INVESTIGATION OF ANTIBIOTIC RESISTANCE GENES AND PANTON-VALENTINE LEUCOCIDIN IN STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM VARIOUS CLINICAL SAMPLES

ESMA KARATAŞ, FAHRIYE EKŞİ, YASEMIN ZER
Department of Medical Microbiology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

ABSTRACT

Introduction: Staphylococcus aureus is the etiological factor of one of the most widespread infections. This study aimed to investigate the relationship between antibiotic resistance and the presence of Panton-Valentine leucocidin (PVL) in S. aureus strains.

Materials and Methods: The methicillin resistance of the various strains was measured using a cefoxitin disc diffusion test. Presence of the ermA, ermC, mecA, tetK, tetM, gyrA, parC, aacA, and vanA genes and PVL (lukF/lukS) was investigated using the hybridization method following a multiplex polymerase chain reaction (PCR).

Results: In this study, 63.5% of the 96 strains were identified as methicillin-sensitive S. aureus (MSSA) and 36.5% as methicillin-resistant S. aureus (MRSA). In 15.6% of the S. aureus strains, only ermA was detected; in 10.4%, only ermC; in 2.1%, both ermA and ermC; in 36.5%, only mecA; in 11.5%, only tetK; in 18.8%, only tetM; in 1.0%, both tetK and M; in 1.0%, only the gyrA mutation; in 7.3%, only the parC mutation; in 14.6%, both gyrA and parC mutations; in 31.3%, aacA; in 5.2%, the PVL gene. The VanA gene was not detected in any strain.

Conclusion: According to our analysis, there may be S. aureus strains that carry but do not express known resistance genes, so other genes that may be responsible for resistance should be researched.

Keywords: S. aureus, MRSA, Resistance genes, MecA, PVL.

DOI: 10.19193/0393-6384_2017_2_051

Received October 30, 2016; Accepted January 02, 2017

Introduction

Staphylococcus aureus is known to be an important agent of severe infections with high morbidity and mortality. In vitro, methicillin-resistant S. aureus (MRSA) are also considered to be resistant to all other beta-lactam-group antibiotics. There are five penicillin-binding proteins (PBP) in methicillin-sensitive S. aureus (MSSA) isolates. In addition to these, MRSA isolates have a different PBP, known as PBP2a. PBP2a has lower resistance to beta-lactam-group antibiotics, and thus, it maintains peptidoglycan synthesis in the presence of these antibiotics. PBP2a is encoded by the mecA gene. A new mecA homolog (with 70% nucleotide similarity) is called mecC (mecA-LGA251) and was found in human and cow MRSA isolates in 2012.

The use of genetic tests for the detection of resistance-related mutations or antimicrobial resistance has various benefits. One is that genetic methods can be used to directly identify resistance-related mutations or antimicrobial resistance genes in clinical samples. A second benefit is that genetic methods can be used to make a decision about minimal inhibitory concentration (MIC) results when values are very close to the resistance limit in bacterial species. In addition, genetic tests are valid tests for use in the screening of epidemiological
distributions of a particular resistance gene in both hospital and societal settings, and these tests are the gold standard for evaluating the validity of new susceptibility-measuring methods.

Panton-Valentine leukocidin (PVL) is a cytotoxin produced by *S. aureus* that causes leukocyte destruction and tissue necrosis through specific lytic activity on leukocytes and human polymorphonuclear cells. It is known that the isolates containing the PVL toxin lead to severe tissue and soft-tissue infections and necrotizing pneumonia. The detection by the polymerase chain reaction of the gene region that encodes PVL is an accepted method for determining PVL presence.

This study aimed to determine the resistance to certain antibiotics of *S. aureus* strains isolated from blood, wound, and respiratory tract samples of inpatients and outpatients by phenotypical methods. The presence of resistance genes, resistance-related mutations, and PVL were investigated using molecular methods.

Materials and methods

**Samples**

This study was conducted with the approval of the Ethics Committee of the Gaziantep University Faculty of Medicine. A total of 96 *S. aureus* strains used in the study were obtained from blood, wound, and respiratory tract samples from inpatients and outpatients at the Gaziantep University Sahinbey Research and Application Hospital Microbiology Laboratory. Three different sample groups with similar sample sizes were used in our study: 37 of the samples (38.5%) were blood samples, 36 (37.5%) were wound samples, and 23 (24%) were respiratory tract samples (11 sputum, 11 tracheal aspirates, and 1 throat swab). While 26 of the strains (27.1%) were obtained from inpatients, the remaining 70 (72.9%) came from outpatients.

**Processing of samples**

Samples were cultivated in 5% sheep blood agar (Becton Dickinson, Germany) and coagulase-negative methylene Blue agar (EMB; Becton Dickinson, Germany). Blood culture flasks were monitored with a BD BACTEC 9240 (Becton Dickinson, USA) device. Strains were identified by conventional methods (1) and an automated identification system (Phoenix; Becton Dickinson, Germany).

**Antibiotic susceptibility test**

For investigation of methicillin resistance, cefoxitin discs (30µg; Bioanalyse, Turkey) were used. The antimicrobial susceptibility tests were performed using the disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) protocol. Resistance to the following antibiotics (sourced from Bioanalyse, Turkey) was tested: vancomycin (30 µg), penicillin (10 IU/ml), ampicillin (10 µg), gentamicin (10 µg), cefazolin (30 µg), amoxicillin/clavulanic acid (20/10 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), teicoplanin (30 µg), rifampicin (5 µg), linezolid (30 µg), tetracycline (30 µg), clindamycin (2 µg), erythromycin (15 µg), telithromycin (15 µg), levofloxacin (5 µg), moxifloxacin (5 µg), norfloxacin (10 µg), nitrofurantoin (300 µg), fusidic acid (10 µg), meropenem (10 µg), mupirocin (5 µg) and quinupristin-dalfopristin (15 µg). Strains that were moderately susceptible to all antibiotics were considered to be resistant. The double-disc synergy test (D-test) was used to detect the resistant phenotype to macrolide-group antibiotics. ATCC 43300 (MRSA) and ATCC 25923 (MSSA) were used as the standard strains.

**DNA isolation**

Boiling method was performed for DNA isolation from bacterial colonies that were grown as pure cultures.

**Searching for resistance genes and PVL**

To identify *S. aureus* strains genotypically and to identify resistance genes, a MRSA Combi kit (GenID, Germany) was used according to kit instructions. This single-kit assay is based on two multiplex PCRs followed by reverse hybridization using sequence-specific oligonucleotide probes (SSOP). Two different PN-Mix mixtures were prepared for use in the PCR protocol. These mixtures contained different specific biotinylated primer pairs. PN-Mix Macrolide/Quinolone/PVL (M/Q/PVL) detects the following: *Staphylococcus aureus*, *S. epidermidis*, *S. haemolyticus*, ermA and ermC genes responsible for resistance to macrolide antibiotics, the wildtype genes gyrA and parC (mutations in which are responsible for resistance to quinolone antibiotics), the virulence factor PVL with lukF/PvlK genes, and the GAP-DH gene (as an internal amplification control and a control for DNA quantity, DNA quality, or PCR inhibition). PN-Mix BetaLactam/Tetracycline/Aminoglycoside/Vancomycin (B/T/A/V) detects the following: *S.
aureus, S. epidermidis, S. haemolyticus, the mecA gene of S. aureus responsible for resistance to beta-lactam antibiotics, the tetK and tetM genes responsible for resistance to tetracycline antibiotics, the aacA gene responsible for resistance to aminoglycoside antibiotics, the vanA gene responsible for resistance to vancomycin, and the GAP-DH gene.

We used two PN-mixes for amplification reactions; each PN-mixes contained: 22.5 μl of distilled water, 5μl of PCR buffer (10X), 5μl of MgCl2 (25 mM), 4μl of 2mM PN-mix (either M/Q/P or B/T/A/V), 0.5μl (100 pmol/μl) of each primer, and 0.25 μl Taq DNA polymerase (5 U). Total DNA was 5μl, and the total mixture was 50μl for each sample. Prepared tubes were placed in a thermal cycler (ABI 2720), and the amplification protocol was programmed as follows: 1 cycle at 95°C for 5 min, 10 cycles at 95°C for 30 sec, 10 cycles at 60°C for 2 min, 22 cycles at 95°C for 10 sec, 22 cycles at 55°C for 30 sec, 22 cycles at 72°C for 30 sec, and 1 cycle at 72°C for 8 min. PCR products were stored for a short time at 4°C and then transferred to -20°C.

**Hybridization**

Following PCR, the biotinylated ampicons were characterized by a hybridization reaction to their respective sequence-specific oligonucleotide probes and controls were immobilized on nitrocellulose membranes in a distinctive line format. Each strip had a conjugate and an amplification control zone. A reverse-hybridization device (ProfiBlot 48, Tecan, Switzerland) was used in this study. The band pattern was analyzed using the evaluation sheet supplied with the device.

**Statistical analysis**

Statistical Package for the Social Sciences (SPSS) for Windows, Version 22.0 pocket program (IBM Corp., Armonk, NY), was used for statistical analysis, and results with p < 0.05 were considered to be statistically significant. Sensitivity and specificity values were calculated and compared with the gold standard (MedCalc, Version 15.11.0, 64-bit).

**Results**

Of the 96 strains in this study, 61 (63.5%) were identified as MSSA, and 35 (36.5%) were identified as MRSA. Fourteen (38.9%) of the 36 wound samples, 10 (27.0%) of the 37 blood samples, and 11 (47.8%) of the 23 respiratory tract samples were identified as MRSA. There was no statistically significant difference among sample types with regard to MRSA detection rate (p = 0.247). While 43 (70.5%) of the MSSA strains were isolated from inpatients and 18 (29.5%) from outpatients, 27 (77.1%) of MRSA strains were isolated from inpatients and 8 (22.9%) from outpatients. There was no significant difference between inpatients and outpatients with regard to MSSA and MRSA detection rate (p = 0.480).

**mecA gene detection**

MecA gene positivity was found in 35 (36.5%) of the 96 S. aureus strains. While the mecA gene was detected in 11 (18%) MSSA strains, it was detected in 24 (68.6%) MRSA strains. The sensitivity and specificity values of the cefoxitin disc diffusion method were found to be 68.5% and 81.9%, respectively.

**Antibiotic resistance status**

The antibiotic resistance status of the S. aureus strains is shown in Table 1. No MSSA or MRSA strains from inpatients or outpatients were resistant to vancomycin, teicoplanin, linezolid, mupirocin, or quinupristin-dalfopristin. One strain was moderately susceptible to clindamycin, three strains were moderately susceptible to erythromycin, one strain was moderately susceptible to chloramphenicol, and one strain was moderately susceptible to fusidic acid; all of these were categorized as resistant. Results showed that 67.7% of the S. aureus strains were susceptible to erythromycin and clindamycin (S phenotype), 17.7% were constitutively resistant to erythromycin and clindamycin (cMLSB phenotype), 9.4% were resistant to erythromycin and susceptible to clindamycin with a D-test-positive inducible phenotype (IMLSB phenotype), 4.2% were resistant to erythromycin and susceptible to D-test negative clindamycin (MS phenotype), and 1.0% were determined to be erythromycin-susceptible and clindamycin-resistant (Table 2).

**Detection of antibiotic resistance genes and comparison with antibiotic resistance status**

We determined that 15 (15.6%) of the S. aureus strains included only ermA genes, 10 (10.4%) included only ermC genes, and 2 (2.1%) included both ermA and ermC genes. As a response to the tetracycline antibiotic resistance genes, it was determined that 11 (11.5%) of the strains included...
only *tetK*, 18 (18.8%) included only *tetM*, and 1 (1.0%) included both *tetK* and *tetM* genes. *ErmA* alone was detected in 4 (23.5%) of the *S. aureus* strains, *ermC* alone was detected in 3 (17.6%) of the strains, and both *ermA* and *ermC* were found in 1 (5.3%) of the *S. aureus* strains defined phenotypically as cMLSB. In *S. aureus* strains that were defined as iMLSB, *ermA* alone was detected in 1 (1.1%) strain, *ermC* alone was found in 2 (22.2%) strains, and the combination of *ermA* and *ermC* was not detected. In four *S. aureus* strains of the MS phenotype, *ermA* was detected in 1 (25%), and *ermC* was not detected in any. However, among 65 strains of the *S phenotype*, *ermA* alone was detected in 9 (13.8%), *ermC* alone was detected in 4 (6.2%), and the combination of *ermA* and *ermC* was seen in 1 (1.5%).

The gyrA mutation responsible for quinolone resistance was found alone in 1 (1.0%) of the strains, the parC mutation was found alone in 7 (7.3%), and both the *gyrA* and *parC* mutations were found in 14 (14.6%) of strains. The *AacA* gene, responsible for aminoglycoside resistance, was found in 30 (31.3%) of the strains. The *VanA* gene was not detected in any strains.

Detection ratios of the genes (*ermA* alone, *ermC* alone, or both *ermA* and *ermC*) that are active in resistance to macrolide-group antibiotics (e.g., clindamycin, erythromycin, and telithromycin) are reported in Table 3.
The detection ratios of the genes (tetK alone, tetM alone, and both tetK and tetM) that are active in resistance to tetracycline are reported in Table 5. The aacA gene was found in 5 (27.8%) of the 18 gentamicin-resistant S. aureus strains and also in 25 (32.1%) of the 78 gentamicin-susceptible S. aureus strains. The PVL gene was detected in 5 (5.2%) of the strains. The PVL gene was also found in 4 (6.6%) of the MSSA strains and in 1 (2.9%) of the MRSA strains (Table 6).

### Discussion

The detection of the mutations (gyrA alone, parC alone, or both gyrA and parC) that are active in resistance to the quinolone-group antibiotics (e.g. levofloxacin, moxifloxacin, and norfloxacin) are reported in Table 4.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptibility status</th>
<th>Only gyrA mutation n (%)</th>
<th>Only parC mutation n (%)</th>
<th>Both gyrA and parC mutations n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin</td>
<td>Susceptible (n=82)</td>
<td>1 (1.2)</td>
<td>7 (8.5)</td>
<td>9 (11.0)</td>
</tr>
<tr>
<td></td>
<td>Resistant (n=14)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>Susceptible (n=79)</td>
<td>1 (1.3)</td>
<td>7 (8.9)</td>
<td>9 (11.4)</td>
</tr>
<tr>
<td></td>
<td>Resistant (n=17)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>5 (29.4)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>Susceptible (n=78)</td>
<td>1 (1.3)</td>
<td>7 (9.0)</td>
<td>9 (11.5)</td>
</tr>
<tr>
<td></td>
<td>Resistant (n=18)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>5 (27.8)</td>
</tr>
</tbody>
</table>

Table 4: Phenotypic and genotypic resistance comparison for quinolone group antibiotics.

ErmC was detected in one sample with an erythromycin-susceptible, clindamycin-resistant phenotype.

The detection of the mutations (gyrA alone, parC alone, or both gyrA and parC) that are active in resistance to the quinolone-group antibiotics (e.g. levofloxacin, moxifloxacin, and norfloxacin) are reported in Table 4.

Table 3: Phenotypic and genotypic resistance comparison for macrolide group antibiotics.
specification of the test were 98% and 100%, respectively. Bhutia et al.\textsuperscript{(17)} found that in MRSA detection, false negativity and positivity of the cefoxitin disc-diffusion test were 13.7% and 16.7%, respectively, and sensitivity and specificity were 86.27% and 83.33%, respectively.

However, many studies have reported 100% sensitivity and specificity of the cefoxitin disc-diffusion test\textsuperscript{(14, 15)}, in comparison to oxacillin disc-diffusion tests with 100% sensitivity and 56% specificity\textsuperscript{(14)} or 87.5% sensitivity and 100% specificity\textsuperscript{(15)} and oxacillin agar dilution tests with 100% sensitivity and 90% specificity\textsuperscript{(14)}. Olowe et al.\textsuperscript{(18)} reported that when compared to the mecA gene as the gold standard for MRSA detection, methicillin, oxacillin, and cefoxitin tests had sensitivity rates of 70%, 80%, and 100%, respectively, and specificity rates of 76.2%, 69.1%, and 78.5%, respectively. The same researchers determined that the mecA gene prevalence in their MRSA strains was 19.2%\textsuperscript{(18)}.

In our study, the sensitivity and specificity of the cefoxitin disc-diffusion test were found to be 68.57% and 81.97%, respectively. We also saw slightly lower sensitivity and specificity values of the cefoxitin disc-diffusion test than other related studies. We think that this result may be due to in vitro conditions such as incubation temperature, NaCl concentration, medium, bacteria storage conditions, and inoculum density, which can affect resistance expression that may affect the phenotypic tests used in MRSA detection. In addition, it is known that there are susceptible or lower-resistance heteroresistant staphylococcus strains against oxacillin and cefoxitin that include the mecA gene\textsuperscript{(19, 20)}.

Apart from these, the mecC gene\textsuperscript{(5)}, which is also considered to be responsible for MRSA resistance, has not been investigated in our study, representing a serious limitation. It is known that S. aureus infections are serious, and delayed treatment increases the mortality rate\textsuperscript{(21)}. Several DNA-based hybridization assays have been developed for the rapid detection of MRSA\textsuperscript{(22)}. Most of these tests are based on simultaneous detection of an S. aureus-specific target and the mecA gene, and some of these have been used as rapid detection methods in routine laboratory tests\textsuperscript{(23)}. Since the test used in this study (MRSA Combi) is a multiplex PCR test that has the ability to investigate mecA and 9 different additional genes at the same time, it is preferred over other tests. For this reason, we think that the use of this test may be beneficial and that the sensitivity and specificity of the test should be investigated.

Whereas macrolide-, lincosamide-, and streptogramin B (MLSB)-group antibiotics are chemically different from one another, they have similar inhibitory effects on bacterial protein synthesis. Resistance against these antibiotics mostly comes about through modification of the target site, inactivation of the drug, or efflux mechanisms\textsuperscript{(24)}. The most important mechanism causing resistance against clindamycin in staphylococcus occurs by the addition of a methyl group to a ribosomal target region of the methylase enzyme encoded by different \textit{erm} genes\textsuperscript{(25)}. The staphylococcus methylase enzyme is frequently encoded by \textit{erm}A and \textit{erm}C genes, and these genes may be inducible or constitutive\textsuperscript{(26)}. Juyal et al.\textsuperscript{(27)} have found cMLSB, iMLSB, MS, and S phenotype prevalence to be 29.0%, 19.4%, 38.7%, and 12.9%, respectively, in MRSA isolates and 12.5%, 6.3%, 24.5%, and 56.7%, respectively, in MSSA isolates.

In our study, it was found that while 23.5% of the \textit{S. aureus} strains determined to be cMLSB included only \textit{erm}A and 17.6% included only \textit{erm}C, 5.3% included both \textit{erm}A and \textit{erm}C. Of iMLSB strains, 11.1% had only \textit{erm}A, 22.2% had only \textit{erm}C, no strains included \textit{erm}A and \textit{erm}C together. Aydeniz Ozansoy et al.\textsuperscript{(28)} found that 81.9% of the 111 strains with iMLSB phenotype included \textit{erm}A and 10.8% \textit{erm}C, and 10.8% of the strains included \textit{msr}A, 1.8% included \textit{msr}B, and 0.9% of the strains included \textit{erm}B; 57.9% of the 19 strains with the cMLSB phenotype included \textit{erm}A, whereas 75% of the strains included \textit{msr}A, 50% contained \textit{msr}B, and 25% included \textit{erm}C. In a study conducted by Vandendriessche et al.\textsuperscript{(29)} to investigate \textit{erm}A, \textit{erm}C and \textit{msr}A genes in 212 MSSA and 314 MRSA strains, \textit{erm}A genes were determined to be the most common. Since \textit{msr}A and \textit{msr}B genes are not found in the kit used in our study, they were not evaluated. This is another limitation of our study.

In our study, the \textit{aacA} gene was detected in 5 (27.8%) of 18 gentamicin-resistant \textit{S. aureus} strains. Hauschild et al.\textsuperscript{(30)} reported that all of aminoglycoside-resistant \textit{S. aureus} were screened for the presence of three genes encoding the most clinically relevant aminoglycoside-modifying enzymes. The most prevalent resistance gene was \textit{aac}(6')-Ie+aph(2'), found in 8.9% of strains. Twelve (26.7%) isolates carried the \textit{ant}(4')-Ia gene, while the \textit{aph}(3')-IIIa gene was detected in only
15.6% of isolates. Additionally, the ant(6)-Ia and str genes were detected in 31.1% and 4.4% of strains, respectively.

Emaneini et al. stated in their study that among 151 S. aureus isolates recovered from burn patients, the mecA gene was detected in 63.6%. The rate of tetracycline resistance genes associated with mecA was 61%. Forty-nine isolates (32.4%) contained tetM, 26 (17.2%) possessed only tetK, and 21 (13.9%) contained both tetM and tetK. The presence of the aac(6’)-Ie-aph(2’’)-I gene was detected in 18 isolates, the aph(3’)-IIIa in 8 isolates, the aac(6’)-Ie-aph(2’’)-I, aph(3’)-IIIa, and ant(4’)-Ia genes in 69 isolates, both aac(6’)-Ie-aph(2’’)-I and ant(4’)-Ia in 6 isolates, and both the aph(3’)-IIIa and the ant(4’)-Ia genes in 8 isolates. Most of the strains that harbored the mecA gene also contained the tet and AME genes. In our study, it was determined that 14.8% of the tetracycline-resistant strains included only tetK, 29.6% only tetM, and 3.7% had both tetK and tetM genes. Similar to the results obtained from a study conducted by Emaneini et al., in our study, one of the tet genes was determined together in 57.1% of the S. aureus strains containing mecA gene.

Fluoroquinolone-resistance in S. aureus has mainly been attributed to mutations occurring in the quinolone-resistance-determining region of parC, which encodes topoisomerase IV, and gyrA, which encodes DNA gyrase A. Both gyrA and parC mutations were detected together in 35.7% of the levofloxacin-resistant strains, in 29.4% of the moxifloxacin-resistant strains, and in 27.8% of norfloxacin-resistant strains in our study. Kwak et al. stated in their study that “all 62 ciprofloxacin-resistant S. aureus had either gyrA or parC mutations”. The S84L mutation of gyrA (59/62, 95.2%) and the S80F mutation of parC (61/62, 98.4%) were the most common. They also found that 58 (93.6%) of the strains had both the S84L mutation of gyrA and the S80F mutation of parC. Quinolone resistance is generally found with point mutations in gyr and par loci. As in the literature, gyrA and parC mutations were detected together in the same strain in our study.

One of the important virulence factors in S. aureus pathogenesis is Panton-Valentin leucocidin, which is a cytotoxin that increases tissue destruction. This important virulence factor is mostly found in community-based MRSA strains. However, studies show that PVL is also found in hospital-based S. aureus strains.

Chini et al. conducted research on 1058 S. aureus strains in Greece from 2001-2003. In the study, PVL positivity was found to be 27% (n = 287). In addition, they determined that 157 of the strains were community-based MRSA, 65 were hospital-based MRSA, 51 were community-based MSSA, and 14 were hospital-based MSSA. Duman et al. reported PVL positivity in MRSA and MSSA strains as 3% and 9%, respectively. In addition, they determined that 15% of PVL positive strains were community-based and 3% were hospital-based.

In our study, PVL positivity was 5.2%. While 4.3% of our isolates came from inpatients, 7.7% came from outpatients, 5.6% came from wound samples, 5.4% came from blood samples, and 4.3% came from respiratory tract samples. PVL positivity was found in MSSA and MRSA strains at 6.6% and 2.9%, respectively. No statistically significant difference was found in terms of origin, sample type, or strain (MRSA or MSSA) for PVL positivity (p > 0.05). Kilic et al. investigated PVL positivity in 385 MRSA strains, and they found PVL positivity to be 1.3% (n = 5).

In a study conducted by Karahan et al. on 261 MRSA strains (230 hospital-based and 74 community-based) and 43 MSSA strains, PVL positivity was found in 12 S. aureus isolates (1 hospital-based, 11 community-based); 8 PVL-positive isolates were MRSA, and 4 were MSSA. Seven of the PVL isolates came from wound samples, 4 from urine samples, and 1 from synovial fluid. In a study conducted by Ozkul et al., PVL positivity was found in 6 of 79 MSSA isolates. In addition, they did not detect PVL positivity in any of 55 MRSA isolates. Bhutia et al. found PVL gene prevalence to be 46.15% (54/117), 94.44% (51/54), and 5.55% (3/54) among S. aureus, MRSA, and MSSA isolates, respectively.

Further studies conducted with the aim of the detection of resistance gene reservoirs in hospitals and communities are required. These kinds of studies will also help to determine the frequency of the organisms that carry the resistance genes but do not express it.
References


26) Zelazny AM, Ferraro MJ, Glenmen A, Hindler JF, Mann LM et al. Selection of strains quality assessment of the disk induction method for detection of inducible clin-


Acknowledgements
This study, Project No. TF.13.13, was supported by the Gaziantep University Scientific Research Projects Administration Department Commission Directorate.

Corresponding author
Assoc. Prof. Dr. FAHRIYE EKSI
Gaziantep University, Faculty of Medicine, Medical Microbiology Department, Universite Bulvari Gaziantep, 27310 (Turkey)