**Oxacillin Resistance and Antimicrobial Susceptibility Profile of *Staphylococcus saprophyticus* and Other Staphylococci Isolated from Patients with Urinary Tract Infection**

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**Key Words**
Urinary tract infection · Susceptibility profile · Oxacillin resistance · *Staphylococcus saprophyticus* · mecA gene · Internal transcribed spacer-PCR

**Abstract**
**Background:** *Staphylococcus saprophyticus* is the second most frequent community-acquired causative agent of urinary tract infection (UTI). The objective of this study was to evaluate the susceptibility profile and resistance detection in *Staphylococcus* species isolated from patients with UTI. **Materials and Methods:** The isolates were investigated using the disk diffusion method, Vitek I system, E-test®, and detection of the mecA gene. **Results:** Most isolates (76.2%) were resistant to oxacillin by the disk diffusion method, followed by those resistant to penicillin (72.2%). The oxacillin disk diffusion method, E-test, and Vitek I method showed higher sensitivity (94.4%) and lower specificity (28.9, 26.5, and 24.0%, respectively) than the cefoxitin disk diffusion test (sensitivity: 83.5%, specificity: 85.5%) for the detection of oxacillin resistance. **Conclusions:** The large number of oxacillin-resistant isolates indicates that the breakpoint value recommended by the Clinical and Laboratory Standards Institute may overestimate oxacillin resistance in *S. saprophyticus*. Thus, changes in these guidelines are necessary for the correct detection of this resistance.

**Introduction**
Urinary tract infection (UTI) is one of the most common diseases in clinical practice [1–3] and the second most common infection in humans after respiratory tract infections [4, 5]. *Staphylococcus saprophyticus* is the second most frequent community-acquired causative agent of acute UTI after *Escherichia coli* [6, 7]. This microorganism is isolated mainly from urine of sexually active young women [8–10] and induces symptoms that are indistinguishable from those caused by *E. coli*. There are also reports of septicemia and pyelonephritis caused by this microorganism [11, 12]. The pathogenicity of other coagulase-negative staphylococci (CoNS) in the urinary tract is generally uncertain, but the clinical significance of some CoNS species (*S. haemolyticus,* *S. epidermidis,* etc.) has been described.
Susceptibility and Resistance Detection in *S. saprophyticus*

**Materials and Methods**

**Strains**

A total of 101 *Staphylococcus* spp. strains isolated from urine samples of different patients were used. The strains were obtained from wards, outpatient clinics, emergency rooms, and several health centers in Botucatu and the surrounding region between March 10 and November 14, 2008, and were sent to the Laboratory of Microbiology, University Hospital, Botucatu School of Medicine (HC-FMB), São Paulo, SP, Brazil, for identification [36]. The study was approved by the Ethics Committee of the Botucatu School of Medicine, UNESP (permit No. 416/08-CEP).

**Criteria for Inclusion and Exclusion**

Included in the study were patients of both genders and all ages whose urine cultures were positive for *Staphylococcus* spp. compatible with UTI, and with a colony count of ≥100,000 colony-forming units per millilitre urine (≥10⁵ CFU/ml) according to the criteria of Kass [34]. Excluded were strains collected from bladder catheters, suprapubic aspirates, and positive urine cultures with colony counts <10⁵ CFU/ml.

**Calculation of Sample Size**

The sample size was calculated by the formula of Fisher and Belle [35] using a 95% confidence interval and a precision of 5% for the expected prevalence of patients with UTI. The proportion of patients with UTI caused by *Staphylococcus* spp. was used as a basis, which was 5% in a study conducted at the Laboratory of Microbiology, University Hospital of Botucatu School of Medicine. Although sample size calculation indicated 73 strains as the minimum number, all *Staphylococcus* spp. strains isolated during the study period that met the inclusion and exclusion criteria were used (n = 101).

**Antimicrobial Susceptibility Testing by the Disk Diffusion Method and Determination of the Minimum Inhibitory Concentration**

The susceptibility profile of the isolates was evaluated according to criteria by the Clinical and Laboratory Standards Institute (CLSI) [37] using the following antimicrobial agents: 1 µg oxacillin, 30 µg cefoxitin, 30 µg cefalothin, 10 µg gentamicin, 30 µg linezolid, 10 µg norfloxacin, 10 U penicillin G, 25 µg sulfa-
methoxazole/trimethoprim, 30 μg vancomycin, 300 μg nitrofurantoin, and 30 μg amoxicillin/clavulanic acid (Oxoid, Basingstoke, UK).

The minimum inhibitory concentration (MIC) of penicillin G, oxacillin, linezolid, and vancomycin against the *Staphylococcus* spp. isolates was determined using E-test® strips and the Vitek I system.

**Detection of Resistance to Oxacillin**

Oxacillin (1 μg) and cefoxitin (30 μg) disks were used to predict resistance to oxacillin mediated by the *mecA* gene. The diameter of the inhibition halo was interpreted according to CLSI criteria (document M100-S18, 2008), considering diameters of ≤12 mm for oxacillin and ≤21 mm for cefoxitin as breakpoint values for *S. aureus* and *S. lugdunensis*. For CoNS, except *S. lugdunensis*, a breakpoint of ≤517 mm for oxacillin and ≤24 mm for cefoxitin was used.

**Detection of β-Lactamase Production and Overproduction**

The strains were tested using a disk containing 30 μg amoxicillin/clavulanic acid (20 μg amoxicillin and 10 μg clavulanic acid). The formation of an inhibition halo of ≥20 mm after 24 h of incubation at 35 °C was established as the susceptibility breakpoint [38].

Strains that were *mecA* negative, susceptible to amoxicillin/clavulanic acid, resistant to oxacillin and/or cefoxitin, and β-lactamase positive in the two tests (Vitek I and nitrocefin disk) were classified as overproducers of β-lactamase.

Production of β-lactamase was evaluated using the Vitek I GPS-652 card and disks impregnated with nitrocefin (chromogenic cephalosporin).

**Extraction of Nucleic Acid and Genotyping of *Staphylococcus* spp.**

Total nucleic acid was extracted from *Staphylococcus* spp. strains cultured on blood agar, inoculated individually into BHI broth, and incubated at 37 °C for 24 h. Nucleic acid was extracted using the Illustra kit (GE Healthcare, Chalfont, UK) according to manufacturer instructions and the extracted DNA was stored at −20 °C.

The *Staphylococcus* spp. isolates were genotyped using conserved primer sequences adjacent to the 16S and 23S genes. This method described by Barry et al. [39] and Couto et al. [40] is known as internal transcribed spacer-PCR. In the present study, genotyping was performed as described by Couto et al. [40] using the G1 (5′ GAA GTC GTA ACA AGG 3′) and L1 (5′ GAA GGC ATC CAC CGT 3′) primers. In addition, primers coa1 (5′ GTA GAT TGG GCA ATT ACA TTT TGG AGG 3′) and coa2 (5′ CGC ATC AGC TTT GTT ATC CCA TGT 3′) were used for the detection of the coagulase (coa) gene according to the protocol of Kearsns et al. [41].

**Detection of the mecA and coa Gene by PCR**

PCR was carried out according to the protocol of Murakami et al. [42] using the mecA1 (5′ AAA ATC GAT GGT AAA GGT TGG 3′) and mecA2 (5′ AGT TCT GCA GTA CCG GAT TGG 3′) primers. In addition, primers coa1 (5′ GTA GAT TGG GCA ATT ACA TTT TGG AGG 3′) and coa2 (5′ CGC ATC AGC TTT GTT ATC CCA TGT 3′) were used for the detection of the coagulase (coa) gene according to the protocol of Kearsns et al. [41].

**Determination of the SCCmec Type in *S. aureus* and CoNS**

The type of SCCmec in *S. aureus* was determined by multiplex PCR as described by Oliveira and de Lencastre [43] and updated by Milheiro et al. [44]. CoNS was determined by multiplex PCR using four primer pairs for loci A, B, D, and E as described by Oliveira and de Lencastre [43] and modified by Machado et al. [45].

**Statistical Analysis**

The accuracy of the antimicrobial susceptibility tests (disk diffusion, E-test, and Vitek I) was evaluated by determination of sensitivity and specificity according to Fletcher et al. [46] using detection of the *mecA* gene as the gold standard.

### Results

**Antimicrobial Susceptibility Testing by the Agar Disk Diffusion Method**

Among the 101 *Staphylococcus* spp. isolates tested, 76.2% were resistant to oxacillin, 72.2% to penicillin, 26.8% to cefoxitin, 17.9% to sulfamethoxazole/trimethoprim, 13.9% to norfloxacin and gentamicin, 10.0% to amoxicillin/clavulanic acid, and 6.0% to cephalothin. Some strains showed intermediate resistance to sulfamethoxazole/trimethoprim (3.9%), norfloxacin (1.9%), and cephalothin (1.0%). *S. saprophyticus* presented high levels of resistance to oxacillin (98.2%) and penicillin (63.2%) (fig. 1). All 101 strains tested were susceptible to vancomycin, linezolid, and nitrofurantoin.

**Detection of the mecA Gene and SCCmec Classification**

Eighteen mecA-positive staphylococci were identified among the 101 strains tested, including six *S. epidermidis* isolates (SCCmec type III in three and type IV in three), four *S. aureus* isolates (type III in two and type II in two), five *S. haemolyticus* isolates (type II in two and three could not be typed), *S. warneri* type III, and two *S. saprophyticus* type IV isolates (table 1).

Comparison of the tests for the detection of oxacillin resistance showed that 15 (83.3%) of the 18 mecA-positive staphylococci were concomitantly resistant to cefoxitin and oxacillin by the disk diffusion test, two were susceptible to cefoxitin and resistant to oxacillin (*S. saprophyticus*), and one was susceptible to both antibiotics (*S. epidermidis*). Seven (12.2%) of the 57 *S. saprophyticus* isolates were resistant to cefoxitin and non of these strains carried the mecA gene, whereas 56 (98.2%) were resistant to oxacillin and only two (3.5%) of these strains carried the mecA gene (table 2).
Table 1. Determination of the SCCmec type in the staphylococcal isolates studied, n (%)

<table>
<thead>
<tr>
<th>SCCmec</th>
<th>Total</th>
<th>S. saprophyticus</th>
<th>S. epidermidis</th>
<th>S. aureus</th>
<th>S. warneri</th>
<th>S. haemolyticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type II</td>
<td>4 (22.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (11.1)</td>
<td>0 (0.0)</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>Type III</td>
<td>6 (33.3)</td>
<td>0 (0.0)</td>
<td>3 (16.7)</td>
<td>2 (11.1)</td>
<td>1 (5.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Type IV</td>
<td>5 (27.8)</td>
<td>2 (11.1)</td>
<td>3 (16.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Not typable</td>
<td>3 (16.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>Total</td>
<td>18 (100)</td>
<td>2 (11.1)</td>
<td>6 (33.4)</td>
<td>4 (22.2)</td>
<td>1 (5.5)</td>
<td>5 (27.8)</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the cefoxitin (30 µg) and oxacillin (1 µg) disk diffusion method, E-test, Vitek I system, and mecA gene for the detection of oxacillin resistance, n (%)

<table>
<thead>
<tr>
<th>Species</th>
<th>PCR</th>
<th>Phenotypic method</th>
<th>E-test</th>
<th>Vitek I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>oxacillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>susceptible</td>
<td>resistant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>cefoxitin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>susceptible</td>
<td>resistant</td>
<td></td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>mecA+ (n = 2)</td>
<td>0 (0.0)</td>
<td>2 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>mecA– (n = 55)</td>
<td>1 (1.8)</td>
<td>54 (98.2)</td>
<td>48 (87.2)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>mecA+ (n = 4)</td>
<td>0 (0.0)</td>
<td>4 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>mecA– (n = 13)</td>
<td>13 (100)</td>
<td>0 (0.0)</td>
<td>12 (92.3)</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>mecA+ (n = 6)</td>
<td>1 (16.7)</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td></td>
<td>mecA– (n = 10)</td>
<td>7 (70.0)</td>
<td>3 (30.0)</td>
<td>8 (80.0)</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>mecA+ (n = 5)</td>
<td>0 (0.0)</td>
<td>5 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>mecA– (n = 3)</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>S. warneri</td>
<td>mecA+ (n = 1)</td>
<td>0 (0.0)</td>
<td>1 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>mecA– (n = 1)</td>
<td>1 (100)</td>
<td>0 (0.0)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>S. lugdunensis</td>
<td>mecA+ (n = 0)</td>
<td>0 (0.0)</td>
<td>1 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>mecA– (n = 1)</td>
<td>1 (100)</td>
<td>0 (0.0)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>mecA+ (n = 18)</td>
<td>1 (5.6)</td>
<td>17 (94.4)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td></td>
<td>mecA– (n = 83)</td>
<td>24 (28.9)</td>
<td>59 (71.1)</td>
<td>71 (85.5)</td>
</tr>
</tbody>
</table>

*One S. haemolyticus isolate presented an intermediate result, but was included as resistant in the table.

The oxacillin disk diffusion method, E-test, and Vitek I system presented higher sensitivity (94.4%) and lower specificity (28.9, 26.5, and 24.0%, respectively) than the cefoxitin disk diffusion test (sensitivity: 83.5%, specificity: 85.5%) (table 3).

### MIC of Antimicrobials Determined by the E-Test and Vitek I Method

Seventy-seven (76.3%) of the 101 Staphylococcus spp. evaluated by the E-test were resistant to oxacillin (MIC$_{50}$: 0.75 µg/ml and MIC$_{90}$: ≥8.0 µg/ml), one presented intermediate resistance (MIC: 0.38 µg/ml), and 85 (84.1%) were resistant to penicillin G (MIC$_{50}$: 0.25 µg/ml and MIC$_{90}$: >32.0 µg/ml). Using the Vitek I system, 81 (80.2%) of the 101 isolates were resistant to oxacillin (MIC$_{50}$: 0.5 µg/ml and MIC$_{90}$: ≥16.0 µg/ml) and 98 (97.1%) were resistant to penicillin (MIC$_{50}$ and MIC$_{90}$: ≥16.0 µg/ml). All isolates were susceptible to linezolid (MIC: 0.5–4.0 µg/ml) and vancomycin (MIC: 0.5–2.0 µg/ml) by the two methods (E-test and Vitek I).

All 57 S. saprophyticus isolates were resistant to oxacillin (MIC: 0.5–3.0 µg/ml by the E-test and 0.5–4.0 µg/ml by the Vitek I method) (table 4).
positive and mecA gene negative, and the remaining two strains were β-lactamase negative and mecA gene positive. Using the Vitek I system, only five (8.8%) of the isolates were β-lactamase negative.

Seven strains were classified as overproducers of β-lactamase since they tested negative for the mecA gene, were resistant to oxacillin and/or cefoxitin by the disk diffusion method, susceptible to amoxicillin/clavulanic acid, and positive for β-lactamase by the two methods used. Three (43.0%) of these strains were identified as S. saprophyticus, two (28.5%) as S. epidermidis, and two (28.5%) as S. haemolyticus (table 5).

**Discussion**

UTI is one of the most common infectious diseases in clinical practice and the second most prevalent infection in humans after respiratory tract infections. The etiological agents most frequently involved in UTIs are enterobacteria, non-fermenters, fungi, enterococci, and staphylococci. The most common and most important staphylococcal species are S. aureus and S. saprophyticus, but other CoNS species have become important over the past years.

Although UTIs caused by S. saprophyticus are well documented, little is known about the antimicrobial resistance and dissemination of this species. According to the CLSI M100-S20 document [47], routine susceptibility testing of urinary S. saprophyticus isolates is not recommended since this microorganism is normally susceptible to the antimicrobial agents used for the treatment of acute uncomplicated UTIs (nitrofurantoin, sulfamethoxazole/trimethoprim, or a fluoroquinolone). However, some of the S. saprophyticus isolates tested in the present study were resistant to sulfamethoxazole/trimethoprim, with a resistance rate of 17.6% (including strains with intermediate resistance), a fact that may lead to therapeutic failure when UTIs are treated empirically. As observed for other CoNS species, antibiotic resistance seems to have emerged also among S. saprophyticus strains. Thus, in addition to their correct identification, antimicrobial susceptibility testing of these strains is necessary.

The results of antimicrobial susceptibility testing obtained in the present study agree with those reported by Kahlmeter [48], Gupta et al. [49], and Camargo et al. [50]. Nitrofurantoin emerged as a good therapeutic alternative even for multi-resistant S. aureus isolates, which were susceptible only to vancomycin, linezolid, and nitrofurantoin among all antimicrobial agents tested. In addition, nitrofurantoin is an antimicrobial agent specific for UTI which is characterized by few side effects, low toxicity, and low cost.

Higashide et al. [51, 52], investigating the susceptibility of 101 S. saprophyticus isolates, showed that all strains were resistant to 1-µg oxacillin disks and were in the resistance range (MIC: 0.5 µg/ml) by the agar dilution method. The MIC ranged from 0.5 to 4.0 µg/ml in 93 isolates, all of them mecA negative. In contrast, the eight isolates with MICs higher than 64 µg/ml were mecA positive, with oxacillin MIC50 and MIC90 of 0.5 and 1.0 µg/ml, respectively.

In the present study, 56 (98.2%) of the 57 S. saprophyticus isolates were resistant to oxacillin, with an inhibition zone of ≤16 mm in 47 (82.4%) isolates. All strains were found to be resistant by the E-test and Vitek I system, with MICs ranging from 0.5 to 3.0 µg/ml and from 0.5 to 4.0 µg/ml, respectively. Only two (3.5%) isolates carried the mecA gene, in agreement with the findings reported by Higashide et al. [52]. Similar results have been reported by
Ramotar et al. [53] who tested 83 S. saprophyticus strains. All isolates were mecA positive and resistant to oxacillin (inhibition zone diameter of ≤17 mm) by the disk diffusion method, 77 were resistant by the broth microdilution method, 73 by the agar dilution method, and all 83 isolates were resistant by the Vitek I system (MIC: 0.5 µg/ml).

In the study of Higashide et al. [52], the inhibition zone diameter in the cefoxitin (30 µg) disk diffusion test was ≤16 mm for mecA-positive isolates and ranged from 22 to 33 mm for mecA-negative isolates. Considering the current breakpoint, four mecA-negative isolates were resistant by the cefoxitin disk test. The authors therefore recommended a breakpoint of 19 mm to correctly characterize mecA gene-mediated resistance in S. saprophyticus. In the present study, seven (12.2%) of the 57 S. saprophyticus isolates were resistant to cefoxitin, with an inhibition zone diameter of 23–24 mm, except for one isolate which presented a diameter of 16 mm. However, none of these isolates was mecA positive. Thus, only one isolate would be classified as resistant when the breakpoint recommended by these authors is used, a marked improvement in the detection of these isolates.

The cefoxitin disk can be used as an alternative or in combination with oxacillin to improve detection of the mecA gene. The present study identified two S. saprophyticus isolates that were mecA positive and presented an inhibition zone diameter of 27–28 mm (susceptible) for cefoxitin and of 12–17 mm (resistant) for oxacillin. These findings demonstrate that, even when the breakpoint for cefoxitin proposed by Higashide et al. [52] is used, inconsistent results may still be obtained for CoNS such as S. saprophyticus.

With respect to the detection of oxacillin resistance, the oxacillin disk diffusion method, E-test, and Vitek I system showed higher sensitivity (94.4%) than the cefoxitin disk test (83.5%), but low specificity (28.9, 26.5, and 24.0%, respectively) mainly because of the large number of S. saprophyticus isolates (57/101). Better specificity was obtained with the cefoxitin disk test (85.5%). Studies [30, 54, 55] have reported a sensitivity of 92.5–94.5% and a specificity of 96.0–98.8% for the oxacillin disk diffusion test and a sensitivity of 92.5–100% and a specificity of 90.7–98.8% for the cefoxitin disk test. However, no or only a small number of S. saprophyticus isolates were tested in those studies, a fact explaining the low specificity observed in the present investigation.

The difference in the distribution of MIC between mecA-positive and -negative strains demonstrated by Higashide et al. [52] was clearly seen in the present study in which all mecA-positive isolates presented MICs ranging from 64 to 256 µg/ml and mecA-negative isolates had MICs ranging from 0.5 to 4.0 µg/ml. These findings suggest that the MIC could be used to predict oxacillin resistance if the current breakpoint value would be reconsidered. However, the two mecA-positive isolates of this study had oxacillin MICs of 0.75 and 1.0 µg/ml, values much lower than those found by Higashide et al. [52], demonstrating that an increase in the MIC may also fail to detect some isolates.

In summary, the disk diffusion method, E-test, or Vitek I system cannot be recommended for the detection of oxacillin resistance in S. saprophyticus isolates, since species that do not carry the mecA gene will be classified as resistant when the current breakpoint is used, a fact resulting in the overestimation of oxacillin resistance. Similar results have been reported by Ramotar et al. [53] for the broth microdilution method, agar dilution test, Vitek I system, and disk diffusion method, and by Higashide et al. [51, 52] for the agar dilution and disk diffusion methods.

Resistance to β-lactam antibiotics among S. saprophyticus isolates has been reported in Japan [42], Canada [31], United States [56], and Brazil [57]. However, studies investigating resistance mediated by the mecA gene in S. saprophyticus, particularly in urinary isolates, are scarce. The prevalence of mecA-positive S. saprophyticus found in the present study was 3.5% (2/57), a rate lower than that reported by Higashide et al. [52] (7.9%, 8/101). The frequency of isolation of S. saprophyticus has not been reported so far and we believe that the prevalence of oxacillin resistance is increasing among clinical isolates. Therefore, correct identification of S. saprophyticus and susceptibility testing are necessary, in addition to the fast and correct detection of the type of resistance. Further studies are needed to determine the extent of the increase in resistance and the presence of mecA-positive isolates.

The CLSI does not recommend susceptibility testing for S. saprophyticus. However, in laboratories where this test is performed, one of the major problems related to resistance detection in S. saprophyticus is the overestimation of oxacillin resistance. According to the CLSI, when this resistance is detected, the strain should be reported as resistant to all β-lactam antibiotics, except for novel cephalosporins that possess anti-MRSA activity (ceftaroline and ceftobiprole). Thus, automated test systems such as the Vitek I system already display these corrected results, reducing the antimicrobial susceptibility profile of S. saprophyticus and increasing the unnecessary use of broad-spectrum antibiotics which contributes to the increased selection of resistant strains.
The increase in the populations of CoNS with heterogeneous oxacillin resistance is one of the factors that impair their detection in clinical analysis laboratories [31]. However, we found no staphylococcal strain expressing visible heterogeneous resistance by the disk diffusion method or E-test, in contrast to reports of CoNS isolated from other clinical materials. However, one S. epidermidis isolate was mecA positive and showed susceptibility to oxacillin in all tests used and to cefoxitin, suggesting the lack of phenotypic expression of oxacillin resistance or the presence of heterogeneous resistance.

In 2009, Soderquist and Berglund [58] identified mecA-positive S. saprophyticus in Sweden that carried three different types of SCCmec. Five isolates were characterized as SCCmec type III and three isolates could not be typed. In the present study, the two mecA-positive S. saprophyticus isolates were characterized as type IV. These strains were isolated from patients seen at a health center and presented the same susceptibility profile (resistant to oxacillin and susceptible to all other antimicrobial agents tested). SCCmec type IV does not carry resistance genes other than mecA. This type is responsible for most community-acquired infections and presents multiple subtypes. According to Ito et al. [25], since SCCmec type IV is a smaller element, it can be acquired more frequently because the transfer efficiency is higher the smaller the DNA. This fact suggests that the prevalence of diseases caused by clones carrying SCCmec type IV tends to increase. Further studies involving species isolated from urine are needed to clarify this aspect.

Isolates carrying SCCmec type III contain a large number of resistance genes [19]. In the present study, all isolates with SCCmec types II and III were resistant to cefoxitin and oxacillin and showed the highest level of resistance to the antimicrobial agents tested, except for one S. haemolyticus type II strain which was resistant only to oxacillin, cefoxitin, and penicillin and one S. epidermidis type III strain which was resistant only to penicillin.

The nitrocefin disk test (cefinase) for detection of β-lactamase production was positive in only 37 (36.6%) of the Staphylococcus spp. studied. In contrast, Cunha and Lopes [59] found that 71.8% of CoNS isolated from newborns hospitalized in the neonatal unit of HC-FMB produced β-lactamase, all of them being nosocomial strains. According to Hovielius and Mardh [60], chromogenic cephalosporin (nitrocefin) does not detect β-lactamase production in S. saprophyticus. In contrast, Latham et al. [61] identified β-lactamase production by the chromogenic cephalosporin method in 50 (75%) of 67 S. saprophyticus isolates. McDougal and Thornberry [62] evaluated β-lactamase production in 66 S. aureus strains using the nitrocefin disk test and all strains tested positive. However, the color change was weak when the penicillin MIC was low (≤16 µg/ml). In the present study, all S. saprophyticus isolates that were negative in the nitrocefin disk test exhibited penicillin MICs ranging from 0.125 to 0.38 µg/ml, except for one strain with a MIC of 0.94 µg/ml. In contrast, two of the three positive isolates had high MICs (1.0 and 1.5 µg/ml) and the MIC was 0.19 µg/ml for the other strain.

Comparison of the results of β-lactamase production detected by the nitrocefin disk test and Vitek I system revealed a higher rate of detection for the latter (36.6 vs. 87.1%). Therefore, the Vitek I system showed a better correlation with the penicillin MIC determined by the E-test, which detected 84.1% of resistant strains. However, some discrepancies were observed. Seven (6.9%) of the 101 staphylococcal isolates studied were overproducers of β-lactamase. This rate is higher than those reported by Ghoshal et al. [38] who identified four (4.4%) overproducing strains among 90 CoNS, and by Pereira et al. [55] who detected only one overproducing isolate among 100 S. aureus strains tested.

The large number of oxacillin-resistant isolates found in the present study is probably the result of modifications in PBPs other than PBP2a, or might even be due to the fact that the breakpoint value recommended by the CLSI overestimates oxacillin resistance in S. saprophyticus, suggesting the need for changes in these guidelines to permit the correct detection of this resistance.

Acknowledgment

This study was supported by the Fundação de Amparo à Pesquisa do Estado São Paulo (FAPESP), grant 2009/10083-5 and 2012/25108-6.

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Chemotherapy 2012;58:482–491
DOI: 10.1159/000346529


