Research, Society and Development, v. 10, n. 10, e323101018951, 2021
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v10i10.18951

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

Received: 08/01/2021 | Reviewed: 08/04/2021 | Accept: 08/07/2021 | Published: 08/13/2021

Marcelle Aquino Rabelo
ORCID: https://orcid.org/0000-0003-3176-1008
Universidade Federal de Pernambuco, Brazil
E-mail: marcellearabelo@gmail.com
Stephanie Targino da Silva
ORCID: https://orcid.org/0000-0001-6997-2623
Universidade Federal de Pernambuco, Brazil
E-mail: stephanietargino@hotmail.com
Jussyêgles Niedja da Paz Pereira
ORCID: https://orcid.org/0000-0002-6494-0128
Universidade Federal de Pernambuco, Brazil
E-mail: ju-biomed@hotmail.com
Natália Regina Souza da Silva
ORCID: https://orcid.org/0000-0002-7800-5552
Universidade Federal de Pernambuco, Brazil
E-mail: nathregina@gmail.com
Vítor Hugo de Arimatéa Rocha
ORCID: https://orcid.org/0000-0002-0273-7255
Faculdade Frassinetti do Recife, Brazil
E-mail: arimatearocha@gmail.com
Paulo Sérgio Ramos Araújo
ORCID: https://orcid.org/0000-0002-7839-0737
Fundação Oswaldo Cruz, Brazil
E-mail: psergiora@gmail.com
Alzira Maria Paiva de Almeida
ORCID: https://orcid.org/0000-0002-8304-3182
Fundação Oswaldo Cruz, Brazil
E-mail: alzira.almeida@fiocruz.br
Nilma Cintra Leal
ORCID: https://orcid.org/0000-0001-9769-7630
Fundação Oswaldo Cruz, Brazil
E-mail: nilma.leal@fiocruz.br
Maria Amélia Vieira Maciel
ORCID: https://orcid.org/0000-0002-4220-6889
Universidade Federal de Pernambuco, Brazil
E-mail: amelia57@gmail.com

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 (*mec*A, *bla*Z, *erm*A and *erm*C) and virulence (*ica*A, *ica*D 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 *mec*A 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 $MLSB_c$ phenotype and five the $MSLB_i$ phenotype, and one isolate from the OH displayed the constitutive phenotype *erm*C 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 (*mec*A, *bla*Z, *erm*A e *erm*C) e virulência (*ica*A, *ica*D 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 *mec*A positivos do HU. Maior porcentagem de isolados suscetíveis, exceto para oxacilina e cefoxitina, foi encontrada entre os isolados de UH e 27,3% dos isolados resistentes à vancomicina foram identificados através método de *screening*; 41 isolados exibiram o fenótipo MLSBc e cinco o fenótipo MSLBi, e um isolado do OH do fenótipo constitutivo exibiu o gene *erm*C. 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 (*mec*A, *bla*Z, *erm*A y *erm*C) y virulencia (*ica*A, *ica*D 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 *mec*A positivos de la HU. Se encontró un mayor porcentaje de aislados susceptibles, a excepción de oxacilina y cefoxitina, 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 fenotipo MLSBc y cinco el fenotipo MSLBi, y un aislado OH del fenotipo constitutivo exhibió el gen *erm*C. 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 fator 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-trimethropim 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 35ºC 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 *mec*A gene by PCR

The presence of the *mec*A 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, $MgCl_2$ 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 oxacilin and/or cefoxitin phenotypically resistant isolates using the primers described by Milheiriço 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 $MgCl_2$ (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 ate 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 *mec*A 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 $MgCl_2$ (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')
α_1	5'AACCTATATCATCAATCAGTACGT3'
α_2	5'TAAAGGCATCAATGCACAAACACT3'
α_3	5'AGCTCAAAAGCAAGCAATAGAAT3'
β_c	5'ATTGCCTTGATAATAGCCITCT3'
MA1	5'TGCTATCCACCCTCAAACAGG3'
MA2	5'AACGTTGTAACCACCCCAAGA3'
γF	5'CGTCTATTACAAGATGTTAAGGATAA T3'
γR	5'CCTTTATAGACTGGATTATTCAAAAT AT3'

Source: Authors.

Chart 1: PCR products on 2% agarose gel

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

Source: Authors.

Detection of *erm*A and *erm*C genes by PCR technique

The isolates erythromycin and clindamycin resistant (phenotype $MLSB_c$) and erytromycin positive in the D test (phenotype $MLSB_i$) were analysed for the presence of the *erm*A and *erm*C genes using the primers described by Lina et al (1999a): *erm*A F5'GTTCAAGAACAATCAATACAGAG3' and R5'GGATCAGGAAAAGGACATTTTAC3'; *erm*C: F5'GCTAATATTGTTTAAATCGTCAATTCC3' and R5'GGATCAGGAAAAGGACATTTTAC3'. For the detection of *erm*A 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 $MgCl_2$ (25 mM), 0,4µl of Go Taq DNA polimerase (5U) and 14,5µl of sterile milliQ water. For the detection of *erm*C, 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 94ºC, 30

seconds at 49ºC, and 30 seconds at 72ºC. 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 (*erm*A and *erm*C) confirmed by amplification and sequencing were used.

Detection of the *ica*A and *ica*D genes

The amplification of the *ica*A and *ica*D genes from the operon *ica*ADBC, was performed using the primers described by Vasudevan et al (2003) (F5'CCTAACTAACGAAAGGTAG3' and R5'GGCAATATGATCAAGATAC3') for of 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 $MgCl_2$ (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'GCCAATCCGTTATTAGAAAATGC3' 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 $MgCl_2$ (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, x^2 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).

Table 2: Distribution of *Staphylococcus spp.* isolates from the samples and hospital origin.

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

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).

Table 3: Antimicrobial susceptibility of *Staphylococcus spp.* isolates in University and Oncologic Hospitals.

Antimicrobial	UH		OH	
	Sensitive	Resistant	Sensitive	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/ trimethropim	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 *bla*Z and *mec*A 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 *bla*Z and *mec*A genes (Table 5). The isolates analyzed from the University Hospital 45.7% were positive for the *bla*Z gene and 17% for the *mec*A gene (Table 5).

Table 5: Results of PCR for *bla*Z, *mec*A, *ica*AD and *hlg* genes for the two hospitals.

PCR type	Results	Hospital	
		UH	OH
*bla*Z	Positive	16	9
	Negative	19	9
*mec*A	Positive	8	14
	Negative	39	4
*ica*AD	Positive	3	9
	Negative	9	19
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 $MLSB_c$ phenotype (Macrolides, Lincosamides and Streptogramis constituent B) and five $MSLB_i$ phenotype (Macrolides, Lincosamides and Streptogramis inducible B) were identified at the University Hospital. In the Oncology Hospital, eight isolates were identified with the phenotype $MLSB_c$ and and six $MLSB_i$ (Table 6).

Table 6: Results of PCR for *erm*A and *erm*C genes for the two hospitals.

Hospital	Phenotype							
	$MLSB_c$				$MLSB_i$			
	*erm*A positive	*erm*A negative	*erm*C positive	*erm*C negative	*erm*A positive	*erm*A negative	*erm*C positive	*erm*C negative
UH	5	36	16	25	0	5	1	4
OH	0	8	1	7	0	6	0	6

Source: Authors.

The isolates displaying the phenotypes $MLSB_c$ and $MLSB_i$ were analyzed by PCR for the detection of the *erm*A and *erm*C genes. Only one isolate was found harboring the constitutive phenotype *erm*C gene. The *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 *erm*A gene in induced phenotype in the University Hospital (Table 6).

The occurrence of the genes *ica*AD and *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 *bla*Z, *mec*A, *erm*A and *erm*C when compairing 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 *p* value found in the static analysis.

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

Chart 2: Results fot the *p* values for comparisons between the PCR of resistance genes for the two hospitals.

PCR blaZ

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6,129[a]	2	,047
Likelihood Ratio	6,219	2	,045
N of Valid Cases	113		

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 11,50.

PCR mecA

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	27,421[a]	2	,000
Likelihood Ratio	30,998	2	,000
N of Valid Cases	125		

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 8,98.

PCR ermA

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8,098[a]	2	,017
Likelihood Ratio	9,743	2	,008
N of Valid Cases	141		

a. 2 cells (33,3%) have expected count less than 5. The minimum expected count is 1,60.

PCR ermC

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10,147[a]	2	,006
Likelihood Ratio	12,096	2	,002
N of Valid Cases	140		

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 5,97.

Source: Authors.

Chart 3: Results for the *p* values for comparisons between the PCR of virulence genes for the two hospitals.

PCR icaAD

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,234[a]	1	,651		
Continuity Correction[b]	,036	1	,940		
Likelihood Ratio	,238	1	,648		
Fisher's Exact Test				,725	,479
N of Valid Cases	40				

a. 1 cells (25,0%) have expected count less than 5. The minimum expected count is 3,60.

b. Computed only for a 2x2 table

PCR hlg

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2,505[a]	1	,113		
Continuity Correction[b]	1,510	1	,219		
Likelihood Ratio	2,514	1	,113		
Fisher's Exact Test				,163	,110
N of Valid Cases	34				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 5,76.

b. Computed only for a 2x2 table

Source: Authors.

4. Discussion

Most *Staphylococcus* spp. carriers are asymptomatic and related 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 communitarian 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 *bla*Z and *mec*A 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 *bla*Z gene and 7% for the *mec*A 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; Milheiriço 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 $MLSB_c$ phenotype found in 41 isolates (33.6%) from the University Hospital and eight isolates (15.7%) from the Oncology Hospital was predominant over the $MLSB_i$ 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%) $MLSB_c$ and five $MLSB_i$ (3.3%) isolates (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 *erm*C 35.2% and *erm*A 20.4% (Sedaghat et al., 2017) and Saderi, Emadi and Owlia (2011), found the opposite, the *erm*A gene was more frequent than the *erm*C 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 *erm*C gene was higher than the *erm*A gene in the isolates of both Hospitals. In our study, no single positive isolate was found for the *erm*A 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 *erm*A or *erm*C genes, may possess the *erm*B gene. Due to a possible association of these genes the isolates harboring the *erm*A or *erm*C genes may also contain *erm*B. The presence of the *erm*B 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 *erm*B 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 is mediated by the inter cell adhesin *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 *ica*AD, 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 (*bla*Z, *mec*A, *erm*A and *erm*C) when comparing the two hospitals whilst statistically significant difference was found in the presence of the virulence genes (*ica*A, *ica*D 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 *mec*A positive isolates from the University Hospital and the *ccr* type I (*ccr*A1, 695bp) and type II (*ccr*A2, 937 bp) were found with higher frequency of *ccr* type I. Previous studies reported the occurrence of *SCC*mec 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 mumber 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.

Acknowledgments

This work was supported by FACEPE (Fundação de Amparo Ciência do Estado de Pernambuco) grant nº APQ 0579-2.12/06, PROPESQ (Pró-Reitoria para Assuntos de Pesquisa e Pós-Graduação-UFPE) for the Edital PQ-Multiusuário-2010/Propesq nº 23076.024115/2010-91 and CNPq (protocolo number 474131/2011-4). CAPES for the scholarship provided. The Hospital das Clínicas bacteriology laboratory for providing some samples and CIAC laboratory, Hospital do Câncer de Pernambuco, for providing some samples and information records. Centro de Pesquisas Aggeu Magalhães (CPqAM) for providing space for the molecular techniques realization.

References

Aggarwal, S., Jena, S., Panda, S., Sharma, S., Dhawan, B., Nath, G. et al. (2019). Antibiotic susceptibility, virulence pattern, and typing of *Staphylococcus aureus* strains isolated from variety of infections in India. *Frontiers in Microbiology*, 10:2763. 10.3389/fmicb.2019.02763.

Andrade-Figueiredo, M. & Leal-Balbino, T.C. (2016). Clonal diversity and epidemiological characteristics of *Staphylococcus aureus*: high prevalence of oxacillin-susceptible *mec*A-positive *Staphylococcus aureus* (OS-MRSA) associated with clinical isolates in Brazil. *BMC Microbiology*, 16:115. 10.1186/s12866-016-0733-4.

Azmi, K., Qrei, W. & Abdeen, Z. (2019). Screening of genes encoding adhesion factors and biofilm production in methicillin resistant strains of *Staphylococcus aureus* isolated from Palestinian patients. *BMC Genomics*, 20:578. 10.1186/s12864-019-5929-1.

Barbieri, R., Pesce, M., Franchelli, S., Baldelli, I., De Maria, A., Marchese, A. (2015). Phenotypic and genotypic characterization of Staphylococci causing breast peri-implant infections in oncologic patients. *BMC Microbiology*, 15 (1), 26. 10.1186/s12866-015-0368-x.

Bezerra Neto, A. M., Rabelo, M. A., Lima, J. L. C., Loibman, S. O., Leal, N. C., Maciel, M. A. V. (2018). Occurrence of the *van*A gene in *Staphylococcus epidermidis* from nasopharyngeal secretion of Health-Care Workers, Recife, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 51(3), 304-309. 10.1590/0037-8682-0159-2017.

Bride, L. L., Pereira, M. F., Barbosa, M. C., Silva, N. C., Klein, N. M., Nascimento, T. C., & Schuenck, R. P. (2019). Differences in resistance profiles and virulence genes among methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* of different lineages at a public tertiary hospital. *Revista da Sociedade Brasileira de Medicina Tropical*, 52, e20190095. 10.1590/0037-8682-0095-2019

Burnham, C. A. D., Weber, C. J. & Dunne Jr, W. M. (2010). Novel screening agar for detection of vancomycin-nonsusceptible *Staphylococcus aureus. Journal of Clinical Microbiology*, 48(3), 949–951. 10.1128/JCM.02295-09.

Cagol, A. R., Castro Junior, C. G., Martins, M. C., Machado, A. L., Ribeiro, R. C., Gregianin, L. J., &Brunetto, A. L. (2209). Oral vs. intravenous empirical antimicrobial therapy in febrile neutropenic patients receiving childhood cancer chemotherapy. *Jornal de Pediatria*, 85(6), 531-535. 10.2223/JPED.1956.

CLSI (Clinical and Laboratory Standards Institute). (2014). Performance standards for antimicrobial susceptibility testing, twenty-fourth informational supplement, documentM100-S24.Wayne, PA, USA. 2014.

Coutinho, V. L. S, Paiva, R. M., Reiter, K. C., de-Paris, F., Barth, A. L., & Machado, A. B. (2010). Distribution of *erm* genes and low prevalence of inducible resistance to clindamycin among *staphylococci* isolates. *The Brazilian Journal of Infectious Diseases*, 14(6), 564-568. 10.1016/S1413-8670(10)70113-6

Elboshra, M. M. E., Hamedelnil, Y. F., Moglad, E. H., & Altayb, H. N. (2020). Prevalence and characterization of virulence genes among methicillin resistant *Staphylococcus aureus* isolated from Sudanese patients in Khartoum state. *New Microbe and New Infect*. 38, 100784. https://doi.org/10.1016/j.nmni.2020.100784.

Figueiredo, A. M. S., Ferreira, F. A., Beltrame, C. O., & Côrtes, M. F. (2017). The role of biofilms in persistent infections and factors involved in ica-independent biofilm development and gene regulation in *Staphylococcus aureus*. *Critical Reviews in Microbiology*, 43(5), 602-620. 10.1080/1040841X.2017.1282941.

Fontelles, M. J., Simões, M. G., Farias, S. H., & Fontelles, R. G. S. (2009). Scientific research methodology: guidelines for elaboration of a research protocol. Revista Paraense de Medicina, 23(3). https://pesquisa.bvsalud.org/portal/resource/pt/lil-588477.

Jenkins, A., Diep, B. A., Mai, T. T., Vo, N. H., Warrener, P., Suzich, J., Stover, C. K., & Sellman, B. R. (2015). Differential Expression and Roles of *Staphylococcus aureus* Virulence Determinants during Colonization and Disease. *mBio*. 6(1), e02272-14. 10.1128/mBio.02272-14

Jiang, A.M., Shi, X., Liu, N., Gao, H., Ren, M. D., Zheng, X. Q. et al. (2020). Nosocomial infections due to multidrugresistant bacteria in cancer patients: a sixyear retrospective study of an oncology Center in Western China. *BMC Infectious Diseases*. 20, 452. 10.1186/s12879-020-05181-6.

Jones, R. N., Guzman-Blanco, M., Gales, A. C., Gallegos, B., Castro, A. L., Martino, M. D., Vega, S., Zurita, J., Cepparulo, M., & Castanheira, M. (2013). Susceptibility rates in Latin American nations: report from a regional resistance surveillance program (2011). *The Brazilian Journal of Infectious Diseases*, 17(6), 672–681. 10.1016/j.bjid.2013.07.002.

Juyal, D., Shamanth, A. S., Pal, S., Sharma, M. K., Prakash, R., & Sharma, N. (2013). The prevalence of inducible clindamycin resistance among Staphylococci in a tertiary care hospital – A study from the Garhwal Hills of Uttarakhand, India. *Journal of Clinical & Diagnostic Research*, 7(1), 61-65. 10.7860/JCDR/2012/4877.2671.

Kang, C. I., Song, J. H., Chung, D. R., Peck, K. R., Yeom, J. S., Son, J. S., & Wi, Y. M. (2012). Bloodstream infections in adult patients with cancer: clinical features and pathogenic significance of *Staphylococcus aureus* bacteremia. *Supportive Care in Cancer*, 20(10), 2371–2378. 10.1007/s00520-011-1353-z.

Kondo, Y., Ito, T., Ma, X. X., Watanabe, S., Kreiswirth, B. N., Etienne, J., & Hiramatsu, K. (2007). Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. *Antimicrobial Agents and Chemoterapy*, 51(1), 264-274. 10.1128/AAC.00165-06.

Kord, M., Ardebili, A., Jamalan, M., Jahanbakhsh, R., Behnampour, N., & Ghaemi, E. A. (2018). Evaluation of biofilm formation and presence of *Ica g*enes in *Staphylococcus epidermidis* clinical isolates. *Osong Public Health and Research Perspectives*, 9(4), 160−166. 10.24171/j.phrp.2018.9.4.04.

Kumar, S., Bandyopadhyay, M., Bhattacharya, K., Bandyopadhyay, M.K., Banerjee, P., Pal, N., Mondal, S., & Ghosh, T. (2012). Inducible clindamycin resistance in *Staphylococcus* isolates from a tertiary care hospital in eastern India. *Annals of Tropical Medicine and Public Health*, 5(5), 468-470. doi:10.4103/1755-6783.105134.

Leclercq R. (2002). Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clinical Infectious Diseases*, 34(4), 482- 492. 10.1086/324626.

Lim, K. T., Hanifah, Y. A., Yusof, M. Y. M., & Thong, K. L. (2012). Characterisation of the virulence factors and genetic types of methicillin susceptible *Staphylococcus aureus* from patients and healthy individuals. *Indian Journal of Microbiology*, 52(4), 593–600. 10.1007/s12088-012-0286-7.

Lima, D. F., Brazão, N. B., Folescu, T. W., Neves, F. P., Ferreira, A. G., Santos, E. A., Marques, E. A., & Leão, R. S. (2014). Panton-Valentine leukocidin (PVL) gene carriage among *Staphylococcus aureus* strains with SCC*mec* types I, III, IV,and V recovered from cystic fibrosis pediatric patients in Brazil. *Diagnostic Microbiology and Infectious Disease*, 78(1), 59–62. 10.1016/j.diagmicrobio.2013.10.004.

Lina, G., Quaglia, A., Reverdy, M. E., Leclercq, R., Vandenesch, F., & Etienne, J. (1999a). Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among Staphylococci. *Antimicrobial Agents and Chemoterapy*, 43(5), 1062–1066. 10.1128/aac.43.5.1062.

Lina, G., Piémont, Y., Godail-Gamot, F., Bes, M., Peter, M. O., Gauduchon, V., Vandenesch, F., & Etienne, J. (1999b). Involvement of Panton-Valentine Leukocidin- producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clinical Infectious Diseases.* 29(5), 1128-1132. 10.1086/313461.

Milheiriço, C., Portelinha, A., Krippahl, L., de Lencastre, H., & Oliveira, D. C. (2011). Evidence for a purifying selection acting on the betalactamase lócus in epidemic clones of methicillin-resistant *Staphylococcus aureus*. *BMC Microbiology*, 11(1), 76. 10.1186/1471-2180-11-76.

Montassier, E., Batar, E., Gastinne, T., Potel, G., & de La Cochetière, M. F. (2013). Recent changes in bacteremia in patients with cancer: a systematic review of epidemiology and antibiotic resistance. *European Journal of Clinical Microbiology & Infectious Diseases*, 32 (7), 841–50. 10.1007/s10096-013-1819-7.

Muldrew, K. L., Tang, Y. W., Li, H., & Stratton, C. W. (2008). Clonal Dissemination of *Staphylococcus epidermidis* in an Oncology Ward. *Journal of Clinical Microbiology*, 46(10), 3391–3396. 10.1128/JCM.00115-08.

Oliveira, A. A. F., Pinheiro Jr, J. W., Mota, R. A., Cunha, M. L., Lopes, C. A., & Rocha, N. S. (2011). Phenotype characterization of *Staphylococcus* species strains isolated from buffalo (*Bubalus bubalis*) milk. *Journal of Veterinary Diagnostic Investigation*, 23(6), 1208-1211. 10.1177/1040638711428946.

Oliveira, W. L. M., Ferreira, E. L., Mangueira, E. V. C., Vilela, M. A., Almeida, A. M. P., & Leal, N. C. (2014). Coagulase-Negative Staphylococci from hospital-associated infections harboring virulence genes. *Jacobs Journal of Microbiology and Pathology*, 1(2), 013.

Oliveira, W. L. M., Mangueira, E. V. C., Vilela, M. A., Júnior, S. P., Leal, N. C., & Almeida, A. M. P. (2015). Diversity of Sccmec types in methicillin resistant *Staphylococcus* spp. causing hospital-associated infections. *Jacobs Journal of Microbiology and Pathology*, 2(3), 020.

Panesso, D., Planet, P. J., Diaz, L., Hugonnet, J. E., Tran, T. T., Narechania, A. et al. (2015). Methicillin-Susceptible, Vancomycin-Resistant *Staphylococcus aureus*, Brazil. *Emerging Infectious Diseases*, 21(10): 1844-1848. 10.3201/eid2110.141914.

Prabhash, K., Medhekar, A., Ghadyalpatil, N., Noronha, V., Biswas, S., Kurkure, P., Nair, R., & Kelkar, R. (2010). Blood stream infections in cancer patients: a single center experience of isolates and sensitivity pattern. *Indian Journal of Cancer*, 47 (2), 184–188. 10.4103/0019-509X.63019.

Quiles, M. G., Menezes, L. C., Bauab, K. C., Gumplv, E. K., Rocchetti, T. T., Palomo, F. S., Carlesse, F., & Pignatari, A. C. C. (2015). Diagnosis of bacteremia in pediatric oncologic patients by in-house real-time PCR. *BMC Infectious Diseases*, 15, 283. 10.1186/s12879-015-1033-6.

Rabelo, M. A., Bezerra Neto, A. M., Loibman, S. O., Lima, J. L. C., Ferreira, E. L., Leal, N. C., & Maciel, M. A. V. (2014). The occurrence and dissemination of methicillin and vancomycin-resistant *Staphylococcus* in samples from patients and health professionals of a university hospital in Recife, State of Pernambuco, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 47(4), 437-446. 10.1590/0037-8682-0071-2014.

Rabelo, M. A., Bezerra Neto, A. M., Silva, E. C. B. F., Oliveira, W. L. M., Melo, F. L., Lopes, A. C. S., Leal, N. C., & Maciel, M. A. V. (2013). Phenotypic methods for determination of methicillin resistance in *Staphylococcus spp*. from health care workers. *Jornal Brasileiro de Patologia e Medicina Laboratorial*, 49(2), 91-96. 10.1590/S1676-24442013000200003.

Rossi, F., Diaz, L., Wollam, A., Panesso, D., Zhou, Y., Rincon, S. et al. (2014). Transferable Vancomycin Resistance in a Community-Associated MRSA Lineage. *The New England Journal of Medicine*, 370(16), 1524-1531. 10.1056/NEJMoa1303359.

Saderi, H., Emadi, B. & Owlia, P. (2011). Phenotypic and genotypic study of macrolide, lincosamide and streptogramin B (MLSB) resistance in clinical isolates of *Staphylococcus aureus* in Tehran, Iran. *Medical Science Monitor*, 17 (2), 48-53. 10.12659/MSM.881386.

Sangappa, M. & Thiagarajan, P. (2012). Methicillin resistant *Staphylococcus aureus*: resistance genes and their regulation. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(1):658-667.

Sedaghat, H., Esfahani, B. N., Mobasherizadeh, S., Jazi, A. S., Halaji, M., Sadeghi, P., Emaneini, M., & Havaei, A. S. (2017). Phenotypic and genotypic characterization of macrolide resistance among *Staphylococcus aureus* isolates in Isfahan, Iran. *Iranian Journal of Microbiology*, 9(5), 264-270.

Silveira, A., Cunha, G. R., Caierão, J., Cordova, C. M., & d'Azevedo, P. A. (2015). MRSA from Santa Catarina State, Southern Brazil: intriguing epidemiological differences compared to other Brazilian regions. *The Brazilian Journal of Infectious Diseases*, 19(4), 384–389. 10.1016/j.bjid.2015.04.009

Spaan, A. N., Vrieling, M., Wallet, P., Badiou, C., Reyes-Robles, T., Ohneck, E. A. et al. (2014). The staphylococcal toxins γhaemolysin AB and CB differentially target phagocytes by employing specific chemokine receptors. *Nature Communications*, 5, 5438. 10.1038/ncomms6438.

Vasudevan, P., Nair, M. K. M., Annamalai, T., & Venkitanarayanan, K. S. (2003). Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. *Veterinary Microbiology*. 92, 179-185. 10.1016/S0378-1135(02)00360-7.