

# Antimicrobial susceptibility profile and research of *mecA* and *erm* genes in coagulase-negative staphylococci isolated from platelet concentrates bags

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In recent years, several studies have described the clinical impact of bacterial infection associated with transfusion of platelet concentrates (PCs). Among the blood components, PCs are responsible for the highest rates of bacterial contamination as well as septic transfusion reactions. We assessed antimicrobial susceptibility profile, resistance to methicillin (MRCoNS), and resistance to macrolides, lincosamides and streptogramins of group B (MLS<sub>B</sub>) of 16 coagulase-negative staphylococci (CoNS) isolates from an investigation in 691 PCs bags. We then compared conventional and automated phenotypic methods, disc diffusion test (DD) and VITEK® 2, respectively as well as phenotypic and genotypic methods (Polymerase Chain Reaction - PCR). All CoNS were susceptible to vancomycin. The disc diffusion test characterized 18.75% as MRCoNS and 37.5% with inducible resistance to MLS<sub>B</sub> (iMLS<sub>B</sub>), and with VITEK® 2, 6.3% and 31.25%, respectively. The *mecA* gene was detected in 18.75% and the *erm* gene in 31.25% of the isolates. In this study, we found equal percentage values between presence of the *mecA* gene by PCR and resistance to methicillin using cefoxitin by DD test, evidence of the *erm* gene by PCR, and iMLS<sub>B</sub> resistance by automation (VITEK® 2). Moreover, we identified three strains with beta-lactamase overproduction, and the occurrence of a bigger mistake was verified when automation was compared with DD test. And we observed that D-test was the most reliable for the detection of iMLS<sub>B</sub> resistance in *Staphylococcus sp.*

**Uniterms:** Bacterial resistance. *Staphylococcus sp.* Platelet concentrates. *MecA*. *Erm*.

## INTRODUCTION

Bacterial contamination of blood components has probably been the first disease transmitted by transfusion (Blajchman, 1995). Despite bacterial sepsis associated to transfusion being known for a long time, it remains a serious health problem. Several measures have been deployed to prevent and detect bacterial contamination of platelet concentrates (PCs). These actions have reduced but not eliminated the risk of septic transfusion reactions (Eder, Goldman, 2011). Besides the risk of bacterial contamination of blood components, current studies seek to detect and reduce the risk of transfusion-related acute

lung injury (TRALI), a syndrome characterized by acute respiratory distress following transfusion (Eder, Dy, O'Neill, 2016).

PCs are blood components with the highest bacterial contamination rate and usually responsible for most of septic transfusion reactions (Chang *et al.*, 2004). This type of bacterial contamination due to PCs infusion is much higher than other types of transfusion-transmitted infections (Jacobs, Palavecino, Yomtovian, 2001; Goodnough, Sander, Brecher, 2003; Vasconcelos *et al.*, 2004).

Gram-positive cocci are the most common contaminants in PCs and coagulase-negative staphylococci (CoNS) are the main responsible for septic transfusion reactions (Greco *et al.*, 2008). They are frequently associated with hospital infections, particularly in immunocompromised patients (Stoll *et al.*, 2002).

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Although CoNS form part of the normal flora of humans, they are acknowledged as important pathogens, causing a wide variety of infections. They are isolated from skin and soft tissue infections, respiratory and lower urinary tract, and specifically bloodstream infections (Landrum *et al.*, 2012).

In recent years, due to the high rate of hospital infections associated with antimicrobial resistance, resistance patterns of CoNS have become the study source of many researchers worldwide (De Giusti *et al.*, 1999).

The classic mechanism of resistance to methicillin present in CoNS (coagulase-negative staphylococci resistant to methicillin - MRCoNS), encoded by the *mecA* gene is well documented, conferring resistance to all beta-lactam agents. Studies show that there has been a substantial increase in MRCoNS isolation rates in hospital infections, including Brazil, where resistance rates are among 50-80%, varying according to bacterial species and patient's demographic data (Marshall *et al.*, 1998; Hussain *et al.*, 2000; Ferreira *et al.*, 2003; Palazzo, Darini, 2006).

CoNS may also be resistant to macrolides, lincosamides and streptogramins of group B agents (MLS<sub>B</sub>), which may be constitutive (cMLS<sub>B</sub>) or inducible (iMLS<sub>B</sub>). In the constitutive expression, resistance to erythromycin and clindamycin is detected in a normal antibiogram. However, in the inducible form, resistance to erythromycin and false susceptibility to clindamycin are detected. It is recommended that the conventional phenotypic detection of iMLS<sub>B</sub> resistance is performed by D-test (Clinical and Laboratory Standards Institute, 2014). There are three mechanisms of *Staphylococcus* resistance linked to the MLS<sub>B</sub> group: target modification of ribosomes by methylase, mediated by *erm* genes (Nakajima, 1999); efflux mechanisms mediated by *msrA* gene (Matsuoka *et al.*, 1999) and characterized as MS<sub>B</sub> phenotype, conferring resistance to macrolides only (Matsuoka *et al.*, 2002; David, Pimentel, Freire, 2005); and antibiotics enzymatic inactivation (Roberts *et al.*, 1999). Nevertheless, the predominant form is target modification (Roberts *et al.*, 1999; Leclercq, 2002).

A study published by our research group emphasizes the importance of recognizing sepsis caused by contaminated PCs transfusion, specifically in immunocompromised patients, since bacterial sepsis associated to platelet transfusion is nowadays the most common infectious complication of transfusion medicine (Martini *et al.*, 2012).

The aim of this study was to assess the susceptibility profile against antimicrobials as well as to research the typical resistance to methicillin and to MLS<sub>B</sub> group by phenotypic, conventional and automation methods, including *mecA* and *erm* genes.

## MATERIAL AND METHODS

### Inclusion criteria for isolated bacterial and reference standard strains

A total of 16 CoNS were isolated through a study on bacterial investigation carried out in 691 PCs bags, collected during 2009/2010, from the Blood Bank of the city of *Santa Maria, Rio Grande do Sul* State (HEMORGS/SM, RS),

The following strains of international reference standard (*American Type Culture Collection* - ATCC) were used as controls in genotypic and phenotypic tests: *Staphylococcus aureus* ATCC 43300, ATCC 25923, BAA977, BAA976 and ATCC 29213.

### Antimicrobial Susceptibility Testing by disk diffusion test (DD) and VITEK® 2; and determination of minimum inhibitory concentration (MIC) of vancomycin

For the performance of phenotypic tests, strains previously stored in Trypticase Soy Broth 15% glycerol were activated in Mueller Hinton agar and incubated at 35±2 °C for 24 hours.

We performed antimicrobial susceptibility testing using two methods, the conventional method of disk diffusion (DD) and the automated method VITEK® 2. Antimicrobial susceptibility testing was performed using disk diffusion test (DD) following the cutoff points recommended by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2014), and susceptibility testing was performed by VITEK® 2 automated method, according to the manufacturer's standards (bioMérieux). Vancomycin minimum inhibitory concentration (MIC) was made by the broth micro-dilution method, performed in duplicate (Clinical and Laboratory Standards Institute, 2014).

### Analysis of resistance genes

For the genotypic testing, deoxyribonucleic acid extraction (DNA) was carried out by heating shock. Bacterial isolates were grown on Trypticase Soy Agar and then incubated at 35 ± 2°C for 18 to 24 hours. Subsequently, a dense bacterial suspension with 4-5 isolated colonies was prepared in an Eppendorff type microtube containing 500 uL autoclaved Milli Q water. This suspension was homogenized by vortex and placed in water bath at 100 °C for 15 minutes. The microtube was subjected to heating shock (on ice) for 5 minutes

and centrifuged at 11,000 rotations per minute (rpm) for 20 minutes. The supernatant (containing the DNA) was transferred to another microtube which was frozen at -20 °C (Nogueira *et al.*, 2004; Freschi, Carvalho, Oliveira, 2005; Scheffer, 2008; Aguilar, 2009). DNA extraction by heating shock was confirmed by the 16S rRNA gene amplification, obtaining a high quality DNA.

### Amplification of 16S ribosomal RNA (rRNA)

The extracted DNA was subjected to electrophoresis in 1.0% agarose gel to verify DNA presence in the extracted material. The 16S rRNA was amplified by Polymerase Chain Reaction (PCR) and the following primers were used: 5'-GTAGTCCACGCCGTAAC-3' and 5'-TAAACCACATGCTCCACC-3 (Li *et al.*, 2009).

### Identification of MRCoNS

Identification of bacterial species isolated from infected PCs bags was performed by VITEK® 2 automated system. To study phenotypic resistance to methicillin were used discs of cefoxitin (30 µg) and MIC cefoxitin (VITEK® 2). Results were interpreted according to the criteria established by the CLSI (Clinical and Laboratory Standards Institute, 2014).

To identify the presence of *mecA* gene, the following sequences were used: *mecA\_F* AGT TAG ATT GGG ATC ATA GCG and *mecA\_R* CGA TGC CTA TCT CAT ATG CTG (Ludwig Biotec®). The primers used for *mecA* gene testing were designed using the PBP2a protein structure made in the Laboratory of Molecular Biology and Sequencing - LabDros (Lim, Strynadka, 2002). The best mold region for primer design is the non-variable portion of PBP2a - between 27 and 326 amino acids - being in base pairs, corresponding to the region between 81 and 978 nucleotides. The GU301106 reference sequence from GenBank was used as the primer's choice. The size of amplicons generated by the amplification using these primers is 145 bp, and they were checked in 0.8% agarose gel. MRCoNS identification was followed as suggested by Kearns *et al.* (1999).

### Detection of macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>) resistance

Double disk or Diffusion Test (D-test) was performed using the DD technique, following the standards recommended by the CLSI (Clinical and Laboratory Standards Institute, 2014). *ermABC* genes determination was carried out according to Coutinho *et al.* (2010)

Primers' sequence was: *ermA\_F* 5'-GTT CAA GAA CAA TCA ATA CAG AG-3' and *ermA\_R* 5'-GGA TCA GGA AAA GGA CAT TTT AC-3'; *ermB\_F* 5'-CCG TTT ACG AAA TTG GAA CAG GTA AAG GGC-3' and *ermB\_R* 5'-GAA TCG AGA CTT GAG TGT GC-3'; *ermC\_F* 5'-GCT AAT ATT GTT TAA ATC GTC AAT TCC-3' and *ermC\_R* 5'-GGATCA GGAAA GGA CAT TTT AC-3'.

### Statistical analysis

We proceeded to sensitivity assessment and methods specificity tested in the detection of each resistance mechanism (Bonita, Beaglehole, Kjellström, 2010).

### Ethical considerations

This study was approved by the Research Ethics Committee (CEP) of UFSM under the number 0285.0.243.000-09.

## RESULTS AND DISCUSSION

### Automated bacterial identification - VITEK® 2

From the total of 16 isolates of CoNS obtained through a research on bacterial contamination carried out in 691 PCs from HEMORGS/SM, five were identified as *Staphylococcus haemolyticus* (31.25%), four as *Staphylococcus epidermidis* (25%), four as *Staphylococcus warneri* (25%), and three as *Staphylococcus saprophyticus* (18.75%). The bacterial species isolated from contaminated PCs bags were identified by the automated system Vitek® 2. All organisms isolated in contaminated PCs were identified as CoNS, similar to the results found by several authors (Hsueh *et al.*, 2008; Martínez, Tarrand, Lichtiger, 2010; Walther-Wenke *et al.*, 2010). Currently, CoNS represent one of the major nosocomial pathogens and *S. epidermidis* and *S. haemolyticus* are the most significant species. Moreover, the increasing rates of antibiotic resistance are an even greater problem for CoNS than for *Staphylococcus aureus*, limiting our therapeutic options (Becker, Heilmann, Peters, 2014).

### Comparison of susceptibility profile between disk diffusion test (DD) and VITEK® 2

In this study, there was discrepancy in the susceptibility assessment between DD and automation methods with clindamycin, sulfamethoxazole/trimethoprim, erythromycin, gentamicin and norfloxacin antibiotics (in descending order), and agreement was

observed only in three (No. 11, 12 and 14) of the 16 analyzed samples as they showed the same profile of antimicrobial susceptibility (Table I).

Studies by other authors confirm our findings when these two methods are compared (VITEK® 2 and DD test - considered reference methods), reporting the occurrence of much higher error (VME), higher error (ME), and lowest error (MiE) with the VME prevalence profile (Sapino *et al.*, 2012; Paim, Cantarelli, D'Azevedo, 2014), in susceptibility assessed by the automated method.

All CoNS were resistant to penicillin G and penicillin (automated and DD test, respectively), and by DD test 62.5% were resistant to clindamycin and erythromycin against 31.25% and 43.75%, respectively, by automation. Studies have reported CoNS high resistance levels to penicillin, clindamycin and erythromycin (Bernardi, Pizzolitto, Pizzolitto, 2007; Melo *et al.*, 2007; Rigatti *et al.*, 2010). All samples were susceptible to vancomycin, a similar result to that found in the studies mentioned above. In our study vancomycin MIC was between 0.5 µg/mL a 2 µg/mL.

### Assessment of Methicillin-Resistant Coagulase-Negative Staphylococci (MRCoNS)

In relation to methicillin resistance in CoNS, three samples (18.75%) were resistant to cefoxitin in DD, and by VITEK® 2 one sample was resistant (Table II). Regarding CoNS, the standardized tests reporting MRCoNS strains are: agar dilution, DD technique and broth micro-dilution (Clinical and Laboratory Standards Institute, 2014). In the study developed by John *et al.* (2009), authors showed that VITEK® 2 did not detect methicillin resistance against *S. aureus* and most of the CoNS. By the PCR method, considered by many authors the gold standard one for the phenotypic testing comparison (Cafiso *et al.*, 2004; Ruzicka *et al.*, 2004; Gad *et al.*, 2009), the *mecA* gene presence was detected in three samples (18.75%), which were susceptible to cefoxitin in DD test and VITEK® 2 (Table II).

Studies show that methicillin resistance in *Staphylococcus* occurs mainly due to the production of modified penicillin binding protein (PBP2a) encoded by the *mecA* gene. This less frequent resistance, however, may also be due to the beta-lactamase overproduction by oxacillin inactivation, resulting in partial hydrolysis of the beta-lactamase ring (borderline resistance to methicillin - BORSA) or the presence of a PBP with low affinity to oxacillin (MODSA) (Tomasz *et al.*, 1989; Ito *et al.*, 2003; Rossi, Andreazzi, 2005). In these last two resistance mechanisms to methicillin, bacteria do not carry the *mecA* gene (Mohanasoundaram, Lalitha, 2008).

We suggest the identification of three MRCoNS strains with overproduction of beta-lactamase enzyme, which rarely occurs when *Staphylococcus* strains show resistance in DD test and do not have the *mecA* gene. The treatment for infections caused by MRCoNS may be performed with linezolid, teicoplanin, and vancomycin, as well as alternative drugs such as macrolides, clindamycin, and trimethoprim/sulfamethoxazole (Rossi, Andreazzi, 2005).

Resistance to methicillin and beta-lactamase overproduction are the main causes of discordant results among phenotypic and genotypic tests to detect MRCoNS (Ghoshal *et al.*, 2004). Research of this kind of resistance by phenotypic methods can be complex due to the heterogeneous expression of many strains, which may also be influenced by growth conditions (Chambers, 1997).

Recent surveys have detected a rare homologous gene to *mecA* in *S. aureus* strains isolated from humans and animals with phenotypic resistance to methicillin, found through DD test with cefoxitin, which did not show the *mecA* gene when specific primers for this gene were used (Cuny *et al.*, 2011; Shore *et al.*, 2011; Cartwright *et al.*, 2013). This gene was initially called <sup>mecA</sup><sub>LGA251</sub> and later designated as *mecC* (Ito *et al.*, 2012). This *mecC* gene has also been reported in CoNS as *Staphylococcus stepanovicii*, *Staphylococcus xylosus* and *Staphylococcus scirui* (Harrison *et al.*, 2013a, 2013b; Loncaric *et al.*, 2013). As already suggested for the *mecA* gene, we can probably infer that the origin of the *mecC* gene is also among the CoNS (Tsubakishita *et al.*, 2010; Couto *et al.*, 2013). Although *mecC* gene detection is rare and reported only in Europe so far, this gene is a potential diagnosis problem and triggers several issues for future studies (Paterson, Harrison, Holmes, 2014).

### Analysis of macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>) resistance

The conventional phenotypic analysis (DD) of resistance to MLS<sub>B</sub> antimicrobials, carried out with the disk approximation test - D-test, characterized six samples (37.5%) with inducible (iMLS<sub>B</sub>) and two samples (12.5%) with constitutive phenotype (cMLS<sub>B</sub>). On the other hand, the MS<sub>B</sub> phenotype was not detected by DD test (Table III). Coutinho *et al.* (2010) analysis of CoNS through the DD method found three different phenotypes, distributed as follows: 46.7% cMLS<sub>B</sub> and 3.3% of the other two phenotypes (iMLS<sub>B</sub> and MS<sub>B</sub>). Through the automated method, our study detected 31.25% (5 of 16) iMLS<sub>B</sub> and 6.25% (1 of 16) MS<sub>B</sub> (Table III).

**TABLE I** - Comparison of susceptibility profile of the 16 isolates of coagulase-negative staphylococci from Platelet Concentrates, using the qualitative methodologies of Disk Diffusion Test (DD) and VITEK® 2, according to the criteria of CLSI<sup>14</sup>

	Fusidic acid	Amox/Clav	Ampi/Sulb	Azithromycin	Benzylpenicillin	Cefoxitin	Ciprofloxacin	Clindamycin	Ceftriaxone	Erythromycin	Gentamicin	Imipenem	Linezolid	Meropenem	Moxifloxacin	Nitrofurantoin	Norfloxacina	Penicillin	Rifampicin	Sulfa/Trim	Tecoplanin	Tetracycline	Tigecycline	Vancomycin	
1	<i>S. epidermidis</i>	DD	S	S	R	R	S	R	S	R	S	S	S	S	S	S	S	R	R	R	R	S	S	S	
		VITEK® 2	S	S	R	R	S	S	R	S	R	S	S	S	S	S	S	S	R	R	R	R	S	S	S
2	<i>S. epidermidis</i>	DD	S	S	R	R	S	R	S	R	S	S	S	S	S	S	S	R	R	R	R	S	S	S	
		VITEK® 2	S	S	R	R	S	R	S	R	S	S	S	S	S	S	S	R	R	R	R	S	S	S	S
3	<i>S. saprophyticus</i>	DD	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
		VITEK® 2	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
4	<i>S. haemolyticus</i>	DD	S	S	S	S	R	R	I	S	S	S	S	S	S	S	S	R	R	R	R	S	S	S	S
		VITEK® 2	S	S	R	R	S	R	R	I	S	S	S	S	S	S	S	R	R	R	R	S	S	S	S
5	<i>S. saprophyticus</i>	DD	S	S	R	R	S	R	I	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	I	S	R	R	S	R	R	I	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R
6	<i>S. haemolyticus</i>	DD	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	S	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
7	<i>S. saprophyticus</i>	DD	S	S	R	R	S	R	I	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	I	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
8	<i>S. epidermidis</i>	DD	S	S	R	R	S	R	I	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	S	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
9	<i>S. warneri</i>	DD	S	S	I	R	S	R	R	I	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	S	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
10	<i>S. haemolyticus</i>	DD	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	S	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
11	<i>S. haemolyticus</i>	DD	S	S	S	S	S	S	I	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	S	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
12	<i>S. warneri</i>	DD	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	S	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
13	<i>S. haemolyticus</i>	DD	S	S	R	R	S	R	I	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	S	S	R	R	S	R	I	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
14	<i>S. epidermidis</i>	DD	S	S	R	R	S	R	S	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	S	S	R	R	S	R	S	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
15	<i>S. warneri</i>	DD	S	S	R	R	S	R	S	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	S	S	R	R	S	R	S	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
16	<i>S. warneri</i>	DD	S	S	I	R	S	S	I	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R
		VITEK® 2	S	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R

Amox/Clav = Amoxicillin/Clavulanic; Ampi/Sulb = Ampicillin/Sulbactam; Sulfa/Trim = Sulfamethoxazole/Trimethoprim; DD = Qualitative methodology of disk diffusion; Vitek= Automated methodology VITEK® 2; S = Susceptible; I = Intermediate; R = Resistant; “-“= Non tested.

**TABLE II** - Analysis of MRCoNS of the 16 coagulase-negative staphylococci isolates from Platelet Concentrates through the phenotypic tests of Cefoxitin Disk Diffusion (DD), *screening* of cefoxitin by VITEK® 2, research of the gene *mecA* by Polymerase Chain Reaction

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Species	<i>S. epidermidis</i>	<i>S. epidermidis</i>	<i>S. saprophyticus</i>	<i>S. haemolyticus</i>	<i>S. saprophyticus</i>	<i>S. haemolyticus</i>	<i>S. saprophyticus</i>	<i>S. epidermidis</i>	<i>S. warneri</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	<i>S. warneri</i>	<i>S. haemolyticus</i>	<i>S. epidermidis</i>	<i>S. warneri</i>	<i>S. warneri</i>
CFO (DD)	S	S	R	S	S	S	R	S	R	S	S	S	S	S	S	S
Screening of CFO (automation)	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>mecA</i>	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+

\*Research conducted only with strains that showed phenotypic resistance to methicillin; CFO = Cefoxitin; DD = Qualitative methodology of disk diffusion; S = Susceptible; I = Intermediate; R = Resistant; “-“= absence; “+“= presence.

As mentioned in the paragraph above, no CoNS of our research showed an efflux mechanism (MS<sub>B</sub> phenotype) by the D-test result. However, when automation was used (VITEK® 2) one sample showed the MS<sub>B</sub> phenotype: this one showed the iMLS<sub>B</sub> phenotype by DD test.

The *erm* gene was detected in five PCR samples (31.25%). One (6.25%) containing *ermA* gene and four (25%) *ermC* gene; the *ermB* gene was not detected in our study. Coutinho *et al.* (2010) identified the presence of *ermA* gene in 29.6% of samples. Regarding the genotypic test, our index showed 67% of sensitivity and 86% of specificity, when compared to D-test, which was considered a reference in this study.

Lavallée *et al.* (2010) compared detection methodologies for iMLS<sub>B</sub> resistance only, in a collection of 163 *Staphylococcus* with resistant or intermediate profile to erythromycin and susceptible profile to clindamycin. In our study, through the performance of the same phenotypic tests and *erm* genes detection by PCR, 93% and 100% specificity and sensitivity were found, respectively, for VITEK® 2. Therefore, they concluded that automation has failed to detect iMLS<sub>B</sub> resistance in ten strains (10/163), resulting in a negative predictive value (NPV) of 74% (Lavallée *et al.*, 2010). When comparing these same methodologies, in our study we found 73% of NPV.

The authors cited above concluded that D-test is the most simple and reliable technique to detect this resistance in CoNS strains. They also concluded that agar dilution and VITEK® 2 are not as sensitive as D-test within 24 hours for CoNS strains for the detection of iMLS<sub>B</sub> (Lavallée *et al.*, 2010).

The discrepancy between results given by the

different methods, i.e., inhibition in DD test and gene absence in PCR may be justified by the presence of another gene, *ermY*, which was not the subject of this research. However, there are reports of its presence in *Staphylococcus* (Roberts *et al.*, 1999). The other reason for strains showing the iMLS<sub>B</sub> phenotype that do not contain the *erm* gene is still not clear (Roberts *et al.*, 1999), but it is attributed to possible changes in the local targets of primers for these genes (Lavallée *et al.*, 2010). Our study confirmed the superiority of D-test when compared to VITEK®2 in iMLS<sub>B</sub> detection.

## CONCLUSIONS

As for resistance genes showed by the different species studied, it was found that one *S. epidermidis* and one *S. warneri* showed the *mecA* gene and the *erm* gene. These two species also showed the highest resistance rate to methicillin. Through the antimicrobial susceptibility profile, obtained by the DD technique, approximately 50% of strains were characterized as multi-drug resistant (MDR), being resistant to more than three classes of antimicrobials.

The results of this study are a reminder to health professionals regarding the CoNS antimicrobial susceptibility profile, since most of them were resistant to antimicrobials (A and B-CLSI ratings) by the conventional assessment method. Also, half of the isolates were resistant to multiple classes of antibiotics, which directly influence on morbidity and mortality, specifically in immunocompromised patients, who often develop infections from resident microorganisms of the skin flora

**TABLE III** - Analysis of resistance of the 16 isolates of coagulase-negative staphylococci of Platelet Concentrates against antimicrobials of the MLS class of group B through the phenotypic tests of disk diffusion, *screening* of inducible/constitutive resistance of clindamycin by VITEK® 2, and the research of the genes *ermA*, B and C by Polymerase Chain Reaction

Samples	Species	D- Test	DD CLI/ERI	Phenotype DD	Screening of CLI (automation)	<i>ermA</i>	<i>ermB</i>	<i>ermC</i>
1	<i>S. epidermidis</i>	+	R/R	iMLS <sub>B</sub>	MS <sub>B</sub>	-	-	-
2	<i>S. epidermidis</i>	-	R/R	cMLS <sub>B</sub>	-	-	-	-
3	<i>S. saprophyticus</i>	-	R/R	cMLS <sub>B</sub>	iMLS <sub>B</sub>	-	-	-
4	<i>S. haemolyticus</i>	-	R/S	-	-	-	-	-
5	<i>S. saprophyticus</i>	+	R/R	iMLS <sub>B</sub>	iMLS <sub>B</sub>	-	-	-
6	<i>S. haemolyticus</i>	-	I/R	-	-	-	-	-
7	<i>S. saprophyticus</i>	-	I/R	-	iMLS <sub>B</sub>	-	-	-
8	<i>S. epidermidis</i>	+	R/R	iMLS <sub>B</sub>	iMLS <sub>B</sub>	-	-	+
9	<i>S. warneri</i>	-	R/I	-	-	-	-	+
10	<i>S. haemolyticus</i>	-	S/S	-	-	-	-	-
11	<i>S. haemolyticus</i>	-	S/S	-	-	-	-	-
12	<i>S. warneri</i>	-	S/S	-	-	-	-	-
13	<i>S. haemolyticus</i>	+	R/R	iMLS <sub>B</sub>	-	+	-	-
14	<i>S. epidermidis</i>	+	R/R	iMLS <sub>B</sub>	iMLS <sub>B</sub>	-	-	+
15	<i>S. warneri</i>	+	R/R	iMLS <sub>B</sub>	-	-	-	+
16	<i>S. warneri</i>	-	I/I	-	-	-	-	-

S = Susceptible; I = Intermediate; R = Resistant; “-“= absence; “+“= presence; Cli = Clindamycin; Eri = Erythromycin; D-Test Negative = cMLS<sub>B</sub> (resistant to CLI and ERI); D-Test Negative = MS<sub>B</sub> (susceptible CLI and resistant ERI); D-Test Positive = iMLS<sub>B</sub> (resistant to CLI and ERI).

such as CoNS. Restrictive policy with antimicrobials as well as immediate and correct prescription should be encouraged in order to control bacterial resistance.

This is in fact the first study conducted in Brazil that investigates susceptibility profile and resistance mechanisms of microorganisms isolated from PCs bags.

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## CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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