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The Determination of mecA Gene Presence In MRSA Strains Isolated From Intensive Care Unit By Conventional, Automated and PCR Method

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ABSTRACT

Meticillin-resistant *S. aureus* (MRSA) strains are becoming increasingly important as a cause of hospital and community-acquired infections. The aim of this study is to compare PCR, gradient tests and automated system which are the methods for determining methicillin resistance in *S. aureus* strains.

The study included 50 MRSA strains isolated from various samples (wound, blood, sputum, respiration, abscess, osteomyelitis, etc.) from the microbiology laboratory of Van Yüzüncü Yıl University Medical Faculty between 2010-2016. A single isolate was obtained from each patient. In our study, the presence of mecA gene in MRSA strains was investigated by conventional, automated and PCR methods.

The presence of methicillin resistance was found in 49 of 50 MRSA strains which were examined by Oxacillin E-test method (MIC>2). All of the strains which were tested by cefoxitin E-test were found resistant to methicillin (MIC>4). Vitek 2 automated system detected that all of the strains are resistant to methicillin. All samples examined by PCR method were positive, the presence of mecA gene was determined. Sensitivity rate of strains examined by oxacillin E-test method was 98%, while the susceptibility rate of strains examined by cefoxitin E-test, Vitek 2 automated system and PCR methods was 100%.

In order to obtain the necessary precautions in hospitals with high MRSA ratio, mecA investigation is of great importance in achieving the correct results. Since the results of the three methods are almost close to each other, three methods can be used to determine the correct results.

Key Words: *Staphylococcus aureus*, mecA, E -test, Vitek 2 system, PCR

Introduction

Methicillin resistant of *S. aureus* (MRSA) strains as a hospital-based and community-acquired infection is becoming of great significance (1). Since methicillin resistance appeared in 1961 an important global health problem has been created (2). Due to their antibiotic resistance, little children and elderly patients in hospitals and nursing homes are difficult to treat, particularly susceptible to MRSA infection. In hospitalized patients, special applications such as endoscope, catherization, ventilation and tracheostomy applied for therapeutic purposes, creates a suitable environment for the colonization of microorganisms, by breaking the immune system. Furthermore, MRSA causes infection in healthy children and adults out of hospital (3). Since 1990, there has been a growth in the number of community-acquired infections (CA-MRSA) as well as hospital-based (HB-MRSA) MRSA infections (4).

The frequency of presence of MRSA can vary from country to country and even from one unit to another in a hospital. While the rate of this infection is 1% in Scandinavian countries, this rate can reach up to 80% in other countries, such as Italy and Greece (5). In our country, between the years 2003-2005, in an antibiotic resistance study, which determined the ratio of methicillin resistance among *S. aureus* isolates, resistance rates are 43%, 40%, and 35%, respectively (6). In an infection with methicillin-resistant MRSA, treatment options are restricted, also costs, mortality and morbidity increase. For this reason, accurate and rapid determination of methicillin resistance in MRSA strains plays a crucial role in infection prevention and control and avoidance of its spread in hospitals (7).

In this research, methicillin resistance detection of cefoxitin and oxacillin E-test and Vitek-2 automated results in MRSA strains isolated from various clinical specimens of microbiology laboratory of Van Yüzüncü Yıl university research

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hospital are compared with Golden Standard PCR method.

Materials and Method

Bacterial isolation and collecting specimens from clinical samples: 50 MRSA strains isolated from various samples (wound, blood, sputum, respiration, abscess, osteomyelitis, etc.) from the microbiology laboratory of medical faculty of Van Yüzüncü Yıl university, between the years 2010-2016, were included in the study. A single isolate was taken at each hospital. Blood culture samples, Bactec 9642 (Beckton-Dickinson, USA) and BACT/ALERT 3D (BioMerieux) were incubated till maximum of 7 days in automated blood culture devices, till the device gave positive warning. Bottles with positive stimuli and other culture samples, being cultivated in 5% sheep blood agar and EMB agar medium, were incubated in 37 C for 18-24 hours. In bacterial growth colonies, identification and antibiotic susceptibility tests were done using Phoenix automated system (Becton Dickenson, USA) for the strains with positive catalase and plasma coagulase tests and Gram-positive cocci appearance in Gram-staining. Accordingly, 50 MRSA strains were kept in -80 C until runtime.

Phenotypic methicillin resistance in *S. aureus* strains whose catalase and coagulase tests are positive were investigated, using gradient test and strips of oxacillin and cefoxitin. Using Vitek-2 Biometrioux automated system, Oxacillin and Cefoxitin resistance levels were investigated. Later, the presence of mec A gene was examined, using PCR method in our laboratory.

Gradient test (oxacillin and cefoxitin) methods: In accordance with the 0,5 McFarland standard of Bacteria, bacterial suspensions were prepared in serum physiologic and spread over the surface of Mueller Hinton agar. The plates were incubated at 35 C for 24 hours after placing the E-test strips of oxacillin and cefoxitin. MIK values of oxacillin and cefoxitin were determined and compared to the MIK values recommended by CLSI. For oxacillin and cefoxitin, 2 MIK values were accepted as sensitive and 4 MIK values were considered resistant. *S. aureus* ATCC 29213 was used as the control strain.

Resistance and detection in Vitek 2 automated system: Vitek 2 (BioMerieux, France) automated system devices was used for the detection of oxacillin and cefoxitin resistance. For antibiotic susceptibility, the conduction has been done in convenient to device operation procedure.

Investigation of methicillin resistance by molecular method: A colony from the staphylococcus, incubated in blood agar medium at 37 C for 24 hours, was taken into a 1.5 ml sterile tube; After adding 500 ml of distilled water and being vortexed, 300 microliter were taken from the mixture and transferred into another tube; 100 ml lysozyme was added and incubated at 37 C for 30 minutes; Later, isolation process was started by putting it in Fluorion i12 Nucleic acid isolation device. PCR method was performed using primers specific to the mecA as the responsible gene for methicillin resistance in staphylococci (mec A1 5'AAA ATC GAT GGT AAA GGT TGG C 3' and mec A2 5'AGT TCT GCA GTA CCG GAT TTG C3')(8).

Statistical Evaluation: The data obtained were evaluated in the computer environment SPSS 15.0 statistics package program, accepting Genotype MRSA method as Gold Standard the specificity and sensitivity of the phenotype methods were calculated using the related formulas.

Ethics committee approval: This research is approved by the Van Yüzüncü Yıl university noninvasive clinical research ethics committee on 30.11.2016/06.

Results

Of the examined MRSA samples, 17 were isolated from blood (34%), 12 from wound (24%), 9 from respiratory (18%), 9 from abscess (18%) and 3 from other clinical specimens (6%). In the 50 MRSA strains isolated from patient samples, conventional method, automated system and PCR method test results used to determine the methicillin resistance are shown in table 1.

Of 50 *S. aureus* examined with oxacillin E-test method, 49 were discovered as resistant (MIK>2) and one as sensitive (MIK≤2). All the samples examined by cefoxitin E-test method were determined as resistant (MIK>4). When the automated system results of Vitek 2 were probed, all of the examined samples were determined to be resistant to oxacillin and cefoxitin (MIK>4). Using PCR method, existence of mec A gene was determined in all of the samples. Based on these results, the sensitivity of oxacillin E-test method, with reference to PCR method, is 98% and the sensitivity of cefoxitin and Vitek 2 automated

Table 1. Number of strains determined as MRSA according to the method used

Methods		MRSA [mecA (+)]	MSSA [mecA (-)]
PCR		50	0
Gradient	Oxacillin (OX)	49	1
Test	Cefoxitin (FOX)	50	0
Vitek 2 System	Oxacillin (OX)	50	0
	Cefoxitin (FOX)	50	0

Table 2. Comparison of sensitivity and specificity of the methods used in the diagnosis of MRSA

Methods		Sensitivity (%)	Specificity (%)
Gradient	Oxacillin (OX)	98	100
Test	Cefoxitin (FOX)	100	100
Vitek 2 System	Oxacillin (OX)	100	100
	Cefoxitin (FOX)	100	100

system was determined as 100%. The sensitivity and specificity of the methods used in the diagnosis of MRSA are given in table 2.

Discussion

S. aureus is one of the important factors of community and hospital-based infections (9). Nowadays, MRSA strains have become an important health problem due to the limitations of treatment options, the cost of antibiotics used in treatment and infection control measures(10). In a study, between the years 1992-2003, in which infections were examined in intensive care units of the USA, MRSA was detected in 64% of *S. aureus* isolation, and the annual increase rate was discovered to be 3,1% (11). Between years 2006-2007, the rate of MRSA in the USA was reported as 79% (12). In the various studies conducted in Turkey, MRSA visibility is reported at around 30% (13-15).

In their studies with 139 *S. aureus* strains in the susceptible strains of mecA gene with molecular method, John et al. reported the sensitivity and specificity of the disc diffusion method of cefoxitin as 100% and 100%, respectively and for Vitek 2 cefoxitin test as 100% and 98.9% and for vitek 2 oxacillin sensitivity as 100% and 98.9% (16). In 140 *S. aureus* strains, in their study comparing disc diffusion test and vitek 2 oxacillin examining test using molecular method, for cefoxitin 30mg, Acosta-Pere et al. determined the sensitivity and specificity values for disk diffusion test as 97% and 92%, for vitek2 test as 97% and 69%, respectively (17). Tiwari et al. in their study with 237 *S. aureus* isolate, determined the sensitivity and specificity values as 98.5% and 100% for disc diffusion test of cefoxitin, and 77.3% and 84.6% for disc diffusion test of oxacillin (18). Junkins et al. mixed Vitek 2 test and Phonei automated test to detect methicillin resistance in *S. aureus* strains. They detected 100% compatibility between the two methods and in 448 MRSA and 172 methicillin sensitive *S. aureus* isolate, the categorical agreement rates with those obtained with reference methods are 99.7% for vitek 2 and 99.8% for phoenix (19). In their study with the 98 Staphylococcus strains, Uzun et al. detected *S. aureus* carrying the mecA gene in 60 isolated by PCR.

In 59 of these strains, methicillin resistance was defined by using cefoxitin disc diffusion and in 61 by using automated system. Using PCA as a reference method for determination of mecA gene, in determining the resistance of methicillin, the sensitivity and specificity of the tests used by cefoxitin diffusion test were determined as 98.3%, 100%, and for automated system as 100% and 97.4%, respectively (20). Kaya et al. using automatic system and disk diffusion, discovered all of the 19 *S. aureus* strains with positive mecA gene, methicillin resistant (21). Cirit et al. detected the rate of mecA gene as 86.6% in 150 *S. aureus* strains. Also, in the study they recognized that methicillin sensitivity results obtained with Vitek2 are completely compatible with the results obtained with PCR (22). In our research, compatible with the above literature, mecA gene is detected by PCR method, the sensitivity and specificity in *S. aureus* strains were 98% and 100% for oxacillin gradient test, 100% and 100% for cefoxitin gradient test, 100% and 100% for Vitek 2 oxacillin examining test, and 100% and 100% for Vitek 2 cefoxitin examining test.

To sum up, with Gradient test and Vitek 2 automated system, values for the detection of MRSA are close to each other and the sensitivity and specificity of the both methods were detected to be high. Accordingly, for the detection of MRSA, a molecular method, gradient test (Oxacillin and Cefoxitin), and Vitek 2 automated system can be used with confidence.

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References

1. Sancak B. MRSA direnç mekanizmaları: Dünyada ve Türkiye'de epidemiyolojisi. ANKEM Derg 2012; 26: 38-47.
2. Çelik C, Bakıcı MZ, diğ. Kan akımı infeksiyonlarından izole edilen Staphylococcus aureus suşlarında antimikrobiyal direnç paterni, Genel Tıp Dergisi 2013; 23: 109-113.
3. Kloos WE. Bonnerman TL. Staphylococcus and micrococcus. In: Murray PR, Borron EJ, Pfaller MA, Tenover FC, Yolken RH (eds). Manuel of Clinical Microbiology, American Society of Microbiology. Washington DC 1999; 264-282.
4. Banu Sancak Staphylococcus aureus ve Antibiyotik Direnci Mikrobiyol Bul 2011; 45: 565-576.
5. Martins A. Cunha M de L. Methicillin resistance in Staphylococcus aureus and coagulase- negative staphylococci: Epidemiological and molecular aspects. Microbiol Immunol Rev 2007; 51: 787-795.
6. Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE. The molecular evolution of methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect 2007; 13: 222-235.
7. Özen NS, Dağlar D, Özhak Baysan B, Yildirim Ç, Yazisiz H, Öğünç D, Öngüt G, Çolak D, Gültekin M. Metisilin Dirençli Staphylococcus Aureus Suşlarının Saptanmasında MRSA Id Kromojenik Besiyerinin Değerlendirilmesi. ANKEM Derg 2011; 25: 31-34.
8. Siripornmongcolchai T, Chomvarin C, Chaicumpar K, Limpaiboon T, Wongkhum C. Evulation of different primers for detection mecA gene by PCR in comparison with phenotypic methods for discrimination of methicillin-resistant Staphylococcus aureus. The Southeast Asian journal of tropical medicine and public health 2002; 33: 758-763.
9. Başustaoğlu A. Dirençli mikroorganizma enfeksiyonları ve kontrol programları: Gram pozitif bakteriler. Aktüel tıp dergisi 2002; 7: 82-85.
10. Aköğretmen,T. Balıkesir ve yöresinde mental retarde hastalarda nazal metisilin dirençli Staphylococcus aureus (MRSA) taşıyıcılığı oranının moleküler yöntemlerle araştırılması. [Yüksek Lisans Tezi], Balıkesir: Balıkesir Üniversitesi; 2016.
11. Klevens RM, Edwards JR, Tenover FC, McDonald LC, Horan T, Gaynes R. Changes in the epidemiology of methicillin-resistant Staphylococcus aureus in intensive care units in US hospitals, 1992-2003. Clin Infect Dis 2006; 42: 389-391.
12. Kallen AJ, Brunkard J, Moore Z, Budge P, Arnold KE, Fosheim G ve ark. *S. aureus* community-aquired pneumonia during the 2006 to 2007 influenza season. Ann Emerg Med 2009; 53: 358-365.
13. Bozca B, Coşkuner SA, Biçer KÇ, Genç VE, Özgenç O. Stafilokoklarda metisilin direnç oranlarının saptanması. ANKEM Dergisi 2007; 21: 26.
14. Güner S, Gürler N. 2001-2006 yıllarında cerehat ve yara örneklerinden izole edilen stafilokok suşlarında metisilin direnci. ANKEM Derg 2007; 21: 27.
15. Orak F. Mardin Devlet Hastanesi'nde 2011-2013 yılları arasında metisiline dirençli stafilokoklarda direnç profilleri. Turk Hij Den Biyol Derg 2015; 72: 191-198.
16. John MA, Burden J, Stuart JI, Reyes RC, Lannigan R, Milburn S, Diagre D, Wilson B, Hussain Z. Comparison of three phenotypic techniques for detection of methicillin resistance in Staphylococcus spp. reveals a species-dependent performance. J Antimicrob Chemother 2009; 63: 493-496.
17. Acosta-Pérez G, Rodríguez-Ábrego G, Longoria-Revilla E, Castro-Mussot ME. [Evaluation of four methods for detecting methicillin-resistant Staphylococcus aureus isolates from clinical specimens at a regional hospital in Mexico]. Salud Publica Mex 2012; 54: 1-6.
18. Tiwari HK, Sapkota D, Das AK, Sen MR. Assessment of different tests to detect methicillin resistant Staphylococcus aureus. Southeast Asian J Trop Med Public Health 2009; 40: 801-826.
19. Junkins AD, Lockhart SR, Heilmann KP, Dohrn CL, Von Stein DL, Winokur PL, Doern GV, Richter SS. BD Phoenix and Vitek 2 detection of mecA-mediated resistance in Staphylococcus aureus with cefoxitin. J Clin Microbiol 2009; 47: 2879-2882.

20. Uzun B, Karataş Şener AG, Güngör S, Afşar I, Yüksel Ergin O, Demirci M. Staphylococcus aureus Suşlarındaki Metisilin Direncinin Belirlenmesinde Sefoksitin Disk Difüzyon Testi, Otomatize Sistem ve Kromojenik Besiyerinin Karşılaştırılması Mikrobiyol Bul 2013; 47: 11-18.
21. Kaya, E, Aral, M, Orhan, Z, Yalçınkaya, K, Uğurlu, H. Comparison of Conventional PCR Method With Cefoxitin Disc Diffusion, Automated System and Isolation on Chromogenic MRSA Medium Methods for The Detection of Meticillin Resistance In Staphylococcus aureus Strains Isolated From Various Clinical Specimens. KSÜ Tarım ve Doğa Dergisi 1019; 22: 451-455.
22. Cirit OS, Yıldırım T, Çoban AY. Staphylococcus aureus klinik izolatlarında panton-valentin lökosidin varlığının araştırılması. Balkan Med J 2009; 28: 119-124.