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Research Article

Molecular Epidemiological Research on Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolated from Nosocomial Samples of Patients Hospitalized in Turkey

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen responsible for nosocomial and community infections difficult to treat in humans because of its resistance to β-lactam antibiotics. The resistance is associated with *mecA* gene product *PBP2a* and *PVL* genes which are important virulence factors in MRSA. This research was designed to investigate MRSA prevalence in hospital environment and to examine the *mecA* and *PVL* genes in MRSA isolates. Firstly, nasal mucosa specimens were collected from 730 hospital patients in Medical Faculty Hospital. The samples were plated on 5% sheep blood agar and incubated at 37°C overnight in aerobic condition. 20 Methicillin-susceptible *S. aureus* (MSSA) from hospital patients and 710 MRSA were determined from cultured nosocomial infections. *S. aureus* strains were determined with the API test. Methicillin-resistant strains were determined by Disk Diffussion Method and then Methicillin-resistance was confirmed by the E-Test Analysis.

In the second phase of the study, *MecA-1*, *MecA-2*, *PVL-1*, *PVL-2* and *PVL-3* genes in each samples were investigated by PCR method. *MecA-1* (40), *MecA-2* (40), *PVL-1*(22), *PVL-2* (25) and *PVL-3* (33) genes were amplified in MRSA strains by PCR. In 20 MSSA strains, there were no amplifications of these genes. Molecular findings verified the microbiological results. As a result, this study showed high prevalence of MRSA strains in hospital environment and that the application of microbiological and molecular analysis together are useful for detection of these strains. We repeated at least three times for each bacteria strain all of molecular workings in this study.

Keywords: MRSA; *MecA*; PVL; *Staphylococcus aureus*

Introduction

Epidemiology is a discipline investigating the incidence of the disease and the factors affecting the incidence among groups of people. The molecular epidemiology provides biochemical and molecular level identification of genes responsible for the pathogenesis of bacterial agents. Molecular epidemiology is effective on the etiology, spread, and control of bacterial phenotypes. Also, Molecular epidemiology bacterial virulence phenotype and determines which vary in terms of specific strains to be infectious on diseases, and so, it is important in controlling the spread of these and the etiology of infectious diseases. Thus, molecular epidemiology products definitive solution to the prevention of the spread the family and between societies of infectious diseases emerging under the impact of genetic and environmental risk factors at the molecular level. Severe staphylococcal infections, are still a major problem due to complications of being life threatening and lead to high mortality rates. As well as the bacteria increases in recent years, staphylococcal infections have also changed and the resistance profiles were observed in many strains develop resistance to the most effective antibiotics. So that significant reduction in drug options in the treatment of these infections constitute a significant problem in the developing antibiotic resistance.

Methicillin resistant *S aureus* (MRSA) causes outbreaks in hospitals and can be epidemic. In our country, the increasing of multidrug resistance in *S. aureus* bacteria can increase the risk of infections on disease. Some strains of *S. aureus* that carry epidemic character and is known to cause nosocomial epidemics. Methici-

llin resistance in bacteria is an indication of multiple resistance. Methicillin is one of the most common source of infection in the world resistant *Staphylococcus aureus* (MRSA) is becoming a bigger problem every day with antimicrobial resistance mechanisms. In the case of treating a disease linked to Methicillin resistant *Staphylococcus aureus* or MRSA is important to know the antibiotic susceptibility of patients to receive appropriate treatment. Not only to methicillin but also all beta-lactam resistance to antibiotics it should be determined.

The reason for developing resistance in MRSA is PBP2a protein encoded by the mecA gene. In this study, hospitalized patients nasal swab samples were collected and isolated by conventional means by these examples MRSA strains virulence effect showing PVL (Panton-Valentine leukocyte) intended to detect the presence of the gene. Panton-Valentine leukocyte (PVL) gene region which shows the effect of virulence in strains of MRSA (Figure 1-3).

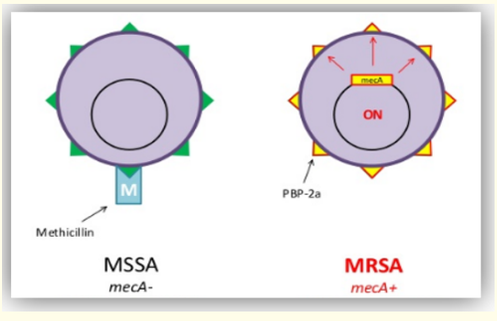


Figure 1: Formation of the MRSA in *S.aureus* bacteria.

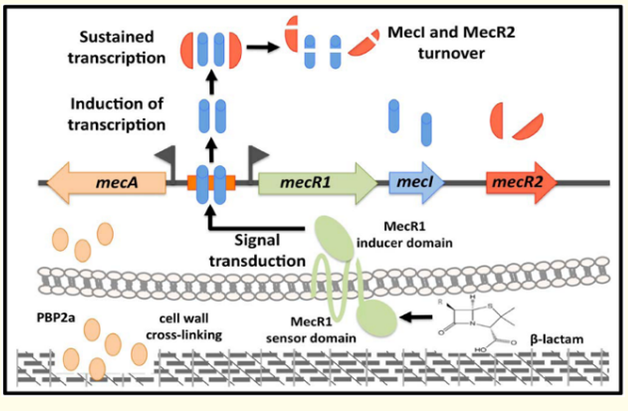


Figure 2: PBP2a protein is encoded by the MecA gene in MRSA

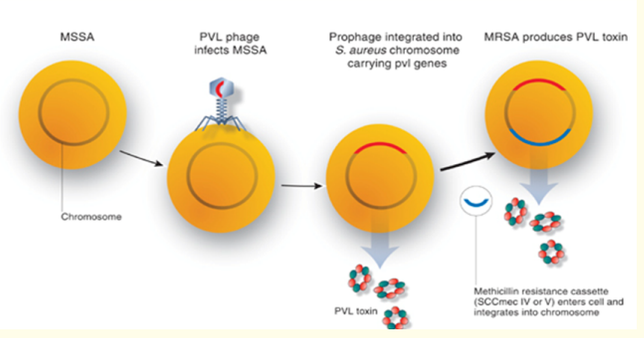


Figure 3: The high virulence of MRSA is associated with PVL toxin gene or MRSA resistance to beta lactams, *Staphylococcal* cassette chromosome genetic elements that can be transferred (SCCmec) is through.

In the control of methicillin resistant *Staphylococcus aureus* (MRSA), it is important to identify these bacteria rapidly and demonstrate their relationship with nosocomial outbreaks. Therefore, in this study, we were examined *MecA-1, MecA-2, PVL-1, PVL-2* and *PVL-3* genes in each samples were investigated. In this study we aimed to investigate epidemiology of MRSA strains by determination with molecular and functional assays of clonal relationships of these bacteria.

Material and Methods

Collection of samples

Nasal mucosa specimens were collected from 730 hospital patients in Medical Faculty Hospital. The samples were plated on 5% sheep blood agar and incubated at 37°C overnight in aerobic condition. 20 Methicillin-susceptible *S. aureus* (MSSA) from hospital patients and 710 MRSA were determined from cultured nosocomial infections. S. aureus strains were determined with the API test (Figure 5). Methicillin-resistant strains were determined by Disk Diffusion Method and then Methicillin-resistance was confirmed by the E-Test Analysis (Table 1). MRSA and MSSA strains were obtained from nosocomial samples of patients and hospital staff in Meram Medicine Faculty by using sterile swap. *S. aureus* ATCC 25923, S. aureus ATCC 29213 and MRSA or PVL-positive control strain LY19990053 were used as positive controls and taken from Microbiology Laboratory of Meram Medicine Faculty in Selcuk University, Turkiye.

Services	Number of Patients
Nephrology	56
Infectious Diseases	39
Endocrinology	26
Neurosurgery	27
Gastroenterology, Hematology	48
Reaminasyon	56
Neurology	49
Oncology	38
Orthopedics-Traumatology	46
Urology	84
Pulmonary Diseases	45
Cardiovascular Surgery	67
Cardiology	48
Neonatal	15
Children's Infectious Diseases	68
General Pediatrics	18
Total	730

Table 1. Distribution of service of samples to be subjected to work.

Microbiological analysis

Resistance to antibacterial agents as microbiological analysis, was determined by Disk Diffusion test and E-test methods. During a 2-years period, from june, 2014, to june, 2016, all clinical specimens of in-patients submitted to microbiology and molecular biology laboratories of Selçuk University of Medical Sciences in Turkey, were screened for the presence of S. aureus isolates by standard microbiology tests.

Molecular analysis

The MRSA isolates were identified by the combination of phenotypic and genotypic methods. The presence of PVL1-3 genes and SCCmec types or MecA1-2 genes was determined by PCR, respectively. The MRSA isolates, in which the presence of mecA gene had been confirmed by PCR. The primers which were used for the detection of MecA and PVL genes of *S. aureus* strain were listed in Table 2.

Genomic DNA isolation

DNA was extracted using alkaline lysis method and EZI - Nucleic Acid Analyzer Biorobot (Qiagen). The concentration of the extracted DNA was confirmed by Nano drop 1000 (NanoDrop, Wilmington, USA). Later, isolated DNA was subjected to electrophoresis

PVL-1 F	5' ACA CACTATGGCAATAGTTATTT 3'
PVL-1 R	5' AA GCAATGCAATTGATGTA 3'
PVL-2 F	5' GAGACTATTTTGTGCCAGAC 3'
PVL-2 R	5' CACCTGACAAGCCGTTA 3'
PVL-3 F	5' TCACAAAATGCCAGTGTTATC CAG 3'
PVL-3 R	5' TTTTGCAGCGTTTTGTTTTCG 3'
MecA-1 F	5' GTAGAAATGACTGAACGTCCGATAA 3'
MecA-1 R	5' CCAATTCCACATTGTTTCGGTCTAA 3'
MecA-2 F	5' GGCAATATTACCGC CCTCA 3'
MecA-2 R	5' GTCTGCCACTTTCTCCTTGT 3'

Table 2. Primers used in the study.

on 1% agarose gel. Biorobot EZ1 (Qiagen) the DNA isolation device isolation method is different from alkali lysis method, 10 ml to each tube growth observed in LB-Broth lysozyme was added. It was allowed at 37°C for 30 min incubation. After incubation biorobot EZ1 (Qiagen) was allowed isolation by DNA isolation device. LB-Broth growth was observed in each tube were centrifuged at 5000 x g for 5 minutes. The supernatant was removed and 190 µl of solution onto the pellets G2 (Qiagen, D-40724) was added. After addition of 10 ml of lysozyme was allowed at 37°C for 30 min incubation. Following this process biorobot examples EZ1 (Qiagen) was placed in a DNA extraction device. The DNA obtained was observed by carrying out a 1% agarose gel (Figure 4).

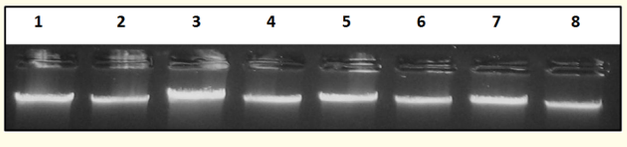


Figure 4. Gel Image of isolated *S. aureus* DNAs from Nasal Mucosa samples of Inpatients.

Amplification of MecA and PVL gene loci by polymerase chain reaction

S. aereus bacteria MecA-1 MecA-2 and PVL-1, PVL-2, the presence of PVL-3 gene was investigated by the PCR method. Nosocomial nasal mucosa samples obtained which enables identification of S. aureus strains and particularly methicillin resistance status of the identified reference genes MecA gene. Also important in the identification of this species as well as genes responsible for the production of toxins and virulence of strains PVL determining the effects were examined.

Statistical analyses

Data were analyzed by the chi-square test using the SPSS 22.0 statistical software. A statistically significant difference was considered as a P value of <0.05.

Results and Discussıon

Antibiotic resistance to be ineffective in clinical infection represents a particular antibiotic. *S. aureus* is one of the most important causes of hospital-acquired infections. *S. aureus* causes rapid infections associated with nosocomial and community winning multiple antibiotic resistance. *S. aureus* because of this feature plays an important role in terms of public health. These strains of S. aureus (MRSA) is today still cause significant difficulty in treatment and continues to be important in hospitals.

Since 1970s, methicillin-resistant *S. aureus* (MRSA) strain of "multidrug resistance" has emerged as a problem. MRSA with the increase of this problem has become a serious health problem that is causing nosocomial epidemic all over the world. MRSA infections seen in patients are exposed to long-term antibiotic treatment. MRSA infections particularly appears to be concentrated in intensive care units, nursing homes, worldwide. According to some studies were performed in Turkey, the ratio between Staphylococcus aureus MRSA strains are reported to be between 35 to 89%. To apply the appropriate treatment of patients is important to know the sensitivity to antibiotic substances. The rapid development of immunological and molecular techniques based on susceptibility testing has gained importance in recent years. Positive results to be obtained from these tests as will enable earlier initiation of antibiotic therapy appropriate to the patients; the early detection of potential outbreaks and allows the isolation of patients in suitable conditions.

Through molecular techniques, the disease resistance and they have developed defense mechanisms against the therapeutic can be resolved in a very short time. The samples used in this study were obtained from the hospitalized patients 730 pieces from the patient's nasal mucosa. Nosocomial service of distribution of nasal mucosa samples are shown in Table 1. 20 MSSA strains and 710 pieces MRSA strains (89%), were isolated from nasal cultures of hospitalized patients.

Conclusions of Microbiologic Analyses

API analysis

This test is based on bacteria isolated sample from the nose 100 (where 80 MRSA, 20 MSSA) was identified as *Staphylococcus aureus.*

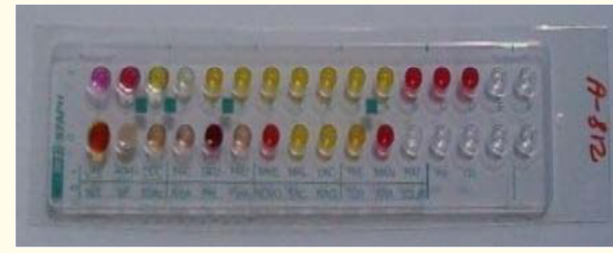


Figure 5: View in API identification test panel of *Staphylococcus aureus*

E-Test findings

Staphylococcus aureus was identified from nasal mucosa and isolated 100 bacteria samples (80 MRSA, 20 MSSA). MIC values of methicillin-susceptible strains were determined by the resistance of oxacillin and vancomycin using E-tests for CLSI measure. MRSA strains were observed as oxacillin resistant and vancomycin susceptible. The antimicrobial susceptibility rates of MRSA and MSSA strains are shown in Table 3.

Conclusions of Molecular Analyses

Identification of MecA and PVL genes by the Polymerase Chain Reaction

We were examined MecA-1, MecA-2, PVL-1, PVL-2 and PVL-3 genes in each samples were investigated.

Confirmation of all *S. aureus* isolates was carried out by PCR for the amplification of variants of MecA genes (380 bp and 280 bp), respectively. The PCR reactions were performed in a final volume of 25 µl with an automated thermal cycler (Eppendorf mastercycler gradient, Germany) using the primers for the genes (Table 2). Negative controls for each primer contained all components except the template DNA. Amplification of the variants of PVL genes (190 bp, 680 bp, and 80 bp) among the isolates of *S. aureus* was performed according to the researcher's previous study (Figure 6A-6B and 7A-B-C)

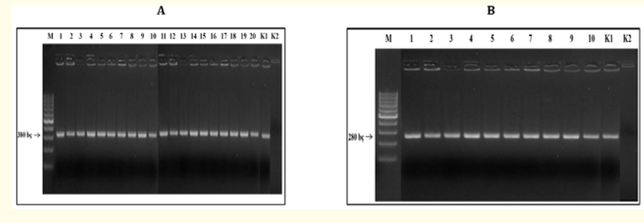
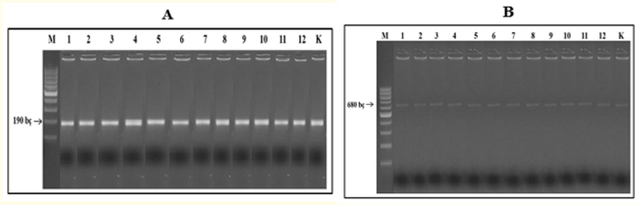


Figure 6A and 6B: Detection of MecA gene in methicillin-resistant *Staphylococcus aureus* isolates.Figure A and B, respectively, MecA-1 and MecA-2 genes PCR amplification results; M: 100 bp DNA Marker (Fermentase); K1: ATCC 29213 *S. aureus* Positive control strains, *Mec* (+) and K2: ATCC 25923 *S. aureus* negative control strains, *Mec* (-).



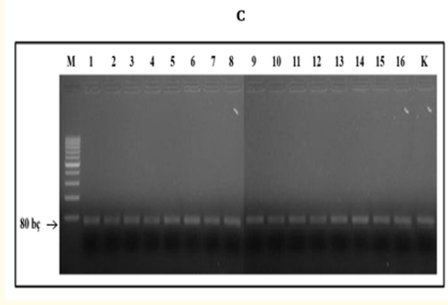


Figure 7A and 7B: Detection of PVL gene in methicillin-resistant *Staphylococcus aureus* isolates. Figure A and B, respectively,PVL-1 and PVL-2 genes PCR amplification results; M: 100 bp DNA Marker (Fermentase); K1 and K2, LY19990053 PVL-positive control strain

Figure 7C: Detection of PVL gene in methicillin-resistant *Staphylococcus aureus* isolates. PVL-3 genes PCR amplification results; M: 100 bp DNA Marker (Fermentase); K LY19990053 PVL-positive control strain.

Control strains used in this study

Quality control of discs with antibiotics was performed using *S. aureus* ATCC 25923, in accordance with the recommendations of CLSI 2014. The presence of negative control strain of MecA gene or Mec (-) and was controlled by *S. aureus* ATCC 25923 and the positive control strain of MecA gene or Mec (+) 29213 *S. aureus,* respectively. In the PCR assay for PVL gene, the native isolate of *S. aureus* with PVL gene and *S. aureus* ATCC 25923 were used as positive and negative controls, respectively. Methicillin resistance was controlled by standard strains: *S. aureus* ATCC 25923 as the negative control and *S. aureus* ATCC 29213 as the positive control. All control isolates of MRSA used as the positive control for (SCCmec) typing were provided by author or researcher. According to API test results, *Staphylococcus aureus* was identified from nasal mucosa and isolated 100 bacteria samples (80 MRSA, 20 MSSA). MIC values of methicillin-susceptible strains were determined by the resistance of oxacillin and vancomycin using E-tests for CLSI measure. MRSA strains were observed as oxacillin resistant and vancomycin susceptible. Vancomycin, caused by methicillin-resistant *Staphylococcus aureus* for the treatment of infections is one of the drugs used options.

Martinez., *et al.* collected nasal swab cultures from 294 healthy volunteers in order to investigate the colonization rates of *S. aureus* in the community in South-Eastern USA. The results showed that 21.4% (63/294) of healthy individuals were carrying Staphylococcus aureus and 0.68% (2/294) of them were carrying MRSA which has MecA gene.

However, Gorwitz., *et al.* reported that the rate of MRSA colonization was 1.5% in the healthy population. In our study, the rate of MRSA was 11.2% (80/710). In the study of Jannati., *et al.* 173 nurses were scanned for S aureus nasal colonization and 8 of them were MRSA carriers. All of the MRSA strains were MecA positive, while 6 were oxacilin resistant and 2 were oxacilin susceptile.

Afroz., *et al.* isolated 9 PVL positive MSSA strains among 33 MSSA and 26 MRSA strains by PCR. While researchers were defining S. aureus, they found that MRSA strains were PVL negative.

According to the researchers; It demonstrated that *S. aureus* are present in the gene of MRSA positive on molecular methods defining MSSA strains. In studies regarding argued that the bacteria need for microbiological identification of molecular genetic methods, as well as in absolute terms. Because resistance varies according to the source is isolated and accuracy of the non-mutated bacteria. PVL virulence factor in the efficacy of gene MSSA strains observed that more effective than MRSA. Considering the overall situation with the control strains, 80 MRSA strains, 20 MSSA strains and control strains were amplified using PCR and five different regions vary in each case. Nosocomial MRSA and MSSA strains studied the distribution of gene variants in percentage relative to the PCR results for each sample are summarized in Table 3 and 4.

Genes	MecA-1	MecA-2	PVL-1	PVL-2	PVL-3
MRSA strains	40 (%100)	40 (%100)	22 (%55)	25 (%62,5)	33 (%82,5)
MSSA strains	-	-	-	-	-

Table 3: Gene amplification rates of MRSA and MSSA strains according to the PCR results.

Control Strains	MecA-1	MecA-2	PVL-1	PVL-2	PVL-3
ATCC 29213 Methicillin-resistant strains Positive Control	+	+	-	-	-
ATCC 25923 Methicillin-sensitive strains negativecontrol	-	-	-	-	-
LY19990053 PVL-positive control strain	+	+	+	+	+

Table 4. PCR amplification results in the control strains

The modes of transmission for reducing the spread of antibiotic resistance genes and resistant strain path must be checked. In addition, techniques developed to prevent infection must be provided to reduce the need for antibiotics [1-20].

Acknowledgements

The authors declare that there is no conflict of interest. This study is supported the Scientific Research Foundation of the Necmettin Erbakan University, Konya, Turkey.

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Volume 2 Issue 9 September 2019