Laboratory detection methods for methicillin resistance in coagulase negative Staphylococcus isolated from ophthalmic infections

Métodos laboratoriais para a detecção da resistência à meticilina nos Staphylococcus coagulase negativos de infeções oculares

Adília Dias Dourado Oliveira\(^1\)
Pedro Alves d’Azevedo\(^2\)
Luciene Barbosa de Sousa\(^3\)
Cristina Viana-Niero\(^4\)
Waldemar Francisco\(^5\)
Cláudio Lottenberg\(^6\)
Marines Dalla Valle Martino\(^7\)
Ana Luisa Höfling-Lima\(^8\)

ABSTRACT

**Purpose:** To evaluate different methods of oxacillin susceptibility testing of ocular isolates, considering polymerase chain reaction (PCR) as the "gold standard", and to compare the *in vitro* susceptibility to oxacillin with that of other antimicrobials used in ophthalmologic practice. **Methods:** The Vitek gram-positive identification card was used to identify ocular coagulase negative *Staphylococcus* species. The presence of the *mecA* gene was determined by the polymerase chain reaction assay with a combination of two primer sets (*mecA* and 16S rRNA) in a single region. Results were analyzed and compared with other oxacillin susceptibility methods: PBP2a detection by rapid slide latex agglutination test (SLA); oxacillin E-test; the Vitek automated gram-positive susceptibility card (GPS-105); the oxacillin salt agar screening test (OSAS) at a concentration of 0.0, 1.0 and 0.75 µg oxacillin per ml and the cefoxitin disk diffusion test (CDD). Automated susceptibility was also determined to other antimicrobial agents (fluoroquinolones, penicillin G, amoxicillin-ampicillin, cefazolin, ampicillin-sulbactam, erythromycin, clindamycin, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, vancomycin and rifampin. **Results:** Of the 69 CoNS isolates tested, 71% were *mecA*-positive and 29% *mecA*-negative. All methods tested had a statistically significant agreement with polymerase chain reaction. There was a tendency of positive polymerase chain reaction predomination among the *S. epidermidis* isolates in comparison to non-epidermidis isolates, although this was not statistically significant (78.3% vs. 56.5%; \( \chi^2 = 2.54; P = 0.11 \)). The oxacillin salt agar screening test (0.75 µg oxacillin/ml) showed the best performance, with 100% sensitivity and negative predictive value; 95% specificity and 98% positive predictive value. Using the E-test, the *mecA*-positive isolates were statistically significantly more resistant to ciprofloxacin, ofloxacin, gatifloxacin and moxifloxacin (\( P = 0.002; P = 0.008; P = 0.002 \) and \( P = 0.003 \), respectively). There was a statistically significant higher proportion of resistance of the coagulase negative *Staphylococcus mecA*-positives for penicillin G, amoxicillin-ampicillin, cefazolin, ampicillin-sulbactam, erythromycin, clindamycin, gentamicin and tetracycline (\( P \leq 0.05 \)). All coagulase negative *Staphylococcus* species were susceptible to vancomycin and there was no statistically significant correlation between the *mecA*-positive isolates and resistance to trimethoprim-sulfamethoxazole or to rifampin. **Conclusions:** In the present study, we found that the E-test and the oxacillin salt agar screening test S (0.75 µg oxacillin per ml), when compared with polymerase chain reaction, were the most accurate currently available methods to phenotypically detect oxacillin resistance of coagulase negative *Staphylococcus* species. This study demonstrated that a good option for screening of ocular isolates for oxacillin resistance in the microbiology laboratory is the cefoxitin disk diffusion test and the automated Vitek system. We believe it is important to have available methods that accurately detect methicillin resistance of the less commonly encountered species, chiefly because of their increasing importance as opportunistic pathogens.

**Keywords:** Methicillin resistance; Sensitivity and specificity; Latex fixation tests; Oxacillin; Microbial sensitivity tests; Staphylococcus/isolation & purification; Coagulase; Eye infections, bacterial; Comparative study
Laboratory detection methods for methicillin resistance in coagulase negative Staphylococcus isolated from ophthalmic infections

INTRODUCTION

Coagulase-negative Staphylococcus (CoNS) species are commonly isolated bacteria, often admixed with more typical ocular microbes, and can lead to major infections, including keratitis, conjunctivitis and endophthalmitis. Staphylococcus epidermidis, the predominant species of the CoNS, is the most commonly cultured intraocular pathogen, and accounts for an average of 40% postoperative and posttraumatic endophthalmitis cases.

Methicillin-resistant coagulase negative Staphylococcus (MRCoNS) is just one species of intraocular pathogens and one report in the literature has described patients with MR-CoNS among the preoperative ones seen at a Japanese eye clinic. Resistance to methicillin is associated with in vitro crossing resistance to other antimicrobials. The laboratory data from the Ophthalmological Department of the University of São Paulo (UNIFESP) has documented a recent increase in infections due to this microorganism. In contrast to infections at other sites, the ocular therapeutic difficulties posed by the MRCoNS have been virtually unrecognized.

Two basic mechanisms are responsible for the resistance of staphylococci to the beta-lactamic antimicrobials: first, the beta-lactamase production that destroys these agents, and, second, alteration of proteins located in the cellular wall of the bacteria, the so-called penicillin-binding proteins (PBPs). Most resistance to oxacillin by staphylococci is mediated by the mecA gene, which codes for the production of a supplemental penicillin-binding protein, PBP2a or 2', which is expressed either homogeneously or heterogeneously. PBP2a has a low affinity for beta-lactamic antimicrobials. Homogeneous resistance is easily detected with standard testing methods, whereas heterogeneous expression is more difficult to detect with some methods, because only a fraction of the population (e.g., 1 in 100,000 cells) expresses the resistance phenotype.

Tests for mecA or for the protein encoded by mecA, PBP2a, are the most accurate methods for prediction of resistance to oxacillin, and could be used to confirm results of staphylococci isolates from serious infections. Oxacillin is no longer the agent recommended by the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee of Clinical and Laboratory Standards - NCCLS) for CoNS phenotypic tests to predict resistance to penicillinase-stable penicillins (PSPs). Antimicrobial susceptibility tests using oxacillin are often difficult to interpret, despite changes that have improved discrimination between oxacillin-resistant and -susceptible strains. Minimum inhibitory concentration (MIC) panels in which oxacillin is tested must be examined carefully to detect any growth that may be indicative of resistance. Several groups of investigators have reported that the results of ceftoxitin (30 μg) disk diffusion tests correlate better with the presence of mecA than do the results of disk diffusion tests using oxacillin, and the cefoxitin disk test is now the preferred method for testing CoNS.

METHODS

Bacterial isolates and bacteriologic methods

Fresh and cryopreserved bacterial isolates were used. They were collected between July 2002 and July 2004 and processed according to standard microbiologic protocols in a reference ophthalmological laboratory. Samples included 69 CoNS isolates from clinically diagnosed keratitis, conjunctivitis or endophthalmitis. The laboratory had identified the organisms as CoNS by colony morphology, Gram stain characteristics, and by catalase and coagulase tests. S. aureus (ATCC 25923) was used as the mecA gene negative control and S. aureus (ATCC 43300) was used as the mecA gene positive control.

Inoculum preparation

Inocula for all tests were prepared using trypticase soy agar (TSA) (Difco, Le Pont de Claix, France) supplemented with 5% sheep blood that had been streaked with a single colony from an initial subculture plate and incubated for 18 to 24 h. The test inoculum was prepared by removing growth from the agar plate, inoculating it directly into Mueller-Hinton broth (MHB) (Difco, Le Pont de Claix, France), and adjusting the inoculum to equal 0.5 McFarland turbidity standard.

Identification of species

The Vitek (BioMerieux, Hazelwood, MI) gram-positive identification card was used to identify the CoNS species. Card inoculation, sealing, incubation, and reading were performed according to the instructions of the manufacturer. The species were then classified into two groups: epidermidis (S. epidermidis CoNS) and non-epidermidis (CoNS non-S. epidermidis).

Polymerase chain reaction (PCR) detection of mecA gene

The mecA gene, in combination with the 16S rRNA gene, was detected with a PCR technique based on the procedure described previously. The multiplex assay combined two...
primer sets (mecA and 16S rRNA) in a single region, and presence of the mecA gene was considered the ‘gold standard’ for oxacillin resistance.

**PBP2a latex slide agglutination test (LSA)**

Penicillin-binding protein 2a detection by the rapid slide latex agglutination test (SlideX MRSA Detection Test, BioMerieux, Paris, France) was performed according to the manufacturer’s instructions with slight modification: the resulting agglutination pattern was read at 10. Bacterial cells (approximately 5 µl) were obtained from a fresh subculture and suspended in 4 drops of extraction reagent 1 and boiled for 3 min. The suspension was allowed to cool to room temperature, after which 1 drop of extraction reagent 2 was added, and the mixture vortexed thoroughly. The suspension was then centrifuged at 1500 x g for 5 min. A 50 µl aliquot of the supernatant was mixed with 1 drop of anti-PBP2a monoclonal antibody-sensitized latex beads. A negative control test was performed by using 50 µl of supernatant mixed with 1 drop of negative control latex. The isolates were then placed on a shaker and gently mixed for up to 15 mins.

**Automated susceptibility test (AS)**

The AS test was performed with the Gram-Positive Susceptibility card (GPS-105) of the Vitek system (BioMerieux, Hazelwood, MI), according to the manufacturer’s recommendation. The susceptibility of all CoNS isolates was determined for oxacillin and the other antimicrobials used in ophthalmology: penicillin G, amoxicillin-ampicillin, cefazolin, ampicillin-sulbactam, erythromycin, clindamycin, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, vancomycin and rifampin.

**Oxacillin salt agar screen susceptibility test (OSAS)**

Agar screen tests for susceptibility to oxacillin were performed by a method described previously[23]. The OSAS test was performed with agar plates produced by PROBAC (São Paulo, Brazil). A total of 10⁶ CFU was spot inoculated onto Mueller-Hinton agar (MHA) (PROBAC, São Paulo, Brazil) with 4% NaCl supplementation containing 6, 1 or 0.75 µg of oxacillin per ml. Plates were read after a 24 h incubation at 35°C. If any colonies of growth were detected, the test was considered to be positive for resistance.

**Disk susceptibility test (DD)**

The CLSI reference method for disk diffusion was used to test cefoxitin (30 µg) (Oxoid, Basingstore, England)[23]. MHA plates were incubated at 35°C, and zone diameters were read at 18 h to 24 h.

**E-test**

The minimum inhibitory concentrations (MIC) for oxacillin, ciprofloxacin, ofloxacin, gatifloxacin and moxifloxacin were each determined using the E-test (AB Biodisk, Solna, Sweden). Plates were incubated at 35°C for 18 to 24 hours. The MIC value (E-test) was read at the point where the edge of the growing culture intersects the strip. The antimicrobial susceptibility of each bacterial isolate was determined by comparing the MIC with that of the Clinical and Laboratory Standards Institute (CLSI)[35] when available. Each isolate was classified as susceptible or nonsusceptible (intermediate or resistant).

**β-Lactamase test**

β-Lactamase production was identified using nitrocefin disks (Difco, Le Pont de Claix, France). This test was performed with all mecA-negative isolates that were resistant by E-test or by the automated method.

**Amoxicillin-clavulanic acid disk diffusion test**

The amoxicillin-clavulanic acid disk test (20 µg amoxicillin and 10 µg clavulanic acid) (Oxoid, Basingstore, England) was performed with all mecA-negative isolates that were found to be resistant by E-test or by the automated method. The breakpoint for susceptibility was a zone of inhibition ≥20 mm in diameter after 24 h of incubation at 30°C.

**Data analysis**

Evaluation of data comparing results of the mecA gene PCR assay and results from the other susceptibility tests required a matched 2-by-2 table. Evaluation of data was calculated using the SPSS (Statistical Package for Social Sciences, 11.0 version, SPSS Inc, Chigaco). Difference in susceptibility methods and significance of the results was calculated by the Chi-square test or Fisher exact test. Statistical significance was accepted when the P-value was <0.05. Validity tests (sensitivity, specificity, positive predictive value and negative predictive value) were also calculated. Sensitivity was defined as the percentage of mecA-positive isolates determined to be nonsusceptible by phenotypic testing, and specificity was defined as the percentage of mecA-negative isolates determined to be susceptible by phenotypic testing. The isolates were designated as susceptible or nonsusceptible (resistant and intermediate) based on the 2006 CLSI document.

**RESULTS**

Of 69 CoNS tested, 49 (71%) were mecA-positive and 20 (29%) were mecA-negative. They were classified into one of two groups based on species identification: 46 (66.7%) were *S. epidermidis* and 23 (33.3%) were *non-epidermidis* (9 *S. auricularis*; 4 *S. haemolyticus*; 4 *S. capitis*; 2 *S. simulans*; 1 *S. hominis*; 1 *S. sciuri*; 1 *S. cohnii* and 1 *S. saprophyticus*). For the mecA-negative isolates only the 16S rRNA specific band was observed.

All methods tested had a statistically significant agreement with PCR (Table 1). Table 2 shows the results of oxacillin susceptibility tests for each of the 69 CoNS isolates.

All mecA-positive isolates were oxacillin resistant by the E-test, although three isolates that were resistant by the E-test did not show the mecA gene. In the automated method, four
Laboratory detection methods for methicillin resistance in coagulase negative Staphylococcus isolated from ophthalmic infections

resistant isolates did not show the mecA gene, although they were positive by the nitrocefin disk test and were susceptible to amoxicillin-clavulonic acid disks. Only 46 mecA-positives were detected by the agar screen test with 0.75 oxacillin \( \mu \text{g/ml} \), although, one and six isolates had not being detected by the agar screen test with 1 and 6 oxacillin \( \mu \text{g/ml} \) respectively (\( \kappa = 0.96; \kappa = 0.97; \kappa = 0.87; P < 0.001 \) respectively). The cefoxitin disk diffusion test did not show resistance in 5 mecA-positive isolates (\( \kappa = 0.84; P < 0.001 \)).

Validity of results for all methods tested is shown in Table 3. The agar screen oxacillin test (0.75 \( \mu \text{g/ml} \)) showed the best performance with results of 100% sensitivity and negative predictive value, and 95% specificity and 98% positive predictive value. When comparing the epidermidis with the non-epidermidis groups, the latter had the worst values regarding sensitivities in the latex, automated, agar screen (6 and 1 \( \mu \text{g/ml} \)) and cefoxitin disk diffusion tests, and in specificities of the E-test and agar screen (0.75 \( \mu \text{g/ml} \)) tests (Table 4).

There was a tendency toward mecA predominance among the S. epidermidis compared to non-epidermidis groups, although the difference was not statistically significant (78.3% vs. 56.5%; \( \chi^2 = 2.54; P = 0.11 \) (Figure 1). For the non-epidermidis group, there were more false-positives in the E-test, automated method and the agar screen test with 0.75 oxacillin \( \mu \text{g/ml} \) and more false-negatives in the automated method, cefoxitin disk diffusion test and the agar screen test with 1 oxacillin \( \mu \text{g/ml} \) than for the epidermidis group. For the epidermidis group, there were more false negatives for the latex agglutination test (Table 5).

By the E-test, the mecA-positive isolates were statistically significantly more resistant to ciprofloxacin, ofloxacin, gatifloxacin and moxifloxacin (\( P = 0.002; P = 0.008; P = 0.002 \) and \( P = 0.003 \) (Figure 2).

There was a statistically significant higher proportion of resistance of the CoNS mecA-positives to penicillin G, amoxicillin-ampicillin, cefazolin, ampicillin-sulbactam, erythromycin, clindamycin, gentamicin and tetracycline (\( P \leq 0.05 \)). Resistance to vancomycin was not observed. There was no statistically significant correlation between the mecA-positive isolates and resistance for trimethoprim-sulfamethoxazole or rifampin (although with no statistical significance).

Table 1. Comparison of PCR and others oxacillin susceptibility tests (agreement)

<table>
<thead>
<tr>
<th>Test</th>
<th>Agreement (( \kappa ))</th>
<th>( \rho )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex agglutination</td>
<td>0.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oxacillin E-test</td>
<td>0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Automated susceptibility</td>
<td>0.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Agar screen (0.75 ( \mu \text{g/ml} ))</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Agar screen (1.0 ( \mu \text{g/ml} ))</td>
<td>0.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Agar screen (6.0 ( \mu \text{g/ml} ))</td>
<td>0.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cefoxitin disk diffusion</td>
<td>0.84</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2. Results of all oxacillin susceptibility tests for the 69 CoNS isolates

<table>
<thead>
<tr>
<th>Test</th>
<th>Negative/ Susceptible</th>
<th>Positive/ Non-susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>29.0%</td>
<td>71.0%</td>
</tr>
<tr>
<td>Latex agglutination</td>
<td>33.3%</td>
<td>66.7%</td>
</tr>
<tr>
<td>Oxacillin E-test</td>
<td>24.6%</td>
<td>75.4%</td>
</tr>
<tr>
<td>Automated susceptibility</td>
<td>26.1%</td>
<td>73.9%</td>
</tr>
<tr>
<td>Agar screen (0.75 ( \mu \text{g/ml} ))</td>
<td>27.5%</td>
<td>72.5%</td>
</tr>
<tr>
<td>Agar screen (1.0 ( \mu \text{g/ml} ))</td>
<td>30.4%</td>
<td>69.6%</td>
</tr>
<tr>
<td>Agar screen (6.0 ( \mu \text{g/ml} ))</td>
<td>34.8%</td>
<td>65.2%</td>
</tr>
<tr>
<td>Cefoxitin disk diffusion</td>
<td>36.2%</td>
<td>63.8%</td>
</tr>
</tbody>
</table>

By the E-test, the mecA-positive isolates were statistically significantly more resistant to ciprofloxacin, ofloxacin, gatifloxacin and moxifloxacin (\( P = 0.002; P = 0.008; P = 0.002 \) and \( P = 0.003 \) (Figure 2).

There was a statistically significant higher proportion of resistance of the CoNS mecA-positives to penicillin G, amoxicillin-ampicillin, cefazolin, ampicillin-sulbactam, erythromycin, clindamycin, gentamicin and tetracycline (\( P \leq 0.05 \)). Resistance to vancomycin was not observed. There was no statistically significant correlation between the mecA-positive isolates and resistance for trimethoprim-sulfamethoxazole or rifampin (although with no statistical significance).

Figure 1 - PCR results: presence of mecA gene in the epidermidis and non-epidermidis groups

Figure 2 - Comparison between the mecA-positive and fluoroquinolone nonsusceptible vs. mecA-negative and fluoroquinolone nonsusceptible isolates percentages
Laboratory detection methods for methicillin resistance in coagulase negative Staphylococcus isolated from ophthalmic infections

DISCUSSION

Staphylococci are the most commonly pathogens isolated from ocular tissues. Because of their ubiquitous nature and relatively low virulence, staphylococci, other than *S. aureus*, in the past were often simply reported by the microbiology laboratory as CoNS. However, over the past 15 years, there has been increased documentation of ocular infections caused by CoNS. Because the antimicrobial susceptibility of CoNS is unpredictable, and because multiresistant isolates are common, the recommendation now is to perform antimicrobial susceptibility testing in all cases of clinically significant ocular infections caused by these organisms.

Identification of methicillin-resistant staphylococci in the laboratory is complicated by the heterogeneous nature of the resistance and by the variables that influence its expression (i.e., medium, inoculum size, pH, temperature, and salt concentration). Only a few cells in a population of bacteria may be PBP2a positive because of heterogeneity of the *mecA* gene.
expression, especially in CoNS [29]. Furthermore, methicillin resistance can involve non-PBP2a-dependent mechanisms, such as hyperproduction of β-lactamase [30].

Several studies have demonstrated that PCR is a sensitive method for the detection of methicillin resistance in CoNS [25-27]. Unfortunately, most laboratories, especially ophthalmologic ones, are not in a position to perform this test. The percentage of mecA-positive cultures and the predominance of S. epidermidis among CoNS isolated from ocular infections is similar to that reported in the literature, which describes the identification of methicillin-resistant strains [29-31]. A multicenter study in Brazil showed methicillin resistance in 87.7% of CoNS isolated from infections of the bloodstream [32].

Previous studies that examined results of the GPI card (automated Vitek system), have reported CoNS identification to vary from 67.3% to 89%. These discrepant results in identification are related to species that are less commonly isolated, while the system was quite reliable for the most commonly isolated and more clinically relevant species: S. epidermidis [33-36].

All of the oxacillin susceptibilities tests performed in this study were in overall agreement with PCR results, and the phenotypic methods evaluated in the present study also appeared to perform well for the detection of oxacillin resistance. The LSA test was done with extension of the reaction time to 10 min, with resultant 93.9% sensitivity; this is not as good as the oxacillin disk test for CoNS (46). The MIC breakpoints were found to be attributable to the high oxacillin MICs for mecA-positive isolates with 100% sensitivity. However, these tests were not satisfactory to assess methicillin resistance to other CoNS species. Our study showed 100% sensitivity of the E-test and OSAS (0.75 µg/ml) for both groups (epidermidis and non-epidermidis), but there were differences in the level of specificity (78%) for the E-test, similar to what we noted. By the E-test and OSAS oxacillin (0.75 µg/ml), 3 (1 S. epidermidis, 1 S. auricularis and 1 S. haemolyticus) of 20 (15%) and 1 (S. auricularis) of 20 (5%) mecA-negative isolates appeared to be resistant. Again, the new breakpoints values, reported by Tenover et al. [27], in 1999, were chosen because they were the best for maximizing the sensitivity of detection of mecA-positive S. epidermidis isolates without severely compromising specificity. However, for CoNS other than S. epidermidis, the actual breakpoints are less effective in differentiating mecA-positive from mecA-negative isolates. Rowe et al. [23], indicated that, for S. epidermidis, the E-test and the OSAS (0.6 µg/ml) correctly discriminated mecA-positive from mecA-negative isolates with 100% sensitivity. However, these tests were not satisfactory to assess methicillin resistance to other CoNS species. Our study showed 100% sensitivity of the E-test and OSAS (0.75 µg/ml) for both groups (epidermidis and non-epidermidis), but there were differences in OSAS 1 and 6 µg/ml for the two groups (100% vs. 91.7% and 94.6% vs. 83.3%, respectively). There were also differences in the latex agglutination, automated and cefoxitin disk diffusion tests. The present study used only 23 of the non-epidermidis CoNS species, and a greater number would be required for validation of the interpretation.

Four of the false-positive isolates were β-lactam hyperproducers, which was confirmed by the nitrocefin and amoxicillin-clavulonic acid disk diffusion tests.

Although it has been reported [27] that OSAS (6.0 µg/ml) is ineffective for CoNS, the OSAS (0.75 µg/ml) in the present study showed the best performance of all of the used tests, including the other two concentrations (1.0 and 6.0 µg/ml), and it could thus be considered an accurate method for confirmation of resistance. Comparative studies to assess the OSAS and disk diffusion test have shown that OSAS has good sensitivity for the identification of methicillin-resistant strains [29,33,36,45-52].

The E-test and OSAS (0.75 µg/ml) were equally reliable in their detection of mecA-positive isolates. However, these methods proved to be less accurate in discerning isolates that lacked the mecA gene. Louie et al. [45] found a relatively low level of specificity (78%) for the E-test, similar to what we noted. By the E-test and OSAS oxacillin (0.75 µg/ml), 3 (1 S. epidermidis, 1 S. auricularis and 1 S. haemolyticus) of 20 (15%) and 1 (S. auricularis) of 20 (5%) mecA-negative isolates appeared to be resistant. Again, the new breakpoints values, reported by Tenover et al. [27], in 1999, were chosen because they were the best for maximizing the sensitivity of detection of mecA-positive S. epidermidis isolates without severely compromising specificity. However, for CoNS other than S. epidermidis, the actual breakpoints are less effective in differentiating mecA-positive from mecA-negative isolates. Rowe et al. [23], indicated that, for S. epidermidis, the E-test and the OSAS (0.6 µg/ml) correctly discriminated mecA-positive from mecA-negative isolates with 100% sensitivity. However, these tests were not satisfactory to assess methicillin resistance to other CoNS species. Our study showed 100% sensitivity of the E-test and OSAS (0.75 µg/ml) for both groups (epidermidis and non-epidermidis), but there were differences in OSAS 1 and 6 µg/ml for the two groups (100% vs. 91.7% and 94.6% vs. 83.3%, respectively). There were also differences in the latex agglutination, automated and cefoxitin disk diffusion tests. The present study used only 23 of the non-epidermidis CoNS species, and a greater number would be required for validation of the interpretation.

The false-negative results in the tests evaluated may be due to an extremely heterogeneous expression of resistance [53]. On the other hand, the false-positive results may have been the result of overproduction of penicillinase, or of superexpression/alterations of constitutive PBPs. It is known that penicillinase-resistant penicillins may show some degree of lysis when such enzymes are present. The superexpression or alteration of constitutive PBPs generates a higher concentration of free transpeptidase, which synthesize the cell wall of the bacteria [40]. The expression of resistance is enhanced by passage in β-lactam antimicrobials, because the susceptible subpopulation is eliminated and the highly resistant subpopulation is selected out. These antimicrobial-selected cells are therefore more uniformly resistant than is the parent strain, but the trait is unstable. With repeated subculturing in drug-
free medium, the culture reverts to its heterogeneous pattern of resistance\(^{(52)}\). Attempts to increase sensitivity for detection of methicillin resistance may do so at the cost of reduced specificity. Factors that enhance such resistance (48-h incubation, 5% NaCl, 30°C incubation, and high doses of inoculum) may also enhance β-lactamase production\(^{(53)}\).

Methicillin-resistant staphylococci are typically resistant to a variety of antimicrobials, including quinolones, tobramycin, clindamycin, trimethoprim-sulfamethoxazole, tetracycline, and erythromycin\(^{(52,54)}\). Therefore, before any drug other than vancomycin is used, susceptibility of the isolate must be confirmed\(^{(54)}\). Hussain et al.\(^{(34)}\), in 2002, affirmed that CoNS with the \(mecA\) gene were resistant to erythromycin, clindamycin and co-trimoxazole, but that tetracycline was equally active against \(mecA\)-positive and \(mecA\)-negative isolates; no resistance was observed for vancomycin\(^{(54)}\). It has also been reported that in vitro susceptibility of CoNS to fluoroquinolones antimicrobials is higher for the methicillin-resistant group than for the methicillin-susceptible group, and both groups of staphylococci are more susceptible to gatifloxacin and moxifloxacin than to ciprofloxacin and ofloxacin\(^{(55)}\). The data show higher potency of the fourth generation of fluoroquinolones to treat these infections than that of the second generation\(^{(56,58)}\).

CONCLUSION

In conclusion, because the E-test and the OSAS using 0.75 μg oxacillin per ml, compared with PCR, were the most accurate available phenotypic methods of detecting oxacillin resistance in CoNS ocular isolates, we recommend these tests to confirm results on ocular specimens, since many laboratories, especially those dedicated to ophthalmologic purposes cannot afford to perform PCR. A good option for the screening of oxacillin resistance is the cefoxitin disk diffusion test and the automated test. The differences observed in the oxacillin susceptible tests for CoNS may be explained by the fact that CLSI/NCCLS does not take into consideration the species of the isolated bacterium. Because of their increasing importance, clinically significant CoNS should be identified to the species level and should have their antimicrobial susceptibility profiled. Further studies also may better define the breakpoints for methicillin resistance in CoNS other than \(S.\) \(epidermidis\). These steps should improve our knowledge of the role played in clinical ophthalmology diseases by this group of bacteria, and aid clinicians in the treatment and alleviation of patient suffering and morbidity. The generic identification “coagulase negative staphylococci” may be inappropriate, as it ‘lumps’ together species that respond differently to the same experimental conditions with respect to \(mecA\) gene detection. Not surprisingly, \(mecA\)-positive CoNS were more resistant to the other antimicrobials than were \(mecA\)-negative CoNS that are widely used in ophthalmology and were tested in the present study.

RESUMO

Objetivos: Avaliar os diferentes métodos de suscetibilidade à oxacilina, em isolados oculares, considerando a reação em cadeia da polimerase (PCR) como “padrão-ouro” e compara- rar a suscetibilidade in vitro para outros antimicrobianos de uso oftalmológico. Métodos: O sistema automatizado Vitek foi utilizado para identificar as diferentes espécies de \(Staphylococcus\) coagulase negativo (CoNS). A presença do gene \(mecA\) foi determinada pela reação em cadeia da polimerase com a combinação de 2 “primer” sets (\(mecA\) e 16S rRNA) em uma única região. Estes resultados foram analisados e comparados com outros métodos de suscetibilidade à oxacilina: detecção da proteína \(PBP2a\) pelo teste de aglutinação em látex (SLA); E-test oxacilina; o sistema automatizado Vitek (GPS-105); o teste de triagem em ágar (OSAS) com oxacilina nas concentrações de 6,0, 1,0 e 0,75 μg oxacilina por ml e o teste de disco difusão com cefotaxima (CDD). A suscetibilidade automatizada foi obtida para os seguintes agentes antimicrobianos: fluoroquinolonas, penicilina G, amoxicilina-ampicilina, cefalozina, ampicilina-sulbactam, eritromicina, clindamicina, gentamicina, tetraciclina, sulfametoxazol-trimetoprima, vancomicina e rifampicina. Resultados: Dos 69 \(Staphylococcus\) coagulase negativo testados, 71% foram \(mecA\)-positivos e 29%, \(mecA\)-negativos. Todos os métodos testados apresentaram concordância estatisticamente significante com a reação em cadeia da polimerase. Houve tendência à predominância da positividade da reação em cadeia da polimerase entre os \(S.\) \(epidermidis\) comparado aos não-\(epidermidis\), embora sem significância estatística (78,3% vs. 56,5%; \(\chi^2=2,54; p=0,11\)). O teste de triagem em ágar (0,75 μg oxacilina/ml) apresentou a melhor performance com resultados de: 100% de sensibilidade e valor preditivo negativo, 95% de especificidade e 98% de valor preditivo positivo. Os isolados \(mecA\)-positivos foram estatisticamente significativamente mais resistentes para ciprofloxacina, ofloxa cina, gatifloxacina e moxifloxacina, no E-test (p=0,002; p=0,008; p=0,002 e p=0,003). Houve maior proporção estatisticamente significativa de resistência entre os \(Staphylococcus\) coagulase negativo \(mecA\)-positivos para: penicilina G, amoxicilina-ampicilina, cefalozina, ampicilina-sulbactam, eritromicina, clindamicina, gentamicina e tetraciclina. (p=0,05) Todos os \(Staphylococcus\) coagulase negativo foram suscetíveis à vancomicina e não houve correlação estatisticamente significativa entre as amostras \(mecA\)-positivas e a resistência para sulfametoxazol-trimetoprima e rifampicina. Conclusões: No presente estudo, foi observado que o E-test e o OSAS (0,75 μg oxacilina por ml), comparado à reação em cadeia da polimerase, foram os métodos fenotípicos mais acurados em detectar a resistência à oxacilina nos \(Staphylococcus\) coagulase negativos. Foi demonstrado que os testes de disco difusão com cefotaxima e o método automatizado (Vitek) são boas opções para a triagem da resistência à oxacilina em laboratórios de microbiologia ocular. Destacou-se a importância de métodos acurados para detectar a resistência à meticilina dentre as espécies menos
Laboratory detection methods for methicillin resistance in coagulase negative Staphylococcus isolated from ophthalmic infections

frequentemente encontradas, considerando a crescente importância destes patógenos oportunistas.

Descritores: Resistência a meticilina; Sensibilidade e especificidade; Testes de fixação do látex; Oxacilina; Testes de sensibilidade microbiana; Staphylococcus/isolemento & purificação; Coagulase; Infecções oculares bacterianas; Estudo comparativo

REFERENCES


Laboratory detection methods for methicillin resistance in coagulase negative Staphylococcus isolated from ophthalmic infections