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

Adriano M. Ferreira^{a, b} Mariana F. Bonesso^{a, b} Alessandro L. Mondelli^c
Carlos H. Camargo^c Maria de Lourdes R.S. Cunha^{a, b}

^aDepartment of Microbiology and Immunology, Botucatu Biosciences Institute, and Departments of

^bTropical Diseases and ^cInternal Medicine, Botucatu School of Medicine, UNESP – Universidade Estadual Paulista, Botucatu, Brazil

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 rec-

ommended 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.

Copyright © 2013 S. Karger AG, Basel

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 undistinguishable 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. epi-*

dermidis, *S. simulans*, *S. sciuri*, *S. capitis*, *S. xylosus*, *S. warneri*, *S. cohnii*, *S. lentus*, and *S. hominis*) in UTIs has been demonstrated [13, 14].

The production of PBP2a, a penicillin-binding protein with extremely low affinity for β -lactam antibiotics, certainly is the main mechanism responsible for resistance to oxacillin. However, other mechanisms such as modifications in other PBPs and overproduction of β -lactamases have been described [15, 16]. The gene encoding PBP2a, the *mecA* gene, and its regulatory genes are located on a mobile genetic element, called the staphylococcal cassette chromosome *mec* (SCC*mec*) [17, 18], which serves as a vehicle for the exchange of resistance genes between *Staphylococcus* spp. and is found in a variety of CoNS species and *S. aureus* [19–21]. At least 11 types of SCC*mec* are known [22], which differ in terms of the number of genes they contain [23]. Strains carrying SCC*mec* types I, IV, and V are generally more susceptible to antibiotics, since these types do not contain resistance genes other than *mecA* [24]. SCC*mec* types I, II, and III mainly cause nosocomial infections, and type IV is the main causative agent of community-acquired infections [25, 26].

Although resistance mediated by the *mecA* gene is found intrinsically in all cells of a resistant population, the gene might be expressed in only a small percentage of cells, a fact impairing the interpretation of tests for resistance detection. This phenomenon is called heterogeneous resistance. Resistance to methicillin has also been described for species that do not carry the *mecA* gene and is called borderline resistance. Two mechanisms have been proposed to explain this type of resistance: the first involves the inactivation of oxacillin mediated by the overproduction of β -lactamase, which results in the partial hydrolysis of the β -lactam ring. These strains are known as borderline oxacillin-resistant *S. aureus*. The second mechanisms involve the production of modified PBPs with low affinity for oxacillin, and these strains are called modified penicillin-binding protein *S. aureus* [27–30].

The increase in the population of CoNS with heterogeneous oxacillin resistance is one of the reasons that make the detection of these strains in clinical analysis laboratories difficult. In some cases, even automated test systems are unable to detect this type of resistance [31, 32]. The disk diffusion test is the least reliable method for the detection of oxacillin resistance [33]. Nevertheless, this method is widely used in clinical microbiology laboratories because of its easy and rapid application and low cost. Although oxacillin is usually not indicated for the treatment of UTIs, the drug is used to predict resistance or

susceptibility to β -lactam antibiotics. Therefore, the incorrect detection of this type of resistance may result in the unnecessary use of broad-spectrum antibiotics and consequently contributes to the selection of resistant strains.

The detection of oxacillin resistance mediated by the *mecA* gene continues to be one of the major challenges faced by clinical microbiology laboratories, which require rapid, reliable and low-cost detection. The objective of the present study is to evaluate the detection of oxacillin resistance and the antimicrobial susceptibility profile of *S. saprophyticus* and other staphylococci isolated from patients with UTI.

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 $\geq 100,000$ colony-forming units per milliliter urine ($\geq 10^5$ 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 $<10^5$ 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 μ g oxacillin, 30 μ g cefoxitin, 30 μ g cephalothin, 10 μ g gentamicin, 30 μ g linezolid, 10 μ g norfloxacin, 10 U penicillin G, 25 μ g 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 ≤17 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' CAA 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 Kearns 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 TTG 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 Kearns 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 Milheirico 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 none 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).

Fig. 1. Antimicrobial susceptibility profile of *S. saprophyticus* determined by the disk diffusion method. Antimicrobial agents: FOX = ceftiofur; OX = oxacillin; KF = cephalothin; P = penicillin; SXT = sulfamethoxazole/trimethoprim; NOR = norfloxacin; CN = gentamicin; F = nitrofurantoin; LZD = linezolid; VA = vancomycin; AMC = amoxicillin/clavulanic acid.

Table 1. Determination of the SCCmec type in the staphylococcal isolates studied, n (%)

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

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

Species	PCR	Phenotypic method							
		disk diffusion				E-test		Vitek I	
		oxacillin		ceftiofur		oxacillin		oxacillin	
		susceptible	resistant	susceptible	resistant	susceptible	resistant	susceptible	resistant
<i>S. saprophyticus</i>	<i>mecA</i> + (n = 2)	0 (0.0)	2 (100)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	2 (100)
	<i>mecA</i> - (n = 55)	1 (1.8)	54 (98.2)	48 (87.2)	7 (12.8)	0 (0.0)	55 (100)	0 (0.0)	55 (100)
<i>S. aureus</i>	<i>mecA</i> + (n = 4)	0 (0.0)	4 (100)	0 (0.0)	4 (100)	0 (0.0)	4 (100)	0 (0.0)	4 (100)
	<i>mecA</i> - (n = 13)	13 (100)	0 (0.0)	12 (92.3)	1 (7.7)	12 (92.3)	1 (7.7)	12 (92.3)	1 (7.7)
<i>S. epidermidis</i>	<i>mecA</i> + (n = 6)	1 (16.7)	5 (83.3)	1 (16.7)	5 (83.3)	1 (16.7)	5 (83.3)	1 (16.7)	5 (83.3)
	<i>mecA</i> - (n = 10)	7 (70.0)	3 (30.0)	8 (80.0)	2 (20.0)	7 (70.0)	3 (30.0)	7 (70.0)	3 (30.0)
<i>S. haemolyticus</i>	<i>mecA</i> + (n = 5)	0 (0.0)	5 (100)	0 (0.0)	5 (100)	0 (0.0)	5 (100)	0 (0.0)	5 (100)
	<i>mecA</i> - (n = 3)	1 (33.3)	2 (66.7)	1 (33.3)	2 (66.7)	1 (33.3)	2 (66.7) ^a	0 (0.0)	3 (100)
<i>S. warneri</i>	<i>mecA</i> + (n = 1)	0 (0.0)	1 (100)	0 (0.0)	1 (100)	0 (0.0)	1 (100)	0 (0.0)	1 (100)
	<i>mecA</i> - (n = 1)	1 (100)	0 (0.0)	1 (100)	0 (0.0)	1 (100)	0 (0.0)	1 (100)	0 (0.0)
<i>S. lugdunensis</i>	<i>mecA</i> + (n = 0)	0 (0.0)	1 (100)	0 (0.0)	1 (100)	0 (0.0)	1 (100)	0 (0.0)	1 (100)
	<i>mecA</i> - (n = 1)	1 (100)	0 (0.0)	1 (100)	0 (0.0)	1 (100)	0 (0.0)	1 (100)	0 (0.0)
Total	<i>mecA</i> + (n = 18)	1 (5.6)	17 (94.4)	3 (16.7)	15 (83.3)	1 (5.6)	17 (94.4)	1 (5.6)	17 (94.4)
	<i>mecA</i> - (n = 83)	24 (28.9)	59 (71.1)	71 (85.5)	12 (14.5)	22 (26.5)	61 (73.5)	20 (24.1)	63 (75.9)

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

Table 3. Sensitivity and specificity of the phenotypic methods used for the detection of oxacillin resistance in the *Staphylococcus* spp.

Accuracy test	Cefoxitin (disk)	Oxacillin (disk)	Oxacillin (E-test)	Oxacillin (Vitek I)
Sensitivity, %	83.3	94.4	94.4	94.4
Specificity, %	85.5	28.9	26.5	24.0

Table 4. Comparison of the MIC for *S. saprophyticus* determined by the E-test and Vitek I system

Antimicrobial agent	Sensitive, n (%)	Resistant, n (%)	MIC ₅₀ , µg/ml	MIC ₉₀ , µg/ml	MIC, µg/ml (range)
Oxacillin (E-test)	0 (0)	57 (100)	0.75	1.0	0.5–3.0
Oxacillin (Vitek I)	0 (0.0)	57 (100)	0.5	0.5	0.5–4.0
Penicillin G (E-test)	9 (15.8)	48 (84.2)	0.19	0.38	0.19–1.5
Penicillin G (Vitek I)	0 (0.0)	57 (100)	≥16	≥16	≥16
Linezolid (E-test)	57 (100)	0 (0.0)	0.75	1.0	0.5–1.0
Linezolid (Vitek I)	57 (100)	0 (0.0)	≤2.0	4.0	≤2.0–4.0
Vancomycin (E-test)	57 (100)	0 (0.0)	1.0	1.0	0.5–1.5
Vancomycin (Vitek I)	57 (100)	0 (0.0)	2.0	2.0	1.0–2.0

Sensitivity and Specificity of the Phenotypic Methods for the Detection of Oxacillin Resistance in *Staphylococcus* spp.

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 (MIC₅₀: 0.75 µg/ml and MIC₉₀: ≥8.0 µg/ml), one presented intermediate resistance (MIC: 0.38 µg/ml), and 85 (84.1%) were resistant to penicillin G (MIC₅₀: 0.25 µg/ml and MIC₉₀: >32.0 µg/ml). Using the Vitek I system, 81 (80.2%) of the 101 isolates were resistant to oxacillin (MIC₅₀: 0.5 µg/ml and MIC₉₀: ≥8.0 µg/ml) and 98 (97.1%) were resistant to penicillin (MIC₅₀ and MIC₉₀: ≥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).

Fig. 2. Detection of β-lactamase production by the nitrocefin disk test and Vitek I system.

β-Lactamase Production and Overproduction

The nitrocefin disk test was positive in only 37 (36.6%) strains, whereas the Vitek I system detected 88 (87.1%) β-lactamase-producing strains (fig. 2). Using the nitrocefin disk test for the detection of β-lactamase production, 52 (91.2%) of the 57 *S. saprophyticus* isolates were β-lactamase and *mecA* gene negative, three were β-lactamase

Table 5. Strains overproducing β -lactamase

Species	<i>mecA</i>	Disk, mm			E-test, $\mu\text{g/ml}$		β -Lactamase	
		oxacillin	cefoxitin	amoxicillin/clavulanic acid	oxacillin	oxacillin	nitrocefin	Vitek I
<i>S. saprophyticus</i>	Negative	R (16)	S (28)	S (26)	R (0.75)	R (4.0)	positive	positive
<i>S. saprophyticus</i>	Negative	R (14)	S (28)	S (22)	R (1.0)	R (0.5)	positive	positive
<i>S. saprophyticus</i>	Negative	R (12)	S (25)	S (30)	R (0.75)	R (0.5)	positive	positive
<i>S. epidermidis</i>	Negative	R (0)	R (10)	S (27)	R (1.0)	R (4.0)	positive	positive
<i>S. epidermidis</i>	Negative	R (0)	R (15)	S (27)	R (1.0)	R (≥ 8.0)	positive	positive
<i>S. haemolyticus</i>	Negative	R (10)	R (17)	S (21)	R (2.0)	R (≥ 8.0)	positive	positive
<i>S. haemolyticus</i>	Negative	R (11)	R (20)	S (26)	I (0.38)	R (≥ 8.0)	positive	positive

S = Susceptible; I = intermediate; R = resistant.

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 $\mu\text{g/ml}$) by the agar dilution method. The MIC ranged from 0.5 to 4.0 $\mu\text{g/ml}$ in 93 isolates, all of them *mecA* negative. In contrast, the eight isolates with MICs higher than 64 $\mu\text{g/ml}$ were *mecA* positive, with oxacillin MIC₅₀ and MIC₉₀ of 0.5 and 1.0 $\mu\text{g/ml}$, 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 $\mu\text{g/ml}$ and from 0.5 to 4.0 $\mu\text{g/ml}$, 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 $\mu\text{g/ml}$).

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 $\mu\text{g/ml}$ and *mecA*-negative isolates had MICs ranging from 0.5 to 4.0 $\mu\text{g/ml}$. 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 $\mu\text{g/ml}$, 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 SCC*mec*. Five isolates were characterized as SCC*mec* 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). SCC*mec* 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 SCC*mec* 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 SCC*mec* type IV tends to increase. Further studies involving species isolated from urine are needed to clarify this aspect.

Isolates carrying SCC*mec* type III contain a large number of resistance genes [19]. In the present study, all isolates with SCC*mec* 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 (cefinaise) 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 Hovelius 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 Thornsberry

[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 $\mu\text{g/ml}$). 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 $\mu\text{g/ml}$, except for one strain with a MIC of 0.94 $\mu\text{g/ml}$. In contrast, two of the three positive isolates had high MICs (1.0 and 1.5 $\mu\text{g/ml}$) and the MIC was 0.19 $\mu\text{g/ml}$ 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.

References

- 1 Stamm WE, Hooton TM: Management of urinary tract infections in adults. *N Engl J Med* 1993;329:1328–1334.
- 2 Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE: Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). *Clin Infect Dis* 1999;29:745–758.
- 3 Lopes HV, Tavares W: Diagnóstico das infecções do trato urinário. *Rev Assoc Med Bras* 2005;51:301–312.
- 4 Valiquette L: Urinary tract infections in women. *Can J Urol* 2001;8:6–12.

- 5 Hörner R, Vissotto R, Mastella A, Salla A, Meneghetti B, Dal Forno NL, Righi RA, Oliveira LO: Prevalência de microrganismos em infecções do trato urinário de pacientes atendidos no Hospital Universitário de Santa Maria. *Rev Bras Anal Clin* 2006;38:147–150.
- 6 Henry D, Ellison W, Sullivan J, Mansfield DL, Magner DJ, Dorr MB, Talbot GH: Treatment of community-acquired acute uncomplicated urinary tract infection with sparflaxacin versus ofloxacin. The Sparflaxacin Multi Center UUTI Study Group. *Antimicrob Agents Chemother* 1998;42:2262–2266.
- 7 Gupta K, Scholes D, Stamm WE: Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. *JAMA* 1999;281:736–738.
- 8 Jordan PA, Iravani A, Richard GA, Baer H: Urinary tract infection caused by *Staphylococcus saprophyticus*. *J Infect Dis* 1980;142:510–515.
- 9 Abrahamsson K, Hansson S, Jodal U, Lincoln K: *Staphylococcus saprophyticus* urinary tract infections in children. *Eur J Pediatr* 1993;152:69–71.
- 10 Svanborg C, Godaly G: Bacterial virulence in urinary tract infections. *Infect Dis Clin North Am* 1997;11:513–529.
- 11 Colledge CL: *Staphylococcus saprophyticus* bacteremia. *J infect Dis* 1989;157:215.
- 12 Lee W, Carpenter RJ, Phillips LE, Faro S: Pyelonephritis and sepsis due *S. saprophyticus*. *J Infect Dis* 1987;155:1079.
- 13 Guirguitova B, Chancova D, Zozikov B: Staphylococci as uropathogens – frequency of isolation in hospitalized patients and sensitivity to antimicrobial agents. *Ann Urol* 2002;36:341–347.
- 14 Alcaráz LE, Satorres SE, Lucero RM, Puig de Centorbi ON: Species identification, slime production and oxacillin susceptibility in coagulase-negative staphylococci isolated from nosocomial specimens. *Braz J Microbiol* 2003;34:45–51.
- 15 De Giusti M, Pacifico L, Tufi D, Panero A, Boccia A, Chiesa C: Phenotypic detection of nosocomial *mecA*-positive coagulase-negative staphylococci from neonates. *J Infect Chemother* 1999;44:351–358.
- 16 Jarlov JO, Sorensen CB, Espersen F, Mortensen I, Hougaard DM, Rosdahl VT: Evaluation of different methods for the detection of methicillin resistance in coagulase-negative staphylococci. *J Antimicrob Chemother* 1997;40:241–249.
- 17 Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG: The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *PNAS* 2002;99:7687–7692.
- 18 Fiolic Z, Bosnjak Z, Snajdar I, Gregorek AC, Kalenic S, Budimir A: The screening of methicillin-resistant *Staphylococcus aureus* in vascular surgery patients: a comparison of molecular testing and broth-enriched culture. *Chemotherapy* 2012;58:330–336.
- 19 Ito T, Katayama Y, Hiramatsu K: Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. *Antimicrob Agents Chemother* 1999;43:1449–1458.
- 20 Katayama Y, Ito T, Hiramatsu K: Genetic organization of the chromosome region surrounding *mecA* in clinical staphylococcal strains: role of IS431-mediated *mecI* deletion in expression of resistance in *mecA*-carrying, low-level methicillin-resistant *Staphylococcus haemolyticus*. *Antimicrob Agents Chemother* 2001;45:1955–1963.
- 21 Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K: Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. *Drug Resist Updat* 2003;6:41–52.
- 22 International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC): Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. *Antimicrob Agents Chemother* 2009;53:4961–4967.
- 23 Hiramatsu K, Longzhu CUI, Kuroda M, Ito K: The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. *Trends in Microbiol* 2001;9:486–493.
- 24 Hanssen A, Sollid JUE: SCC*mec* in staphylococci: genes on the move. *FEMS Immunol Med Microbiol* 2006;46:8–20.
- 25 Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K, Takeuchi F: Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. *Antimicrob Agents Chemother* 2004;48:2637–2651.
- 26 Martins A, Cunha MLRS: Methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci: epidemiological and molecular aspects. *Microbiol Immunol* 2007;51:787–795.
- 27 Martins A, Pereira VC, Cunha MLRS: Oxacillin resistance of *Staphylococcus aureus* isolated from the university hospital of Botucatu Medical School in Brazil. *Chemotherapy* 2010;56:112–119.
- 28 McDougal LK, Thornsberry C: The role of the β -lactamases in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. *J Clin Microbiol* 1986;23:832–839.
- 29 Kereses JS, Gál Z, Barabás G, Benko I, Szabó I: Investigation of beta-lactamases in clinical isolates of *Staphylococcus aureus* for further explanation of borderline methicillin resistance. *Chemotherapy* 2005;51:300–304.
- 30 Caierão J, Musskopf M, Superti S, Roesch E, Dias CG, d'Azevedo PA: Evaluation of phenotypic methods for methicillin resistance characterization in coagulase-negative staphylococci (CNS). *J Med Microbiol* 2004;53:1195–1199.
- 31 Cauwelier B, Gordts B, Descheemaeker VAN, Landuyt H: Evaluation of a disk diffusion method with cefoxitin (30 μ g) for detection of methicillin-resistant *Staphylococcus aureus*. *Eur J Clin Microbiol* 2004;6:389–392.
- 32 Frebourg NB, Nouet D, Lemée L, Martin E, Lemeland JF: Comparison of ATB staph, rapid ATB staph, Vitek, and E-test methods for detection of oxacillin heteroresistance in staphylococci possessing *mecA*. *J Clin Microbiol* 1998;36:52–57.
- 33 York MK, Gibbs L, Chehab F, Brooks GF: Comparison of PCR detection of *mecA* with standard susceptibility testing methods to determine methicillin resistance in coagulase-negative staphylococci. *J Clin Microbiol* 1996;34:249–253.
- 34 Kass EH: Asymptomatic infections of the urinary tract. *Trans Assoc Am Phys* 1956;69:56–64.
- 35 Fisher LD, Belle GV: Biostatistics: a methodology for health science. New York, John Wiley, 1993.
- 36 Cunha MLRS, Sinzato YK, Silveira LVA: Comparison of methods for the identification of coagulase-negative staphylococci. *Mem Inst Oswaldo Cruz* 2004;99:855–860.
- 37 Clinical and Laboratory Standards Institute (CLSI): Performance Standards for Antimicrobial Susceptibility Testing, 18th Information Supplement. M100-S18. Clinical and Laboratory Standards Institute, Wayne, Pa., 2008.
- 38 Ghoshal U, Prasad KN, Singh M, Tiwari DP, Ayyagari A: A comparative evaluation of phenotypic and molecular methods for the detection of oxacillin resistance in coagulase-negative staphylococci. *J Infect Chemother* 2004;10:86–89.
- 39 Barry T, Colleran G, Glennon M, Dunican LK, Gannon F: The 16S/23S ribosomal spacer region as a target for DNA probes to identify eubacteria. *PCR Methods Appl* 1991;1:51–56.
- 40 Couto I, Pereira S, Miragaia M, Sanches IS, de Lencastre H: Identification of clinical staphylococcal isolates from humans by internal transcribed spacer PCR. *J Clin Microbiol* 2001;39:3099–3103.
- 41 Kearns AM, Seiders PR, Wheeler J, Freeman R, Steward M: Rapid detection of methicillin-resistant staphylococci by multiplex PCR. *J Hosp Infect* 1999;43:33–37.
- 42 Murakami K, Minamide K, Wada K, Nakamura E, Teraoka H, Watanabe S: Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol* 1991;29:2240–2244.
- 43 Oliveira DC, de Lencastre H: Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002;46:2155–2161.
- 44 Milheirico C, Oliveira DC, de Lencastre H: Update to the multiplex PCR strategy for assignment of *mec* element types in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2007;51:3374–3377.

- 45 Machado ABMP, Reiter KC, Paiva RM, Barth AL: Distribution of staphylococcal cassette chromosome *mec* (SCC*mec*) types I, II, III, and IV in coagulase-negative staphylococci from patients attending a tertiary hospital in southern Brazil. *J Medical Microbiol* 2007;56:1328–1333.
- 46 Fletcher RH, Fletcher SW, Wagner EH, Diagnóstico; in: Fletcher RH, Fletcher SW, Wagner EH (eds): *Epidemiologia Clínica*, ed 3. Porto Alegre, Artes Médicas, 1991, pp 68–107.
- 47 Clinical and Laboratory Standards Institute (CLSI): *Performance Standards for Antimicrobial Susceptibility Testing*, 20th Information Supplement. M100-S20. Clinical and Laboratory Standards Institute, Wayne, Pa., 2010.
- 48 Kahlmeter G: An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS project. *J Antimicrob Chemother* 2003;51:69–76.
- 49 Gupta K, Sahm DF, Mayfield D, Stamm WE: Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis. *Clin Infect Dis* 2001;33:89–94.
- 50 Camargo CBS, Pedro CC, Lourenço DS, Gironi RHA, Martinez R: Infecção de vias urinárias na comunidade de Ribeirão Preto – SP: etiologia, sensibilidade bacteriana a antimicrobianos e implicações terapêuticas. *Medicina* 2002;35:173–178.
- 51 Higashide M, Kuroda M, Omura CTN, Kumano M, Ohkawa S, Ichimura S, Ohta T: Methicillin-resistant *Staphylococcus saprophyticus* isolates carrying staphylococcal cassette chromosome *mec* have emerged in urogenital tract infections. *Antimicrob Agents Chemother* 2008;52:2061–2068.
- 52 Higashide M, Kuroda M, Ohkawa S, Ohta T: Evaluation of a cefoxitin disk diffusion test for the detection of *mecA*-positive methicillin-resistant *Staphylococcus saprophyticus*. *Int J Antimicrob Agents* 2006;27:500–504.
- 53 Ramotar K, Woods W, Toye B: Oxacillin susceptibility testing of coagulase-negative staphylococci using the disk diffusion method and the Vitek GPS-105 card. *Diagn Microbiol Infect Dis* 2002;42:291–294.
- 54 Palazzo ICV, Darini ALC: Evaluation of methods for detecting oxacillin resistance in coagulase-negative Staphylococci including cefoxitin disc diffusion. *FEMS Microbiol Lett* 2006;257:299–305.
- 55 Pereira VC, Martins A, Rugolo LMSS, Cunha MLRS: Detection of oxacillin resistance in *Staphylococcus aureus* isolated from the neonatal and pediatric units a Brazilian teaching hospital. *Clin Med Ped* 2009;3:23–31.
- 56 Swenson JM, Tenover FC: Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. *J Clin Microbiol* 2005;43:3818–3823.
- 57 Souza AL, Secchi C, Reiter KC, Perez LRR, Freitas ALP, d'Azevedo PA: Evaluation of oxacillin and cefoxitin disks for detection of resistance in coagulase negative staphylococci. *Mem Inst Oswaldo Cruz* 2007;102:719–723.
- 58 Soderquist B, Berglund C: Methicillin-resistant *Staphylococcus saprophyticus* in Sweden carries various types of staphylococcal cassette chromosome *mec* (SCC*mec*). *Eur Soc of Clin Microbiol Infect Dis* 2009;15:1170–1191.
- 59 Cunha MLRS, Lopes CAM: Estudo da produção de beta-lactamase e sensibilidade às drogas em linhagens de estafilococos coagulase-negativos isolados de recém-nascidos. *J Bras Patol Med Lab* 2002;38:281–290.
- 60 Hovelius B, Mardh P: *Staphylococcus saprophyticus* as a common cause of urinary tract infections. *Rev Infect Dis* 1984;6:328–337.
- 61 Latham RH, Zeleznik D, Minshew BH, Schoenkecht FD, Stamm WE: *Staphylococcus saprophyticus* β -lactamase production and disk diffusion susceptibility testing for three β -lactam antimicrobial agents. *Antimicrob Agents Chemother* 1984;26:670–672.
- 62 McDougal LK, Thornsberry C: The role of the β -lactamases in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. *J Clin Microbiol* 1986;23:832–839.