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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

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ABSTRACT

Meticillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important infectious agents. Therefore, fast and accurate diagnosis of MRSA is utmost important. Although *mecA* gene detection by PCR is reference method, conventional methods are preferred in routine practices due to simplicity. As conventional methods last 48-96 hours, several chromogenic media have been developed. Our objective was to compare the methods used for meticillin resistance detection with PCR. Forty-eight *S. aureus* strains isolated from various clinical specimens were included. Of the 48 S. aureus strains, 19 were *mecA* gene-positive and 29 were negative. *mecA* gene-positive 19 strains were also meticillin-resistant by automated system and disk diffusion. On chromogenic agar, 15 of 19 MRSA strains were meticillin-resistant and 4 were meticillin-sensitive. Twentynine *mecA* gene-negative strains were susceptible to meticillin by automatized system and disk diffusion. Among 29 *mecA* gene-negative MSSA strains inoculated on chromogenic agar, 17 were methicillin-resistant. According to our study, chromogenic media would be ineffective to detected to meticillin-resistance because of low sensitivity and specificity in routine.

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Çeşitli Klinik Örneklerden Izole Edilen *Staphylococcus aureus* Suşlarında Metisilin Direncinin Araştırılmasında Konvansiyonel PCR Yöntemi ile Sefoksitin Disk Difüzyon, Otomatize Sistem ve Chromogenic MRSA Agar Yöntemlerinin Karşılaştırılması

ÖZET

Metisiline dirençli *Staphylococcus aureus* (MRSA) en önemli enfeksiyöz ajanlardan biridir. Bu nedenle MRSA'nın hızlı ve doğru tanısı çok önemlidir. PCR ile *mecA* gen tespiti referans metodu olmakla birlikte, basit olmasından dolayı rutin uygulamada geleneksel yöntemler tercih edilmektedir. Geleneksel yöntemler ile tanının koyulması 48-96 saat sürdüğü için çeşitli kromojenik besiyerleri geliştirilmiştir. Çalışmamızdaki amacımız metisilin direncinin tespitinde kullanılan çeşitli yöntemleri PCR ile karşılaştırmaktır. Çeşitli klinik örneklerden izole edilen 48 *S. aureus* suşu çalışmamıza dahil edilmiştir.48 *S.aureus* suşunun 19'u *mecA* gen-pozitif ve 29'u negatiftir. *mecA* gen-pozitif 19 suş da otomatik sistem ve disk difüzyonu ile dirençli bulunmuştur. Kromojenik agarda, 19 MRSA suşunun 15'i metisilin dirençli ve 4'ü duyarlı bulunmuştur.29 *mecA* gen-negatif suş, otomatik sistem ve disk difüzyonu ile duyarlı bulunmuştur. *mecA* geni negatif olan 29 MSSA suşunun kromojenik agarda 17'si dirençli olarak değerlendirmiştir.

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INTRODUCTION

Staphylococcus aureus is encountered as the cause of a wide range of diseases from mild skin infections to life-threatening conditions (Xu et al., 2016, Prosper et al., 2013, Feng et al., 2008). While it could be treated with penicillin previously, due to natural selection of penicillinase-producing strains, *S. aureus* isolates are 95% penicillin-resistant nowadays (Xu etal., 2016). Although penicillinase resistant beta lactam antibiotics have been developed in this period, meticillin resistant *Staphylococcus aureus* (MRSA) strains have been reported since 1960 (DeLeo et al., 2009). In *S. aureus*, the meticillin resistance is encoded by the *mecA* gene and causes PBP2a expression by a change in the penicillin-binding protein (PBP). Because of its very low affinity, it leads to resistance to this group of antibiotics and its derivatives (Roisin et al., 2008, Zhu et al., 2006). In recent years, reporting of the MRSA has become increasingly important problem (DeLeo et al., 2009).

While detection of the *mecA* gene by Polymerase Chain Reaction (PCR) for the diagnosis of MRSA infection is the best standard, most of the laboratories use phenotypic methods (cefoxitin disc diffusion, automatized systems). The identification of MRSA takes 48-96 hours by routine phenotypic methods (Kluytmans et al., 2007, Strulens et al., 2006, Paule et al., 2007). However, the faster identification of MRSA is crucial for faster infection control (Malhotra-Kumar et al., 2008). For this reason, various chromogenic media have been developed to identify MRSA strains recently (Perry et al., 2007, Uzun et al., 2013, Cesur et al., 2014, Özen et al., 2011). The basic mechanism of chromogenic media is based on the principle of chromogenic substrate being cut with specific enzymes of the target microorganism, making the chromogen insoluble and remaining in the bacterial wall and gaining original color (Uzun et al., 2013). The aim of using these media is to diagnose MRSA infection in one step and to start the treatment as soon as possible. The first developed chromogenic medium for MRSA identification is Chromagar ™ MRSA (Chromagar microbiology, Paris, France) (Xu et al., 2016).

The aim of our study was to compare cefoxitin disk diffusion, Phoenix automated system and isolation on Chromogenic MRSA medium methods to *mecA* gene detection with PCR as reference test.

MATERIAL and METHODS

Forty-eight *Staphylococcus aureus* strains isolated from various clinical specimens delivered to the Medical Microbiology Laboratory of Kahramanmaraş Sütçü İmam University Medical Faculty at January-October 2014 were included in our study. The samples from which *S. aureus* strains were isolated are shown in Table 1.

Table 1. Samples from where *S. aureus* strains isolated.

Sample	MRSA*	MSSA**
Wound	10	17
Blood	5	4
Nose	2	2
Urine	1	4
Sputum	1	0
Throat	0	2
	19	29

* MRSA: Meticillin-resistant *Staphylococcus aureus.*
** MSSA: Meticillin-susceptible *Staphylococcus aureus*

Samples were inoculated onto sheep-blood agar and incubated at 37°C for 24 hours. In bacterial identification, gram-positive cocci in the form of bunch of grapes were accepted as *S. aureus* if they were additionally catalase and tube coagulase test-positive. Cefoxitin disk diffusion test, Phoenix automated system (Becton Dickinson, USA), Chromogenic MRSA (RTA Laboratories, Turkey) were used to determine meticillin resistance. Conventional PCR was performed for the detection of *mecA* resistance gene (Strommenger et al., 2003).

Cefoxitin disc diffusion test (30 µg, Beckton Dickinson, USA) was performed by Kirby-Bauer disc diffusion method in accordance with CLSI (Clinical and Laboratory Standards Institute) recommendation. Strains with an inhibition zone ≤21 mm were considered meticillin resistant and those with an inhibition zone >22 mm were considered susceptible to meticillin (CLSI, M100-S24).

The *mecA* gene was detected by conventional PCR method. In briefly, bacterial DNA was extracted with QIAamp DNA Mini Kit (Qiagen, Germany). A 532 base pair region of the *mecA* gene was amplified using primers mecA1 and mecA2 (Table 2). Samples were considered to be *mecA* gene-positive if an amplicon with appropriate base length was detected by gel electrophoresis (Strommenger et al., 2003).

Table 2. Primers used in the detection of *mecA* gene.

Target gene	Resistance phenotype	Primer Sequence (5' → 3')	Amplicon size (bp)	Reference
mecA	oxacillin, penicillin	mecA1: AAAATCGATGGTAAAGGTTGGC mecA2: AGTTCTGCAGTACCGGATTTGC	532	Strommenger et al., 2003.

All isolates were inoculated onto Chromogenic MRSA medium and incubated at 37°C in aerobic atmosphere. After 24 or 48 hours, isolates producing pink-red colony on the medium were accepted as MRSA in accordance with the manufacturer's recommendation. The isolates did not grow or form colorless colonies on Chromogenic MRSA medium were accepted as MSSA. ATCC 29213 for MRSA and ATCC 25923 for MSSA were used as standard strains.

Sensitivity, specificity, positive and negative predictive values of the tests used to determine methicillin resistance were calculated with the acceptance of PCR as reference test.

RESULTS

Of the 48 *S. aureus* strains, 19 were found to be positive for *mecA* by conventional PCR method. These 19 strains harboring *mecA* gene were also found to be meticillin-resistant by cefoxitin disc diffusion method and Pheonix automated system. After 24 hour-incubation in Chromogenic MRSA medium, 15 of 19 MRSA strains were found to have resistance to meticillin and the other four samples were evaluated as susceptible. The evaluation was the same after 48 hours of incubation (Table 3). The 29 MSSA strains in which *mecA* gene was not detected by PCR were also susceptible to cefoxitin by disc diffusion and automated

Table 3. Comparison of the methods with reference PCR method.

	RESULT			
	mecA-Positive		*mecA*-Negative	
METHOD	MRSA	MSSA	MRSA	MSSA
Cefoxitin disc diffusion	19	0	0	29
Phoenix automated system	19	0	0	29
Chromogenic MRSA (at 24th hour)	15	4	17	12
Chromogenic MRSA (at 48th hour)	15	4	12	17
Cefoxitin disc diffusion	MRSA	MSSA	MRSA	MSSA

Phoenix system. After 24-hour incubation of Chromogenic MRSA medium, 17 and 12 of the 29 MSSA strains were considered meticillin resistant and meticillin susceptible, respectively. On the contrary, after 48 hours of incubation 12 strains were resistant to meticillin and 17 strains were susceptible (Table 3).

Sensitivity, specificity, PPD (positive predictive value) and NPD (negative predictive value) of the Chromogenic MRSA medium after 24 hours of incubation were 78.9%, 41.3%, 46.8% and 75% respectively. Sensitivity, specificity, PPD and NPD were found as 78.9%, 58.6%, 55.5% and 80.9% respectively after 48 hours incubation of the medium (Table 4).

DISCUSSION and CONCLUSION

MRSA is one of the major causes of morbidity and mortality in hospital and community-acquired infections (Von Eiff et al., 2008, Lodise et al., 2005). Therefore, rapid diagnosis of MRSA infection is of great importance for initiation of treatment in a short term and prevention of the spread of the disease (Malhotra-Kumar et al., 2008). Detection of the *mecA* gene by PCR in identification of MRSA is the gold standard method.

Table 4. Sensitivity, specificity, positive and negative predictive values of the methods compared to PCR.

Method	Sensitivity (%)	Specificity (%)	PPV* (%)	NPV** (%)
Cefoxitin disc diffusion	100	100	100	100
Phoenix automated system	100	100	100	100
Chromogenic MRSA (at 24th hour)	78.9	41.3	46.8	75
Chromogenic MRSA (at 48th hour)	78.9	58.6	55.5	80.9

*PPV: Positive predictive value.
**NPV: Negative predictive value.

Although PCR yields results in a short time, it is expensive and difficult to apply in every laboratory (Marlowe et al., 2011, Cesur et al., 2010). For this reason, accurate identification of MRSA diagnosis by conventional methods is of great importance.

In routine laboratories oxacillin disk diffusion test and automated systems are used according to CLSI criteria in diagnosing MRSA. As well as these methods, cefoxitin disc diffusion test, agar dilution method and latex agglutination methods are also used because of heterogeneous resistance (Broekema et al., 2009).

Chromogenic media have been preferred because they are faster than conventional methods and appropriate to use statistically according to many articles in the literature (Xu et al., 2016, Malhotra-Kumar et al.,

2008, Sürücüoğlu et al., 2011, Denys et al., 2013). There are many studies that have investigated whether various chromogenic media are comparable and suitable for use (Strulens et al., Uzun et al., 2013, Cesur et al., 2014Von Eiff et al., 2008, Denys et al., 2013) .

In Van Hal and colleagues' study of swab samples from axilla, nose and groin, the susceptibilities and specificities of MRSA ID, MRSASelect and CHROMAGAR MRSA media at 24th hour were 71%, 64%, 63% and 98%, 95%, 99% respectively. At the end of the 48th hour, their sensitivities were 82%, 69%, 71% and specificities were 53%, 74%, 67% respectively. Prolongation of the incubation time to 48 hours resulted in increase in susceptibility but significant decrease in specificity. It was also found that chromogenic media yieded the most accurate results in nasal swabs in this study (van Hal et al., 2007).

In a study by Perry and colleagues with 747 swab samples taken from various body regions, the media of MRSA ID, CHROMagar MRSA and ORSAB were compared. Their sensitivities and specificities after 24 hours were 80%, 59%, 62% and 99.5%, 99.3%, 97.9%, respectively. Sensitivities and specificities at 48 hours were 89%, 72%, 78% and 85.6%, 92.1% and 93.1%, respectively. Extension of the incubation to 48 hours resulted in a significant increase in susceptibilities and a decrease in specificities. In addition, the MRSA ID medium was superior to the other two media in MRSA detection (Perry et al., 2007).

MRSA-ID, CHROMagar MRSA and MRSA-Select media were used in the study of Nahimana et al. Their sensitivities and specificities were found to be 51%, 59%, 65% and 100%, 99%, 100% respectively after 18 hours incubation. Sensitivities and specificities for 42-hour incubation were 82%, 75%, 80% and 98%, 97% and 98%, respectively (Nahimana et al., 2006).

In Kumar and his colleagues' study, the sensitivity and specificity of MRSA agar, ChromID, MRSASelect, CHROMagar and BBL-CHROMAGAR at 24-hour were 89.9%, 82.8%, 80.7%, 81.9%, 82.9% and 86.9%, 96.3%, 97.2%, 99.1%, 99.2%, respectively. The sensitivity and specificity at the end of 48 hours were 96.4%, 93.5%, 92.6%, 93.1%, 93.5% and 69.0%, 89.7%, 92.1%, 97.4%, 97.8% respectively (Malhotra-Kumar et al., 2008).

The sensitivity and specificity, PPD and NPD of CHROMagar MRSA medium were found to be 97.1%, 99.2%, 98.5% and 98.4%, respectively in the study conducted by Datta and colleagues with 130 *mecA* gene-negative and 70 *mecA* gene-positive *S. aureus* strains (Data et al., 2011).

Uzun et al. found the sensitivity, specificity, PPD and NPD of 60 *mecA* gene-positive and 38 *mecA* gene-negative strains as 91.7%, 89.5%, 93.2% and 87.2% in CHROMagar MRSA medium. The values obtained after 48 hours were 96.7%, 81.6%, 89.2% and 93.9% (Uzun et al., 2013).

In a study with 45 MRSA and 130 MSSA isolates, Cesur and their colleagues found the sensitivity, specificity, PPD and NPD values of CHROMagar MRSA and ORSAB medium as 95.5%, 37.6%, 35.7%, 96.1% and 97.8%, 40%, 36.5%, 98.1%, respectively. It has been argued in this study that, although the specificity is low, due to its high sensitivity this medium maybe used for screening in laboratories where the intensity of work is high (Cesur et al., 2010).

In our study, the sensitivity of Chromogenic MRSA at the end of incubation for 24 hours and 48 hours was 78.9%, and no increase in sensitivity was detected with the extension of the incubation period. While the specificity was 41.3% at 24th hour, it was found to be 58.6% when the incubation period was extended to 48 hours.

Most studies revealed that the use of chromogenic media for MRSA identification can generally, provide acceptable diagnostic performance, although specificity and sensitivity of chromogenic media varies amongst suppliers. The performance of chromogenic media is influenced by several variables, including specimen type, incubation time, broth enrichment step, or investigator perception (Xu Z, et al., 2016).

As a result of our study, cefoxitin disk diffusion test and Pheonix automated system could be used in the laboratories where the PCR method is not available for the detection of MRSA. On the contrary, it can be oncluded that the use of chromogenic medium for MRSA in routine would be ineffective because of its low sensitivity and specificity.

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