Brazilian Journal of Microbiology (2003) 34 (Suppl.1):35-37
ISSN 1517-8382

CONVENTIONAL *VERSUS* MOLECULAR TESTS (MULTIPLEX PCR AND PCR *MECA* GENE) FOR DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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This paper corresponds to an "extended abstract" selected for oral presentation in the 22nd Brazilian Congress of Microbiology, held in Florianópolis, SC, Brazil, in November 17-20, 2003

ABSTRACT

In this study, for detection of methicillin-resistant *S. aureus* (MRSA), a *mec*A multiplex PCR-based amplification was compared with the 1 μg oxacillin disk diffusion test, detection of minimal inhibitory concentration (MIC), and screening in agar with 4% NaCl and 6 μg/mL oxacillin. Among 24 isolates obtained from blood, *mec*A gene was detected in only 16 (66.7%) isolates by multiplex PCR. The MIC test showed a range of resistance to oxacillin from 0.19 to 512 μg/mL, among these isolates. Data obtained by screening and dilution tests showed that sensitivity to methicillin was 80.0% and 72.8%, respectively, when compared with the presence of *mec*A gene (multiplex). All isolates, including the negatives, when revaluated for *mec*A gene by PCR were positive. β-lactamase production was positive for 20/25 isolates (80.0%). About ¼ of patients died dispite most of them (83.3%) were adequately treated. The simultaneous identification of the bacteria and determination of this susceptibility to antibiotics are necessary for the choice of empiric antibiotic therapy in suspected staphylococcal sepse, but is important to considering the sensibility, specificity and validation of the available kits.

Key words: multiplex PCR; MRSA; β-lactamase; *mec*A gene.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen worldwide (1) and several classical methods have been used to detect the MRSA isolates including the 1 μg/mL oxacillin disk diffusion test, agar plate screen, agar dilution and the E'*test* (NCCLS) (2,3). Alternatively, molecular techniques mostly based on PCR amplification of *mec*A gene were used for rapid detection of MRSA (1). In the present study we compared the *mec*A multiplex PCR-based amplification and agar dilution, E'test, oxacillin agar screen results with the 1 μg disk diffusion test results that initially classified 24 isolates of MRSA.

MATERIALS AND METHODS

Hospital and design of the study

The 24 MRSA strains used in the present study were isolated by the microbiology laboratory of the Clinic Hospital of Uberlandia Federal University.

Oxacillin disk diffusion method and agar screening method for detection of MRSA

The 1 μg oxacillin disk diffusion method was performed on Mueller-Hinton agar. All isolates were also plated on Mueller-Hinton agar containing 4% NaCl and 6 μg/mL (4) oxacillin.

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E'test for determining the MICs of oxacillin against *S. aureus*

For confirmation of *mec*A negative isolates, the E-test was used to determine the MICs of oxacillin.

Detection of β-lactamase production

β-lactamase production was tested using nitrocefin (Oxoid-Hampshire, UK).

Multiplex PCR

PCR was performed with six complementary primers: mecA1 (5'- CTC Agg TAC TgC TAT CCA CC–3'); mecA2 (5' – CAC TTg gTA TAT CTT CAC C-3'); mupA1 (5' – TgA CAA TAg AAA Agg ACA gg–3'); mupA2 (5' – CTC TAA TTC AAC Tgg TAA Gcc–3'); 16S rRNA1 (5' – ggA ATT CAA A (T/g)g AAT TgA Cgg ggg C–3') and 16S rRNA2 (5' – Cgg gAT CCC Agg CCC ggg AAC gTA TTC AC– 3').

RESULTS

The results for the 25 MRSA isolates characterised by disk diffusion test are presented in Table 1. They are listed according to their level of MICs. Correlations between susceptibility testing and oxacillin disk test, oxacillin agar screening and presence of *mec*A gene are showed in Table 2. All isolates showed resistance to 1 μg/mL oxacillin disk diffusion test and 91.7 % of isolates were considered resistant by the agar dilution test. Using the multiplex PCR-based amplification, *mec*A gene was detected in 16 of the 24 (66.7%) isolates. The negative isolates, revaluated for the *mec*A gene by PCR, were all *mec*A positive. β-lactamase production was detected in 20 of the 24 (83.3%) isolates. Among the four β-lactamase negative strains, two were positive for *mec*A.

DISCUSSION

We have compared multiplex PCR assays for the detection of antibiotic resistance genes with classical methods for the determination of susceptibility to antibiotics. In this study the 24 isolates initially classified as methicillin resistant based on disk diffusion showed discrepant results when tested with the multiplex PCR. The *mec*A was positive in 16 of 24 (66.7%) isolates and oxacillin MIC of ≥ 128 μg/mL was found in 22 (91.7%) isolates. The discrepant findings in our study can be attributed to techinical problems. Investigators reported that the conventional susceptibility testing methods, including disk diffusion and agar dilution tests, are not always reliable in detecting methicillin resistant staphylococci (4). The staphylococcal strains showing discrepancies between the genotype ant the phenotype were revaluated by PCR of the *mec*A gene only, and all were positive. Some studies reported *S. aureus* strains carrying the *mec*A gene but susceptible to oxacillin. York *et al.* (5) observed that staphylococci strains with MIC less than 2 μg/mL may be methicillin resistant. In this work, two isolates with MIC ≤ 0.25 μg/mL carried *mec*A gene. Although the expression of the *mec*A gene is considered an important mechanisms, of resistance, other mechanism alone or in combination have been detected in *Staphylococcus* strains. In our study, 83.3% of the *S. aureus* isolates were producers of β-lactamase.

Table 1. Overall summary of tests results for the 24 *S. aureus* isolates initially characterized as methicillin resistant by the disk diffusion.

Isolate number	MICs (μg/mL)	1μg oxacillin disk diffusion	Agar screening	β-lactamase	*MecA* (multiplex)	*Meca* (PCR)
1	512	+	+	+	-	+
2	512	+	+	-	-	+
3	0.19	+	-	+	-	+
4	512	+	+	+	-	+
5	512	+	+	+	+	NT
6	512	+	+	+	+	NT
7	512	+	+	+	+	NT
8	512	+	+	+	+	NT
9	512	+	+	+	+	NT
10	512	+	+	+	+	NT
11	512	+	+	+	+	NT
12	512	+	+	+	+	NT
13	256	+	+	+	+	NT
14	512	+	+	+	+	NT
15	512	+	+	+	-	+
16	128	+	+	+	-	+
17	256	+	+	-	-	+
18	0.25	+	+	+	-	+
19	512	+	+	-	+	NT
20	256	+	+	-	+	NT
21	512	+	+	+	+	NT
22	32	+	+	-	+	NT
23	512	+	+	+	+	NT
24	256	+	+	+	+	NT

NT, Non tested.

Table 2. Correlation of susceptibility testing with MIC, oxacillin disk test, oxacillin agar screening, β-lactamase and with the presence of *mec*A gene for the 24 *S. aureus* isolates.

MIC (μg/mL)	Nº isolates	Disk diffusion	Agar Screening	β-lactamase	*mecA* positive (multiplex)	*mecA* negative
> 256	22	22	21	20	15	7
32	1	1	1	0	1	0
≤ 4	1	1	1	0	0	1

RESUMO

Testes convencionais versus moleculares (Multiplex PCR e PCR Gene *mecA*) na detecção de *Staphylococcus aureus* resistente a meticilina

Nesse estudo, para detecção de *S. aureus* resistente à meticilina (MRSA), a amplificação do gene *mec*A baseada no multiplex PCR foi comparada com os testes de difusão com disco para 1 µg/mL de oxacilina, detecção da concentração inibitória mínima (CIM), meio de triagem com 4% de NaCl e 6 µg/mL de oxacilina. Na investigação de 24 isolados obtidos de sangue, o genótipo *mec*A foi detectado em apenas 16 (66,7%) dos isolados pelo multiplex PCR. A CIM apresentou valores variando de 0,19 a 512 µg/mL entre os isolados de MRSA. Os dados obtidos pelos testes de triagem e diluição em ágar apresentaram sensibilidades de 80,0% e 72,8% respectivamente, quando comparados com a presença do gene *mecA* (multiplex). Todos os isolados, incluindo os negativos, quando reavaliados com a técnica de PCR exclusivo para este gene, o resultado foi positivo. A produção de β-lactamase foi positiva em 20/25 (80,0%) dos isolados. Cerca de ¼ dos pacientes evoluiu para óbito apesar da maioria (83,3%) ter sido tratada adequadamente. A identificação simultânea da bactéria e sua susceptibilidade aos antibióticos é necessária para a escolha de uma terapia adequada para casos de sepse estafilocócica, mas é importante considerar a sensibilidade, especificidade e validação dos kits disponíveis.

Palavras-chave: multiplex PCR; MRSA; β-lactamase; gene *mec*A.

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