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EVALUATION OF DIFFERENT METHODS FOR DETECTING METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* ISOLATES IN A UNIVERSITY HOSPITAL LOCATED IN THE NORTHEAST OF BRAZIL

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ABSTRACT

Many methods have been described for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA), but the heterogeneous expression of methicillin resistance affects the reliability of these methods. The aim of the present study was to evaluate some methods for detecting methicillin resistance in *Staphylococcus aureus* isolates in a university hospital located in the Northeast of Brazil. Among the isolates, 15 were methicillin-susceptible and 45 were methicillin-resistant, including low-level heterogeneous resistance strains. Both the 30 µg-cefoxitin disk and PBP2a test had 100% sensibility/specificity and appear to be good options for the detection of MRSA in the clinical laboratory.

Key words: MRSA, methicillin resistance, heterogeneous resistance, cefoxitin, PBP2a.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become the leading nosocomial pathogen worldwide and seems to have spread into the community (10). Methicillin resistance in staphylococci is caused by the expression of PBP2a encoded by the *mec*A gene that is located on a genetic element called the staphylococcal cassette chromosome *mec*- SCC*mec* (9).

There are several methods available to laboratories for detecting methicillin resistance. These include oxacillin disk test, automated susceptibility testing systems, and oxacillin agar screen plate. In addition, the cefoxitin disk test was recently recommended by the Clinical and Laboratory Standards Institute for prediction of *mec*A–mediated resistance (5). Finally, there are also *mec*A-specific tests such as *mec*A polymerase chain reaction and PBP2a latex agglutination test (21). However, detection of methicillin resistance in routine clinical laboratories has been problematic ever since the emergence of MRSA during the 1960s (19). The difficulties are associated mainly with heterogeneous expression of methicillin resistance in most MRSA strains currently prevalent (11, 24).

Errors in the detection of methicillin resistance can have serious adverse clinical consequences. False-susceptibility results may result in treatment failure and the spread of MRSA

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if appropriate infection control measures are not applied. Conversely, false-resistance results may increase healthcare costs following unnecessary isolation precautions and may lead to overuse of glycopeptides (3, 23). Thus, as some controversy still exists over the inaccuracy of the recommended methods for identification of MRSA, we evaluated some of these methods for detecting methicillin resistance in *Staphylococcus aureus* isolates in a university hospital located in the Northeast of Brazil.

MATERIAL AND METHODS

Bacterial strains

A total of 60 strains of *S. aureus* isolated from clinical samples in a university hospital in Natal city, RN, located in the Northeast of Brazil were used in the study. The isolates were identified as *S. aureus* using routine tests (Gram's stain, catalase and free coagulase tests) and stored at -70 ºC in TSB containing 10% (w/v) glycerol. The study was approved by the Research Ethics Committee of the Universidade Federal do Rio Grande do Norte (UFRN), according to protocol no. 109/2006. The methicillin-susceptible strain of *S. aureus* ATCC 25923 and the MRSA isolate BMB9393 were used to control.

Detection of the *mec*A gene

We considered the presence of the *mec*A gene as the reference or "gold standard" method for establishing the sensitivity and specificity of each of the techniques studied. The DNA extraction was performed as described by Pacheco *et al.* (13) and the *mec*A gene was detected with the polymerase chain reaction (PCR) technique based on the procedure described by Oliveira and de Lencastre (12), with the following primers: *mec*A-F AAA ACT AGG TGT TGG TGA AGA TAT ACC and *mec*A-R GAA AGG ATC TGT ACT GGG TTA ATC AG, which amplify an internal region of 585bp of this gene. Amplicons were visualized following electrophoresis on agarose gels stained with ethidium bromide.

Susceptibility tests

The 1 µg-oxacillin and 30 µg-cefoxitin disk tests (DME, Araçatuba, SP, Brazil) and 6 µg/mL oxacillin screen test (OST) were performed by observing the recommendation of the Clinical and Laboratory Standards Institute (5). The 25 µg/mL methicillin test (25-Met) was carried using trypticase soy agar plates and heavy bacterial inoculums (10^9-10^{10} c.f.u.), as described previously (14).

Detection of penicillin-binding protein 2a (PBP2a test)

The Slidex® MRSA Detection (Biomérieux, Paris, France), which is based on the agglutination of latex particles sensitized with monoclonal antibodies against PBP2a, was carried out and interpreted according to the manufacturer's instructions.

Population analysis profiling (PAP)

The expression of methicillin resistance was analyzed by PAP for strains that yielded isolated colonies on 25-Met. Overnight cultures grown in TSB at 35ºC, containing 10^9–10^{10} c.f.u./mL, were plated at four dilutions (10^0, 10^{-1}, 10^{-3} and 10^{-5}) on to agar plates containing serial (two-fold) dilutions of methicillin at concentrations of 0 and 0.75 to 800 mg/mL. Colonies were counted after 48 h incubation at 35ºC. A graphic representation was constructed by plotting colony forming units per millilitre against the concentration of methicillin (22).

RESULTS

Among the 60 *S. aureus* isolates, 45 were MRSA (*mec*A positive) and 15 were MSSA (*mec*A negative; Fig. 1). Of the 45 MRSA isolates, 73% (33/45) belonged to the Brazilian Epidemic Clone (ST239- SCC*mec*IIIA), 7% (3/45) to the pediatric clone (ST5- SCC*mec*IV), and 20% (9/45) to the sporadic clones (20).

Of all the *mec*A-positive isolates, one (NT42) displayed inhibition zone diameters of 13mm by a 1µg-oxacillin disk test (resistance breakpoint ≤10mm) and two (NT05 and NT80) showed small individual colonies or light growth within the inhibition zone >10mm and could have been erroneously classified as methicillin-susceptible isolates (sensibility 93.3%, specificity 100%). The NT42 strain yielded a very hazy growth on the surface of OST (sensibility 97.8%, specificity 100%). In addition, these strains (NT42, NT05 e NT80) yielded isolated

colonies on 25-Met and were classified as low-level heterogeneous resistance to methicillin by PAP (Fig. 2). The 30 µg-cefoxitin disk test and PBP2a test showed 100% de sensibility e specificity. The sensibility and specificity of the phenotypic tests as compared with *mec*A detection are presented in Table 1.

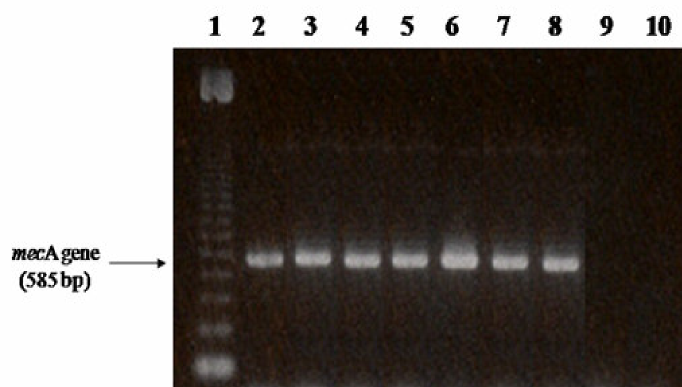

Figure 1. Gel image of representative PCR *mec*A gene products (585bp), Lane 1: molecular size marker (123bp); Lanes 2-10: isolates BMB9393 (a positive control), NT05, NT11, NT42, NT76, NT80, NT99, NT21 and ATCC 25923 (a negative control).

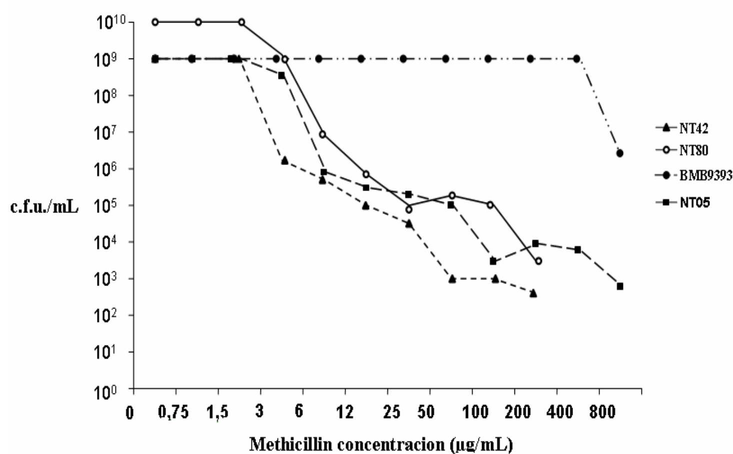

Figure 2. Population analysis profile of three low-level heterogeneous isolates (NT42, NT80 and NT05) and one homogeneous isolate (BMB9393).

Table 1. Sensibility and specificity of the phenotypic tests as compared with *mec*A detection

Test method	Detected as MRSA	Sensibility (%)	Specificity (%)
1 µg-oxacillin disk test	42	93.3	100
30 µg-cefoxitin disk test	45	100	100
6µg/mL oxacillin screen test	44	97.8	100
PBP2a test	45	100	100

DISCUSSION

Rapid and accurate identification of MRSA is required to help clinicians select appropriate antibiotic treatment and to avoid the spread of these strains (16, 18). However, there is no optimal phenotypic method for detecting methicillin resistance in *S. aureus* (2, 3). Furthermore, genotypic tests involving *mec*A gene detection by PCR, which are considered to be the reference (1, 21), are not practical for routine use in clinical laboratories.

In the paper presented here, we evaluated some methods for detecting methicillin resistance in *Staphylococcus aureus* isolates and observed that the 1 µg-oxacillin disk test and oxacillin screen test may occasionally result in misidentification, especially by a microbiology technician with no previous practice with MRSA detection (17). Similar results were found by other authors (2, 6, 11, 24). Then, special attention should be given to these strains because these, as well as community-acquired MRSA (CA-MRSA) strains, have been described in Brazil and throughout the world (15, 17, 24).

In this study the 30 µg-cefoxitin disk test was found to be as sensitive as the *mec*A gene detection by PCR. The greater reliability of the test with the cefoxitin disk confirmed earlier studies which showed that the cefoxitin disk test, without modification to conditions to improve expression of resistance, is more reliable than the oxacillin disk test for the detection of methicillin resistance in *S. aureus* (7, 16, 18). According to Caulwelier *et al.* (4), because the cefoxitin would be a better inducer of the expression of the *mec*A gene, this could explain

why heterogeneous MRSA populations that variably express the *mec*A gene are better detected by disk diffusion with cefoxitin than with oxacillin, which is a weak inductor of PBP2a production.

The PBP2a test was 100% sensitive and did not misclassify any MRSA with low-level resistance as MSSA. Our findings are consistent with many other studies (4,6,8). In addition to being easy to interpret and showing 100% correlation with *mec*A detection, the PBP2a test has the advantage that suspected colonies could be tested from the primary cultures before the accomplishment of the bacterial identification (17). However, this test is rather expensive for routine application.

Although the number of isolates tested in our study was low, our results support the evidence that the cefoxitin disk test and the PBP2a test are good options for MRSA detection in the clinical laboratories, as both showed 100% correlation with *mec*A detection, including low-level resistance to methicillin strains.

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