50	0	50	0	0	0.5	FEHAN	50	50	0	50	0	0	0.5
S.warneri	Janaúba	100	100	0	0	0	0	0.3	VistaAlegre	100	100	0	0	0	0	0.3
P valeu	0.3917	0.5712	0.5712	0.3564	0.0881	N.V	N.V	0.5712	0.1546	0.4442	0.4442	0.1326	0.421	N.V	N.V	0.4442

Note:CFO=cefoxitin;SBA=subactam-ampicilin,OXA=oxacilin;VAN=vancomYcin;IPM=imipenem;MER=meropenem; AMR= antimicrobial multiresistance index. P values through the chi-square test ($p < 0.05$); NV= There was no significant variation.

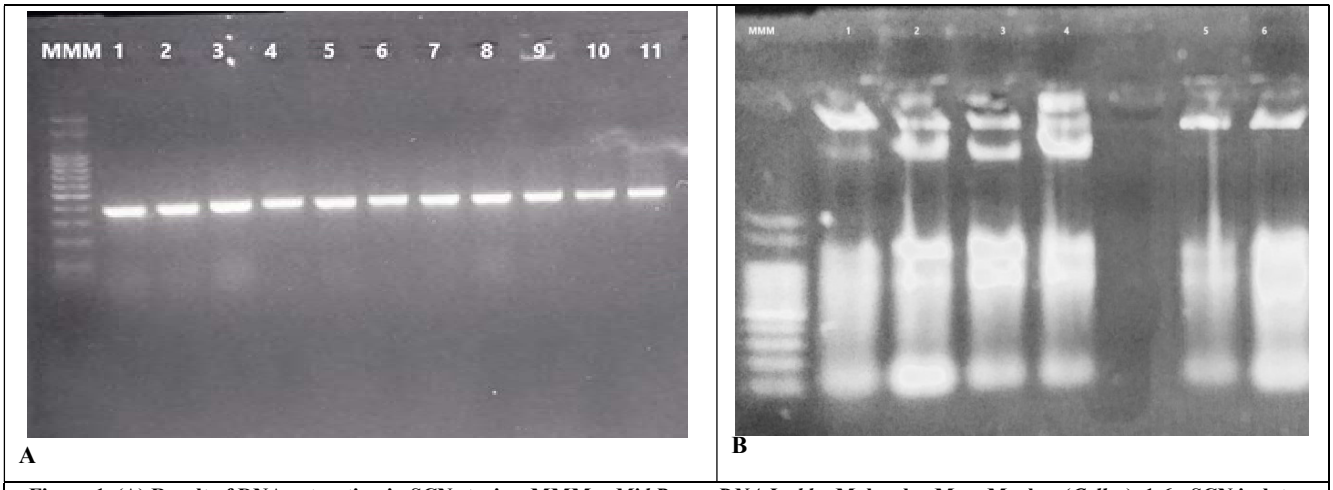
Table 2. Phenotypic characterization of antibiotic susceptibility, biochemistry, Gram staining, proteomic and genomic profile analysis of seventeen coagulase-negative *Staphylococcus* strains isolated from cows with subclinical mastitis at the North of Minas Gerais

Farm*	Isolation date	Municipalities	Antimicrobial resistance profile	Microbiological analysis - Gram	Biochemical coagulase test	Proteomic analysis MALDI-TOF	*16SrDNA gene universal of bacteria*	Genomic Profile	
								*Gene bla*Kpc	Gene *bla*OXA23
MUG1	16/02/2018	Porteirinha	CFO,OXA,VAN	*Staphylococcusspp*	-	*S.epidermidis*	+	NT	NT
MUG2	16/02/2018	Porteirinha	Sensitive	*Staphylococcusspp*	-	*S.epidermidis*	+	NT	NT
NP1	16/02/2018	Janaúba	CFO,OXA,MER	*Staphylococcusspp*	-	*S.epidermidis*	+	-	-
NP2	16/02/2018	Janaúba	CFO, OXA	*Staphylococcusspp*	-	*S.epidermidis*	+	NT	NT
NP3	16/02/2018	Janaúba	CFO,OXA,VAN	*Staphylococcusspp*	-	*S.epidermidis*	+	NT	NT
NP4	16/02/2018	Janaúba	CFO, OXA	*Staphylococcusspp*	-	*S.haemolyticus*	+	NT	NT
VA1	16/02/2018	Janaúba	CFO, OXA	*Staphylococcusspp*	-	*S.warneri*	+	NT	NT
VA2	16/02/2018	Janaúba	Sensitive	*Staphylococcusspp*	-	*S.chromogenes*	+	NT	NT
TR1	16/02/2018	Bocaiuva	CFO,OXA,MER	*Staphylococcusspp*	-	*S.crhomogenes*	+	-	-
TR2	16/02/2018	Bocaiuva	CFO,OXA,MER	*Staphylococcusspp*	-	*S.chromogenes*	+	-	-
GU1	16/02/2018	IcaraídeMinas	CFO. OXA	*Staphylococcusspp*	-	*S.epidermidis*	+	NT	NT
GU2	16/02/2018	IcaraídeMinas	CFO,OXA,VAN	*Staphylococcusspp*	-	*S.chromogenes*	+	NT	NT
FEHAN 1	16/02/2018	MontesClaros	CFO,OXA,MER	*Staphylococcusspp*	-	*S.crhomogenes*	+	-	-
FEHAN 2	16/02/2018	MontesClaros	CFO,OXA,MER	*Staphylococcusspp*	-	*S.auriculares*	+	-	-
FEHAN 3	16/02/2018	MontesClaros	sensitive	*Staphylococcusspp*	-	*S.chromogenes*	+	NT	NT
FEHAN 4	16/02/2018	MontesClaros	CFO,OXA,MER	*Staphylococcusspp*	-	*S.haemolyticus*	+	-	-
FEHAN 5	16/02/2018	MontesClaros	sensitive	*Staphylococcusspp*	-	*S.auriculares*	+	NT	NT

[a]CFO=Cefoxitin;OXA=Oxacillin;MER=Meropenem;VAN=Vancomycin;SBA=Subactam-ampicilina;IMP=Imepenem.
*MUG=Muganga;NP=NovaPrima;VA=VistaAlegre;TR=Triunfo;GU;FEHAN:FazendaExperimentalProfessorHamiltondeAbreu Navarro.
NT= not treated;(-)= negative result;(+)= positive result.



Graph 1- Frequency of multidrug-resistant *Staphylococcus* spp. species identified by MALDI-TOF MS in cows with subclinical mastitis



A B

Figure 1. (A) Result of DNA extraction in SCN strains. MMM = *Mid Range DNA Ladder* Molecular Mass Marker (*Cellco*). 1-6= SCN isolates under study. (B) PCR analysis with universal bacterial 16S rDNA gene primers.MMM= 100bp DNA Molecular Mass Marker (Ludwig). Lane 1-8: SCN strains. Line 9-10: *Staphylococcus aureus* strains. Row 11: positive control (*Klebsiella pneumoniae* strain). The expected 370bp amplicom corresponding to the 16S rDNA as indicated on the right side of the gel

35.3% of the isolates, followed by *S. auriculares*, *S. haemolyticus* and *S.warneri* (graph 1). Studies report the presence of the same SCN species identified here, although using different methodologies for identification. Mello *et al*.,(2017), Silva *et al*., (2014) also observed a similar frequency of *S. chromogenes* and *S. epidermidis* in milk from teats with subclinical mastitis. Lange *et al*. (2015) observed *S. chromogenes* (38.5%) at a higher frequency than *S. epidermidis* (13.1%). The aforementioned authors also observed to a lesser extent frequency *S. haemolyticus, S. auricular, S. warneri*, in addition to other CNS. In other countries, the frequency of SCN species is variable, as described by Pyörälä; Taponen (2009); Piessens *et al*., (2011); Piessens *et al*., (2012); De Vliegher *et al*., (2012); Thorberg *et al*., (2009) also observed a higher frequency *of S. chromogenes* and *S. epidermidis* in milk from teats with subclinical mastitis and Taponen *et al*., (2016) who identified *S. epidermidis* (30.7%) as the second most common agent. frequency in subclinical mastitis. These searches used genetic identification of SCN. On the other hand, Tomazi *et al*. (2014) used MALDI-TOF and Frey *et al*. (2013) also found a diversity of species in milk from teats with subclinical mastitis, partly corroborating the study, as *S. chromogenes* (74.07%) was the most prevalent, followed by *S. haemolyticus* and *S. epidermidis* among other SCN. The efficiency of MALDI-TOF MS used in bovine milk isolates contaminated by subclinical mastitis was 92% (n=102/111), corroborating the work of Schaubauer *et al*., (2014) who, using the same technique, obtained 90.5% accuracy in twenty-one samples of milk isolates from cows with subclinical mastitis.The MALDI-TOF MS technology, compared to other laboratory techniques for the identification of microorganisms, has the advantage of agility and accuracy in the results. Between the preparation of the deposit until the final reading, an isolated result can be obtained in less than thirty minutes (Croxatto; Prod'Hom and Greub, 2012). When evaluating epidemiologically between the participation of SCN among the herds studied, the frequency of coagulase-negative *Staphylococcus* species identified was similar between farms (Graph 2) (p= 0.1546) and municipalities (Graph 3) (p= 0.3917). The prevalence of SCN species in the etiology of bovine mastitis varies with the study regions (ISAAC *et al*., 2017; NYMAN *et al*., 2017; VANDERHAEGHEN *et al*., 2015,) and with an epidemiological diversity between herds and between species (PIESSEN *et al*., 2012), as observed in this work. The evaluation of clonality among these isolates may indicate more clearly the transmission between and within herds, as observed by Piessens *et al* (2012) when detecting similar genotypes of SCN in milk contaminated by mastitis, from different herds, as well as in the environment. Resistance of the coagulase-negative *Staphylococcus* group (Tab.1) was observed in thirteen strains of the seventeen identified. The *Staphylococcus* species: *S. epidermidis, S. chromogenes, S. haemolyticus, S. auricularis* and *S. warneri* obtained high resistance to the bases cefoxitin, oxacillin, meropenem and vancomycin, except for the antimicrobials subactam-ampicillin and imepenem, according to (Table 2).

All municipalities and properties evaluated showed multiresistance to beta-lactams tested. The municipality of Janaúba presented the highest multiresistance index (MAR) with an average value of 0.6 (n= 4/6). of 0.5 (n=3/6), however, all municipalities were multidrug resistant due to resistance to more than two antibiotics used. As in the properties, in which the Nova Prima farm obtained the highest MAR, with an average value of 0.6 (n=4/6) the other properties evaluated such as Muganga, Triunfo, Gu and FEHAN, MAR values were observed at 0.5 (n=3/6). The results found between the municipalities and properties in relation to MAR and antimicrobial resistance were similar, according to Table 1, corroborating the work of Xavier *et al*., (2017) who observed antimicrobial resistance in *S. aureus* strains, found in dairy herds with subclinical mastitis, in the municipalities of Janaúba, Bocaiuva and Icaraí de Minas. As were similar to those of Mahato *et al*., (2017) Taponen *et al*., 2016 who observed higher rates of resistance to cefoxitin and oxacillin bases, respectively, by CNS isolated from teats of cows with subclinical mastitis. The participation of multidrug-resistant SCNs to antimicrobials is described in studies in several countries, including Brazil (Bansal *et al*., 2015; Mahato *et al*., 2017, Tapponen, *et al*., 2016). In relation to multi-resistance to betalactams, similar results were observed Bansal *et al*., (2015) when evaluating SCN isolated from teat with subclinical mastitis, however, when evaluating antimicrobial bases, higher rates of resistance to penicillin, ampicillin and amoxicillin . The authors observed 50.9% resistance to amoxicillin + sulbactam, different from the results obtained in this work, which was 100% of sensitive strains. It should be considered that the authors carried out a broader study with a greater number of SCN isolates and the species under study were not mentioned in the work.Likewise, Soares *et al*., (2012) observed higher rates of multiresistant SCN to beta-lactams ampicillin (79%) and penicillin (79%), but also observed 40% resistance to oxacilia and cephalothin, similar to the results here obtained, although the SCN species are not the same as those observed in this study. Frey *et al*., (2013) observed 43.9% of oxacillin-resistant SCN in subclinical mastitis. Regarding beta lactam-resistant SCN species, studies by Sawant *et al*., 2009 and Waller *et al*., (2011) observed high frequencies of beta-lactam-resistant *S. epidermidis, S. chromogenes* and *S. haemolyticus*, but in none of these studies was resistance to oxacillin and cefoxtin observed, which characterizes resistance to methicillin. Few studies describe the SCN species observed in this study for methicillin indicated by resistance to oxacillin and cefoxitin. In Brazil, Santos *et al*. (2016) observed Methicillin-resistant *S. epidermidis* in milk from teats with subclinical mastitis, observed phenotypically by resistance to oxacillin and cefoxitin, as well as genetically. Kibli *et al*., (2018), Bandyopadhyay *et al*., (2015) and Frey *et al*., (2013) also describe the diagnosis of Methicillin-resistant *S. epidermidis* in other countries, corroborating the results obtained here. As themethicillin-resistant *S. chromogenes*, the results obtained here are corroborated by Xu *et al*., (2015) report 75% of methicillin-resistant *S. chromogenes* strains in milk from teats with subclinical mastitis in China and Taponem *et al*., (2015) , however, have already observed oxacillin-resistant *S. epidermidis, S. chromogenes* and *S. haemolyticus* isolates in milk from teats with subclinical mastitis.

All these authors highlight the importance of resistant methylicin SCN in both human and animal health, through the possibility of transferring resistance genes, this group being an important reservoir of mobile genetic elements. These may participate not only in resistance to beta-lactams but also to other classes of antimicrobials (Vanderhaeghen *et al*., 2015), in addition to the possibility of transmission of strains between animals and humans (Beyene, *et al*., 2017). Santos *et al*., (2016), in studies with methicillin-resistant *S. epidermidis* isolated from Brazilian herds, showed that this pathogen might be a reservoir of beta-lactam resistance genes for other staphylococci species. The authors emphasize the importance of knowledge about the phenotypic resistance of Staphylococcus only in a given region so that preventive and therapeutic procedures were adopted, aiming at reducing the spread of resistance genes in herds and between animals and humans. The resistance to the carbapenem meropenem observed in *S. epidermidis*, *S. auricularis*, *S. haemolyticus* and *S. chromogenes* isolates (Table 1) wasn't find in the consulted literature. Some studies report the presence of carbapenem tolerance genes in clinical isolates and in products of animal origin, indicating a potential risk for humans when associated with transmission by food of animal origin (Michael *et al*., 2015).Researchers call attention to the selection of carbapenemase marker genes in conditions of excessive use of beta lactams, as is practiced in cattle, since the risk of resistance to carbapenems, associated with resistance to other beta lactams, is not excluded (Poirel *et al*. , 2014).

The use of carbapenems in production animals isn't allowed, but when used in companion animals it is possible that in some common situation they are used in production animals for an inappropriate indication or by the owner (FDA, 2013). There are no legislations wich establish research or residue limits of these antimicrobials in food, which does not guarantee that they are absent. The potential risk of foods of animal origin as carriers of carbapenem resistance is discussed by Morisson; Rubin, (2015). These authors cite the importance of transmission of resistance genes by non-pathogenic bacteria, which can be transmitted to humans, animals and other bacteria, including Gram positive ones. Webb *et al*., (2016) detected reduced resistance to carbapenems in bacteria isolated from cow feces and the authors conclude that the possibility of spreading carbapenem-resistant bacteria in cattle herds will have serious implications for human and animal health, requiring constant monitoring of the transmission of these strains between herds and humans.

Detection of *bla* OXA-23 and *bla* KPC resistance genes in coagulase negative *Staphylococcus* strains: The DNA extracted from the strains under study indicated good quality, allowing its use in later stages. The confirmation of the DNA extracted in Figure 1 (A) as bacterial DNA was obtained in the PCR shown in Figure 1 (B). SCN isolates under study. (B) PCR analysis with universal bacterial 16S rDNA gene primers.MMM= 100bp DNA Molecular Mass Marker (Ludwig). Lane 1-8: SCN strains. Line 9-10: *Staphylococcus aureus* strains. Row 11: positive control (*Klebsiella pneumoniae* strain). The expected 370bp amplicom corresponding to the 16S rDNA as indicated on the right side of the gel. Although meropenem-resistant strains were phenotypically observed (Table 1), the presence of $bla_{OXA-23}$and bla_{KPC} genes were not identified in the PCR analyses. The quality of the DNA obtained and the confirmation of the 16S ribosomal rDNA of bacteria ensure that there were no possible technical failures in the analysis of PCRs, as well as the positive results obtained for the genes present in *A. baumanni* and *K. penumoniae*. Aguirre-Quiñonero and Martinez *et al*., (2015) also found divergence between results of phenotypic resistance to carbapenems and research of genes in enterobacteria obtained in clinical isolates, corroborating the results achieved here. The absence of amplification of these genes in the strains does not prove that these microorganisms do not have other mechanisms of resistance to cabarpenems. Other types of resistance genes have been detected in research, as well as VIM, IMP, NDM, KPC and OXAs (Morisson; Rubim, 2015). Otel and Aracil (2015) report that the development of molecular methodologies for diagnosing carbapenem resistance should allow the detection of target genes in different variants related to the production of enzymes involved in resistance mechanisms. The authors cited by them report that the selection of an appropriate methodology depends on factors to be considered, such as the epidemiological situation, laboratory availability and other confirmatory tests.

Mechanisms of Gram-negative resistance to Class D beta-lactams, oxacylinases (OXAs) are well known (Monge *et al*., 2013). The production of these enzymes by Gram positive bacteria was recently described in Bacillus sp (Toth *et al*., 2015). Studies indicate that the resistance of S. aureus to carbapenems is associated with the expression of mecA and related to PBP 2a, a low-affinity protein (Pendentlon *et al*., 2015). KPC's carbapenemases are widespread and outbreak-causing worldwide and 12 variants are described, although a single genetic element (transposon Tn4401) has been found. In the researched literature, there were no reports of the presence of this class in Gram positive bacteria, according to current data described by

Bush (2018). Subsequent research is needed to ascertain the presence of genes that are related to the meropenem resistance phenotypic profiles found in this work, as the main concern with carbapenemases is their ability to rapidly change and expand their spectrum of activity (Codjoe and Donkor, 2018; Nowak and Paluhowska, 2016).

CONCLUSION

- Among the SCN present in milk from teats with subclinical mastitis, *S. epidermidis* and *S. chromogenes* showed a higher frequency of isolation.
- The CNS analyzed in this study showed multidrug resistance to the beta-lactam group of antimicrobials.
- *S. epidermidis* and *S. chromogenes* showed resistance to the carbapenem meropenem in the disk-diffusion test.
- The $bla_{OXA-23}$and bla_{KPC}genes weren't identified by PCR in meropenem-resistant isolates.

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