Antimicrobial Resistance and Virulence of *Staphylococcus* spp. in patients from oncologic and non oncologic hospitals of Recife City/PE

Resistência antimicrobiana e virulência de *Staphylococcus* spp. em pacientes de hospital oncológico e não oncológico da Cidade de Recife / PE

Resistencia a los antimicrobianos y virulencia de *Staphylococcus* spp. en pacientes de un hospital oncológico y no oncológico de la Ciudad de Recife / PE

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Abstract

*Staphylococcus* spp. is one of the major infection-associated bacteria within health care, especially in intensive care units, and one of principal cause of complication in cancer patients. This study compared the antimicrobial susceptibility profile and frequency of resistance (*mecA, bluZ, ermA* and *ermC*) and virulence (*icaA, icaD* and *hlg*) genes in *Staphylococcus* spp. from patients of Oncology Hospital (OH) and University Hospital (UH). The type of the *ccr* complex was assessed by PCR among the *mecA* positive isolates from the UH. Higher percentage of susceptible isolates, except for oxacillin and cefoxitin was found among the UH isolates and 27.3% vancomycin-resistant isolates were identified through the screening spot; 41 isolates displayed the MLSB _, phenotype and five the MSLB _i_ phenotype, and one isolate from the OH displayed the constitutive phenotype *ermC* gene. The *ccr* types I and II were identified with a higher frequency of *ccr* type I. No statistically significant difference was found in the frequency of the genes between the two groups of patients or in the two hospitals. Regarding the virulence genes, there was statistically significant difference when comparing the two hospitals.

**Keywords:** *Staphylococcus*; Antibiotic resistance; Oncology; Virulence.
Resumo

Staphylococcus spp. é uma das principais bactérias associadas a infecções na área de saúde, especialmente em unidades de terapia intensiva, e uma das principais causas de complicações em pacientes com câncer. Este estudo comparou o perfil de susceptibilidade aos antimicrobianos e a frequência de genes de resistência (mecA, blaZ, ermA e ermC) e virulência (icaA, icaD e hlg) em Staphylococcus spp. de pacientes de um Hospital Oncológico (OH) e Hospital Universitário (HU). O tipo de complexo ccr foi avaliado por PCR entre os isolados mecA positivos do HU. Maior porcentagem de isolados suscetíveis, exceto para oxacilina e cefotaxima, foi encontrada entre os isolados de UH e 27,3% dos isolados resistentes à vancomicina foram identificados através de screening; 41 isolados exibiram o fenótipo MLSBc e cinco o fenótipo MSLBi, e um isolado do OH do fenótipo constitutivo exibiu o gene ermC. Os ccr tipos I e II foram identificados, sendo maior frequência do ccr tipo I. Não foi encontrada diferença estatisticamente significativa na frequência dos genes entre os dois grupos de pacientes ou nos dois hospitais. Em relação aos genes de virulência, houve diferença estatisticamente significativa na comparação dos dois hospitais.

Palavras-chave: Staphylococcus; Resistência a antibióticos; Oncologia; Virulência.

Resumen

Staphylococcus spp. es una de las principales bacterias asociadas a las infecciones en la asistencia sanitaria, especialmente en las unidades de cuidados intensivos, y una de las principales causas de complicaciones en los pacientes oncológicos. Este estudio comparó el perfil de susceptibilidad antimicrobiana y la frecuencia de genes de resistencia (mecA, blaZ, ermA y ermC) y virulencia (icaA, icaD y hlg) en Staphylococcus spp. de pacientes de un Hospital Oncológico (OH) y Hospital Universitario (HU). El tipo de complejo ccr se evaluó mediante PCR entre los aislados mecA positivos de la HU. Se encontró un mayor porcentaje de aislados susceptibles, a excepción de oxacilina y cefotaxima, entre los aislados de UH y el 27,3% de los aislados resistentes a la vancomicina se identificaron mediante el método de cribado; 41 aislamientos exhibieron el fenótipo MLSBc y cinco el fenótipo MSLBi, y un aislado del OH del fenotipo constitutivo exhibió el gen ermC. Se identificaron CCR tipo I y tipo II, con mayor frecuencia de CCR tipo I. No hubo diferencia estadísticamente significativa en la frecuencia genética entre los dos grupos de pacientes o en los dos hospitales. En cuanto a los genes de virulencia, hubo una diferencia estadísticamente significativa al comparar los dos hospitales.

Palabras clave: Staphylococcus; Resistencia antibiótica; Oncología; Virulencia.

1. Introduction

Bacterial infections are a major cause of complications in patients with hematological neoplasia and chemotherapy-induced neutropenia and bacteremia is the leading cause of death, either directly or by interfering with the chemotherapy chronogram (Montassier, et al., 2013; Quiles et., al, 2015; Jiang et al., 2020). Risk factors for infection in this group of patients are more expressive, because the immunocompromised related by disease itself, they are subjected to surgical treatment, chemotherapy and/or radiotherapy, where neutropenia is a major risk for infection in this population (Jiang et al., 2020).

In recent decades, the success of the treatment of malignancies in children and teenagers has increased, with up to 70 – 80% of cases. Furthermore, the immunosuppression from the treatment makes these patients more susceptible to infections, which are the main cause of death among them. In these patients, bacteremia often results from infection by one or more microorganisms, mainly members of the Enterobacteriaceae family, Pseudomonas aeruginosa, Streptococcus and Staphylococcus (Quiles et., al, 2015). Over the last three decades, the proportion of Gram-positive microorganisms has increased because of the widespread use of permanent catheters (Montassier, et al., 2013).

It is noteworthy that the intensive care centers concentrate the highest incidence rates of hospital infections, and are emergency epicenters of microbial resistance, due to the hospitalization of seriously ill patients. These patients, in most cases, use a wide spectrum antimicrobials and are subjected to successive invasive procedures (Jiang et al., 2020). Occur more and more evidences supports the idea that neutropenic patients at low risk do not necessarily need hospitalization and antibiotic wide spectrum prolonged to bacterial invasive infections. The strategies for this group include antimicrobial short-term treatment with a consequent reduction in hospitalization and outpatient management with the use of oral antibiotics (Cagol et al., 2009), reinforcing the importance of proper characterization of the resistance profiles of bacterial strains.

S. aureus can evade the host immune system due to the existence of several virulence factors and some studies have shown differences in the production of virulence factors such as gamma-hemolysin (hlg) and biofilm (ica) between the strains...
isolated from different clinical specimens (Kord et al., 2018; Elboshra et al., 2020). However, these comparisons have not been related described in patients of oncology centers. The biofilm production may allow these isolates colonize the catheter and other devices, to evading the immune system and microorganisms might lead to an increased frequency of bacteremia and false-positive or false-negative blood cultures (Figueiredo et al., 2017; Kord et al., 2018).

In a retrospective study in an U.S., oncology hospital it was determined the existence of clones among isolates resistant groups related with the resistance data associated with clinical information suggestive of infection (Muldrew et al., 2008).

Among the several resistance mechanisms of *Staphylococcus*, the most common are the resistance to macrolides, aminoglycosides, beta-lactams and glycopeptides (Sangappa & Thiagarajan, 2012; Jenkins et al., 2015). The importance of these microorganisms depends on the toxins mediated virulence, its invasive nature and its antibiotics resistance profile (Jenkins et al., 2015; Elboshra et al., 2020). These characteristics are related to the diversity of clinical manifestations (Lim et al., 2012; Jenkins et al., 2015), however concerning the oncology patients, this is not yet clear.

The increasing of resistant strains with different virulence factors over the years indicates the potential failure of the use of antimicrobials in the treatment of infections due to the association of the resistance mechanisms of the microorganism and the resistance of the host immune response (Lim et al., 2012). The number of virulence associated genes harbored by a bacterial isolate results from the interaction between gene acquisition rates, the biological maintenance cost and the failure rate of the disease (Leclercq, 2002; Jenkins et al., 2015). Thus, the more severe *S. aureus* infections cannot be explained by the action of a certain virulence factor only, but rather by the action of several virulence factors during the infectious process. The survival and proliferation of a certain pathogen in the host is favored by mechanisms that enable evasion of the host immune mechanisms. Thus, strains that escape more efficiently the host defense mechanisms are the most prevalent. There is evidence that some clonal types are more virulent than others and they occur more frequently among patients than in healthy individuals (Leclercq, 2002; Spaan et al., 2014).

The proposal of this study was to characterize and comparing *Staphylococcus* spp. isolates from oncologic and non-oncologic patients of two hospitals in the city of Recife-PE, regarding antimicrobials susceptibility profile, and the resistance and virulence genes content. This microbiological analysis will allow mapping the bacterial strains through resistance and virulence factors, which will facilitate tracing rational use of antimicrobials and treatment of the infections in the hospitals.

2. Methodology

The observacional study (Fontelles et al., 2009) compared *Staphylococcus* spp. isolates from oncological and non-oncological patients from an oncology (OH) and a university hospital (UH) respectively in Recife, PE, Brazil during one year (2013) period. In the study, patients of male and female gender were included. The samples were collected during routine by the medical and nursing staff of the hospitals. The isolates were identified by macroscopic characteristics on 5% blood agar plates, Gram staining and the catalase, coagulase, DNase and Mannitol salt agar tests (CLSI, 2014).

**Antimicrobial Susceptibility.**

*Staphylococcus* isolates were tested by the disk diffusion technique in Mueller-Hinton Agar (CLSI, 2014) plates using the following antimicrobials and concentrations: penicillin 10U, oxacillin 1µg, vancomycin 30µg, gentamicin 10µg, clindamycin 2µg, sulfamethoxazole-trimethoprim 1.25/23.75µg, ciprofloxacin 5µg, cefoxitin 30µg, teicoplanin 30µg, erytromycin 15µg e tetracyclin 30µg. The Clinical and Laboratory Standards Institute (CLSI) interpretive breakpoints were considered (CLSI, 2014). The erythromycin and clindamycin disks were placed approximate to assess the induced resistance (D test) (CLSI, 2014).
Screening of Oxacillin

Subcultures were made in nutrient agar plates of the isolates that were resistant to oxacillin and/or cefoxitin in disk diffusion technique. It was made direct suspension of colonies to obtain a turbidity to a standard solution of 0.5 McFarland scale. Was dipped into a 1μL inoculation loop in this suspension and proceeding with the inoculum in area with a diameter of 10 to 15 mm on plates containing Mueller Hinton agar medium with NaCl (4% v/v, 0.68 mol/L) and 6 μg/mL of oxacillin. These plates were incubated at 35 °C for 24 hours being considered, after reading the results as: >1 colony = resistant. Were used as quality control standard strains for MRSA and MSSA: *Staphylococcus aureus* ATCC 29213 – Sensitive and *Staphylococcus aureus* ATCC 33591 – Resistant (CLSI, 2014).

Vancomycin Screening

All isolates were subjected to screening of vancomycin. These samples were inoculated into BHI broth and incubated at 35°C to reach a 2 McFarland turbidity scale. One 1μL loop of this grow was spread in area of 10 to 15mm diameter on plates containing BHI agar supplemented with 6 μg/mL of vancomycin (OXOID) and incubated at 35 °C for 24 and 48 hours considering resistance as > 1 colony (Burnham, Weber & Dunne Jr, 2010; CLSI, 2014).

*Enterococcus faecalis* ATCC 29212 – Sensitive e *Enterococcus faecalis* ATCC 51299 – Resistant were used as quality control standard strains (CLSI, 2014).

Total DNA Extraction

Total DNA was extracted from the isolates following Oliveira et al (2015) and the DNA obtained was quantified using nanodrop 2000 (Thermo Scientific).

Identification of the mecA gene by PCR

The presence of the mecA gene was assessed by PCR among the oxacillin and/or cefoxitin phenotypically resistant isolates using the primers described by Oliveira et al (2015). The PCR reactions were individually prepared in a final volume of 25μL containing: 20ng of genomic DNA, 20pmol of each primer, MgCl2 1,5mM, dNTP 200µM, 1U of Go Taq DNA polimerase (Promega, Brasil) and 5μL of Green Go Taq DNA polimerase buffer (Promega, Brasil). The reactions were performed in a thermocycler (Biometra), set for 35 cycles of one minute at 94°C, one minute at 55°C and two minutes at 72°C followed by a final-step of 15 minutes at 72°C. As negative control, a tube containing all the components of the mixture without DNA and for positive control DNA from the strain *Staphylococcus aureus* ATCC 33591 – Resistant were used.

Identification of the beta – lactamase gene (*blaZ*) by PCR

The presence of the *blaZ* gene was assessed by PCR among the oxacillin and/or cefoxitin phenotypically resistant isolates using the primers described by Milheiroço et al (2011). The PCR reactions were individually prepared in a final volume of 25μL containing: 20ng of genomic DNA, 20pmol of each primer, 2,5μL of MgCl2 (25mM), 2,5μL dNTP (200µM), 1U of Go Taq DNA polimerase (Promega, Brasil) and 5μL of Green Go Taq DNA polimerase buffer (Promega, Brasil). The reactions were performed in a thermocycler (Biometra), set for 30 cycles composed of one minute at 92°C, one minute at 55°C and two minutes at 72°C followed by a final step of seven minutes at 72°C. A tube containing all the components of the mixture without DNA was used as negative control and DNA from the isolate code 1171 from the Culture Collection from the Microbiology Departament from the FIOCRUZ-PE, positive for the gene *blaZ* confirmed by sequencing, was used as positive control.
Determining the type of SCCmec

The type of the ccr complex was assessed by multiplex PCR among the meca positive isolates from the UH following a protocol based on Kondo et al (2007) as follows: PCR reactions were individually prepared in a final volume of 50µL containing: 20ng of genomic DNA, 10µM of each primer, 6,4µL of MgCl2 (25mM), 4,0µL dNTP (2,5mM), 1U of Go Taq DNA polimerase (Promega, Brasil) and 5µL of Green Go Taq DNA polimerase buffer (Promega, Brasil). The primers used are described in Table 1 and the PCR products are described in Chart 1. The reactions were performed in a termocycler (Biometra), programmed for 30 cycles composed of one minute at 95°C, one minute at 57°C and two minutes at 72°C followed by a step of seven minutes at 72°C.

### Table 1: Primer sequence to be used for multiplex PCR reactions to determine the ccr complex (Kondo et al., 2007).

| Primer | Sequences (5'–3') |
|--------|-------------------|
| α1     | 5'AACCTATATCATCAATCAGTG3' |
| α2     | 5'TAAAGGCATCAATGCACAAACT3' |
| α3     | 5'AGCTCAAAAGCAAGCAATAGAT3' |
| βc     | 5'ATTGCGCTTGATAATAGCCTC3' |
| MA1    | 5'TGCTATCCACCCTCAAGAG3' |
| MA2    | 5'AACGTTGTAAACCACCCCAAGA3' |
| γF     | 5'CGTCTATTACAGATGTTAAGGATAA T3' |
| γR     | 5'CCTTTATAGACTGGATTATCAAAT AT3' |

Source: Authors.

### Chart 1: PCR products on 2% agarose gel

| ccr complex | bp   |
|-------------|------|
| 1           | 695  |
| 2           | 937  |
| 3           | 1791 |
| 5           | 518  |

Source: Authors.

Detection of ermA and ermC genes by PCR technique

The isolates erythromycin and clindamycin resistant (phenotype MLSBc) and erytromycin positive in the D test (phenotype MLSB) were analysed for the presence of the ermA and ermC genes using the primers described by Lina et al (1999a): ermA: F5'GTTCAGAAACATCAATACAGG3' and R5'GGATCAGGAAAAGGACATTTTAC3'; ermC: F5'GCTAATATTGTTTAAATCGTCAATTCC3' and R5'GGATCAGGAAAAGGACATTTTAC3'. For the detection of ermA the amplification reaction was prepared in a final volume of 25µl for tube including: 1µl (40ng) of total DNA, 1µl (20pmol) of each primer, 0,6µl of deoxyribonucleotide triphosphate (dNTP) (8 mM), 5,0µl of buffer (5x), 1,5µl of MgCl2 (25 mM), 0,4µl of Go Taq DNA polimerase (5U) and 14,5µl of sterile milliQ water. For the detection of ermC, the amplification reaction was prepared as above, except for using 0,3µl (5U) of Go Taq DNA polimerase and 14,6µl sterile milliQ water. Amplification reactions were performed in a thermocycler under the following conditions: 30 cycles of one minute at 94°C, 30
seconds at 49°C, and 30 seconds at 72°C. As negative control a tube containing all the components of the mixture without DNA and as positive control DNA from one isolate positive for the genes (ermA and ermC) confirmed by amplification and sequencing were used.

**Detection of the icaA and icaD genes**

The amplification of the icaA and icaD genes from the operon icaADBC, was performed using the primers described by Vasudevan et al (2003) (F5’CCTAACTACGAAAGGTAG3’ and R5’GGCAATATGATCAAGGATAC3’) for a 1.585bp amplification. PCR reactions were individually prepared in a final volume of 25µL, containing: 20ng of genomic DNA, 20pmol of each primer, 1,75µL of MgCl2 (25 mM), 0,63µL dNTP (8 mM), 1U of Go Taq DNA polimerase (Promega, Brasil) and 5µL of Green Go Taq DNA polimerase buffer (Promega, Brasil). The reactions were performed in a thermocycler (Biometra), programmed for 30 cycles of one minute at 94°C, one minute at 50°C and one minute at 72°C followed by 15 minutes at 72°C. As negative control a tube containing all the components of the mixture without DNA and as positive control the DNA from the isolate code 047 from the Culture Collection from the Microbiology Departament from the FIOCRUZ-PE, positive for the genes confirmed by sequencing, was used as positive control.

**Detection of the hlg gene**

For amplification of the hlg gene the protocol described by Lina et al (1999b) was employed using the primers F5’GCCAATCCGTATTAGAAAATGC3’ and R5’CCATAGACGTAGCAACGGAT3’ for a 937bp fragment. PCR reactions were individually prepared in a final volume of 25µL containing: 20ng of genomic DNA, 20 pmol of each primer, 1,75µL of MgCl2(25mM), 0,63µL dNTP (8mM), 1U of Go Taq DNA polimerase (Promega, Brasil) and 5µL of Green Go Taq DNA polimerase buffer (Promega, Brasil). The amplification reaction was programmed for 30 cycles composed of one minute at 94°C, one minute at 50°C one minute at 72°C followed by 15 minutes at 72°C. As negative control, a tube containing all the components of the mixture without DNA and as positive control the DNA from the isolate code 412 from the Culture Collection from the Microbiology Departament from the FIOCRUZ-PE, positive for the gene hlg confirmed by sequencing, was used.

The products of all amplifications were electrophoresed in 1% agarose gels, stained with Blue-Green (LGC Biotechnology), visualized in UV transilluminator and scanned by Kodak 1D software version 3.5.2 (Scientific Imaging Systems, USA). For the products of the ccr complex amplification 2% agarose gels were used. The molecular weight marker 100bp DNA ladder (Invitrogen) was used.

**Data Analysis**

Clinical and microbiological data were introduced in SPSS Statistics 20 software for statistical analysis through frequency distribution, x² test and Fisher’s Test.

**3. Results**

Total of 173 isolates (104 S. aureus and 69 CoNS) were obtained, 51 (47 S. aureus and 4 CoNS) from patients of the OH and 122 (57 S. aureus and 65 CoNS) from patients of the UH in Recife, PE, Brazil were obtained during the year 2013. The more frequent source of the samples was blood culture, followed by catheter tip in both hospitals (Table 2).
Source: Authors.

Related with the antimicrobial profile there was a higher percentage of susceptible isolates, except for oxacillin and cefoxitin among the 122 isolates from the UH (Table 3). 54 (51.9%) isolates were resistant in the screening spot of oxacillin, however, observing each hospital, the UH has 54.8% (46/84) of resistant isolates and OH 40% (8/20). 24 (27.3%) isolates were resistant vancomycin screening spot (Table 4).

| Sample type             | UH  | OH  | TOTAL |
|-------------------------|-----|-----|-------|
| Blood                   | 41  | 13  | 54    |
| Catheter tip            | 21  | 7   | 28    |
| Peritoneal fluid        | 1   | 1   | 2     |
| Tracheal aspirates      | 16  | 6   | 22    |
| Wounds                  | 7   | 3   | 10    |
| Diverse secretions      | 16  | 12  | 28    |
| Urine                   | 4   | 2   | 6     |
| Fist tumor              | 0   | 1   | 1     |
| Ascites fluid           | 1   | 2   | 3     |
| Tissue fragment         | 6   | 1   | 7     |
| Chest wall              | 0   | 1   | 1     |
| Abscess                 | 4   | 2   | 6     |
| Sperm                   | 1   | 0   | 1     |
| LCR                     | 3   | 0   | 3     |
| Bronchoalveolar lavage  | 1   | 0   | 1     |
| **TOTAL**               | **122** | **51** | **173** |

*Table 2: Distribution of *Staphylococcus* spp. isolates from the samples and hospital origin.*
Table 3: Antimicrobial susceptibility of *Staphylococcus spp.* isolates in University and Oncologic Hospitals.

| Antimicrobial  | UH Sensitive | UH Resistant | OH Sensitive | OH Resistant |
|----------------|--------------|--------------|--------------|--------------|
| Oxacillin      | 54           | 68           | 40           | 11           |
| Cefoxitin      | 43           | 79           | 39           | 12           |
| Vancomycin     | 120          | 2            | 51           | 0            |
| Teicoplanin    | 118          | 4            | 43           | 8            |
| Gentamicin     | 71           | 51           | 45           | 6            |
| Erytromycin    | 54           | 68           | 33           | 18           |
| Clindamycin    | 66           | 56           | 43           | 8            |
| Tetracyclin    | 94           | 28           | 41           | 10           |
| Sufamethoxazole/trimethoprim | 84 | 38 | 49 | 2 |
| Ciprofloxacin  | 107          | 15           | 42           | 9            |

Source: Authors.

Table 4: Antimicrobial susceptibility to Oxacillin and Vancomycin of *Staphylococcus spp.* isolates in University and Oncologic Hospitals.

| Screening type | Test Results | Hospital | Total |
|----------------|--------------|----------|-------|
|                |              | UH       | OH    |
| Oxacillin      | Resistant    | 46       | 8     | 54    |
|                | Sensitive    | 38       | 12    | 50    |
|                | Total        | 84       | 20    | 104   |
| Vancomycin     | Resistant    | 11       | 13    | 24    |
|                | Sensitive    | 33       | 31    | 64    |
|                | Total        | 44       | 44    | 88    |

Source: Authors.

The occurrence of the *blaZ* and *mecA* genes was assessed by PCR among the methicillin and cefoxitin and/or oxacillin phenotypically resistant isolates. Among these from the Oncology Hospital 50% and 77.7%, respectively were positive for the *blaZ* and *mecA* genes (Table 5). The isolates analyzed from the University Hospital 45.7% were positive for the *blaZ* gene and 17% for the *mecA* gene (Table 5).
Table 5: Results of PCR for \textit{bla}Z, \textit{mec}A, \textit{icaAD} and \textit{hlg} genes for the two hospitals.

| PCR type | Hospital | Results |
|----------|----------|---------|
|          | UH       | OH      |
| \textit{bla}Z | Positive  | 16      | 9  |
|           | Negative  | 19      | 9  |
| \textit{mec}A | Positive  | 8       | 14 |
|           | Negative  | 39      | 4  |
| \textit{icaAD} | Negative | 3       | 9  |
| \textit{hlg}  | Positive  | 6       | 14 |
|           | Negative  | 8       | 6  |

Source: Authors.

Related another antimicrobial, the profile of susceptibility to erythromycin and clindamycin was also analyzed through the disk diffusion method, 41 isolates with MLSB\textsubscript{c} phenotype (Macrolides, Lincosamides and Streptogramin constituent B) and five MLSB\textsubscript{i} phenotype (Macrolides, Lincosamides and Streptogramin inducible B) were identified at the University Hospital. In the Oncology Hospital, eight isolates were identified with the phenotype MLSB\textsubscript{c} and six MLSB\textsubscript{i} (Table 6).

Table 6: Results of PCR for \textit{erm}A and \textit{erm}C genes for the two hospitals.

| Hospital | Phenotype | MLSB\textsubscript{c} | MLSB\textsubscript{i} |
|----------|-----------|------------------------|-----------------------|
|          | \textit{erm}A | \textit{erm}A | \textit{erm}C | \textit{erm}C | \textit{erm}A | \textit{erm}C | \textit{erm}C |
| UH       | Positive   | 5        | 36       | 16       | 25       | 0        | 5        | 1        | 4        |
| OH       | Positive   | 0        | 8        | 1        | 7        | 0        | 6        | 0        | 6        |

Source: Authors.

The isolates displaying the phenotypes MLSB\textsubscript{c} and MLSB\textsubscript{i} were analyzed by PCR for the detection of the \textit{erm}A and \textit{erm}C genes. Only one isolate was found harboring the constitutive phenotype \textit{erm}C gene. The \textit{erm}A gene wasn’t observed in Oncology hospital (Table 6). It was observed positive isolates for all genes in the studied phenotypes, except for the \textit{erm}A gene in induced phenotype in the University Hospital (Table 6).

The occurrence of the genes \textit{icaAD} and \textit{hlg} was observed respectively in 25\% and 42.8\% in the isolates from the University Hospital and in 32.1\% and 70\% respectively in the isolates from the Oncology Hospital. Table 5 shows the distribution according to the source of origin.

In this study, there was no statistically significant difference in the occurrence of the resistance genes \textit{bla}Z, \textit{mec}A, \textit{erm}A and \textit{erm}C when comparing the two hospitals. Regarding the virulence genes, there was statistically significant difference when comparing the two hospitals. Chart 2 and 3 shows the results of the \textit{p} value found in the static analysis.

Out of the five \textit{mec}A positive isolates from the University Hospital analyzed by PCR for determination of the \textit{ccr} complex type, four showed Type I \textit{ccr} (\textit{ccr}A1, 695bp) and one was Type II (\textit{ccr}A2, 937 bp).
4. Discussion

Most Staphylococcus spp. carriers are asymptomatic and related to the process of infection is usually associated with some factor which decreases the immune response of the individual, as invasive medical procedures, being this microorganism referred to as a main agents of bacteremia, often acquired in hospital environment (Muldrew et al., 2008). Among the users of central vascular catheter, S. aureus and CoNS are the more frequent microorganisms, accounting for 30% of the isolates (Muldrew et al., 2008; Montassier et al., 2013) in our study, the most frequent samples were blood culture and catheter tip with percentages of respectively 33.6% and 17.2% for the UH and 25.5% and 13.7% for OH, similar a study in public hospital in Brazil (Bride et al., 2019).

The frequency of isolation of S. aureus and its relationship with hospital infections reach high values in many Brazilian hospitals, related of prevalence of MRSA strains varies from 40 to 80% (Lina et al., 1999b; Jones et al., 2013; Bride et al., 2019) and data from the Antimicrobial Surveillance Program show that MRSA reached 31% among the nosocomial and community infections agents and are considered the more common among the most prevalent pathogens (Jones et al., 2013; Silveira et al., 2015). A survey in Brazilian hospitals showed 80% (Silveira et al., 2015) resistance of CoNS in blood cultures. In our study, this was the most frequent type of sample; however, S. aureus was more prevalent in both hospitals.

Concerning the detection of the genes blaZ and mecA by PCR 50% and 77.7% of positive samples were found respectively in the OH while in the UH there was 45.7% of occurrence for the blaZ gene and 7% for the mecA gene. Some studies show that the rate of beta-lactamase production ranges from 55.7% to 92.6% for Staphylococcus (Oliveira et al., 2011; Rabelo et al., 2013) and our results fit within these limits. Oliveira et al (2011) found high prevalence of beta-lactamase in their
isolates (83%) and they drew attention to the widespread use of drugs in the clinical practice which may trigger resistance to several antimicrobial classes.

A study in India by Prabhash et al (2010) reported a rate of 18% of methicillin-resistant S. aureus from cancer patients, similar to our results in the University Hospital in Recife. Another study conducted in South Korea by Kang et al (2012), reported a prevalence of 48.4% of MRSA associated with bacteremia in cancer patients, a percentage lower than that from our work (77.7%) for oncology patients.

Another study conducted earlier in the same University Hospital, reported percentage slightly lower than ours, about 10% of the isolates were considered MRSA (Rabelo et al., 2014), suggesting that the control measures of this type of microorganism have not been fully effective. In this study, was noted the presence of strains resistant to methicillin only by phenotype methods for both hospitals, suggests the presence of other mechanisms of resistance or non – expression of genes researched (Sangappa & Thiagarajan, 2012; Milheiroço et al., 2011; Andrade-Figueiredo & Leal-Balbino, 2016).

The profile of resistance to vancomycin in the present study among the isolates from the University and Oncology Hospitals described the percentage rate of 25% and 29.5% respectively. Staphylococci resistant to glycopeptides were described in Brazil, and isolation of vancomycin resistant S. aureus registered in São Paulo (Rossi et al, 2014; Panesso et al, 2015). Other study previously conducted in the University Hospital (Rabelo et al., 2014), reported 11% of resistant isolates using the screening spot method, but only among MRSA samples and the same hospital vanA gene was detected of isolates of S. epidermidis from nasopharyngeal secretion of health care workers (Bezerra Neto et al., 2018).

In the present study, the MLSBc phenotype found in 41 isolates (33.6%) from the University Hospital and eight isolates (15.7%) from the Oncology Hospital was predominant over the MLSB phenotype found in five isolates (4.1%) from the University and six isolates (11.8%) from the Oncology Hospital. These findings are different from those from another study in Brazil that identified 71 (46.7%) MLSB phenotype found in 41 isolates (33.6%) from the University Hospital and eight isolates (15.7%) from the Oncology Hospital (Coutinho et al., 2010).

Antimicrobial susceptibility data are essential for appropriate therapy making imperative to perform the D test (Coutinho et al., 2010; Juyal et al., 2013). Categorizing a Staphylococcus spp. isolate as clindamycin sensitive without verifying if there is inducible resistance may result inadequate therapy. On the other hand, a negative result for inducible clindamycin resistance confirms the antimicrobial sensitivity and allow a correct therapeutic (Kumar et al., 2012).

Two studies conducted in Iran related with erythromycin-resistant genes described the frequency of ermC 35.2% and ermA 20.4% (Sedaghat et al., 2017) and Saderi, Emadi and Ovilia (2011), found the opposite, the ermA gene was more frequent than the ermC gene, 76 (60.3%) and 69 (54.8%) respectively among the S. aureus isolates. Different results were obtained in our study, the presence of the ermC gene was higher than the ermA gene in the isolates of both Hospitals. In our study, no single positive isolate was found for the ermA gene in Oncology Hospital for the studied phenotypes. No single positive isolate was found for the induced phenotype at the University Hospital. The isolates that do not harbor the ermA or ermC genes, may possess the ermB gene. Due to a possible association of these genes the isolates harboring the ermA or ermC genes may also contain ermB. The presence of the ermB gene was not researched in our work because it occurs mainly among streptococci and enterococci (Leclercq, 2002). Furthermore, Coutinho et al (2010), reported a low frequency of the ermB gene.

In the present work, it was observed 42.8% and 70% of positive isolates for the hlg gene, respectively in the UH and OH, a higher frequency of 98.2% was found in hospital of India (Aggarwal et al., 2019), the data of UH was similar of Oliveira et al (2014) found 41.86% of methicillin-resistant CoNS isolates from blood cultures at another University Hospital of the same region and a study in Malaysia reported a percentage of 45% of methicillin-resistant isolates from patients and students from an university hospital (Lim et al., 2012). The gene hlg codes for the gamma toxin that has pro-inflammatory activity and is capable of lysing erythrocytes and leukocytes, which increases the toxigenic potential of the bacteria and the severity of the infections (Oliveira et al., 2014; Spaan et al., 2014).
S. aureus and CoNS are able to produce biofilms and this process starts with the adhesion of N-acetyl-glicosamine polimeric (PNAG) which is synthesized by enzymes encoded by genes present in the ica locus (Barbieri et al., 2015; Figueiredo et al., 2017). In our work, there was 25% and 32.1% of positive isolates for icaAD, respectively at the UH and OH. Higher positivity (83.5%) were detected in Palestinian hospital in isolates of S. aureus (Azmi, Qrei & Abdeen, 2019), positivity (79%) was found among inpatients and students in a hospital in Malaysia (Lim et al., 2012). Another study reported 75% of positive isolates in samples from oncology patients with breast implants (Barbieri et al., 2015).

In this study, there was no statistically significant difference in the presence of the resistance genes (blaZ, mecA, ermA and ermC) when comparing the two hospitals whilst statistically significant difference was found in the presence of the virulence genes (icaA, icaD and hlg). This findind suggests that the oncology plays no role on the presence of resistance genes between the groups. On the other hand, the characteristics of the patient groups seems to be associated with the toxigenic and pathogenicity potential of the isolates studied.

The type of the ccr complex was assessed by PCR among the mecA positive isolates from the University Hospital and the ccr type I (ccrA1, 695bp) and type II (ccrA2, 937 bp) were found with higher frequency of ccr type I. Previous studies reported the occurrence of SCCmec types I, II, III, IV and V in Brazilian hospitals (Lima et al., 2014; Oliveira et al., 2015; Andrade-Figueiredo & Leal-Balbino, 2016). Due to the small number of isolates, it was not be possible to make inferences about these findings. It is known that SCCmec types I and II are associated with strains of hospital origin, characterized by multiple antibiotic resistance besides to the betalactams, such as macrolides, aminoglycosides, tetracyclines, quinolones and rifampicin (Andrade-Figueiredo & Leal-Balbino, 2016).

5. Conclusion

The study thus suggests that Staphylococcus strains are drug resistant, virulent, and diverse irrespective of sources and place of isolation. These findings necessitate the continuous surveillance of multidrug-resistant and virulent S. aureus and monitoring of the transmission of infection. The antimicrobial monitoring is essential for the treatment of infections in each hospital.

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