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Comparison between Cefoxitin Disc Diffusion and Phoenix Automated System with *mecA/mecC* PCR for Determination of Methicillin Resistance in *Staphylococcus aureus* Isolates and Investigation of the Presence of *PVL* Gene

Staphylococcus aureus İzolatlarında Metisilin Direncinin Belirlenmesinde Sefoksitin Disk Difüzyon ve Phoenix Otomatize Sisteminin *mecA/mecC* PZR Yöntemi ile Karşılaştırılması ve *PVL* Gen Varlığının Araştırılması

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Abstract

Introduction: A fast and accurate determination of methicillin resistance in *Staphylococcus aureus* strains is vital. This study aimed to compare the sensitivity and specificity of the cefoxitin disc diffusion (CDD) test and BD Phoenix automated system considering *mecA/mecC* positivity as the gold standard and to investigate the presence of Panton-Valentine leukocidin (*PVL*) toxin gene, a crucial virulence factor of *S. aureus* strains.
Materials and Methods: Overall, 179 *Staphylococcus aureus* strains from various clinical samples were included. Antibiotic sensitivity was tested using the Phoenix automated system and by applying the Kirby-Bauer disc diffusion method for cefoxitin (30 µg). The *mecA, mecC,* and *PVL* presence was determined using the conventional multiplex polymerase chain reaction method. mecA/*mecC* positivity was considered as the gold standard. Statistical analysis was performed using SPSS 15.0 for Windows (Chicago, IL, USA).
Results: Overall, 91 strains (50.8%) were *mecA* positive and identified as methicillin resistant *Staphylococcus aureus* (MRSA). No isolates containing the *mecC* gene were detected. The Phoenix automated system falsely identified six methicillin-sensitive *S. aureus* (MSSA) isolates, which were *mecA* and *mecC* negative as MRSA. The sensitivity and specificity of the CDD test were found to be 100% in determining MRSA, and the sensitivity and specificity the Phoenix automated system were 100% and 93.2%, respectively. The *PVL* positivity rate in MRSA and MSSA strains was 6.5% and 7.4%, respectively. All *PVL*-positive strains were isolated from the skin and soft tissues.
Conclusion: The CDD test is a reliable method for routine procedures. Methicillin-sensitive strains can be determined as MRSA via the Phoenix automated system. Nevertheless, *mecC*-controlled MRSA should not be excluded from methods used for determining methicillin resistance. Panton-Valentine leukocidin toxin gene should be determined to enable clinicians to understand the infection severity.
Keywords: *mecA*, *mecC*, ORSA, Kirby-Bauer test, *molecular epidemiology*

Öz

Giriş: *Staphylococcus aureus* kökenlerinde metisilin direncinin hızlı ve doğru bir şekilde belirlenmesi hayati öneme sahiptir. Bu çalışmada, *mecA/mecC* pozitifliği altın standart kabul edilerek, sefoksitin disk difüzyon (CDD) testinin ve BD Phoenix otomatize sisteminin duyarlılık ve özgüllüklerinin karşılaştırılması ve *S. aureus* kökenlerinin önemli bir virülans faktörü olan Panton-Valentine lökosidin (*PVL*) toksin geni varlığının araştırılması amaçlandı.
Gereç ve Yöntem: Çeşitli klinik örneklerden elde edilmiş toplam 179 *S. aureus* suşu çalışmaya dahil edildi. Antibiyotik duyarlılığı, Phoenix otomatize sistemi ve sefoksitin (30 µg) için Kirby-Bauer disk difüzyon yöntemi uygulanarak test edildi. *mecA, mecC* ve *PVL* genlerinin varlığı konvansiyonel

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multipleks polimeraz zincir reaksiyonu yöntemi kullanılarak belirlendi. *mecA/mecC* pozitifliği altın standart olarak kabul edildi. İstatistiksel analiz için SPSS 15.0 (Chicago, IL, USA) for Windows programı kullanıldı.

Bulgular: *mecA* geni içeren doksan bir köken (%50,8) metisiline dirençli *Staphylococcus aureus* (MRSA) olarak tanımlandı. *mecC* geni içeren hiçbir izolat tespit edilmedi. Phoenix otomasyon sistemi *mecA* ve *mecC* negatif olan altı metisiline duyarlı *S. aureus* (MSSA) izolatını yanlışlıkla MRSA olarak tanımladı. MRSA'nın belirlenmesinde CDD testinin duyarlılığı ve özgüllüğü %100, otomatize sistemin duyarlılığı ve özgüllüğü ise sırasıyla %100 ve %93,2 olarak bulundu. MRSA ve MSSA suşlarında *PVL* pozitifliği sırasıyla %6,5 ve %7,4 idi. Panton-Valentine lökosidin pozitif tüm suşlar deri ve yumuşak doku örneklerinden izole edildi.

Sonuç: Sefoksitin disk difüzyon testi, rutin prosedürde kullanılabilecek güvenilir bir yöntemdir. Metisiline duyarlı suşlar Phoenix sistemi ile metisiline dirençli saptanabilir. Metisilin direncinin belirlenmesinde kullanılan yöntemlerin *mecC* ilişkili MRSA'ları gözden kaçırmaması gerekir. Panton-Valentine lökosidin toksin geni, klinisyeni enfeksiyonun ciddiyeti konusunda uyarması açısından belirlenmelidir.

Anahtar Kelimeler: *mecA, mecC, ORSA*, Kirby-Bauer testi, moleküler epidemiyoloji

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains cause significant health problems worldwide, thereby warranting a fast and accurate method to determine methicillin resistance[1]. Methicillin resistance in *Staphylococcus aureus* lineages is associated with mutated penicillin-binding protein 2a, which is coded by the *mecA* gene[2]. However, after the discovery of a new *mecA* homolog gene (with 70% nucleotide homology), named *mecC* (*mecA*-LGA251), the detection of *mecA* alone is not considered as the gold standard in determining methicillin resistance[3,4]. In various studies, animals like cows and sheep that were infected with MRSA containing *mecC* were identified as the new zoonotic source of infection for humans[5,6]. Since it was was impossible to detect this gene using the conventional and molecular methods employed to determine methicillin resistance through *mecA*, it was necessary to develop commercial and conventional polymerase chain reaction (PCR) methods that determine both genes[7,8].

Nevertheless, because all laboratories do not have access to molecular techniques, various phenotypic methods were used to determine methicillin resistance. Among these, the cefoxitin disc diffusion test (CDD, 30 µg, Oxoid, England) has been reported to be suitable in determining methicillin resistance by the Clinical and Laboratory Standards Institute (CLSI)[9], and supported by various publications[1,2,10].

Furthermore, automatized diagnostic systems that are currently being used in several microbiology laboratories were investigated for their sensitivity in the determination of MRSA. A significant advantage of the automated system is that it saves time in identifying staphylococcal species and determining the methicillin resistance[11].

Panton-Valentine leukocidin (*PVL*) toxin, which is an important virulence factor of *S. aureus,* may cause serious necrotizing infections with high mortality (56%-63%) in healthy and young individuals[12]. Therefore, epidemiological studies uncovering the clonal spread of *S. aureus* strains in the hospital and community settings are crucial[13,14].

In our study, by referencing *mecA* and *mecC* presence in *S. aureus* strains as the golden standard, we aimed to evaluate the performance of the CDD test and Phoenix automated system in accurately detecting MRSA, as well as contribute to the limited epidemiological data regarding isolates containing *mecC*. Moreover, we analysed the *PVL* toxin gene presence in these strains by using multiplex PCR.

Materials and Methods

Bacterial isolates: Overall, 179 *S. aureus* strains isolated from various clinical samples between January 2009 and October 2014 at our laboratory, which were kept at -80 °C in Tryptic soy broth medium and were nonrecurrent (first sample of each patient), were included. Among these samples, 33.5% were collected from clinics (n=60), 25.1% from outpatients (n=45), 18.5% from intensive care units (n=33), 17.9% from the emergency room (n=32), 3.9% from the hemodialysis unit (n=7), and 1.1% from the burn unit (n=2). Of all strains, 26.3% (n=47) were from blood cultures, 20.7% (n=37) from wound swabs, 14% (n=25) from abscesses, 8.4% (n=15) from bronchoalveolar lavage samples, 8.4% (n=15) from urine cultures, 5.6% (n=10) from biopsy samples, 3.9% (n=7) from catheter cultures, 3.9% (n=7) from tracheal aspirates, 3.9% (n=7) from phlegm, 3.4% (n=6) from cerebrospinal fluid, 1.1% (n=2) from synovial fluid, and 0.6% (n=1) from peritoneal fluid.

Bacterial identification and antimicrobial susceptibility testing (AST): Bacteria were identified using matrix-assisted laser desorption-ionization–time of flight mass spectrometry (Bruker Biotyper, Germany). Methicillin resistance was determined using the CDD test (30 µg, Oxoid, England) and automated system (Phoenix, Becton Dickinson, USA). The CDD test was studied and evaluated using the Kirby-Bauer disc diffusion method per the CLSI guidelines[9]. Evaluation of the the Phoenix System (Becton Dickinson, Sparks, MD) was performed based on the manufacturer's instructions.

Molecular detection of staphylococcal protein A (*spa*), *mecA*, *mecC*, and *PVL* genes: In our study, conventional multiplex PCR was used as the molecular method to determine *spa, mecA,*

mecC, and *PVL* genes, as implemented by Stegger et al.[8]. For deoxyribonucleic acid (DNA) extraction, InstaGene Matrix (Bio-Rad®, USA) was used. Two to three *S. aureus* colonies reproduced on sheep blood agar after a 24-h incubation at 37 °C were added to 100 µl lysis buffer (InstaGene Matrix, Bio-Rad, USA), vortexed for 15 s, and heated for 1 h at 56 °C on a heat block. After being reloaded in the vortex mixer, the sample was incubated for another hour at 95 °C and centrifuged at 13200 rpm for 5 min. The supernatant was stored at -20 °C for DNA sampling. Forward and reverse mixtures were prepared from *spa, mecA, mecC*, and *PVL* primers (Table 1), and the product was added to the DreamTaq™ Green PCR Master Mix (Thermo Scientific, Lithuania), which allows the product to be loaded to gel electrophoresis after amplification. The master mix was divided into 23-µl aliquotes in PCR microtubes, and 2 µl of extracted DNA product was put in each tube. For amplification, PCR cycles were configured to 5 min at 94 °C for outset denaturation, followed by 30 cycles for 30 s at 94 °C, 1 min at 58 °C, 1 min at 72 °C, and finally 10 min at 72 °C for the ultimate elongation. The PCR product obtained was set in gel electrophoresis at 130 V for 60 min. The results revealed 162 base pair (bp) DNA fragments as *mecA*-positive, 138 bp fragments as *mecC*-positive, and 85 bp fragments as *PVL*-positive. Deoxyribonucleic acid fragments between 180 and 600 bp in different sizes based on the strain were considered as *spa.* In the study, *S. aureus* American Type Culture Collection (ATCC) 29213 *mecA* (-), *S. aureus mecC* (+) Culture Collection University of Gothenburg (CCUG) 63582, *S. aureus* ATCC 49476 *mecA* (+), and *S. aureus* ATCC 49775 *PVL* (+) were used as control strains (Figure 1).

Statistical Analysis

Descriptive statistics were calculated as count and percent. McNemar test was used for comparison of the dependent proportions, and the Pearson chi-square test was employed for comparison of the independent proportions. The agreement between the protocols used in the study was evaluated using the Kappa coefficient. Moreover, the sensitivity and specificity of methods and their positive (PPV) and negative (NPV) predictive values were calculated. The statistical significance level was accepted as $p<0.05$. All the statistical methods were performed using Statistical Package for the Social Sciences version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Ethics committee approval was obtained for this study from the Ethics Committee of Şişli Hamidiye Etfal Training and Research Hospital (protocol no: 14.01.2013/280). Informed consent was not received.

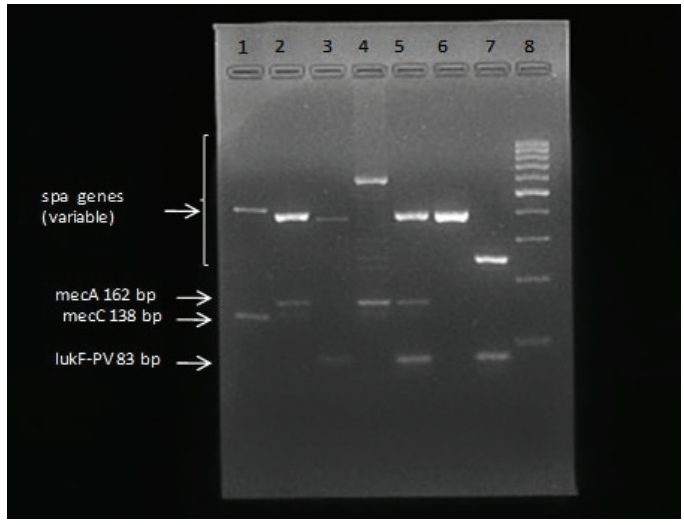

Figure 1. Gel electrophoresis of staphylococcal protein A (*spa*), *mecC*, *mecA* and *Panton-Valentine* leukocidin (*PVL*) genes by multiplex polymerase chain reaction.

Lane 1: *mecC* (+) *S. aureus* CCUG 63582 (138 bp).

Lane 2: *mecA* (+) *S. aureus* ATCC 49476 (162 bp).

Lane 3: *PVL* (+) *S. aureus* ATCC 49775 (85 bp).

Lane 4: *mecA* (+) *S. aureus.*

Lane 5: *mecA* (+), *PVL* (+) *S. aureus.*

Lane 6: *mecA* (-) *S. aureus.*

Lane 7: *mecA* (-), *PVL* (+) *S. aureus.*

Lane 8: 100 bp DNA marker.

ATCC: American Type Culture Collection, CCUG: Culture Collection University of Gothenburg, *S. aureus: Staphylococcus aureus,* bp: Base pair

Table 1. The primer sequences and expected amplicon sizes of each gene investigated using polymerase chain reaction in this study

Gene name	Primer name	Sequence	Amplicon size (bp)	References
spa	spa-1113F	5′ – TAAAGACGATCCTTCGGTGAGC – 3′	180-600	Stegger et al.[8]
	spa-1514R	5′ – CAGCAGTAGTGCCGTTTGCTT – 3′		
mecA	*mecA* P4	5′ – TCCAGATTACAACTTCACCAGG – 3′	162	Stegger et al.[8]
	mecA P7	5′ – CCACTTCATATCTTGTAACG – 3′		
PVL	*PVL*-F	5′ – GCTGGACAAAACTTCTTGGAATAT – 3′	85	Stegger et al.[8]
	PVL-R	5′ – GATAGGACACCAATAAATTCTGGATTG – 3′		
mecC	$mecA_{LGA251}$ MultiFP	5′ – GAAAAAAAGGCTTAGAACGCCTC – 3′	138	Stegger et al.[8]
	$mecA_{LGA251}$ MultiRP	5′ – GAAGATCTTTTCCGTTTTCAGC – 3′		

bp: Base pair, *PVL:* Panton-Valentine leukocidin

Results

Based on *mecA/mecC* presence, 50.8% (n=91) of the isolates were MRSA and 49.2% (n=88) were methicillin-sensitive *S. aureus* (MSSA). All isolates determined to be *mecA*-positive through PCR were also determined to be resistant when tested using the CDD method. The Phoenix automated system falsely identified six MSSA isolates as MRSA, and it was statistically significant (p=0.031) (Table 2). Moreover, these six MSSA isolates were determined to be resistant by using the CDD test. Considering *mecA/mecC* positivity as the gold standard, the sensitivity, specificity, PPV, and NPV were 100% for the CDD method and 100%, 93.2%, 93.8%, and 100%, respectively, for the Phoenix automated system (Table 3).

Among the 179 *S. aureus* strains that were included, 12 *PVL*-positive strains were detected, of which six were from MRSA strains and six from MSSA strains. Based on this data, the *PVL* positivity rate was found to be 6.5% among MRSA and 7.4% among MSSA. MRSA strains were isolated from the skin and soft tissue samples, including abscesses, wounds, and biopsy samples. PVL-positive MRSA strains were collected from patients hospitalized under the following services: orthopedics, otorhinolaryngology, general surgery, pediatric surgery, pediatric oncology, and pediatric infectious diseases. All *PVL*-positive MSSA strains were isolated from the skin and soft tissue samples of outpatients. Notably, no strains with *mecC* were detected among the MRSA and MSSA strains.

Table 2. Number of methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *Staphylococcus aureus* strains and percentages determined using cefoxitin disc diffusion and Phoenix automated sytem

		mecA/mecC PCR				
		MRSA		MSSA		
		n	%	n	%	p
CDD	MRSA	91	100	0	0.0	1.000
	MSSA	0	0.0	88	100	
Phoenix	MRSA	91	93.8	6	6.2	0.031
	MSSA	0	0.0	82	100	

MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-sensitive *Staphylococcus aureus*, CDD: Cefoxitin disc diffusion, PCR: Polymerase chain reaction

Table 3. Sensitivity, specifity, positive predictive value, and negative predictive value rates for cefoxitin disc diffusion and Phoenix automated system

	Sensitivity	Specificity	PPD	NPD
CDD	1,000	1,000	1,000	1,000
Phoenix	1,000	0,932	0,938	1,000

PPV: Positive predictive value, NPV: Negative predictive value, CDD: Cefoxitin disc diffusion

Discussion

Considering the multiple antibiotic resistance in MRSA, it becomes imperative to rapidly and accurately ascertain methicillin resistance to choose the appropriate antibiotic therapy[15]. MRSA isolates falsely identified as sensitive may cause treatment failures, whereas MSSA isolates incorrectly identified as resistant may cause unnecessary glycopeptide antibiotic use, toxic effect exposure, and increased treatment costs[2].

In 2011, a new gene homolog named *mecC* on SCCmec XI (staphylococcal cassette chromosome mec) element was detected in human and bovine MRSA isolates[15]. After this discovery, several publications stated that detecting *mecA* alone cannot be considered to be the golden standard in the determination of methicillin resistance[3,4,16,17]. Several European countries started retrospective research on *mecC* positive MRSA isolates that could not be detected using *mecA*-based molecular methods. In a study conducted in Spain between 2008 and 2013, overall, seven (0.1%) strains containing *mecC* were determined among the study strains[18]. When *mecC* (+) MRSA was detected in a hospitalized patient in Slovenia, 395 community-origin MRSA isolates were scanned retrospectively between 2006 and 2013, and six more *mecC* (+) MRSA isolates were detected[19]. In a prospective study conducted in England by Paterson et al.[20], nine *mecC*-positive isolates were detected among 2010 MRSA strains, and the prevalence was 0.45%. A study by Basset et al.[7] in Switzerland reported only one isolate (0.06%) carrying this gene, whereas a study conducted in the USA[21] detected no *mecC*-positive strains. Notably, only few studies are available regarding *mecC* in Turkey, and no MRSA strains were reported to carry this gene[22,23]. In a seven-year retrospective study by Kılıc et al.[22], overall, 1700 *S. aureus* isolates comprising 1177 MSSA and 523 MRSA strains were screened for *mecC*, while no *mecC*-positive strains were found. They suggested that considering the regional epidemiological data in Turkey can rapidly change, multicenter studies should be conducted. In a recent multicenter study conducted by Cikman et al.[23], 494 MRSA strains isolated from seven geographical regions in Turkey were investigated, and no *mecC*-positive strains were detected. Similarly, we did not observe any *mecC*-positive isolates among the 179 *S. aureus* strains, of which 91 were MRSA and 88 were MSSA. The number and percentages of *mecC*-harboring *S. aureus* strains detected in various studies worldwide are presented in Table 4. Our study significantly contributes to *mecC* epidemiology in Turkey. However, considering worldwide studies, we believe that the number of strains included in our study was limited, and therefore, extensive studies are warranted to obtain the prevalence data in Turkey.

Molecular-based methods are the golden standards in the determination of methicillin resistance, but every laboratory does not have the required facilities[10]. Therefore, more feasible and cheaper phenotypic methods were developed over the years. In a study by Panda et al.[24] on CDD, which is one of the phenotypic methods, the sensitivity was determined to be 96.7% and specificity 100%. In a study conducted by Iraz et al.[25] in Turkey, the sensitivity and specificity of the disc diffusion method were 96.5% and 98.4%, respectively. In another study by Uzun et al.[2], these values were 98.3% and 100%, respectively. On the other hand, in a study by Kriegeskorte et al.[26] that included 111 *mecC*-positive *S. aureus* strains, CDD and oxacillin broth microdilution methods were used, and the sensitivity of these tests was determined to be 100% and 61.3%, respectively. They concluded that the results emphasized the superiority of cefoxitin in the determination of even the *mecC* MRSA. The sensitivity and specificity results of various studies regarding the CDD test and Phoenix automated system are presented in Table 5. In our study, the sensitivity and specificity of the CDD method were both 100%. Hence, we consider CDD to be an accurate test for determining *mecA*-mediated resistance in *S. aureus* that can be employed as an alternative to PCR in resource constraint laboratories.

Automated diagnostic systems that are currently used in several microbiology laboratories have adapted their products to optimize the detection of *mecA*-mediated resistance. The Phoenix system offers panels that include both oxacillin and cefoxitin-as an improvement from the initial version. The instrument's expert system interprets any *S. aureus* isolate that is tested positive by the cefoxitin screen (MIC>4 µg/ml) to be oxacillin resistant. Mencacci et al.[27] tested the performance of this system with 1066 *S. aureus* strains and determined its sensitivity and specificity to be 100% and 99.8%, respectively. In a study by Junkins et al.[28], the sensitivity and specificity of the Phoenix automated system were determined to be 99.8% and 100%, respectively. A study in Turkey by Cekin et al.[11] compared the determination of methicillin resistance of 206 *S. aureus* isolates by using the Phoenix automated system and real-time PCR. They observed that the sensitivity and specificity were both 100%, and based on these results, they concluded that the automated system was a practical and reliable method that can be used in routine microbiology laboratories. Iraz et al.[25] determined the sensitivity and specificity of the automated system to be 98.8% and 97.6%, respectively. In our study, the Phoenix automated system falsely identified six *mecA*-negative MSSA isolates as MRSA. Based on this data, the sensitivity and specificity of the automated system were 100% and 93.2%, respectively. Our results were in concordance with the other studies, revealing that the sensitivity of automated systems is high, and their specificity is relatively low. Notably, false-positive detection of resistance results in unnecessary use of glycopeptides, particularly vancomycin. Consequently, such an increase in glycopeptide usage may increase vancomycin-intermediate *S. aureus* and vancomycin-resistant *S. aureus* strains. Therefore, it is recommended to determine the presence of *mecA/mecC* genes through molecular tests in the strains that are identified as MRSA by the automated system, and if this is impossible, the CDD test should be performed. Even

Table 4. Number and percentages of *mecC*-harboring methicillin-resistant *Staphylococcus aureus* strains detected in different studies

References	Country	Total number of *S. aureus* isolates	*mecC*-MRSA (n; %)
García-Garrote[18]	Spain	5502	7; 0.1%
Paterson[20]	England	2010	9; 0.45%
Ganesan[21]	USA	102	0; 0%
Basset[7]	Switzerland	1617	1; 0.06%
Kılıc[22]	Turkey	1700	0; 0%
Cıkman[23]	Turkey	494	0; 0%

MRSA: Methicillin-resistant *Staphylococcus aureus*

Table 6. Percentages of Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* strains detected in different studies

References	PVL-positive MRSA (%)	PVL-positive MSSA (%)
Kilic[22]	1.7%	1.9%
Demir[31]	0%	9.1%
Gulmez[32]	0%	2.2%
Hu[14]	28.6%	No data
van der Mee-Marquet[30]	33.8%	No data

PVL: Panton-Valentine leukocidin, MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-sensitive *Staphylococcus aureus*

Table 5. Sensitivity and specificity results of cefoxitin disk diffusion test and Phoenix automated system in different studies

	CDD test		Phoenix automated system		
References	Sensitivity	Specifity	Reference	Sensitivity	Specifity
Panda[24]	96.7%	100%	Junkins[28]	99.8%	100%
Iraz[25]	96.5%	98.4%	Mencacci[27]	100%	99.8%
Uzun[2]	98.3%	100%	Iraz[25]	98.8%	97.6%

CDD: Cefoxitin disk diffusion

though automated systems are frequently used in routine laboratories for AST owing to their ease of use, there is negligible evidence regarding their ability to classify *mecC*-positive MRSA accurately[29]. A study by Kolenda et al.[29] compared three automated AST systems regarding their ability to detect a large number of *mecC*-MRSA isolates (n=111) and observed that the phenotypic detection rate for *mecC*-MRSA by using the Phoenix system was low at 75%, while they concluded that this automated system might fail to detect *mecC*-encoded methicillin resistance. Kriegeskorte et al.[26] investigated the accuracy of the Phoenix system in determining *mecC*-positive *S. aureus* strains and recommended that the categorization as methicillin-susceptible by using the AST systems should be verified using the molecular assays or CDD.

The risk of the prevalent *PVL*-positive healthcare-associated MRSA (HA-MRSA) strains is a serious concern that can result in the emergence of multidrug-resistant HA-MRSA isolates with increased virulence[13,14]. A study by Hu et al.[14] reported the *PVL* positivity rate among MRSA strains to be 28.6%. In another study by van der Mee-Marquet et al.[30], this rate was determined to be 33.8%. However, in studies conducted in Turkey, the rate ranged from 0% to 2%[22,31,32]. Kılıc et al.[22] determined 9 of 523 (1.7%) MRSA isolates and 23 of 1177 (1.9%) MSSA isolates as *PVL*-positive. In a study by Demir et al.[31], 22 of 165 (9.1%) MSSA isolates were *PVL*-positive, whereas none of the 77 MRSA isolates carried this gene. Similarly, in the study by Gülmez et al.[32], no *PVL*-positive strains were detected among the MRSA strains, and the positivity rate was 2.2% among the MSSA isolates. Percentages of *PVL*-positive MRSA and MSSA strains detected in different studies are presented in Table 6. In our study, the *PVL* positivity rate was 6.5% among the MRSA and 7.4% among the MSSA strains. The *PVL* positivity rate among MSSA strains, which varied between 2% and 9% in studies conducted in Turkey, is compatible with our results. However, the rate of *PVL*-positive MRSA strains, which was found to range between 0% and 2% in other studies, is lower than our result. Similarly, in our study, all *PVL*-positive isolates were obtained from the skin and soft tissue samples, as reported in some other studies[14,31,32]. Moreover, it was remarkable that all *PVL*-positive MRSA strains were isolated from inpatients while all *PVL*-positive MRSA strains were isolated from outpatients. However, we did not classify *S. aureus* strains according to the Center for Disease Control and Prevention criteria of whether they were hospital or community-acquired and the lack of mortality data for patients with *PVL*-positive *S. aureus* growth can be considered as some of the shortcomings of this study.

Nonetheless, this study had several additional limitations. The sample size for detecting *mecC*-harboring MRSA was ralatively small. On the other hand, we did not investigate the *mecB* gene in *S. aureus* isolates by using PCR. In addition, we did not perform the origin analysis of these strains by using the Pulsed-field gel electrophoresis method.

Conclusion

We conclude that the CDD method is easy to apply and reliable. Thus, it is suitable for routine laboratory use to determine *mecA*-controlled methicillin resistance in *S. aureus.* Furthermore, we suggest confirming the Phoenix automated system findings by using an additional method, such as CDD. Despite low prevalence (0.06%-0.5%), *mecC* should not be overlooked, particularly in cases where MRSA is unresponsive to treatment, and molecular methods used to detect this gene should be more frequently included in laboratory research in Turkey.

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Ethics

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Şişli Hamidiye Etfal Training and Research Hospital (No: 14.01.2013/280).

Informed Consent: Informed consent was not received since this was a retrospective bacteriological strain based study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: NA, BB, Design: NA, BB, Data Collection or Processing: NA, BB, Analysis or Interpretation: NA, BB, Literature Search: NA, BB, Writing: NA.

Conflict of Interest: No conflict of interest was declared by the authors.

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