Original Article

DOI: 10.14235/bas.galenos.2021.5363
Bezmialem Science 2022;10(2):226-30

Screening of *mecC* Gene in Methicillin Resistant *Staphylococcus aureus* Isolates

Metisiline Dirençli *Staphylococcus aureus* İzolatlarında *mecC* Geni Taraması

Ayşe Nur CEYLAN[1], Bilge SÜMBÜL[2], Mehmet Ziya DOYMAZ[2]

[1]Başakşehir Çam and Sakura City Hospital, Clinic of Medical Microbiology, İstanbul, Turkey
[2]Bezmialem Vakıf University Faculty of Medicine, Department of Basic Medical Sciences, İstanbul, Turkey

ABSTRACT

Objective: The diagnosis and treatment of *mecC* positive methicillin resistant *Staphylococcus aureus* (MRSA) isolates pose a significant problem in clinical microbiology and infectious disease practices. The studies on the frequency of *mecC* positive isolates in Turkey is rather scarce. In the present study, we aimed to investigate the presence of *mecA*, *mecC*, *spa* and *pvu* genes in MRSA strains isolated from various clinical specimens submitted to Clinical Microbiology Laboratories of Bezmialem Vakıf Hospital.

Methods: We performed nucleic acid extraction and multiplex polymerase chain reaction (PCR) to 126 MRSA strains to detect *mecC*, *mecA*, *spa* and *pvl* genes.

Results: According to the multiplex PCR results of 126 MRSA strains studied, 126 (100%) had *mecA*, 107 (85%) had *spa*, and 3 (2%) had *pvl* genes. We performed another polymerase chain reaction protocol and *spa* genes were identified in 19 of specimens, which were found negative priorly.

Conclusion: Considering the factors that a university medical center where the study was conducted provided a tertiary healthcare service to a large metropolitan area in Istanbul and none of the isolates carried *mecC* gene might indicate that *mecC* gene carrying MRSA isolates did not pose a significant public health threat in Turkey.

Keywords: *Staphylococcus aureus*, MRSA, *mecA*, *mecC*, *mec*ALGA251, *spa*

ÖZ

Amaç: *mecC* pozitif metisiline dirençli *Staphylococcus aureus* (MRSA) izolatları hem tanı hem de tedavide önemli problemlere yol açan patojenler olarak değerlendirilmektedir. Türkiye'de *mecC* pozitif izolatlar üzerine yapılan çalışmalar nispeten sınırlı kalmıştır. Bu çalışmanın amacı Bezmialem Vakıf Hastanesi'ne teslim edilen klinik örneklerden izole edilip arşivlenen MRSA suşlarında *mecA*, *mecC*, *spa* ve *pvl* genlerinin varlığını araştırarak ülkemizde *mecC* geni taşıyan MRSA izolatları ile ne sıklıkta karşılaşıldığı konusunda bilgi edinmektir.

Yöntemler: Yüz yirmi altı adet MRSA suşuna nükleik asit ekstraksiyonu ve *mecC*, *mecA*, *spa* ve *pvl* genlerinin tespit edilmesine yönelik multipleks polimeraz zincir reaksiyonu (PZR) işlemi uygulanmıştır.

Bulgular: Çalışılan 126 MRSA suşunun multiplex PZR sonuçlarına göre 126'sında (%100) *mecA*, 107'sinde (%85) *spa*, 3'ünde (%2) *pvl* geni tespit edilmiştir. *Spa* geni tespit edilemeyen 19 suşa faklı bir yöntemle tekrar PZR yapılarak bu suşlarda *spa* genleri tespit edilebilmiştir.

Sonuç: Geniş metropolitan bir yerleşim yerine sağlık hizmeti sunan, üçüncü seviye servis ve eğitim hastanesinde izole edilen 126 MRSA suşunun tamamında *mecA* geni tespit edilirken hiçbir suşta *mecC* genine rastlanmamıştır. Bu sonuçlar, ülkemizde *mecC* geni taşıyan MRSA izolatlarının ciddi halk sağlığı problemi oluşturmadığı yönündeki değerlendirmeleri desteklemektedir.

Anahtar Sözcükler: *Staphylococcus aureus*, MRSA, *mecA*, *mecC*, *mec*ALGA251, *spa*

Address for Correspondence: Ayşe Nur CEYLAN, Başakşehir Çam and Sakura City Hospital, Clinic of Medical Microbiology, İstanbul, Turkey
E-mail: aysenurceylan1011@gmail.com **ORCID ID:** orcid.org/0000-0002-0049-6873

Received: 11.12.2020
Accepted: 15.07.2021

Cite this article as: Ceylan AN, Sümbül B, Doymaz MZ. Screening of *mecC* Gene in Methicillin Resistant *Staphylococcus aureus* Isolates. Bezmialem Science 2022;10(2):226-30

Introduction

In the 2014 report of the World Health Organization, it was reported that when compared to methicillin susceptible *Staphylococcus aureus* (MSSA), infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) were associated with a significantly higher risk of septic shock, all-cause mortality and bacterial-related mortality, and that they were associated with a longer hospital and intensive care unit stay (1). Considering these data, it is very important to detect the presence of MRSA quickly and accurately in patients.

In clinical practice, MRSA strains are considered resistant to all beta-lactam antibiotics (2). This is because MRSAs produce penicillin-binding proteins (PBP2a') that bind with lower affinity to beta-lactams. PBP2a's are encoded by the *mecA* gene located on the staphylococcal chromosome cassette (*SCCmec*) genetic region (3,4).

The presence of MRSA is detected by phenotypic and genotypic methods in clinical microbiology laboratories. The most common method is to look at the susceptibility of oxacillin/cefoxitin. With this method, the presence of MRSA can be demonstrated the next day. Genotypic methods are used in cases where phenotypic methods are not conclusive (eg, limit values in disc diffusion) or when rapid results are required (5). PCR tests to show the presence of the *mecA* gene in bacteria can give results in hours, and quick card tests can give results in minutes.

In 2011, isolation of *S. aureus* (LGA251) strains isolated from milk and different clinical specimens, which were phenotypically resistant to methicillin but could not be detected by tests for the *mecA* gene, and which were determined to have a different mecA gene, was reported for the first time (5). This different *mecA* gene ($mecA_{LGA251}$) was later named the *mecC* gene (6). These studies have shown that MRSA strains with the *mecC* gene can be overlooked in clinical practice. After this date, similar studies have been carried out from different countries and MRSA strains carrying *mecC* have been reported from many European countries (7).

Studies on the presence of *mecC* in our country have been relatively limited. It is clear that the presence and frequency of MRSA isolates carrying the *mecC* gene is also important in the development of public health policies, as it affects the treatment. Bezmialem Vakıf Hospital as a university hospital providing tertiary healthcare with a central location in Istanbul, accepts patients from all over Turkey. Therefore, it would not be wrong to assume that the data obtained from this hospital will also include some country-wide clues.

In this study, the presence of *mecC* was investigated in MRSA strains isolated and archived in the cultures of various clinical samples in the microbiology laboratory between November 2014 and March 2018 in order to identify MRSA isolates carrying the *mecC* gene in our hospital and to obtain information on the distribution of similar isolates throughout the country.

Methods

Bacterial Isolates and Antibiotic Susceptibility Tests

One hundred and twenty six consecutive MRSA strains isolated and stocked from various clinical samples of patients admitted to the outpatient clinics of Bezmialem Vakıf Hospital and hospitalized between November 2014 and March 2018 were included in the study. These strains were not previously passaged for any reason.

Antibiotic susceptibility of bacteria was determined with the Vitek-2 automated system (bioMerieux, France) in accordance with the manufacturer's recommendations.

DNA Isolation

The MRSA strains in the stocks were passaged on sheep blood agar and incubated overnight. After the incubation, 4-5 colonies were taken from the cultures that grew and placed in 1 mL saline filled Eppendorf tubes and thoroughly mixed with vortex. Bacterial suspensions were incubated at 95 °C for 5 minutes in a heat block. Afterwards, the eppendorfs were centrifuged at 3,000 rpm for 1 minute, and the supernatants were separated to be used in the PCR process and stored at -20 °C.

Detection of *mecC, mecA, spa* and *pvl* Genes

According to the protocol published by the European Union Reference Laboratories National Food Institute in 2012, the primer mix used for the detection of *mecC, mecA, spa* and *pvl* genes was prepared and multiplex PCR was performed according to this protocol (8). PCR conditions were set at 94 °C for 5 minutes, 30 cycles (94 °C 30 seconds, 59 °C 1 minute, 72 °C 1 minute), and 72 °C for 10 minutes. *S. aureus* ATCC 43300 for *mecA* and *S. aureus* NCTC 13552 for *mecC* were used as positive controls.

The PCR for *spa* detection was performed according to the protocol published by Votintseva et al. (9) in 2014 for the strains of which *spa* gene could not be detected.

Results

According to the results of multiplex PCR of 126 MRSA strains studied, *mecA* gene was detected in 126 (100%), *spa* gene in 107 (85%) and *pvl* gene in 3 (2%), and *mecC* gene was not detected in any isolate. An example gel image is shown in Figure 1.

The PCR was applied to 19 strains in which the *spa* gene could not be detected, with a different method for *spa* detection, and *spa* gene could be detected in all 19 isolates (Figure 2) (9).

According to the historical VITEK 2 minimum inhibitory concentration results, all isolates were resistant to cefoxitin and oxacillin, and susceptible to vancomycin and linezolid. Inducible clindamycin resistance was reported in 24 isolates. The susceptibility results of some antibiotics are given in Table 1.

Discussion

The MRSA is one of the most important causes of community and hospital-acquired infections. MRSA outcomes reported from intensive care units are increasing day by day. MRSA is

Table 1.

	The number of susceptible strains	Susceptibility results of MRSA strains for some antibiotics	The number of resistant strains	The percentage of susceptibility
Levofloxacin	32	2	12	70%
Erythromycin	22	53	36	21%
Clindamycin	78		40	66%
Tetracycline	52		66	45%
Trimethoprim/sulphamethoxazole	99		19	84%

MRSA: Methicillin resistant Staphylococcus aureus

an important pathogen due to its multi-antibiotic resistance. In addition to being resistant to all beta lactam antibiotics, it also shows resistance to lincosamide, aminoglycoside and macrolides (10). It is very important to detect MRSA in the laboratory in order to select an appropriate and effective antibiotic.

The PCR to detect the mecA gene in bacteria and rapid agglutination tests for the presence of PBP2a may miss the presence of mecC (5). Although mecC has not yet been detected in human samples in our country, it has been detected in many European countries. Since the number of studies conducted in our country is very small, it can be said that isolates carrying mecC have not yet been detected in human samples in our country. In this case, it may be recommended to carry out studies looking for MRSA isolates with the mecC gene at regular intervals, to continue the scans, and to carry out studies with a large number of samples and necessary arrangements in order not to miss the possible mecC gene presence in the laboratory diagnosis of MRSA (7). Our study exemplifies such a survey.

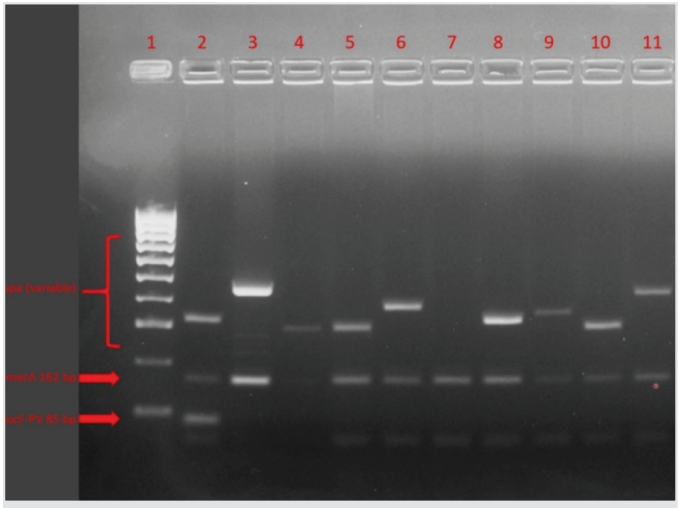

Figure 1. Multiplex PCR gel image
Row1: 100 bp marker.
Row 2: MRSA positive strains for *spa*, *mecA(162bp)* and *pvl(85 bp)* genes from top to bottom.
Row 7: One of the MRSA strains in which the spa gene could not be detected.
Rows 3-6, 8-11: MRSA strains found positive for the spa and mecA genes.
PCR: Polymerase chain reaction, MRSA: Methicillin resistant Staphylococcus aureus

In a study published in 2013, 896 *S. aureus* strains were studied with the VITEK 2 susceptibility system and it was aimed to detect MRSA carrying *mecC*. Of the 455 MRSA *mecA* positive strains, 98% were resistant to oxacillin and cefoxitin, 0.9% were susceptible to cefoxitin, while 1.1% were resistant to oxacillin and susceptible to cefoxitin, and no *mecA* MRSA strains were found to be susceptible to cefoxitin and oxacillin. Of the 62 *mecC* positive MRSA strains, 88.7% were susceptible to oxacillin, resistant to cefoxitin, and 11.3% were found to be resistant to both antibiotics, and similarly, no *mecC* MRSA strains were found to be susceptible to cefoxitin and oxacillin. Of 379 methicillin-susceptible strains (*mecA* and *mecC* negative), 98.8% were found to be sensitive to both antibiotics, while 1.1% were resistant to oxacillin, but sensitive to cefoxitin (11). All 126 MRSA strains in our study were found to have *mecA* gene and according to VITEK 2 results, none of the strains were sensitive to oxacillin or cefoxitin. VITEK 2 antibiotic susceptibility system is a frequently used method in our country. Work needs to be done with other commercial systems as well.

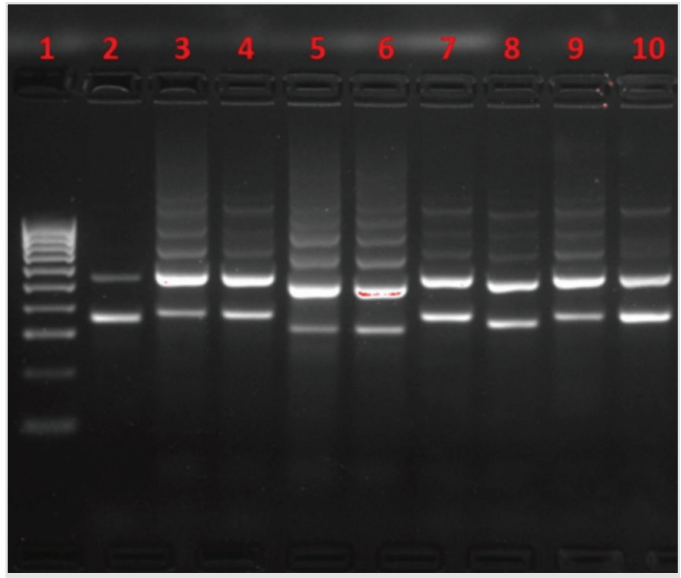

Figure 2. PCR gel image of MRSA strains that were previously found not to have *spa* gene, by using multiplex PCR with new spa primers
Row 1: 100 bp marker
Rows 2-10: *spa* gene regions
PCR: Polymerase chain reaction, MRSA: Methicillin resistant Staphylococcus aureus

Panton-Valentine leukocidin, a cytotoxin known to be secreted from community-acquired *S. aureus*, which causes skin and soft tissue infections and pneumonia, is an important virulence factor (12). Detection of *pvl* gene in a MRSA strain suggests that that strain may be a community-acquired MRSA (13). Data on the origin of the MRSA strains examined in this study were not collected. Community-acquired MRSA strains are often susceptible to clindamycin, tetracycline, trimethoprim-sulfamethoxazole gentamicin, fluoroquinolones, and chloramphenicol, unlike hospital-acquired strains (10). The two MRSA strains we found to have *pvl* gene were also susceptible to these antibiotics. The result of antibiotic susceptibility and *pvl* positivity suggest that these strains may be of community origin.

One of the many virulence factors of *S. aureus* is staphylococcal protein A (*spa*). This protein binds to immunoglobulin (Ig)G and protects the bacteria from phagocytosis. Changes in the IgG binding region of the *spa* gene may cause the *spa* gene not to be detected by classical methods (9). In the multiplex PCR we performed in our study, we could not detect the *spa* gene, although it was repeated twice in 19 of 126 samples. Thereupon, we performed PCR again for the *spa* gene on these 19 isolates according to the protocol published by Votintseva et al. (9) in 2014, and we were able to detect the *spa* gene in all strains (Figure 2).

In many studies conducted in different countries around the world, the presence of *mecC* gene was investigated in *S. aureus* growing from human and animal samples, while *mecC* was not found in some of them (5,7,14). In our country, studies have begun to detect the presence of *mecC* gene in MRSA strains. In the study of Kılıç et al. (15) in 2015, the *mecC* gene was investigated in 1,177 MSSA and 523 MRSA strains isolated from humans, and no *mecC* gene was found in any of the strains. In the study conducted by Cikman et al. (16) in 2018, the presences of *mecA* and *mecC* genes were investigated in 494 MRSA strains isolated from humans, and *mecA* was detected in 315 of them by using PCR, but the presence of *mecC* was not found. In the study conducted by Sayın et al. (17) on cows with mastitis, the presences of *mecA* and *mecC* genes were investigated, and the *mecA* gene was found in 21 of 150 strains and *mecC* gene was found in 7 of 150 strains. In this study, in addition to the previous studies, we discussed MRSA strains from a different region. While we detected *mecA* gene in 126 of 126 MRSA strains isolated from humans in our study, we did not find *mecC* gene.

Studies show that especially molecular methods that look for *mecA* gene in MRSA detection and rapid tests for PBP2a' may miss MRSA due to *mecC* gene, and these methods should be revised to catch *mecC* gene (5). Therefore, laboratories should review the methods by which they detect MRSA to make sure that they do not miss a possible *mecC* positive MRSA with quality control strains. Currently, the number of studies conducted in our country to detect the presence of *mecC* gene in humans and animals is limited. With these data, it would not be correct to say that *mecC* gene was not shown in human samples. In order to show the *mecC* gene status in our country, it is necessary to conduct studies with larger sample numbers from different locations of the country.

Study Limitations

In our study, since the hospital data of the MRSA strains in the stock could not be reached, from which patient samples it was reproduced or outpatient/inpatient interpretation could not be made.

Conclusion

While the *mecA* gene was detected in all of the 126 MRSA strains studied, no *mecC* gene was found in any of the strains. These data support the conclusion that MRSA isolates carrying the *mecC* gene have not yet emerged as an important public health problem in our country.

Ethics

Ethics Committee Approval: Approval was obtained from the Bezmialem Vakıf University Non-Invasive Clinical Research Ethics Committee (02.02.2021/2011-KAEK-42).

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: A.N.C., B.S., M.Z.D., Concept: A.N.C., B.S., M.Z.D., Design: A.N.C., B.S., M.Z.D., Data Collection or Processing: A.N.C., B.S., M.Z.D., Analysis or Interpretation: A.N.C., B.S., M.Z.D., Literature Search: A.N.C., B.S., M.Z.D., Writing: A.N.C., B.S., M.Z.D.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Antimicrobial Resistance Global Report on Surveillance; World Health Organization, 2014. https://apps.who.int/iris/bitstream/handle/10665/112642/9789241564748_eng.pdf?sequence=1
2. Patel JB. M100 Performance standards for antimicrobial susceptibility testing. 27th Edition. Clinical and laboratory standards institute; 2017.
3. Reynolds PE, Brown DF. Penicillin-binding proteins of beta-lactam-resistant strains of Staphylococcus aureus. Effect of growth conditions. FEBS Lett 1985;192:28-32.
4. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. Antimicrob Agents Chemother 2000;44:1549-55.
5. Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, et al. Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 2011;11:595-603.
6. Petersen A, Stegger M, Heltberg O, Christensen J, Zeuthen A, Knudsen LK, et al. Epidemiology of methicillin-resistant Staphylococcus aureus carrying the novel mecC gene in Denmark

corroborates a zoonotic reservoir with transmission to humans. Clin Microbiol Infect 2013;19:E16-E22.

7. Kerschner H, Harrison EM, Hartl R, Holmes MA, Apfalter P. First report of mecC MRSA in human samples from Austria: molecular characteristics and clinical data. New Microbes New Infec 2014;3:4-9.
8. National Food Institute. Protocol for PCR Amplification af mecA, mecC (mecALGA251), spa and PVL. 2012. https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/279_pcr-spa-pvl-meca-mecc-sept12.pdf
9. Votintseva AA, Fung R, Miller RR, Knox K, Godwin H, Wyllie DH, et al. Prevalence of Staphylococcus aureus protein A (spa) mutants in the community and hospitals in Oxfordshire. BMC Microbiol 2014;14:63.
10. Sancak B. *Staphylococcus aureus* ve antibiyotik direnci [*Staphylococcus aureus* and antibiotic resistance]. Mikrobiyol Bul 2011;45:565-76.
11. Cartwright EJ, Paterson GK, Raven KE, Harrison EM, Gouliouris T, Kearns A, et al. Use of Vitek 2 antimicrobial susceptibility profile to identify mecC in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2013;51:2732-4.
12. Duman Y, Tekerekoğlu MS, Otlu B. Toplum ve hastane kökenli *Staphylococcus aureus* klinik izolatlarında Panton-Valentine lökosidin varlığının ve klonal ilişkinin araştırılması. [Investigation of the presence of Panton-Valentine leukocidin and clonal relationship of community- and hospital-acquired clinical isolates of Staphylococcus aureus]. Mikrobiyol Bul 2013;47:389-400.
13. Cirit OS, Yıldırım T, Çoban AY. Staphylococcus aureus Klinik İzolatlarında Panton-Valentin Lökosidin Varlığının Araştırılması. Balkan Med J 2011;28:119-24.
14. Kriegeskorte A, Ballhausen B, Idelevich EA, Köck R, Friedrich AW, Karch H, et al. Human MRSA isolates with novel genetic homolog, Germany. Emerg Infec Dis 2012;18:1016-8.
15. Kılıç A, Doğan E, Kaya S, Baysallar M. Yedi yıllık sürede klinik örneklerden izole edilen Staphylococcus aureus suşlarında mecC ve Panton-Valentine lökosidin gen varlığının araştırılması. [Investigation of the presence of mecC and Panton-Valentine leukocidin genes in Staphylococcus aureus strains isolated from clinical specimens during seven years period]. Mikrobiyol Bul 2015;49:594-9.
16. Cikman A, Aydin M, Gulhan B, Karakecili F, Kurtoglu MG, Yuksekkaya S, et al. Absence of the mecC gene in methicillin-resistant Staphylococcus aureus isolated from various clinical samples: The first multi-centered study in Turkey. J Infect Public Health 2019;12:528-33.
17. Sayın Z, Sakmanoğlu A, Uçan US, Pınarkara Y, Uslu A, Aras Z, et al. Türkiye'de mastitisli inek sütlerinde mecC geni taşıyan metisilin dirençli Staphylococcus aureus varlığının belirlenmesi. Eurasian J Vet Sci 2016;32:182-7.