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COMPARISON OF PBP2a LATEX AGGLUTINATION TEST WITH DISK DIFFUSION, mecA PCR AND VITEK FOR THE DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES

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SUMMARY

To determine the most accurate genotypic or phenotypic method for the detection of MRSA, oxacillin susceptibility of 111 S. aureus isolates recovered from various clinical specimens were studied by 4 different methods: 1. NCCLS disk diffusion test; 2. Susceptibility determination by the Vitek GPS 101 (bioMerieux, France) cards; 3. Direct detection of PBP2a with MRSA-Screen (Denka Seiken, Japan) test; 4. mecA gene detection by PCR.

The numbers of isolates found to be methicillin resistant were 80 by the disk diffusion test, 85 by the Vitek system, 81 by the MRSA-Screen test, and 76 by the mecA gene analysis. According to mecA gene analysis, sensitivity and specificity of disk diffusion, Vitek, and MRSA-Screen tests were found as 100 % and 90 %;100 % and 80 %; 100 % and 88 %, respectively. When the isolates which had shown discrepancies with both phenotypic and genotypic methods were re-studied, it was observed that all results were in complete agreement with MRSA-Screen test.

NCCLS disk diffusion test and Vitek system, providing that all recommendations were followed carefully, are suitable tests to be used in routine laboratories to detect MRSA isolates, whereas MRSA-Screen test can be preferred as a verification tool due to its being a fast, easy and dependable method.

Key words: *disk diffusion, mecA gene, MRSA-Screen, Staphyloccocus aureus, Vitek*

ÖZET

Metisiline Dirençli Staphylococcus aureus İzolatlarında PBP2a Lateks Aglutinasyon Testinin Disk Difüzyon ve Vitek ile Karşılaştırılması

Metisiline dirençli Staphylococcus aureus (MRSA) izolatlarının saptanmasında genotipik ve fenotipik yöntemlerin güvenilirliği araştırılmıştır. Bu amaçla çeşitli klinik örneklerden izole edilen 111 S.aureus izolatının oksasiline duyarlılıkları dört farklı yöntem [1- NCCLS disk difüzyon testi, 2- Vitek GPS 101 (bioMerieux, Fransa) kartları ile duyarlılık saptanması, 3- MRSA-Screen (Denka Seiken, Japonya) ile direkt PBP2a belirlenmesi ve 4- PCR ile mecA geni varlığının gösterilmesi] kullanılarak incelenmiştir.

Metisiline dirençli izolatların sayısı disk difüzyon yönteminde 80, Vitek sistemi ile 85, MRSA-Screen test ile 81 ve mecA gen analizi ile 76 olarak bulunmuştur. mecA gen analizi sonuçlarına göre disk difüzyon, Vitek ve MRSA-Screen testinin duyarlılık ve özgüllükleri sırasıyla % 100 ve % 90; % 100 ve % 80; % 100 ve % 88 olarak bulunmuştur. Gerek fenotipik gerekse genotipik yöntemler arasında sonuçların uyumsuz olduğu örnekler yeniden çalışıldığında, sonuçların MRSA-Screen ile örtüşecek şekilde değiştiği gözlenmiştir.

NCCLS disk difüzyon yönteminin ve Vitek sisteminin inokulum miktarına dikkat edilerek kullanıldığı takdirde rutin laboratuvarlarda MRSA belirlenmesinde kullanılabilecek güvenilir yöntemler olduğu saptanmıştır. Ayrıca, MRSA-Screen testinin hızlı, kolay ve güvenilir bir yöntem olduğu, doğrulama testi olarak tercih edilebileceği düşünülmüştür.

Anahtar sözcükler: *disk difüzyon, mecA, MRSA-Screen, Staphyloccocus aureus, Vitek*

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INTRODUCTION

Staphylococcus aureus is an important human pathogen causing various kinds of infections in a spectrum of simple soft tissue inflammation to septicemia[1]. This species is also one of the leading pathogens in nosocomial infections. The strains isolated from nosocomial infections are frequently found to be resistant to methicillin. Methicillin resistance is associated with a new penicillin binding protein (PBP2a) which is the product of *mec*A gene[9,16]. Methicillin resistant *S.aureus* (MRSA) isolates are resistant to virtually all beta-lactam antibiotics due to the low affinity of PBP2a for these drugs[9]. Moreover, these methicillin resistant strains also exhibit multiple resistance to other groups of antibiotics, compelling clinicans to use glycopeptide antibiotics as the sole solution[3]. As a result, accurate and rapid detection of methicillin resistance in *S.aureus* is essential for the institution of appropriate antimicrobial therapy, and for the restriction of the unnecessary usage of glycopeptide antibiotics. Unfortunately, many strains are heterogenous in the expression of methicillin resistance, which makes the detection of MRSA by routine tests difficult.

Oxacillin disk diffusion method is the most frequently employed test for the detection of methicillin resistance[11]. Additionally, automatized sensitivity tests and commercially available agglutination tests that detects PBP2a are preferred by some laboratories[15]. However, *mec*A gene analysis stands as the ''gold standard'' in the determination of methicillin resistance[6].

In our study, we investigated the reliability of routine methods in determining the methicillin resistance in *S.aureus* isolates by comparing the results with *mec*A detection by polymease chain reaction (PCR).

MATERIALS AND METHODS

Isolates: In the present study, a total of 111 clinical *S. aureus* isolates collected in 2000 were used. Four clinical *mec*A positive strains (one homogenously and 3 heterogenously resistant strains were kindly provided by Dr. S. Kocagöz, Sabancı University) and *S.aureus* ATCC 25923 (*mec*A negative) were used as reference strains.

Methicillin resistance was determined using four different methods in order to compare their performance:

1-*mec*A detection by PCR
2-Disk diffusion
3-Vitek 1 system (bioMerieux, France)
4-MRSA-Screen (Denka Seiken, Japan).

PCR for *mec*A gene detection was performed as reported previously[13]. Briefly, isolates are grown in Mueller-Hinton broth for 24 hours and centrifuged at 4000 rpm for 20 minutes. The supernatant is discarded and one ml TE (10 mM Tris, 1 mM EDTA) buffer was added. After centrifugation, the cells were suspended in 50 µl of lysostaphin (100 µg/ml) (Sigma Comp, Germany) for 10 minutes at 37°C. Tube contents were treated by proteinase K (100 µg/ml) (Sigma Comp, Germany) at 37°C for 10 minutes, after which the suspension was left for 15 min in a boiling water bath. In the next step, centrifugation at 14000 rpm for 20 minute was performed.
The supernatant is collected and used in PCR.

PCR for detection of the *mec*A gene was performed using primers:

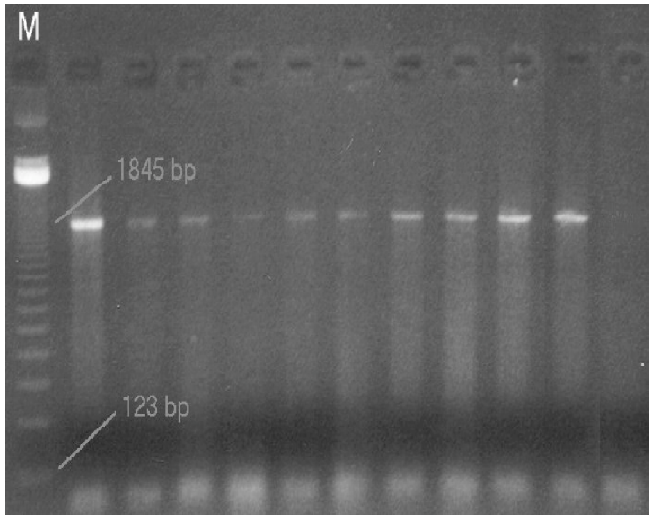
(forward) 5' GTTGTAGTTGTCGGGTTT 3' , and,
(reverse) 5' CCACCCAATTTGTCTGCCAGTTTCTCC 3'.

DNA mixture for PCR is prepared by adding 5 µl of DNA into 45 µl of reaction mixture of: Taq buffer (Promega, USA); 0.1 mM of each dNTP's; 2.5 mM $MgCl_2$; 12 pmol each primer; 2.5 U Taq polymerase.

Amplification programme was as follows:

Denaturation	94°C	30 seconds	
Annealing	55°C	30 seconds	} x 30 cycles
Extension	72°C	2 minutes	
Final extension	72°C	5 minutes.	

Amplification products 1.8 kb were detected by electrophoresis on a 1 % agarose gel containing ethidium bromide, and were evaluated under UV light (Picture 1).



Picture 1: *Lane 1; DNA ladder, lane 2-10; MRSA isolates, lane 11; positive control, lane 12; negative control*

Disk diffusion was performed according to the National

Committee for Clinical Laboratory Standards (NCCLS) recommendations[11]. Testing with Vitek 1 system (version WSVTK-R06.01) was performed according to the manufacturer's instructions. GPS-101 Gram positive test panel was used.

MRSA-Screen is a rapid slide latex agglutination test, which detects PBP2a. The test was performed according to the manufacturer's instructions. Briefly, the isolates were subcultured onto blood agar at 37°C for 18 hours to obtain fresh growth. A loopful of cells was suspended in four drops of extraction reagent 1 and was placed in a boiling water bath for 3 minutes. After allowing the suspension to cool to room temperature (approx.10 minutes), one drop of extraction reagent 2 was added and the mixture was vortexed thoroughly. The suspension was then centrifuged at 4500 rpm for 5 minutes. The latex agglutination test was performed with the supernatant, and 50 μl of the supernatant was mixed with a drop of sensitized latex. For the negative control, 50 μl of the supernatant was mixed with 1 drop of negative control latex. The samples were mixed for 3 minutes on a shaker and the results were evaluated according to the following plan:

Sensitized latex	Control latex	Results
+	-	MRSA
-	-	MSSA
-	+	Undetermined

RESULTS

Results obtained from four methods are as follows (Table 1). According to *mec*A gene analysis, sensitivity and specificity of disk diffusion, Vitek, and MRSA-Screen tests are 100 % and 90 %; 100 % and 80 %; 100 % and 88 %, respectively .

Table 1: *The comparison of mecA gene analysis results with 3 different methods.*

	MRSA-Screen		Disk diffusion		Vitek	
	+	-	+	-	+	-
mec A (+)	76	0	76	0	76	0
mec A (-)	5	30	5	30	9	26

When samples, which yielded contradictory results (Table 2) from phenotypic test (Vitek), was re-examined, they were observed to have similar results with the MRSA-Screen test. Quality control isolates yielded the expected results for each one of the four methods (Table 2).

The discrepancy of the Vitek system with the other methods was thought to be due to the inoculum concentration, while the disconcordance of the *mec*A PCR analysis with the other methods was attributed to inappropriate DNA extraction.

Tablo 2: *Methicillin susceptibility by the application of 4 different methods on contradictory isolates and quality control strains (S=Sensitive, R=Resistant).*

	Disk diffusion	Vitek	MRSA- Screen	mecA
1	R	R	R	S
2	R	R	R	S
3	R	R	R	S
4	R	R	R	S
5	R	R	R	S
6	S	R	S	S
7	S	R	S	S
8	S	R	S	S
9	S	R	S	S
Quality control strains				
MSSA	S	S	S	S
MRSA	R	R	R	R
HMRSA*	R	R	R	R

(Heterogeneously resistant S.aureus strains n=2)*

DISCUSSION

Methicillin resistance of *S.aureus* remains to be a significant problem. Rapid and accurate determination of methicillin resistance is important for initiation of appropriate antimicrobial therapy. Misdiagnosing this resistance leads to treatment failures and spread of infections with these resistant strains. The increasing reports about vancomycin resistance not only among enterococci but also among *S.aureus* isolates mandate us to use the glycopeptide antibiotics, which yet appear to be the only choice, sparingly. Therefore, striving for the identification of methicillin resistance as soon as bacterial growth is observed, is the only way to limit the superfluous use of glycopeptide class on sensitive isolates.

Disk diffusion and microdilution methods are employed in routine laboratories for the detection of methicillin resistance. However, both of the above methods require 24 hours to evaluate the results. Newer methods to detect the PBP2a which is the product of the gene *mec*A appeared in recent years [15,10]. MRSA-Screen test, being one of them, is a rapid and simple to perform method that is completed in only 20 minutes.

Griethuysen et al.[5] compared the results of oxacillin agar screen test and MRSA-Screen test with *mec*A gene analysis results. The sensitivity of MRSA-Screen test were found to be higher than oxacillin agar screen test.

Louie et al[8]. compared the results of MRSA-Screen test, automatized system, and Velegene rapid MRSA identification test with those of oxacillin agar screen test and *mec*A gene test. According to this study, the sensitivity and specificity of Velegene and MRSA-Screen tests were found to be equal 98.5 % and 100 %, respectively. They reported that they obtained faulty results for BORSA isolates from automatized system they used. They concluded that MRSA-Screen test might well be an alternative for *mec*A detection in the identification of BORSA isolates[8]. Likewise, van Leeuwen et al.[14] reported that the MRSA-Screen test had considerably high sensitivity and specificity, which could enable it to be used in routine laboratories.

Cavassini et al.[2], in a study carried out on 200 *S.aureus* isolates, reported that disk diffusion test, applied according to NCCLS regulations, had low sensitivity (61.3 %) compared with that of *mec*A gene test. In another study with 155 *S.aureus* and 261 coagulase negative staphylococci isolates using *mec*A gene analysis and oxacillin disk diffusion with addition of 2 % NaCl, they reported that *mec*A results, especially with *S.aureus* isolates, were compatible with disk diffusion results[7].

Skulnick et al.[12] compared the standard and commercially available sensitivity methods with *mec*A gene analysis, and found that Vitek had 14.2 % very major error in 254 oxacillin resistant isolates, and 0.4 % major error in 252 oxacillin sensitive isolates. They concluded that the Vitek system was incapable in determining the oxacillin resistance. However, another study comparing Vitek systems against *mec*A gene analysis and other methods, revealed that Vitek system was quite reliable in detecting methicillin resistance[4].

Another study performed by Yamazumi et al.[15] compared the results of Vitek GP susceptibility 106 card and MRSA-Screen test with the results of microdilution, oxacillin agar screen test, and *mec*A gene detection. The sensitivity and specificity of the results of MRSA-Screen, GPS card, and oxacillin agar screen, and microdilution tests were found as 96.9 %, 98 %, 98 %, 99 %; 100 %, 100 %, 98 % and 99 %, respectively. As a conclusion, they state that MRSA-Screen test, which is easy to perform and can be completed in 15 to 20 minutes, can be employed in routine microbiology laboratories[15].

Likewise, we also found that MRSA-Screen test was superior to phenotypic tests in both sensitivity and specificity. In five isolates, which were classified as methicillin resistant by the phenotypic methods and MRSA-Screen test, *mec*A gene could not be detected by PCR although the reaction was repeated twice. Although it is not completely clear, the reason for the failure of detection of *mec*A in these five isolates, is thought to be inadequate DNA extraction. In these circumtances, the use of multiplex PCR method which also detects *nuc*A gene is recommended. (After this study was concluded a multiplex PCR method which detects *mec*A, 16SrRNA, and *nuc* genes was applied to the above mentioned five isolates. These isolates were found to possess *nuc* and *mec*A genes confirming the problem with DNA extraction).

In our study, we conclude that the NCCLS disk diffusion method and Vitek system, when inoculum amount is taken into consideration, are reliable methods for routine laboratories. Being a rapidly performed test, which is usually completed in just 20 minutes, MRSA-Screen test is thought to be the method of choice due to its reliability.

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