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Accuracy of Cefoxitin Disk Testing for Characterization of Oxacillin Resistance Mediated by Penicillin-Binding Protein 2a in Coagulase-Negative Staphylococci

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The Clinical and Laboratory Standards Institute (CLSI) proposed, beginning in 2004, the use of cefoxitin disks to predict resistance mediated by the *mecA* gene in all species of coagulase-negative staphylococci (CoNS). The aim of this work was to evaluate the efficiency of the cefoxitin disk and of oxacillin-salt agar screening (MHOX) to characterize the oxacillin resistance mediated by the *mecA* gene in CoNS. One hundred seven CoNS isolates from different clinical samples were studied. Detection of the *mecA* gene by PCR was considered the "gold standard." The susceptibility to oxacillin and cefoxitin was detected by the disk diffusion and agar dilution tests, as described by the CLSI. MHOX was also performed with 6 μg/ml of oxacillin and 4% NaCl. The sensitivities of the oxacillin and cefoxitin disks for all CoNS species were 88% and 80%, respectively, whereas the specificities were 63% and 100%, respectively. The sensitivities of the agar dilution test for oxacillin and cefoxitin (for proposed breakpoints of ≥4 μg/ml for resistance and ≤2 μg/ml for susceptibility) were 90% and 85%, respectively, whereas the specificities were 76% and 98%, respectively. MHOX showed a sensitivity of 90% and a specificity of 95% for all CoNS species. Both the MHOX and the cefoxitin disk results indicate that these are appropriate methods for the evaluation of oxacillin resistance mediated by the *mecA* gene in all CoNS species.

Coagulase-negative staphylococci (CoNS) are frequently recovered from blood cultures and are a leading cause of nosocomial infections, especially in neonates, immunocompromised individuals, and patients with prosthetic implants (13). Such infections pose serious therapeutic dilemmas because of the tendency of CoNS to develop resistance to multiple antibiotics.

Nevertheless, when the strains are susceptible to oxacillin, β-lactam drugs are preferred over treatment with vancomycin because these drugs are easily absorbed into body fluids and tissues, cause fewer complications from treatment, and do not select for vancomycin-resistant organisms.

The presence of the *mecA* gene, which encodes penicillin-binding protein (PBP) 2a, correlates with oxacillin resistance in CoNS (2).

In an effort to improve the detection of resistance, methods for the easy and rapid detection of the *mecA* gene have been recommended for routine laboratory use (7, 17).

In CoNS, such as *Staphylococcus epidermidis*, the breakpoints for oxacillin recommended by the Clinical and Laboratory Standards Institute (CLSI) correctly classify strains with and without *mecA* as oxacillin resistant and oxacillin susceptible, respectively (3, 4, 12, 22).

However, for CoNS other than *S. epidermidis*, these breakpoints are less effective in differentiating *mecA*-positive from *mecA*-negative strains (3, 4 12, 22). Besides, many clinical laboratories do not routinely identify the species of clinical isolates of CoNS.

In January 2004, the CLSI proposed the use of cefoxitin disks (30 μg) as a screening method for the prediction of resistance mediated by the *mecA* gene in all CoNS species (3). Up to now, the CLSI has not stated the MIC breakpoints for cefoxitin for the prediction of resistance in CoNS. Considering that in most clinical laboratories it is not possible to characterize oxacillin resistance by genotypic methods, the use of different phenotypic methodologies was encouraged to optimize its detection. The aim of this work was to evaluate the efficiency of the cefoxitin disk test and of oxacillin-salt agar screening (MHOX) to characterize the oxacillin resistance mediated by the *mecA* gene in CoNS.

MATERIALS AND METHODS

Strains. Clinical isolates characterized as CoNS were collected from Hospital de Clínicas José de San Martín of the city of Buenos Aires, Argentina, from July 2001 to July 2002.

One hundred seven strains were retrieved from stocks stored at −70°C in tryptic soy broth with 20% glycerol. The strains were subcultured once on sheep blood agar for susceptibility testing and onto Mueller-Hinton agar for PCR.

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TABLE 1. Comparison of disk diffusion test results, oxacillin and cefoxitin MICs, and MHOX with detection of *mecA* gene

Species (no. of isolates)[a]	No. of isolates with the indicated results by:									
	Oxacillin assays				Cefoxitin assays				MHOX	
	Diffusion		MIC		Diffusion		MIC		Positive	Negative
	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible		
S. epidermidis										
mecA− (34)	5	29	2	32	0	34	0	34	2	32
mecA+	24	3	26	1	24	3	26	1	26	1
S. saprophyticus										
mecA− (15)	13	2	11	4	0	15	0	15	0	15
mecA+ (3)	3	0	2	1	1	2	1	2	1	2
S. haemolyticus										
mecA− (9)	3	6	1	8	0	9	1	8	1	8
mecA+ (4)	4	0	4	0	4	0	4	0	4	0
S. hominis										
mecA− (3)	0	3	0	3	0	3	0	3	0	3
mecA+ (1)	0	1	0	1	0	1	0	1	1	0
S. simulans										
mecA− (0)	0	0	0	0	0	0	0	0	0	0
mecA+ (4)	3	1	3	1	2	2	2	2	3	1
S. capitis										
mecA− (1)	1	0	1	0	0	1	0	1	0	1
mecA+ (1)	1	0	1	0	1	0	1	0	1	0
S. auricularis										
mecA− (1)	1	0	1	0	0	1	0	1	0	1
mecA+ (0)	0	0	0	0	0	0	0	0	0	0
S. sciuri										
mecA− (1)	1	0	0	1	0	1	0	1	0	1
mecA+ (0)	0	0	0	0	0	0	0	0	0	0
S. cohnii										
mecA− (1)	0	1	0	1	0	1	0	1	0	1
mecA+ (0)	0	0	0	0	0	0	0	0	0	0
S. warneri										
mecA− (0)	0	0	0	0	0	0	0	0	0	0
mecA+ (1)	1	0	1	0	1	0	1	0	1	0
S. lugdunensis										
mecA− (1)	0	1	0	1	0	1	0	1	0	1
mecA+ (0)	0	0	0	0	0	0	0	0	0	0

[a] *mecA*−, *mecA* negative; *mecA*+, *mecA* positive.

The isolates were from anatomic sites, as follows: blood (53%); catheters (21%); urine (21%); and other tissues, fluids, and wounds (22%).

Strains were identified to the species level by the method described by Bannerman (1), including colonial appearance (diameter and pigmentation); the presence of hemolysins; and tests for the following: reactions for catalase, coagulase, heat-stable nuclease, oxidase, alkaline phosphatase, pyrrolidonylarylamidase, ornithine decarboxylase, urease, β-glucosidase, and β-galactosidase; acetoin production; esculin hydrolysis; polymyxin B (300 U) and novobiocin (5 μg) susceptibility; and aerobic production of acid from D-threalose, D-mannitol, D-mannose, D-turanose, D-xylose, D-cellobiose, L-arabinose, maltose, α-lactose, sucrose, and raffinose.

Susceptibility testing. (i) Disk diffusion test. The disk diffusion test was performed as described by the CLSI with 1 μg oxacillin and with 30 μg cefoxitin (3). The results were analyzed according to the breakpoints of the CLSI for all CoNS except for those for *S. lugdunensis*, for which the breakpoints were those for *S. aureus* according to the CLSI (3).

(ii) Agar dilution. The MICs of oxacillin and cefoxitin were determined by the agar dilution methodology of the CLSI (4).

For the susceptibility tests, *S. aureus* ATCC 25923 (*mecA* negative) and *S. aureus* ATCC 43300 (*mecA* positive) were included as control organisms. The results were analyzed according to the breakpoints of the CLSI for oxacillin for all CoNS except *S. lugdunensis*, for which the breakpoints were those for *S. aureus* according to the CLSI (4). The cefoxitin MIC breakpoints for prediction of the presence of *mecA* were ≥4 μg/ml for resistance and ≤2 μg/ml for susceptibility.

(iii) MHOX. Mueller-Hinton agar plates with 4% NaCl and 6 μg of oxacillin per ml were inoculated as a streak in an entire quadrant by using a cotton swab dipped into a direct colony suspension equivalent to a 0.5 McFarland standard in tryptic soy broth. The plates were incubated in ambient air at 35°C and were read at 48 h. Any growth was considered a positive test result.

PCR detection of *mecA*. Detection of the *mecA* gene by PCR was considered the "gold standard."

(i) Oligonucleotides. On the basis of the DNA sequences of the *mecA* gene, the following two oligonucleotides were used to amplify staphylococcal DNA: primer M1 (885-5′-TGGCTATCGTGTCACAATCG-904) and primer M2 (1194-5′-CTGGAACTTGTTGAGCAGAG-1175) (23).

TABLE 2. Sensitivities, specificities, and positive and negative predictive values of disk diffusion test, oxacillin and cefoxitin MICs, and MHOX[a]

Test and antibiotic	CoNS				Other than *S. saprophyticus*				*S. saprophyticus*			
	S (%)	SP (%)	PPV (%)	NPV (%)	S (%)	SP (%)	PPV (%)	NPV (%)	S (%)	SP (%)	PPV (%)	NPV (%)
Disk diffusion												
Oxacillin	88	64	60	89	87	78	75	89	13			100
Cefoxitin	80	100	100	89	84	100	100	89	100			88
Oxacillin + cefoxitin	90	100	100	94	89	100	100	93	100			88
MIC												
Oxacillin	90	76	70	93	92	90	88	94	27			80
Cefoxitin	85	98	97	92	89	98	97	93	100			88
Oxacillin + cefoxitin	90	100	100	94	92	100	100	94	100			88
MHOX	90	95	93	94	95	94	92	96	100			88

[a] S, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

The 16S rRNA primers were used as an internal control: primer X (911-5′-GGAATTCAAATGGAATTGACGGGGGC-930) and primer Y (1371-5′-CGGGATCCCAGGCCCGGGAACGTATTCAC-1390) (2).

(ii) Preparation of DNA. Isolates were grown on Mueller-Hinton agar in ambient air at 35°C for 18 to 24 h. An aliquot (0.1 ml) of an overnight culture (10^8 CFU) was pelleted by centrifugation (5,000 × *g* for 5 min). The bacterial pellet was resuspended in 300 μl of lysis buffer (50 mM Tris HCl [pH 8.0], 100 mM EDTA, 150 mM NaCl, 1% [vol/vol] sodium dodecyl sulfate) containing 100 μg of lysostaphin (Sigma Chemical Co., St. Louis, Mo.) and 100 μg of RNase, and the mixture was incubated at 37C for 30 min. Lysis was achieved by incubation at 37°C for 30 min in the presence of 200 μg of proteinase K.

Samples were treated with 1 volume of phenol-chloroform-isoamyl alcohol (25:24:1) and then with 1 volume of chloroform-isoamyl alcohol (24:1) prior to precipitation of the aqueous phase in 2 volumes of 95% ethanol-0.2 M NaCl for 1 h at −20°C. The DNA was pelleted by centrifugation (12,500 × *g* for 10 min), washed with 80% ethanol, air dried, and resuspended in 200 μl of distilled water (23).

(iii) Reaction mixture. Ten microliters of the DNA samples was added to 40 μl of the PCR mixture, which consisted of 250 μM deoxynucleoside triphosphates (1.25 μl), 0.4 μM primer M1 (2 μl), 0.4 μM primer M2 (2 μl), 0.04 μM primer X (2 μl), 0.04 μM primer Y (2 μl), 2 U of *Taq* DNA polymerase (0.4 μl), 5 μl of buffer (10 mM Tris HCl [pH 8.8]), and 1.5 mM $MgCl_2$ (3 μl).

(iv) Amplification. After an initial denaturation step (3 min at 92°C), 30 cycles of amplification were performed as follows: denaturation at 92°C for 1 min, annealing at 56°C for 1 min, and DNA extension at 72°C for 1 min. The reaction was achieved with a final extension at 72°C for 3 min. The amplification was carried out in a Mastercycler 5330 thermal cycler (Eppendorf, Germany).

(v) Electrophoresis. After amplification, 10 μl of the PCR product was loaded onto a 2% agarose gel in TBE buffer (0.089 M Tris-borate, 0.089 boric acid, 0.002 M EDTA [pH 8]) containing 0.5 μg of ethidium bromide per ml. DNA fragments of 310 bp and 479 bp, which corresponded to the *mecA* and the 16S rRNA PCR products, respectively, were visualized on a UV transilluminator at 300 nm. A 100- to 1,500-bp DNA ladder was used as a molecular size marker (Promega).

S. aureus ATCC 29213 was included as a negative control, and *S. aureus* ATCC 43300 was included as a positive control.

PBP 2a immunoblotting. To detect the gene product of *mecA* from eight strains, immunoblot assays for PBP 2a were performed by a previously described method (8). The strains were previously induced with 4% NaCl and oxacillin at subinhibitory concentrations.

S. aureus ATCC 29213 was included as a negative control, and *S. aureus* ATCC 43300 was included as a positive control.

Statistical tests. The sensitivity, specificity, and positive and negative predictive values of the disk diffusion test, the oxacillin and cefoxitin MICs, and MHOX were estimated by considering the PCR detection of *mecA* the gold standard.

RESULTS

The identities of the strains were as follows: *S. epidermidis*, $n = 61$; *S. saprophyticus*, $n = 18$; and *S. haemolyticus*, $n = 13$. Fifteen isolates of other species were also found: four *S. hominis* isolates, four *S. simulans* isolates, two *S. capitis* isolates, one *S. auricularis* isolate, one *S. sciuri* isolate, one *S. cohnii* isolate, one *S. warneri* isolate, and one *S. lugdunensis* isolate.

The *mecA* gene was found in 41 of 107 (38%) coagulase-negative staphylococcal species: 27 of 61 *S. epidermidis* isolates, 3 of 18 *S. saprophyticus* isolates, 4 of 13 *S. haemolyticus* isolates, 1 of 4 *S. hominis* isolates, 4 of 4 *S. simulans* isolates, 1 of 2 *S. capitis* isolates, and 1 of 1 *S. warneri* isolate (Table 1).

Oxacillin resistance was detected by the disk diffusion test in 60 of 107 (56%) of the isolates. The oxacillin disk diffusion test detected 36 of 41 *mecA*-positive isolates (88%) and correctly identified 42 of 66 *mecA*-negative isolates (64%) (Table 1).

Cefoxitin resistance was detected by the disk diffusion test in 33 of 107 (31%) of the isolates. The cefoxitin disk diffusion test detected 33 of 41 *mecA*-positive isolates (80%) and correctly identified 66 of 66 *mecA*-negative isolates (100%) (Table 1).

The sensitivity of the cefoxitin disk test (80%) was slightly lower than that of the oxacillin disk test (88%) (Table 2). However, the specificity of the cefoxitin disk test was 100% for all CoNS species, whereas that of the oxacillin disk test was 64% for all CoNS species. When the results for the *S. saprophyticus* isolates were excluded, the specificity of the oxacillin disk test was 78% (Table 2). The sensitivity of the combination of two disks (90%) was slightly higher than that of the oxacillin

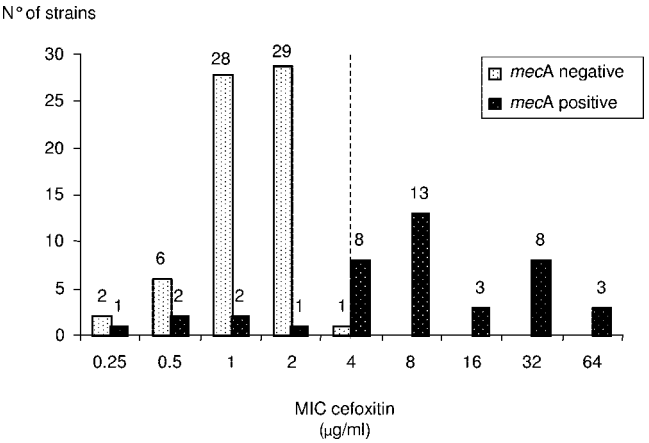

FIG. 1. Distribution of cefoxitin MICs.

disk alone (88%) and that of the cefoxitin disk alone (80%) (Table 2). However, the specificity of the combination of two disks (100%) was higher than that of the oxacillin disk alone (64%) and was the same as that of the cefoxitin disk alone (100%) (Table 2).

Resistance to oxacillin was detected by agar dilution tests in 53 of 107 of the isolates (49%). The oxacillin agar dilution test detected 37 of 41 *mecA*-positive isolates (90%) and correctly identified 50 of 66 *mecA*-negative isolates (76%) (Table 1).

By considering the results observed by the agar dilution test results for cefoxitin for *mecA*-positive and -negative isolates (Fig. 1), the breakpoints of the cefoxitin MICs could be proposed to be ≥4 μg/ml for resistance and ≤2 μg/ml for susceptibility. With these breakpoints, resistance to cefoxitin by the agar dilution method was shown in 36 of 107 of the isolates (34%). The cefoxitin agar dilution test detected 35 of 41 *mecA*-positive isolates (85%) and correctly identified 65 of 66 *mecA*-negative isolates (98%) (Table 1).

The sensitivity of the agar dilution tests for cefoxitin by use of these breakpoints (85%) was similar to for oxacillin (90%) (Table 2). The specificity of the agar dilution tests for cefoxitin (98%) was higher than that for oxacillin (76%) for all CoNS species (Table 2). The sensitivity of the combination of the agar dilution tests for oxacillin and cefoxitin (90%) was slightly higher than that for the test with cefoxitin alone (85%) and the same as that for the test with oxacillin alone (90%) (Table 2). However, the specificity of the combination of the agar dilution tests (100%) was higher than that for the test with oxacillin alone (76%) and similar to that for the test with cefoxitin alone (98%) (Table 2).

MHOX found that 40 of 107 (37%) isolates were resistant to oxacillin (Table 1). This was the most sensitive of the techniques when the results of MHOX were compared to those of the *mecA* PCR, with a sensitivity of 90% and a specificity of 95% (Table 2). The sensitivity of MHOX at 24 h was slightly lower (88%) than that of MHOX at 48 h.

MHOX detected 37 of 41 *mecA*-positive isolates (90%) (Table 1). Of the four isolates not detected, *S. epidermidis* and *S. simulans* showed zone diameters of >20 mm for oxacillin and >27 mm for cefoxitin, whereas two isolates of *S. saprophyticus* showed zone diameters of between 10 and 12 mm for oxacillin and zone diameters of >30 mm for cefoxitin. These four isolates were negative for the *mecA* gene products (PBP 2a), and they did not show any oxacillin phenotypic resistance mediated by PBP 2a.

MHOX correctly identified 63 of 66 *mecA*-negative isolates (95%) (Table 1). Of the three isolates with false-positive results, the two isolates of *S. epidermidis* showed oxacillin zone diameters of 16 mm and 21 mm, respectively. They also showed cefoxitin zone diameters of >30 mm, and both isolates were PBP 2a positive. The third isolate of *S. haemolyticus* showed an oxacillin zone diameter of 11 mm and a cefoxitin zone diameter of 31 mm and was PBP 2a negative.

Interestingly, of all the techniques, only MHOX (at 48 h) was able to detect *mecA* in one strain of *S. hominis*.

DISCUSSION

CoNS are a major cause of bacteremia in hospitalized patients, where the intravascular catheter is the infectious focus (13).

The results of this investigation have shown that both agar dilution and disk diffusion tests with oxacillin can detect *mecA*-positive isolates of CoNS, as described previously (12, 14, 22).

The sensitivity of the cefoxitin disk for the evaluation of oxacillin resistance mediated by the *mecA* gene by use of the breakpoints proposed by the CLSI was slightly lower than that of the oxacillin disk for all CoNS species (sensitivities, 80% and 88%, respectively). Nevertheless, the zone diameters obtained with the cefoxitin disk were easier to read than those obtained with the oxacillin disk, as has been described by the CLSI. In addition, if the results for the *S. saprophyticus* isolates were excluded, the sensitivities of oxacillin and cefoxitin disks were similar: 87% and 84%, respectively, as has also been described by the CLSI. This finding might be because in this work two *mecA*-positive *S. saprophyticus* isolates which were negative for the *mecA* gene product (PBP 2a) were detected; that is, these isolates did not show any phenotypic resistance mediated by PBP 2a. The fact that these isolates showed resistance to oxacillin (not mediated by PBP 2a) by the disk diffusion tests but were susceptible with the use of cefoxitin disks might explain the lower sensitivity of the disk diffusion test for cefoxitin than for oxacillin. Similar to our data, Frigatto et al. also observed that the 30-μg cefoxitin disk failed to detect resistance in *mecA*-positive *S. epidermidis* isolates (6). Likewise, by using the 30-μg cefoxitin disk to predict resistance to oxacillin mediated by PBP 2a, Montgomery et al. described a 100% sensitivity for *S. epidermidis* isolates and 95% sensitivity for non-*S. epidermidis* isolates (J. Montgomery, J. Bywater, and H. King. Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother, abstr. D-64, 2004). The utility of the cefoxitin disk diffusion test was originally proposed in 2001 by Mougeot et al., who described that the 30 μg-cefoxitin disk has a 97% sensitivity for the prediction of resistance to oxacillin mediated by the *mecA* gene in *S. aureus* (15). Likewise, Felten et al. reported that the 30-μg cefoxitin disk has a 100% sensitivity for the detection of all the methicillin resistance classes in *S. aureus* (5). For CoNS, Swenson et al. also found that the 30-μg cefoxitin disk has a 99% sensitivity for the prediction of *mecA*-mediated oxacillin resistance (21). Similar to our data, those authors also found that the results obtained with the cefoxitin disk were much easier to read than those obtained with the oxacillin disk (21). Similarly, Pottumarthy et al. also found that the 30-μg cefoxitin disk demonstrated few very major errors (false susceptibility) when it was used to predict *mecA*-mediated oxacillin resistance (16).

On the other hand, Hederstierna-Johnsen et al. found that the use of a 10-μg cefoxitin disk on Iso-Sensitest agar and interpretive zone diameters of <22 mm for resistance and ≥27 mm for susceptibility had a 100% sensitivity for the prediction of resistance to oxacillin mediated by the *mecA* gene in consecutive isolates of CoNS causing bacteremia (10).

In this study, the sensitivity of the cefoxitin agar dilution test with breakpoints of ≥4 μg/ml for resistance and ≤2 μg/ml for susceptibility was similar to that of the oxacillin agar dilution test (sensitivities, 85% and 90%, respectively). These data seemed to be appropriate for the detection of *mecA*-positive isolates from all CoNS species. Nevertheless, until the CLSI issues the respective guidelines, it would be necessary in future work with a greater number of isolates to evaluate more ade-

quate breakpoints for the agar dilution tests with cefoxitin to predict oxacillin resistance mediated by PBP 2a.

On the other hand, considering the results of this investigation, the disk diffusion and dilution tests for oxacillin overcalled the resistance mediated by PBP 2a mainly in species other than *S. epidermidis*. When the results for the isolates of *S. saprophyticus* were excluded, the specificity of the disk diffusion test rose from 63% to 78% and the specificity of the agar dilution test rose from 76% to 90%. These results were similar to the findings of other authors, such as Tenover et al. (22), Hussain et al. (12), Louie et al. (14), and Horstkotte et al. (11). They reported that the species most affected by the lower specificities of the oxacillin agar dilution and disk diffusion tests were *S. saprophyticus*, *S. cohnii*, *S. warneri*, *S. lugdunensis*, and *S. xylosus* (11, 12, 14, 22). It must be said that the *mecA*-negative isolate corresponding to *S. lugdunensis* did not show false resistance to oxacillin when the breakpoints for *S. aureus* proposed by the CLSI were used. Use of these breakpoints for CoNS would have shown false resistance to oxacillin.

The cefoxitin disk test had a 100% specificity for all CoNS species (*S. saprophyticus* and species other than *S. saprophyticus*). These results were similar to the ones described by Corso et al., who reported that the test had a 100% specificity for CoNS other than *S. saprophyticus* and for *S. saprophyticus* (A. Corso, P. Gagetti, P. Cerlana, M. Rodriguez, S. Corbella, M. Iglesias, and M. Galas, Abstr. XVII Congr. Latinoamericano Microbiol, X Congr. Argentino Microbiol., abstr. A-38, 2004). Likewise, Montgomery et al. described a 100% specificity for *S. epidermidis* and non-*S. epidermidis* isolates when the 30-μg cefoxitin disk was used to predict oxacillin resistance mediated by PBP 2a (Montgomery et al., 44th ICAAC). Besides, the utility of the disk diffusion method was originally proposed in 2001 by Mougeot et al., who described that it had a 100% specificity for *S. aureus* when a 30-μg cefoxitin disk was used to predict resistance to oxacillin mediated by the *mecA* gene (15). Likewise, Felten et al. also reported that the oxacillin disk method had a 100% specificity for *S. aureus* (5). Swenson et al. described a 96% specificity for prediction of *mecA*-mediated oxacillin resistance of CoNS isolates by using the 30-μg cefoxitin disk (21). Similarly, Pottumarthy et al. also reported that the 30-μg cefoxitin disk had 3% major errors when it was used to predict *mecA*-mediated oxacillin resistance (16). Nevertheless, we did not detect any major errors by using this disk.

On the other hand, Hederstierna-Johnsen et al. found that a 10-μg cefoxitin disk on Iso-Sensitest agar and with interpretive zone diameters of <22 mm for resistance and ≥ 27 mm for susceptibility had a 100% specificity for the prediction of resistance to oxacillin mediated by the *mecA* gene in consecutive isolates of CoNS causing bacteremia (10). Besides, Skov et al. described a substantial overlap in the results for CoNS isolates when they used Iso-Sensitest agar with 5- and 10-μg cefoxitin disks and Mueller-Hinton agar with 5- and 10-μg cefoxitin disks (19). However, they reported highly accurate results (81%, 80%, 91%, and 97%, respectively) by avoiding primary interpretation of the overlapping intervals (19).

Interestingly, in our work, the combination of two disks (oxacillin and cefoxitin) and the combination of agar dilution tests for oxacillin and cefoxitin achieved the best sensitivities and specificities for all CoNS species. However, clinical laboratories would find it very easy to read both the oxacillin and cefoxitin disk zones and take advantage of the increased sensitivity and specificity.

MHOX was the most accurate method for determination of the presence of *mecA*. These results were similar to the ones described by Louie et al. (14). Nevertheless, Tenover et al. described a low sensitivity for this method (50% to 70%) (22).

The sensitivity of MHOX was affected by four isolates that were found to be positive for *mecA* by production of a 300-bp fragment of an internal region of the *mecA* gene but that were found to be negative for the *mecA* gene product (PBP 2a). That is, the isolates did not show oxacillin phenotypic resistance mediated by PBP 2a. These isolates were negative for the PCR amplification products of the regulatory genes (data not shown), and the results for these isolates were also confirmed by DNA hybridization assays (data not shown). The possibility of the presence of a deletion in these isolates could be assumed. Graham et al. (9) and Sakumoto et al. (18) also described the lack of expression of the *mecA* gene in CoNS isolates, although it is very rare.

The specificity of MHOX was affected by three isolates that were found to be negative for the 300-bp fragment of an internal region of *mecA* gene. Two of these three isolates were PBP 2a positive. PCR amplification of the regulatory gene and of the *mecA* gene in another region was also positive (data not shown); from this it could be assumed that the lack of amplification in the region of 300 bp could be due to a genetic diversity of the *mec* gene in that region. The third isolate which corresponded to *S. haemolyticus*, was found to be PBP 2a negative; and no PCR amplification of the *mecA* gene or its regulators was seen (data not shown). It could be assumed, then, that this low grade of resistance that allowed its development in oxacillin-salt agar screening might be due to an alteration in normal PBPs that decreased in their amounts or in their affinities to β-lactam antibiotics. Suzuki et al. described alterations in the amounts of PBP 1 and PBP 4 for *S. haemolyticus* and *S. saprophyticus* (20).

For *S. saprophyticus* it would be more adequate to replace the oxacillin disk with the cefoxitin disk, mainly if this germ is isolated from the urine of a woman who is fertile. This is because the oxacillin disk overcalls the resistance mediated by the *mecA* gene of this species.

It may be concluded that the results of MHOX showed that it is the best single test for the evaluation of oxacillin resistance mediated by the *mecA* gene for all CoNS species. In addition, we suggest that screening with both disks is probably necessary for enhanced sensitivity and specificity for the evaluation of all CoNS species for oxacillin resistance.

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