Phenotypic methods for determination of methicillin resistance in *Staphylococcus spp.* from health care workers

Métodos fenotípicos para determinação da resistência à meticilina em Staphylococcus spp. *de profissionais de saúde*

Marcelle Aquino Rabelo[1]; Armando Monteiro Bezerra Neto[1]; Eduardo Caetano Brandão Ferreira da Silva[2]; Wagner Luis Mendes de Oliveira[3]; Fábio Lopes de Melo[4]; Ana Catarina S. Lopes[5]; Nilma Cintra Leal[6]; Maria Amélia Vieira Maciel[7]

ABSTRACT

Introduction: *Staphylococcus spp.* is an important healthcare-associated pathogen and the identification of methicillin-resistant strains in samples of colonization may provide data to assist in the antimicrobial therapy success. **Objectives:** To determine the occurrence of colonization by methicillin-resistant *Staphylococcus spp.* (MRS), through the detection of the mecA gene and to evaluate different phenotypic methods for the presumptive detection of methicillin resistance in samples of the anterior nasal cavity and hands of the health care personnel of a university hospital in the state of Pernambuco, Brazil. **Methods:** We selected the 28 isolates of *Staphylococcus spp.*, which showed an intermediate or resistant phenotypic profile for oxacillin, detected by the Kirby Bauer technique. The methods used were disk-diffusion tests for cefoxitin, minimal inhibitory concentration by E-test for oxacillin, screening for oxacillin resistance and mecA gene detection by polymerase chain reaction (PCR). **Results:** About the phenotypic methods utilized, only the E-test of oxacillin did not show a statistically significant difference in relation to PCR for the mecA gene detection, considered the gold standard. **Conclusion:** The E-test of oxacillin was the best of the phenotypic methods utilized. It is necessary to correctly detect MRS in healthy individuals, because they can act as carriers and can therefore be a potential source of microorganisms involved in hospital infections.

Key words: methicillin; health care professionals; resistance; *S. aureus*; *S. epidermidis*; *Staphylococcus spp.*

INTRODUCTION

In the last decade, *Staphylococcus* has emerged as predominant microorganism in nosocomial infections[6, 21]. The main staphylococcal infections reported include folliculitis and furuncles and even serious toxic shock syndrome and sepsis[6, 18, 21, 22]. Coagulase-negative staphylococci (CNS) can be found in the normal microbiota of the skin and in mucosal membranes and have received special attention as potent pathogens, specifically in catheter-related bacteremias[6, 27, 28].

At the end of the 90, the main change in antimicrobial resistance was the increase in resistance to methicillin among *Staphylococcus aureus* and CNS in all regions. Resistance to methicillin in *Staphylococcus*

results resistance to all beta-lactam antibiotics, leaving few therapeutic options[21]. High rate of isolation of staphylococci resistant to methicillin leads to the need to use on a large scale more expensive or toxic antimicrobials such as vancomycin[7, 21, 26, 27].

Methicillin resistance in *Staphylococcus* is made by two main mechanisms: the betalactamase production and/or the presence of the PBP2a. This latter mechanism encoded by *SCCmec* cassette, a transposon, is the most important and worrying cause of transfer between staphylococcal species. The transposon *SCCmec* has two components, the *mec* to resistance and the *ccr* to mobility of the transposon, and they are used to classify the *SCCmec* types. There are others antibiotics groups of resistance in some types of *SCCmec*, which

makes a multidrug resistant strain. The common element between *SCCmec* types is the *mec*A gene that encodes the PBP2a, therefore the detection of this gene is considered the gold standard for methicillin resistance detection[2, 34]. There is a homologue of this gene, the *mec*A$_{LGA251}$, described in cattle and, more recently, in clinical samples from patients in Germany, which can be ambiguous the *mec*A gene detection in some places[10]. Moreover the detection of the *mec*A gene by polymerase chain reaction (PCR) requires inaccessible and sophisticated equipment to most laboratories.

The methods that stand out among the most utilized or best capable in the identification of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) are: agar dilution method, disk diffusion, screening on agar with methicillin, automated methods (Microscan, Vitek), latex agglutination and the molecular method using PCR for the detection of the gene *mec*A[2, 3, 9, 13-15, 24, 25, 27, 28, 30, 31, 33, 34]. The polymerase chain reaction in real time (RT-PCR) was described as an useful PCR-based diagnostic tool[35].

The Clinical and Laboratory Standard Institute (CLSI) standardized the disk diffusion method for cefoxitin as a presumptive method of the *mec*A gene, but the phenotypic expression of this gene is still a challenge for microbiology laboratories [2, 3, 9, 24, 28] due to the discrepancies observed between laboratories that participate in the Antimicrobial Surveillance Program[23]. Currently, one of the main objectives for the control of hospital infections is the rational use of antimicrobials, which makes the evaluation of the accuracy of phenotypic methods used for determining the susceptibility profile essential for guaranteeing the most appropriate choice of therapeutic antimicrobials[19].

Another important factor in the context of nosocomial infections is the transmission chain in the hospital environment[27], in which health care professional are included as disseminating sources of this microorganism[5, 32].

Thus, considering that *Staphylococcus spp.* are an important pathogenic agent associated with nosocomial infections and that studies about the colonization of health care workers are scarce, our objectives were to determine the occurrence of colonization by methicillin-resistant *Staphylococcus spp.*, through the detection of the *mec*A gene and to evaluate different phenotypic methods for the presumptive detection of methicillin resistance in samples of the anterior nasal cavity and hands of the health care personnel from a university hospital in the state of Pernambuco, Brazil.

MATERIAL AND METHODS

Biological samples

A cross-sectional study, in which, the biological samples of hands and anterior nasal cavity of 202 health workers from surgical clinics, intensive care units (IUCs), hemodyalisis and nephrology services, of the Hospital das Clínicas of the Federal University of Pernambuco, Brazil, were collected in the period of March to July 2007. This study was approved by the Ethics Committee on Research of the Federal University of Pernambuco – CAAE nº 0275.0.172.000-06. A sample of each anatomical site, from each professional, was obtained using sterile and dry swabs and, taken to the bacteriology laboratory, in tubes containing brain heart infusion (BHI) and cultivated in agar sheep blood 5% and incubated at 35ºC for 24 hours. *Staphylococcus spp.* suspect colonies were analyzed by Gram staining, catalase test and coagulase tests, growth in mannitol salty agar and DNAse activity; novobiocin proof for coagulase negative *Staphylococcus*. The antibiogram was performed by disc diffusion test[9], the isolates selected were that showed resistance or intermediate profile to oxacillin and/or cefoxitin, that are considered presumptive techniques by CLSI, of low cost and routine use for methicilin resistance detection. So that, were used 28 isolates for presumptive detection of *mec*A and PCR. The *mec*A gene were detected by PCR.

For quality control in these tests, the standard strain ATCC 33591 *S. aureus* was used for the positive control (MRSA) and the standard strain ATCC 29213 (MSSA) for the negative control.

Methicilin and oxacillin susceptibility

The susceptibility to methicillin was tested by disk-diffusion method: the *Staphylococcus* isolates were cultivated in 5% blood agar for 24 hour at 35ºC, and subsequently, colonies were suspended in saline to a turbidity equivalent to 0.5 on the McFarland scale (10^8 CFU/ml). The bacterial suspension obtained was seeded in a homogeneous manner, with the help of a sterile swab, on the surface of plates containing Mueller-Hinton agar. Next, with the aid of flamed tweezers, a 30 μg cefoxitin disk was placed on the surface, where an inhibition halo ≤ 21 mm for *S. aureus* and ≤ 24 mm for CNS was considered the presumptive test[9].

Determination of the minimal inhibitory concentration (MIC) by the oxacillin E-test

Cultures of *S. aureus* and CNS were grown in nutrient agar plates, and picked colonies were placed in a tube containing sterile saline (0.85% NaCl) to a turbidity of 0.5 on the McFarlands scale[9]. The suspension obtained was evenly seeded with the help of a sterile swab, on the surface of Mueller-Hinton agar plates, after which oxacillin strips were placed on the surface (E-test - Probac do Brazil®), using flamed tweezers. The plates were then incubated at 35ºC for 24 hour. The MIC was determined following the manufacturer's specifications, corresponding to the standards proposed by CLSI[9].

Screening for oxacillin resistance

Colonies from subcultures on nutrient agar plates were resuspended to obtain a turbidity equivalent to 0.5 on the McFarland scale. A platinum wire loop of 1 μl was dipped in the suspension, and the bacteria were seed in an area with a diameter of 10 to 15 mm in plates with Mueller-Hinton agar medium containing NaCl (4% v/v; 0.68 mol/l) and 6 μg/ml oxacillin. These plates were incubated at 35ºC for 24 hour and then read, considering that > 1 colony = resistance[9].

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Deoxyribonucleic acid (DNA) preparation and identification by PCR of the *mec*A gene

Total DNA was extracted from individual colonies after growth in brain heart infusion (BHI) broth for 24 hour at 37ºC, following the protocol described by Freitas *et al.*[16]. PCR was performed utilizing the primers described by Petinaki *et al.*[29]. The amplification reaction mixture was prepared in a total volume of 25 μl containing 50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl$_2$, 200 mM dNTP (Promega), 20 pmol of each primer, 20 ng of genomic DNA, 1U Taq DNA polymerase (Promega). The reactions were carried out in a thermocycler (Biometra), programmed initially for 30 thermal cycles, with denaturation of 1 min at 94ºC, annealing of 1 min at 50ºC and extension of 1 min at 72ºC, followed by a final step of 10 minutes at 72ºC. The negative control contained all the components of the reaction mixture except DNA. The ATCC 33591 *S. aureus* was used for the positive control (MRSA). The amplification product was submitted to 1% agarose gel electrophoresis with ethidium bromide staining, and visualized in a UV transilluminator and digitized (Kodak Digital Science).

Statistical analysis

Differences between the susceptibility methods and the level of significance of the results were calculated by the chi-squared test and Fisher's exact test. $p < 0.05$ was considered statistically significant. Validity tests including sensitivity, specificity, and positive and negative predictive values were calculated. Sensitivity was defined as the percentage of *mec*A-positive isolates determined to be non-susceptible by phenotypic testing, and specificity was defined as the percentage of *mec*A-negative isolates determined to be susceptible by phenotypic methods.

RESULTS

It was obtained 404 samples of 202 health professionals, in which 14.6% (59/404) were *S. aureus* isolates and 6.2% (25/404) were classified as *S. epidermidis* by the novobiocin test. Thus, after the susceptibility testing, twenty-eight (14%) *Staphylococcus spp.* isolates (14 isolates of *S. aureus* and 14 *S. epidermidis*) were selected from the 202 health care professionals, which showed a resistant or intermediate phenotypic profile to methicillin by the disk-diffusion method.

The presumptive tests of detection of the *mec*A gene showed that 71.43% (20/28) of these were from the neonatal intensive care unit (ICU), where 57.14 % (16/28) of the total samples examined were from the professional class of nursing aids and technicians and *mec*A gene was detected in 15 isolates (nine *S. epidermidis* and six *S. aureus*), as can be seen in the **Table 1**.

Of the phenotypic methods utilized, only the oxacillin E-test did not show a statistically significant difference in relation to the gold standard (PCR).

The results of the validity tests for all the techniques studied are presented in **Table 2**. Screening for oxacillin sensitivity showed better results in relation to the other drugs, and the E-test showed the best specificity. Similarly, screening for oxacillin resistance and the disk-diffusion test for cefoxitin showed 11 false positives, while the oxacillin E-test showed five false negatives.

DISCUSSION

The majority of colonized health care professionals, are transitory carriers, but can become persistent carriers, especially when they have skin lesions. The preventive strategy would be the identification and treatment of these colonized individuals, who could act as a reservoir in endemic situation[5, 18]. Various investigators have proposed methods aimed at providing accuracy in the determination of MRS colonization status[15, 20].

Screening for oxacillin susceptibility can be utilized for the detection of *Staphylococcus* resistance, generally with very good accuracy[9, 24]. The advantage of the test is the economic reasons and in diagnostic laboratories due to its reproducibility and cost-effectiveness[31].

Several studies have demonstrated PCR as a sensitive method for the detection of resistance in staphylococci[1, 11, 24, 25]. With the advantage of PCR over phenotypic methods with rapid turnaround time, measures to control infection can be taken more quickly[31]. Some studies have shown discrepancies in accuracy between the phenotypic tests and molecular detection of the *mec*A gene by PCR, considered the gold standard method[3, 24, 30].

In our study, the oxacillin E-test was shown to be the most efficacious in the phenotypic presumptive detection of the *mec*A gene, when compared to PCR. In some studies[14, 31], the presence of the *mec*A gene was correlated with E-test, which showed variations in the conditions of analysis or pointed to other mechanisms utilized by bacteria for resistance to methicillin, for example betalactamases production[10, 29, 34].

By other hand, in another study, the cefoxitin disk diffusion test was the most effective among those analyzed[33]. For the routine detection of methicillin resistance in *Staphylococcus spp.*, the disk-diffusion tests are the most widely utilized, where the cefoxitin disk test is preferred than the oxacillin disk test to detect this resistance, because the latter is comparable in accuracy and shows a better reading[9]. Cefoxitin is regarded as the most reliable way to identify even strains that show a level of methicillin resistance, which is not possible using a 1-μg methicillin disk[28, 34]. In our study, the methicillin disk was utilized as selection criterion of the samples, showing in this manner false positive samples when compared to PCR results, reinforcing the possibility of other mechanisms of resistance.

Antunes *et al.*[3] considered that the treatment of staphylococcal infections depends on the detection of methicillin resistance, usually done using drug susceptibility tests with oxacillin/methicillin disks, but as the results of these assays are inconsistent, complications during the treatment of these infections is to be expected due to inadequate therapy. A false-positive result would lead to an increase in the costs and unnecessary use of some last generation antibiotics, for example, vancomycin[8].

TABLE 1 – Phenotypic test results and *mec*A gene detection

Isolate number	Sector	Professional activity	Species	Disk-diffusion method for cefoxitin	Oxacillin E-test	Oxacillin Screening	PCR *mec*A
1M	Neonatal ICU	Nurse	*S. epidermidis*	R	R	R	+
2NF	Neonatal ICU	Nurse technician	*S. epidermidis*	R	R	R	-
3M	Neonatal ICU	Nurse technician	*S. aureus*	R	R	S	-
4M	Neonatal ICU	Nurse technician	*S. aureus*	R	S	R	-
8NF	Neonatal ICU	Nurse	*S. aureus*	R	S	R	+
9M	Neonatal ICU	Physiotherapist	*S. aureus*	R	S	R	+
10NF	Neonatal ICU	Physician	*S. aureus*	R	S	R	-
12M	Neonatal ICU	Nurse technician	*S. aureus*	R	S	R	-
12NF	Neonatal ICU	Nurse technician	*S. aureus*	R	S	R	-
13M	Neonatal ICU	Physician	*S. epidermidis*	R	R	R	+
13NF	Neonatal ICU	Physician	*S. epidermidis*	R	R	R	+
15NF	Neonatal ICU	Physician	*S. epidermidis*	S	R	R	-
17NF	Neonatal ICU	Physician	*S. epidermidis*	R	R	R	+
20NF	Neonatal ICU	Nurse technician	*S. aureus*	R	S	R	+
21M	Neonatal ICU	Nurse technician	*S. aureus*	R	R	S	+
22M	Neonatal ICU	Nurse technician	*S. aureus*	R	R	R	+
26M	Neonatal ICU	Nurse technician	*S. aureus*	R	S	S	-
30M	Neonatal ICU	Nurse	*S. epidermidis*	R	S	R	+
32NF	Neonatal ICU	Nurse technician	*S. aureus*	R	S	R	+
47M	Surgical clinics	Nurse technician	*S. epidermidis*	R	S	R	-
48M	Surgical clinics	Nurse technician	*S. epidermidis*	R	R	R	+
53NF	Surgical clinics	Nurse	*S. epidermidis*	R	R	R	+
54NF	Surgical clinics	Nurse technician	*S. aureus*	R	S	R	-
60NF	Surgical clinics	Nurse technician	*S. epidermidis*	S	R	R	-
66NF	Surgical clinics	Physiotherapist	*S. epidermidis*	S	R	R	+
92M	Neonatal ICU	Physician	*S. epidermidis*	S	R	R	+
93NF	Surgical clinics	Nurse technician	*S. epidermidis*	R	R	R	-
149NF	General ICU	Nurse technician	*S. aureus*	R	R	R	-

PCR: polymerase chain reaction; M: sample from the microbiota of hands; NF: sample from the microbiota of the nasopharynx; R: resistant; S: sensitive; +: mecA gene positive; -: mecA gene negative; ICU: intensive care unit.

TABLE 2 – Validity test results (PCR is the gold standard)

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Disk-diffusion method for cefoxitin	87	15	54.2	50
Oxacillin E-test	67	54	62.5	58.3
Oxacillin screening	93	15	56	66.6

PCR: polymerase chain reaction; PPV: positive predictive value; NPV: negative predictive value.

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According to some authors, due to the existence of other mechanisms of resistance, the test to determine the MIC of oxacillin for staphylococci by the agar gradient diffusion test (E-test®) should be utilized as a confirmatory and/or adjuvant test on bacterial isolates in which the susceptibility pattern is doubtful or difficult to interpret[33]. Other authors believe that the detection of methicillin resistance in coagulase-negative *Staphylococcus* is a challenge for clinical laboratories because many false negative results can be attributed to heterogeneous resistance to methicillin expressed by these microorganisms[8, 15, 27]. Baddour and coworkers[4] suggest the combination of two phenotypic methods for greater sensitivity.

In cases of heterogeneous resistance, subpopulations of resistant and sensitive *S. aureus* can coexist in the same culture, because all bacteria with resistance genes in their genomic DNA have been shown to express them in routine susceptibility tests performed in the laboratory[7].

In Brazil, data on the prevalence of methicillin-resistant *Staphylococcus* (MRS) in health care professionals are still few, mainly with regard to preventive measures. The estimated frequency of MRSA varies between 4.1% and 60.9%[12, 17]. The study of other resistant species of *Staphylococcus* in health care professionals is rare.

CONCLUSION

To evaluate a prevalence of MRS among health care professionals, it is important to determine preventive measures against hospital infections, including making these professionals aware of precautionary measures for the control of infections, considering them as a potential source of infection, especially those colonized by MRSA.

The oxacillin E-test for phenotypic detection of resistance to methicillin combined with PCR for detection of the *mec*A gene, which encodes PBP2a, is recommended for the correct determination of the susceptibility to methicillin in samples of staphylococci. The rapid and correct identification of the gene of methicillin resistance assures the appropriate planning of antibiotic therapy and of epidemiological control measures for MRS.

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RESUMO

Introdução: Staphylococcus spp. *é um importante patógeno associado aos cuidados em saúde, e a identificação de isolados resistentes à meticilina em amostras de colonização pode fornecer dados para auxiliar no sucesso da terapia antimicrobiana.* **Objetivos:** *Determinar a ocorrência de colonização por* Staphylococcus spp. *resistentes à meticilina (MRS) por meio da detecção do gene* mecA *e avaliar diferentes métodos fenotípicos para a detecção presuntiva da resistência à meticilina em amostras da cavidade nasal anterior e das mãos de profissionais de saúde de um hospital universitário no Estado de Pernambuco, Brasil.* **Métodos:** *Foram selecionados 28 isolados de* Staphylococcus spp. *que mostraram perfil intermediário ou resistente à oxacilina, detectado pela técnica de Kirby Bauer. Os métodos utilizados foram o teste de disco difusão de cefoxitina, concentração inibitória mínima pelo E-test de oxacilina,* screening *para avaliação da resistência à oxacilina e reação em cadeia da polimerase (PCR) para detecção do gene* mecA. **Resultados:** *Dos métodos fenotípicos utilizados, apenas o E-test de oxacilina não mostrou diferença estatística significante em relação à PCR para a detecção do gene* mecA, *considerado o método padrão- -ouro.* **Conclusão:** *O E-test de oxacilina foi o melhor método fenotípico utilizado. É necessário detectar corretamente o MRS em indivíduos saudáveis, pois eles podem atuar como portadores, sendo uma fonte potencial de microrganismos envolvidos em infecções hospitalares.*

Unitermos: meticilina; profissionais de saúde; resistência; S. aureus*;* S. epidermidis*;* Staphylococcus spp*.*

REFERENCES

1. ABU HUJIER, N. S; SHARIF, F. A. Detection of methicillin-resistant *Staphylococcus aureus* in nosocomial infections in Gaza Strip African. *J Microbiol Res*, v. 2, p. 235-41, 2008.

2. ANAND, K. B. *et al*. Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for *mec*A gene for detection of MRSA. *Indian J Med Microbiol*, v. 27, n. 1, p. 27-9, 2009.

3. ANTUNES, A. L. S. *et al*. Evaluation of oxacillin and cefoxitin disks for detection of resistance in coagulase negative staphylococci. *Mem Inst Oswal Cruz*, v. 102, n. 6, p. 719-23, 2007.

4. BADDOUR, M. M.; ABUELKHEIR, M. M.; FATANI, A. J. Comparison of *mec*A polymerase chain reaction with phenotypic methods for the detection of methicillin-resistant *Staphylococcus aureus*. *Curr Microbiol*, v. 55, p. 473-9, 2007.

5. BEN-DAVID, D.; MERMEL, L. A.; PARENTAL, S. Methicillin-resistant *S. aureus* transmission: the possible importance of unrecognized health care worker carriage. *Am J Infect Control*, v. 36, n. 2, p. 93-7, 2008.

6. CASEY, A. L.; LAMBERT, P. A.; ELLIOTT, T. S. J. Staphylococci. *Int J Antimicrob Agents*, v. 29, n. 3, p. 23-32, 2007.

7. CHAMBERS, H. F.; DELEO, F. R. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat Rev Microbiol*, v. 7, p. 629-41, 2009.

8. CHANDRAN, A. U.; RENNIE, R. Routine antimicrobial susceptibility testing of coagulase-negative Staphylococci isolated from blood cultures: is it necessary? *Clin Microbiol Infect Dis*, v. 11, p. 1037-40, 2005.

9. CLSI (Clinical and Laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing, sixteenth informational supplement, documentM100-S20.Wayne, PA, USA: CLSI, 2010.

10. CUNY, C. *et al*. Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mec*A homologue in humans in Germany. *PLoS ONE*, v. 6, n. 9, p. e24360, 2011.

11. DALLA VALLE, C. *et al*. Control of MRSA infection and colonisation in an intensive care unit by GeneOhm MRSA assay and culture methods. *BMC Infect Dis*, v. 9, p. 137, 2009.

12. DE CARVALHO, M. J. *et al*. Prevalence of methicillin-resistant and methicillin-susceptible *S. aureus* in the saliva of health professionals. *Clinics*, v. 64, n. 4, p. 295-302, 2009.

13. DE PAULIS, A. N. *et al*. Five-Test Simple scheme for species-level identification of clinically significant coagulase-negative Staphylococci. *J Clin Microbiol*, v. 41, n. 3, p. 1219-24, 2003.

14. ERCIS, S.; SANCAK, B.; HASCELIK, G. A comparison of PCR detection of *mec*A with oxacillin disk susceptibility testing in different media and sceptor automated system for both *Staphylococcus aureus* and coagulase-negative staphylococci isolates. *Indian J Med Microbiol*, v. 26, p. 21-4, 2008.

15. FERREIRA, R. B. R. *et al*. Coagulase-negative Staphylococci: comparison of phenotypic and genotypic oxacilin susceptibility tests and evaluation of the Agar screening test by using different concentration of oxacilin. *J Clin Microbiol*, v. 41, n. 8, p. 3609-14, 2003.

16. FREITAS, M. F. L. *et al*. Staphylococcal toxin genes in strains isolated from cows with subclinical mastitis. *Pesq Vet Bras*, v. 28, n. 12, p. 617-21, 2008.

17. GRUNDMANN, H. *et al*. Emergence and resurgence of methicillin-resistant *S. aureus* as a public-health threat. *Lancet*, v. 368, p. 874-85, 2006.

18. HAAMANN, F.; DULON, M.; NIENHAUSINT, A. MRSA as an occupational disease: a case series. *Arch Occup Environ Health*, v. 84, p. 259-66, 2011.

19. Hidron, A. I. *et al*. National Healthcare Safety Network Team; participating national healthcare safety network facilities. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. *Infect Contr Hosp Epidemiol*, v. 29, p. 996-1011, 2008.

20. HULETSKY, A. *et al*. Identification of methicillin-resistant *S. aureus* carriage in less than 1 hour during a hospital surveillance program. *Clin Infect Dis*, v. 40, n. 7, p. 976-81, 2005.

21. KIM, J. Understanding the evolution of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol New*, v. 31, n. 3, p. 17-23, 2009.

22. LE LOIR, Y.; BARON, F.; GAUTIER, M. *Staphylococcus aureus* and food poisoning. *Genet Mol Res*, v. 2, n. 1, p. 63-76, 2003.

23. MENDES, E. R. *et al*. Ability of Latin America Laboratories to detect antimicrobial resistance patterns: experience of the SENTRY Antimicrobial Surveillance Program (1997-2000). *Braz J Infect Dis*, v. 7, p. 282-9, 2003.

24. MIMICA, M. J. *et al*. Detection of methicillin resistance in *Staphylococcus aureus* isolated from pediatric patients: is the cefoxitin disk diffusion test accurate enough? *Braz J Infect Dis*, v. 43, n. 6, p. 399-406, 2007.

25. MOUSSA, I.; SHIBL, A.M. Molecular characterization of methicillin-resistant *Staphylococcus aureus* recovered from outpatient clinics in Riyadh, Saudi Arabia. *Saudi Med J*, v. 30, p. 611-7, 2009.

26. PALAZZO, I. C.V.; ARAUJO, M. L. C.; DARINI, A. L. C. First report of vancomycin-resistant Staphylococci isolated from healthy carriers in Brazil. *J Clin Microbiol*, v. 43, p. 179-85, 2005.

27. PALAZZO, I. C. V.; DARINI, A. L. C. Evaluation of methods for detecting oxacilin resistance in coagulase-negative staphylococci including cefoxitin disc diffusion. *FEMS microbial Lett*, v. 257, p. 299-305, 2006.

28. PEREZ, L. R. R.; D'AZEVEDO, P. A. Evaluation of the accuracy of various phenotypic tests to detect oxacilin resistance in coagulase-negative staphylococci. *Braz J Infect Dis*, v. 12, n. 3, p. 210-2, 2008.

29. PETINAKI, E. *et al*. Detection of *mecA*, *mecR1* and *mecI* genes among clinical isolates of methicillin-resistant staphylococci by combined polymerase chain reactions. *J Antimicrob Chemother*, v. 47, n. 3, p. 297-304, 2001.

30. RIBAS, R. M.; GONTIJO-FILHO, P. P.; DARINI, A. L. C. Conventional versus molecular tests (multiplex PCR and PCR *mec*A gene) for detection of methicillin resistant *Staphylococcus aureus*. *Braz J Microbiol*, v. 34, n. 1, p. 35-7, 2003.

31. SHARIATI, L. *et al*. Comparison of real-time PCR with disk diffusion, Agar screen and E-test methods for detection of methicillin-resistant *Staphylococcus aureus*. *Curr Microbiol*, v. 61, p. 520-4, 2010.

32. SILVA, E. C. B. F. *et al*. Epidemiological surveillance and susceptibility of *Staphylococcus aureus* among healthcare workers at a reference hospital: preliminary assessment. *Rev Inst Adolfo* Lutz, v. 69, n. 1, p. 126-30, 2010.

33. SOUSA JÚNIOR, F. C. *et al*. Evaluation of different methods for detecting methicillin resistance in *Staphylococcus aureus* isolates in a university hospital located in the northeast of Brazil. *Braz J Microbiol*, v. 41, p. 316-20, 2010.

34. SWENSON, J. M. *et al*. Detection of *mec*A-mediated resistance using reference and commercial testing methods in a collection of *Staphylococcus aureus* expressing boderline oxacillin MICs. *Diag Microbiol Infect Dis*, v. 38, n. 5, p. 1346-50, 2007.

35. YADAV, M. K. *et al*. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from nasal samples by multiplex real-time PCR based on dual priming AT-rich primers. *Folia Microbiol*, v. 57, p. 37-45, 2012.

MAILING ADDRESS

Marcelle Aquino Rabelo
Universidade Federal de Pernambuco; Centro de Ciências da Saúde; Departamento de Medicina Tropical; Av. Prof. Moraes Rego, s/n; Cidade Universitária; CEP: 50670-901 – Recife-PE, Brazil; Tel.: (81) 2126-8527; e-mail: marcelleaquino@yahoo.com.br.