

Investigation of Various Virulence Factors and SCCmec Types in the Healthcare-associated and Community-associated Methicillin Resistant Staphylococcus aureus Strains

Süreyya Gül Yurtsever¹, Abdurrahman Aygül², İsmail Öztürk³, Salih Atakan Nemli⁴, Selçuk Kaya¹, Şafak Ermertcan⁵

¹Department of Medical Microbiology, İzmir Katip Çelebi University School of Medicine, İzmir, Turkey

²Department of Pharmaceutical Microbiology, Çukurova University Faculty of Pharmacy, Adana, Turkey

³Department of Pharmaceutical Microbiology, İzmir Katip Çelebi University Faculty of Pharmacy, İzmir, Turkey

⁴Department of Infectious Diseases, İzmir Katip Çelebi University School of Medicine, İzmir, Turkey

⁵Department of Pharmaceutical Microbiology, Ege University Faculty of Pharmacy, İzmir, Turkey

ABSTRACT

Objective: The objective of this study was to investigate some virulence genes and SCCmec types of methicillin-resistant Staphylococcus aureus (MRSA) isolates and to determine their relationship with virulence factors.

Methods: A total of 100 MRSA strains, 64 from healthcare-associated and 36 from community-associated infections, were included in the study. The presence of *mecA* gene was investigated by PCR. SCCmec types and *efb*, *clfB*, *agrA* gene were detected by multiplex PCR and their relationship with virulence factors has been analysed.

Results: All of the isolates contain the *mecA* gene. At the same time, in 66 strains (66%) *agrA* gene, in 58 strains (58%) *clfB* gene, and in 47 strains (47%) *efb* gene were positive. In terms of SCCmec types, the distribution of these types among the 64 HA-SA strains was 53% similar-to-type-III, 16% type IV, 2% type I and 30% unclassified. The distribution of the types among the 36 CA-SA strains was 19% similar-to-type-III, 25% type IV, 8% type I and 47% unclassified, respectively. When SCCmec types were evaluated according to clinical sample type, similar-to-type-III isolates were found to be dominant in wound samples. *Efb* (78%), *clfB* (85%), *agrA* (88%) were the dominant genes in similar-to-type-III strains, whereas *clfB* (74%), *agrA* (100%) were the main genes detected in the type IV strains.

Conclusion: It is of clinical and epidemiological importance to know the origin of MRSA strains because this affects the empirical treatment choice.

Keywords: Methicillin-resistant Staphylococcus aureus, PCR typing, SCCmec, virulence factors

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium that causes epidemics and endemics worldwide and leads to infections with high morbidity and mortality (1).

S. aureus is the most commonly isolated bacterium in both community-associated (CA-SA) and healthcare-related (healthcare-associated-HA-SA) infections. *S. aureus* can cause serious infections, such as life-threatening pneumonia and toxic shock syndrome from skin and soft tissue infections (2). The first MRSA strains associated with healthcare delivery were identified in

1960 and CA-MRSA was first described in 1980. These strains are primarily associated with skin and soft tissue infections, but now also cause health-related infections (1, 3).

In addition to the increase in the prevalence of hospital infections caused by CA-MRSA, there is the issue that it cannot be easily differentiated from HA-MRSA, based on clinical and epidemiological criteria. For this reason, the use of genetic indicators in their classification has gained importance (4). MRSA strains isolated from community-associated infections, have been observed to be different from HA-MRSA both genotypically and phenotypically (3).

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Corresponding Author: Süreyya Gül Yurtsever **E-mail:** sgul71@yahoo.com

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Polymerase chain reaction (PCR) is frequently used in the detection of methicillin resistance, genotyping of MRSA strains and determining virulence factors (5).

The *mecA* gene encoding the methicillin resistance is located on a mobile genetic element called *mec* (*SCCmec*) of the staphylococcal cassette chromosome. *SCCmec* consists of *mec* gene complex (*mecA* and regulating genes) and *ccr* complex. To date, 13 (I–XIII) main types have been defined in different *SCCmec* types. Of these, *SCCmec* type I, II and III were mostly detected in HA-MRSA; Type IV, V, VI, and VII were associated with CA-MRSA strains (3, 6).

In *S. aureus* infections, virulence factors that are found on the cell surface and secreted out of the cell also play an important role. The virulence of *S. aureus* strains does not depend on any of these biological factors alone but is caused by several effects (7). Different virulence markers are observed at different stages of staphylococcal infections. *Agr* (accessory gene regulator) is a core sensor system that plays a critical role in the systemic infection process. *Agr*, a quorum-sensing system, plays a role in the regulation of transcription of genes encoding some surface proteins and enzymes released outside the cell (8).

Whether all MRSA strains have an equal potential for disease or whether invasive and chronic diseases are associated with virulent genotypes is still unknown. The identification of virulence genes may explain this issue (1).

The aim of this study was to determine the *SCCmec* types of MRSA strains isolated from outpatients and outpatients by multiplex PCR and to determine the distribution of some important virulence genes among these types.

METHODS

Patient Groups and Bacterial Strains

One hundred MRSA strains from various polyclinics/services from the Microbiology Laboratory in 2014–2016 were included in our study. The strains were identified by the Phoenix™ 100 system (Becton Dickinson, USA) and confirmed by the coagulase test. If more than one MRSA was isolated from one patient, only one was included in the study. The strains were stored at -80 °C in a Brain-Heart Infusion medium with 10% glycerin.

Infection origin types were defined according to CDC criteria (9). Thirty-six of all strains were isolated from patients admitted to

various polyclinics of the hospital and these strains were accepted as ‘community-acquired’. The other 64 strains were isolated from the different infection sites of patients in the hospital and in the intensive care units, and these strains were accepted as ‘healthcare-associated’. The clinic, sample type and date information of patients were recorded. When evaluated in terms of sample type, 58 of the strains were blood, 20 were wound, 9 were nasal, 7 were urine and 6 were sputum samples.

Determination of Methicillin Resistance

Methicillin resistance in strains was determined by detecting the minimum inhibitory concentration (MIC) of ceftiofex by the Phoenix™ 100 (Becton Dickinson, USA) automated system. Data were confirmed by the ceftiofex disc diffusion (DD) test according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (10) standards. *S. aureus* ATCC 25923 was used as a control group.

Antibiotic Susceptibility Testing

The in-vitro susceptibilities of the strains to gentamicin, levofloxacin, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, rifampicin, vancomycin, teicoplanin, daptomycin, quinupristin-dalfopristin, and linezolid were also determined by the Phoenix™ 100 (Becton Dickinson, USA) automated system.

DNA Isolation

Prior to PCR experiments, genomic DNA was isolated as in the Merlino et al. (11) study.

Investigation of Virulence Genes and *MecA* Gene

The presence of the *mecA* gene in resistant strains was investigated. In addition, the presence of the *agrA* regulatory gene, responsible for the regulation of various toxins, enzymes and surface proteins and *efb* (extracellular fibrinogen binding protein) *clfB* (cloning factor B) virulence genes were investigated using the multiplex PCR method. The thermal cycling conditions were applied using the primers indicated in Table 1 for 1 minute at 94°C, 1 minute at 42°C and 1.5 minutes at 72°C, following 5 minutes initial denaturation at 94°C. Finally, multiplex PCR was completed with final elongation for 10 minutes at 72°C.

Molecular Typing with Multiplex PCR

In order to determine the *SCCmec* types of strains, primers given in the study performed by Milheiricho et al. (12) were used. Thermal cycling conditions were also applied as given in the same study. *S. aureus* HPV107 (*SCCmec* type IA), BK2464 (*SCCmec* type II), HUSA304 (*SCCmec* type III), HSJ216 (*SCCmec* type IIIA) and GRE14 (*SCCmec* type IV) were used as a positive control.

Analysis of Results

Statistical significance was analyzed by Fisher's exact chi-square test using the GraphPadPrism (California, USA) program. If *p*-value ≤ 0.05, the results were considered significant.

RESULTS

45 (45%) of the strains included in the study were isolated from females and 55 (55%) were from males. The mean age was 62.25 (24–99) years. *S. aureus* strains were isolated from blood (58; 58%), wound (20; 20%), nasal (9; 9%), urine (7; 7%) and sputum (6; 6%) samples. Hospital-associated strains were most frequently isolat-

Main Points:

- All of the isolates (100 isolates) were *mecA* positive.
- In 66 strains *agrA* gene, in 58 strains *clfB* gene, and in 47 strains *efb* gene were positive.
- The distribution of the *SCCmec* types among the 64 HA-SA isolates was 53% similar-to-type-III, 16% type IV, 2% type I and 30% unclassified.
- The distribution of *SCCmec* types among the 36 CA-SA isolates was 19% similar-to-type-III, 25% type IV, 8% type I and 47% unclassified.

Table 1. Virulence genes and primers used in searching the *mecA* gene

Target gene	Length (bp)		Primers	Reference
clfB	596	F	5'-TGCAAGATCAAACCTGTTCT-3'	11
		R	5'-TCGGTCTGTAAATAAAGGTA-3'	
efb	434	F	5'-TAACAATAGCGGCAATAGGT-3'	This study
		R	5'-CAATTCGCTCTTGTAAAGACCA-3'	
agrA	193	F	5'-TCACAGACTCATTGCCCAT-3'	12
		R	5'-CACCGATGCATAGCAGTGTT-3'	
<i>mecA</i>	300	F	5'-TGCTATCCACCCTCAAACAGG-3'	13
		R	5'-AACGTTGTAACCAACCCCAAGA-3'	

Table 2. Distribution rate of genes in terms of being hospital and community associated

	HA(%)	CA(%)
efb	42	48
clfB	56	59
agrA	56	70

Figure 1. An example of SCCmec typing results of isolates. M: DNA marker, lane 75: similar-to-type-III, lane 76: similar-to-type-III, lane 77: similar-to-type-III, lane 78: only *mecA*, lane 80: type IV, lane 81: subtype IVe, lane 82: similar-to-type-III, lane 83: only *mecA*, lane 84: similar-to-type-III, lane 85: type I pattern without J3 region (342 bp), lane 86: similar-to-type-III, lane 87: similar-to-type-III, lane 88: similar-to-type-III, lane 89: similar-to-type-III, lane 90: similar-to-type-III, lane 92: only *mecA*, lane 93: similar-to-type-III, lane 94: type I pattern without J3 region (342 bp)

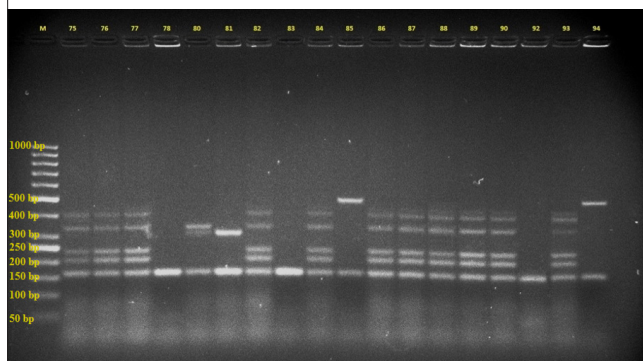


Figure 2. Multiplex PCR for virulence genes (*clfB*, *efb*, *agrA*) and *mecA*.

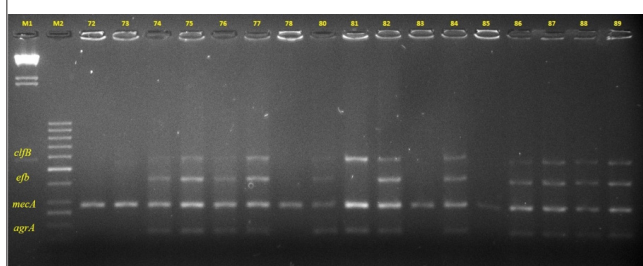


Table 3. Multiplex Amplification Patterns of 36 CA-MRSA Origin Multiplex Pattern and Interpretation

Number of Strains	Multiplex Pattern
22	Only <i>mecA</i> band
5	Type I pattern without J3 region (342 bp)
4	Type III pattern without a J1 region (243 bp) and <i>mecA</i> complex (209 bp)
2	J1 region (495 bp), <i>ccr</i> complex (449 bp), J3 region (414 bp) and <i>mecA</i> band (162 bp) together
1	Type III pattern with a J1 region (495 bp)
1	J1 regions (495 bp), <i>ccr</i> complex (449 bp) and <i>mecA</i> band (162 bp) together
1	<i>ccr</i> complex (449 bp), a <i>ccr</i> complex (311 bp) and <i>mecA</i> band (162 bp) together

ed from blood samples (50/64, 78.12%) and community-acquired strains were isolated from wound samples (20/36, 55.55%). 29 (29%) of the strains were from intensive care units (anesthesia, neurology, internal medicine, brain surgery, cardiology), 24 (24%) from internal medicine clinic/polyclinic, and 22 (22%) other services / polyclinics (neurology), dermatology, chest, infectious diseases, 17 (17%) from surgical services / polyclinics (general surgery, neurosurgery, urology, orthopedics, otolaryngology, cardiovascular surgery) and 8 (8%) from emergency services obtained from clinical samples sent from the observation unit.

Prevalence of Virulence Genes and *mecA* Resistance Gene

According to the multiplex PCR results, it was observed that all of the strains (100) included in the study carried the *mecA* resistance gene. The distribution rates of virulence genes investigated in HA and CA strains are given in Table 2.

Statistically, there was no significant difference in terms of the distribution rates of these virulence genes between the two groups ($p > 0.05$).

Distribution of SCCmec Types

In 41 (41%) of all strains, in addition to bands of 414, 243, 209 and 162 bp, which form the type III pattern, a band of the size of 342 bp,

which normally corresponds to the dcs region of type I, II, IV and V was observed. Gülmez et al. (13) who benefited from the previous study of the same author group (14) identified 342 band size bands corresponding to the dcs region and named the similar- to- type III pattern. In addition, 19 (19%) strains were classified as type IV and 4 (4%) as type I, 4 of whom belonged to type IVe subtype.

In this study, a variety of patterns have been observed in 14 of the 36 strains with non-detectable *SCCmec* types, such as the absence of some loci that cause the pattern of a particular type to be missing or the combination of specific loci of different types, a band with only 162 bp internal positive control amplicon (*mecA*) was observed. The patterns shown by non-groupable strains are shown in Table 3.

The distribution of *SCCmec* types in 64 hospital-associated strains was determined as 53% similar-to-type-III, 16% type IV, 2% type I and 30% non-groupable. The distribution of *SCCmec* types in 36 community associated strains was determined as 19% similar to type III, 25% type IV, 8% type I and 47% non-groupable.

Upon examining the distribution of types in terms of strain type, similar-to-type-III strains were found to be frequent in the wound samples ($p < 0.05$), and there was no statistically significant difference between the groups in the distribution of other types ($p > 0.05$).

Similar-to-type-III strains were found to have *efb* (78%), *clfB* (85%) and *agrA* (88%) genes. For type IV strains, on the other hand, *clfB* (74%) and *agrA* (100%) genes was detected. The distribution of these genes was found to be much less frequent in Type I strains and non-grouped strains.

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Antibiotic susceptibility testing

All *S. aureus* strains were susceptible to vancomycin, teicoplanin, daptomycin, quinupristin-dalfopristin and linezolid. Other antibiotic resistance rates of *S. aureus* strains with HA/CA were; gentamicin 50.0%/33.3%, levofloxacin 64.06%/26.7%, tetracycline 46.7%/33.3%, erythromycin 64.06%/53.3%, clindamycin 46.7%/20%, trimethoprim-sulfamethoxazole 34.4%/33.3%, rifampicin 48.4%/20%.

DISCUSSION

Methicillin-resistant *S. aureus* infections continue to be a significant threat to human health in the second decade of the 21st century. Despite significant progress in understanding MRSA infections and virulence mechanisms, there are continuing challenges that need to be addressed. Knowledge of the prevalence of genetic markers and virulence factors contributing to the success of MRSA will be useful for the control and treatment of community and hospital induced *S. aureus* infections.

HA-MRSA is seen to occur in different rates in our country in relation to the regions. Alp et al. (15) reported that HA-MRSA prev-

alence ranged between 12-75% in a multicentered study they carried out in Turkey, in eight university hospitals, in six different geographic regions, where they had isolated MRSA. The prevalence of CA-MRSA was reported to be 1-3% in our country (16).

In addition to the genotypic differences between HA-MRSA and CA-MRSA, the strains affect different populations and cause different clinical syndromes. CA-MRSA infections tend to occur in healthy children and young adults and are associated with skin and soft tissue infections including necrotizing fasciitis and severe invasive infections such as pneumonia and sepsis. In contrast, HA-MRSA strains are most commonly seen in patients who are under antibiotic treatment, have a weakened immune system and who have been treated using invasive medical devices. HA-MRSA strains commonly cause pneumonia, bacteremia and invasive infections (17). In our study, MRSA strains were first isolated from blood (58%) and secondly from wounds (20%) according to sample types. HA-strains were most frequently isolated 78.12% from blood samples and 55.55% CA -strains were isolated from wound samples.

The risk of bacteremia due to MRSA in inpatients varies according to the services. The highest risk is stated to be in intensive care units (18). In our study, when the distribution of MRSA strains according to services was examined, it was seen that 29% of them were isolated from intensive care. This may be due to a more invasive procedure, more severe underlying disease and / or the presence of immunosuppression.

The monitoring of the *SCC mec* has been carried out around the world for many years. In MRSA strains, it has been confirmed that methicillin resistance gained through the *ccr* and *mec* gene complex (*mecA* and new homologs; *mecB*, *mecC*, *mecD*), influences the concentration of beta-lactam antibiotic resistance and antimicrobial minimal inhibitor, which has been found to lead to multiple drug resistance (19). HA-MRSA strains are resistant to multiple drugs due to drug-resistant genes integrated into *SCCmec* (20, 21). CA-MRSA strains are generally susceptible to non-beta-lactam antibiotics because they do not carry the resistance gene other than *mecA* (3).

A thorough understanding of the prevalence and occurrence of *SCCmec* may help further to identify, control, prevent and treat staphylococcal-mediated human diseases (19). In the studies in order to type *SCCmec* in MRSA strains in Turkey, more than 80% of HA-MRSA strains were reported to be *SCCmec* type III, rarely *SCCmec* type IIIb, CA-MRSA strains were reported to be *SCCmec* type IV and V more frequently, *SCCmec* type I and II have been reported less frequently (15). Karahan et al. (22), reported *SCCmec* type I or II or III and subtypes in 99% of HA-MRSA strains, *SCCmec* type IV in 1%; In 60% of CA-MRSA strains, *SCCmec* type I or II or III and 40% *SCCmec* type IV or V; Tekeli et al. (23) reported 84% *SCCmec* type III in MRSA strains isolated from blood cultures of hospitalized patients; Kılıç et al. (16), reported *SCCmec* type I or II in 3.6% of MRSA strains, *SCC mec* type III in 82.1%, *SCCmec* type IV in 5.1% and *SCCmec* type V in 5.1% of MRSA strains; Gülmez et al. (13) *SCCmec* type IVa in CA-MRSA strains, similar to *SCCmec* type III in HA-MRSA strains; Akoğlu et al. (5), 61.8% *SCCmec* type III in HA-MRSA strains, 34.5% *SCCmec* type IIIb and 2.7% *SCC-*

mec typeIV; Baran et al. (24) found *SCCmec* type III in 24 (85.7%) of the HA-MRSA strains and 100% *SCCmec* type IV in all CA-MRSA strains, 1 (3.6%), *SCCmec* type IV, and 3 (10.7%) HA-MRSA strain could not be typed by the method used. In 2013, Oksuz et al. (25) *SCCmec* type III in MRSA clones; Yilmaz et al. (26) in the HA-MRSA strains, 90% *SCCmec* type III, 2.2% (1 isolate) *SCCmec* type IV and 40% *SCCmec* type IV were detected in CA-MRSA strains and they could not make the typing of the remaining strains. Similar to other studies conducted in our country, 53% *SCCmec* type III strains were found most frequently in HA-MRSA, while 25% type IV strains and 47% non-groupable strains were found to be the most common in CA-MRSA. Similar-to-type-III strains were found to be predominant in wound samples.

It was thought that community-associated MRSA was initially from nosocomial strains and was spreading from hospitals to the community. However, paradoxically, the sensitivity of CA-MRSA to non-beta-lactam antimicrobial agents and their association with clinical syndromes typical for Methicillin-sensitive *Staphylococcus aureus* (MSSA) are strong evidence that CA-MRSA is different from the strains seen in health care units. After the introduction of genotypic differences that differentiate CA-MRSA from HA-MRSA, the idea that CA-MRSA develops from MSSA, which is endemic in the community, has started to be accepted generally. The beta-lactams, once effective in community-associated *S. aureus* strains, have transformed into unreliable therapeutic agents. CA-MRSA is often more sensitive than HA-MRSA to non-beta-lactam antibiotics such as clindamycin, TPM / SMX, and doxycycline (2). Many MRSA clones have gained resistance to antibiotics such as erythromycin, clindamycin, ciprofloxacin, tetracycline. Multi drug resistance exists (27). Most of the CA-MRSA strains were not resistant to additional antibiotics except for the limited outbreaks of multidrug-resistant CA-MRSA (28). In studies conducted in our country, Akoğlu et al. (5) reported a high (> 90%) resistance to gentamicin, ciprofloxacin, and rifampicin, sensitivity to TMP-SMX 90%, clindamycin 53% and erythromycin 32%. Öksüz et al. (25) found penicillin 100%, tetracycline 100%, rifampicin 100%, kanamycin, tobramycin, 93%, levofloxacin 93%, erythromycin 75%, lincomycin 49%, phosphomycin 58% and fusidic acid 4% multidrug resistance. Baran et al. (24) found susceptibility to vancomycin, linezolid and TMP-SMX in all strains, whereas in HA-MRSA / CA-MRSA strains respectively; rifampicin 89.3% / 0%, ciprofloxacin 89.3% / 50%, gentamicin 89.3% / 0%, erythromycin 50% / 50% and clindamycin 28.6 / 0% rates of resistance were found. Tekeli et al. (29); found susceptibility to vancomycin in all strains, 97.7% in tetracycline, 97% in ciprofloxacin, 100% in rifampicin and 94.7% in gentamicin.

In our study, all *S. aureus* strains were found to be sensitive to glycopeptides. Other antibiotic resistance rates of *S. aureus* strains in hospital/community associated strains were 50.0% / 33.3% gentamicin, 64.06%/26.7% levofloxacin, 46.7% / 33.3% tetracycline, 64.06% / 53.3% erythromycin, 46.7% / 20% clindamycin 34.4% / 33.3%, 48.4% / 20%. TMP-SMX rifampicin. It was observed that our resistance rates were lower than other centers.

In recent years, CA-MRSA strains may also have played a role as a nosocomial infectious agent (30). Type IV *SCCmec* is primarily as-

sociated with MRSA infections in patients without risk factors for HA-MRSA. However, according to recent data on patients who do not have risk factors for HA-MRSA, most of the hospitalized patients in the US now have *SCCmec* IV (31). Gonzalez et al. (32) found *SCCmec* type IV in 60% of the HA-MRSA strains isolated from blood.

The virulence factors in *S. aureus* infection have gained importance. There are studies on the activity of *agr*, *clf*, *efb* virulence genes (28,33,34). The host factors that affect the severity of the disease remain an unexplained subject. In understanding virulence factors regulators more research is needed to determine how virulence factors are transmitted between MRSA strains (1).

In our study, it was observed that in type III strains *efb*78%, *clfB* 85% and *agrA* 88% genes, whereas in type IV strains *clfB* and 74% and *agrA* 100% genes were found to be frequent respectively. The distribution of these genes was found to be much less frequent in Type I strains and non-grouped strains.

CONCLUSION

The selective pressure of antimicrobial agents, together with the acquisition of genetic markers, allows MRSA to adapt to different environmental conditions, leading to a global spread of MRSA. Genetic backgrounds that can allow for the development of the MRSA clones can be determined through population studies and post-genomic investigations. The response of regulatory systems to external signals is a significant element in the epidemiologic success of a particular clone. Thus, as has been suggested by many recent studies, these events could be major areas for the development of future therapeutic interventions. Despite the fact that the relationship between endurance, virulence and antibiotic resistance is still to be fully understood, an understanding of how molecular markers can allow the spread of pathological processes will help in the development of prevention and treatment strategies aimed at overcoming the growing challenges associated with MRSA.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Non-Interventional Ethics Committee (1700049757/31.07.2017).

Informed Consent: Due to the in-vitro design of the study, informed consent was not taken.

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