ACCURACY OF OXACILLIN SUSCEPTIBILITY TESTING METHODS FOR *STAPHYLOCOCCUS AUREUS*

OLIVIA DORNEANU[1*], TEODORA VREMERĂ[1], EDUARD NĂSTASE[2], CĂTĂLINA LOGIGAN[2], LUMINIŢA IANCU[1], EGIDIA MIFTODE[2]

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Abstract. Since oxacillin is the first-choice drug in the treatment of methicillin-susceptible *Staphylococcus aureus* infections, correct assessment of oxacillin susceptibility is mandatory. METHODS: One hundred and fifty-six *S.aureus* strains have been used to evaluate the accuracy of disk diffusion method (1 µg oxacillin disk and 30 µg cefoxitin disk), detection of minimum inhibitory concentration of oxacillin (E-test), and a latex-agglutination test for detection of PBP2a compared with detection of *mecA* gene by a real-time PCR technique as gold standard. RESULTS: The *mecA* gene was detected in 68 strains. Sensitivities of the evaluated methods were 98.5%, 100%, and 100%, while specificities were 100% for all three of them. CONCLUSIONS: Accuracy of phenotypic methods for assessment of oxacillin resistance approaches that of detection of *mec*A for *S.aureus* isolates.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) frequently associates multidrug resistance and causes serious nosocomial and community-associated infections worldwide. Thus, evaluation of the accuracy of the phenotypic methods that are used to determine the profile of antimicrobial resistance is essential to ensure that the most appropriate therapy is chosen.

Rapid and accurate identification of MRSA strains is essential in both implementation of infection control measures and prevention of the nosocomial spread of this microorganism.

Because methicillin resistance mediated by PBP 2a can be heterogeneously expressed, detection of *mecA* gene using polymerase chain reaction (PCR) is the “gold standard” for the detection of MRSA (Mohanasoundaram and Lalitha, 2008). Real-time PCR (RT-PCR) technique allows for rapid detection of target sequences and, in the case of severe infections, it is useful to provide quick identification. The objective of our study is to compare oxacillin disk diffusion test, oxacillin E-test, and a PBP2a latex agglutination test, using real-time PCR for *mecA* as the "gold standard" comparison assay.

MATERIALS AND METHODS

We isolated 156 *S.aureus* clinical strains from Infectious Diseases Hospital Iasi between 1.01.2008 and 31.12.2009. The strains were tested for oxacillin susceptibility by use of the oxacillin (1µg) and cefoxitin (30µg) disk diffusion test according to CLSI guidelines (2008) and a latex agglutination test for detection of penicillin-binding protein PBP2a (bioMerieux, France). Oxacillin minimum inhibitory concentrations (MICs) were determined using E-test method (AB Biodisk, Sweden).

MRSA isolates were confirmed by the detection of *mecA* gene with a single-target TaqMan PCR assay. The RT-PCR assay was performed with genomic DNA extracts from broth culture lysates of 156 clinical isolates and the reference strains *S. aureus* ATCC 29213 and *S. aureus* ATCC 33592, using *GenElute Bacterial Genomic DNA kit* (Sigma Aldrich, Germany).

The 20µl PCR mixture contained: 10µl of 1× *HotStar Taq Master Mix kit* (Finnzymes, Finland), 4µl *mecA* specific primers, 0.2µl dual-labeled *mec*A probe with FAM as the 5′ reporter and 3′ TAMRA as the quencher, 2 µl of DNA sample, used as the target in the PCR and 3,8 µl PCR water.

Primer and probe sequences for *mecA* gene are described in the Table 1. The sets of primers were previously reported by McDonald *et al.* (2005).

Reactions were performed in triplicate, under the following thermal cycling conditions: 7 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s.

Data for the PCR assay were collected using Stratagene MX 3005P real-time PCR system.

The amplification conditions were optimized using reference strains *S. aureus* ATCC 29213 and *S. aureus* ATCC 33592.

Table 1: Primer and probe sequences (as described previously by McDonald *et al*.., 2005)

Primer and probe name	Sequence (5′→3′)	Reaction concentration (μM)
mecA For	GGCAATATTACCGCACCTCA	0.30
mecA Rev	GTCTGCCACTTTCTCCTTGT	0.30
mecA Probe	AGATCTTATGCAAACTTAATTGGCAAATCC	0.10

RESULTS AND DISCUSSION

Out of 156 *S. aureus* clinical isolates, 68 strains (43.6%) were methicillin-resistant by PCR detection of *mecA* gene.

Oxacillin MICs varied between 0,06-256 μg/ml. Oxacillin disk diffusion method correctly identified 67 of 68 MRSA strains, with a 98.5% sensitivity compared to RT-PCR *mec*A detection. All of 68 *mecA*-positive *S. aureus* isolates were correctly determined as methicillin-resistant by PBP2a latex agglutination test and oxacillin E-test (sensitivity 100%). Specificities were 100% for all three evaluated methods. All methods tested had a statistically significant agreement with real-time PCR.

Phenotypic methods for the detection of MRSA are problematic because of the heterogeneous resistance. PCR is more accurate and is considered as the gold standard assay for the detection of MRSA, but it is an expensive method and it is not available for most of the routine laboratories. In our study, we have evaluated different phenotypic tests for the detection of MRSA. Our results are comparable to those of other studies.

Shariati *et al.* (2010), in a study on 196 *S. aureus* isolates found that E-test was 100% sensitive and specific for *mecA* presence, while the sensitivity and specificity of oxacillin disk diffusion method were 95 and 93%, respectively and proposed oxacillin E-test as the best phenotypic method (5).

A study performed by Baddour *et al.* (2007) showed that PBP2a latex agglutination methods were more sensitive than oxacillin and cefoxitin disk-diffusion methods, but cefoxitin disk diffusion was the most specific. They also found that a combination of oxacillin disk diffusion with PBP2a latex agglutination improved sensitivity and specificity and concluded that the use of more than one screening method is necessary to detect all MRSA isolates in clinical settings.

Use of two phenotypic methods is also recommended by Kaiser *et al.* (2010) in order to improve accuracy, especially when a diagnostic laboratory only uses an automated system or oxacillin disk diffusion test.

Mohanasoundaram and Lalitha (2008), in a study on 150 isolates found a 100% concordance between the oxacillin disk screening and MIC methods and also that the specificity and sensitivity of latex agglutination test was similar to PCR for *mecA* detection.

A study performed by Ercis *et al.* (2008) showed 100% sensitivity and specificity of disk diffusion and E-test compared to PCR and concluded, based on the analysis of 248 *S. aureus* strains, that disk diffusion is superior to other phenotype-based susceptibility testing methods in detecting MRSA.

Out of the phenotypic methods, the VITEK2 system is highly reliable for methicillin resistance detection at the routine level. Since most of the strains misclassified as oxacillin-

susceptible are resistant to aminoglycosides and/or fluoroquinolones, oxacillin-susceptible classified clinical strains with associated resistance patterns required attention (Berner *et al.*, 2010).

CONCLUSIONS

Accuracy of phenotypic methods for assessment of oxacillin resistance approaches that of detection of *mec*A for *S.aureus* isolates.

Oxacillin E-test and PBP2a latex agglutination methods were more sensitive than oxacillin disk-diffusion method.

Our results showed that for detection of MRSA, PBP2a latex agglutination test is an accurate method that can be easily performed in routine microbiology laboratories and it is faster than oxacillin MIC determination.

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1 - University of Medicine and Pharmacy “Gr. T. Popa” Iaşi, Microbiology Department
2 - University of Medicine and Pharmacy “Gr. T. Popa” Iaşi, Infectious Diseases Department

* Corresponding author: Olivia Dorneanu, odorneanu@yahoo.com, 16, Universităţii street, Iaşi 700115, Romania, phone +40747413919

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