

## Antibiotic resistance genes & susceptibility patterns in staphylococci

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**Background & objectives:** This study was carried out to evaluate the association between the antibiotic susceptibility patterns and the antibiotic resistance genes in staphylococcal isolates obtained from various clinical samples of patients attending a teaching hospital in Hatay, Turkey.

**Methods:** A total of 298 staphylococci clinical isolates were subjected to antimicrobial susceptibility testing. The genes implicated in resistance to oxacillin (*mecA*), gentamicin (*aac(6')/aph(2'')*), *aph(3')-IIIa*, *ant(4')-Ia*), erythromycin (*ermA*, *ermB*, *ermC*, and *msrA*), tetracyclin (*tetK*, *tetM*), and penicillin (*blaZ*) were amplified using multiplex PCR method.

**Results:** Methicillin resistance rate among 139 *Staphylococcus aureus* isolates was 16.5 and 25.9 per cent of *S. aureus* carried *mecA* gene. Of the 159 CoNS isolates, methicillin resistance rate was 18.9 and 29.6 per cent carried *mecA* gene. Ninety four isolates identified as gentamicin resistant phenotypically, contained at least one of the gentamicin resistance genes [*aac(6')/aph(2'')*, *aph(3')-IIIa*, *ant(4')-Ia*], 17 gentamicin-susceptible isolates were found as positive in terms of one or more resistance genes [*aac(6')/aph(2'')*, *aph(3')-IIIa*, *ant(4')-Ia*] by multiplex PCR. A total of 165 isolates were resistant to erythromycin, and contained at least one of the erythromycin resistance genes (*ermA*, *ermB*, *ermC* and *msrA*). Phenotypically, 106 staphylococcal isolates were resistant to tetracycline, 121 isolates carried either *tetK* or *tetM* or both resistance genes. The majority of staphylococci tested possessed the *blaZ* gene (89.9%).

**Interpretation & conclusions:** The present results showed that the phenotypic antibiotic susceptibility patterns were not similar to those obtained by genotyping done by multiplex PCR. Rapid and reliable methods for antibiotic susceptibility are important to determine the appropriate therapy decisions. Multiplex PCR can be used for confirmation of the results obtained by conventional phenotypic methods, when needed.

**Key words** Antibiotic resistance genes - antibiotic susceptibility - *mecA* gene - staphylococci

The resistance to antimicrobial agents is an increasingly global problem worldwide, especially among nosocomial pathogens. Staphylococci have become one of the most common causes of nosocomial

infections. Multidrug-resistant staphylococci pose a growing problem for human health. The rise of drug-resistant virulent strains of *Staphylococcus aureus*, particularly methicillin-resistant *S. aureus* (MRSA)

is a serious problem in the treatment and control of staphylococcal infections<sup>1,2</sup>.

Methicillin-resistant staphylococci (MRS) cause hard-to-treat infections because these are resistant to most of the antibiotics such as beta-lactams, aminoglycosides, and macrolides. The most important mechanism of resistance to penicillin is production of beta-lactamase which inactivates penicillin by hydrolysis of its beta-lactam ring. Another mechanism is associated with penicillin-binding protein 2a (PBP2a), encoded by *mecA*<sup>2</sup>. Another gene involved in penicillin resistance in staphylococci is *blaZ* which encodes  $\beta$ -lactamase<sup>2</sup>.

Aminoglycoside modifying enzymes (AMEs) are major factors responsible for resistance to aminoglycoside in staphylococci. Until now, three classes of AMEs have been identified: acetyltransferase (AAC), aminoglycoside phosphotransferase (APH), and aminoglycoside nucleotidyltransferase (ANT)<sup>3,4</sup>. The most important mechanism of aminoglycoside resistance in staphylococci is drug inactivation by AMEs like aminoglycoside nucleotidyltransferases (APHs). AMEs can be plasmid or chromosome encoded. In staphylococcal strains, the most commonly found AME is *aac(6')/aph(2'')*. The bifunctional enzyme *aac(6')/aph(2'')* is encoded by the *aac(6')/aph(2'')* gene. In addition, APH(3')-III is encoded by *aph(3')-IIIa* gene and the ANT(4')-I by *ant(4')-Ia* gene, are also found in staphylococcal isolates<sup>5-8</sup>.

The accurate and rapid diagnosis of antibiotic resistance genes in the treatment of staphylococcal infections is extremely important in preventing the spread of infections. PCR-based molecular methods are often preferred for determination of antibiotic resistance genes<sup>9</sup>. The present study was aimed to investigate the relation between the antibiotic susceptibility patterns and the antibiotic resistance genes (*mecA*, *aac(6')/aph(2'')*, *aph(3')-IIIa*, *ant(4')-Ia*, *erm(A)*, *erm(B)*, *erm(C)*, *tet(K)*, *tet(M)*, *msrA*, *blaZ*) by multiplex PCR assay in staphylococci isolates obtained from clinical specimens of patients at a teaching hospital in the South of Turkey.

### Material & Methods

The study was carried out in Mustafa Kemal University, Faculty of Medicine, Department of Medical Microbiology (Hatay, Turkey) between July 2007 and August 2009. A total of 298 clinical isolates of staphylococci were isolated from blood (29, 9.7%), wounds (145, 48.7%), urine (42, 14.1%), pus

(59, 19.8%), and other sources samples (23, 7.7%). Of the 298 staphylococcal isolates, 159 (53.4%) were coagulase-negative staphylococci (CONS) and 139 (46.6%) isolates were identified as the *S. aureus*.

The samples were placed into modified amines charcoal transport medium (Difco Laboratories, USA), and were sent immediately to the microbiology laboratory. The samples were inoculated onto 5 per cent sheep blood agar plates (Difco Laboratories, USA), and incubated at 37°C for 48 h. The identification of staphylococci was on the basis of colony morphology, Gram staining, biochemical tests such as catalase and coagulase tests<sup>10</sup>. Isolates were stored at -70°C in Mueller-Hinton Broth (Merck, Germany) supplemented with 40 per cent glycerol (v/v).

### Susceptibility testing

Disc diffusion - Antimicrobial susceptibilities of the isolates were tested by the agar disk diffusion method on Mueller-Hinton agar (Tiantan Biotechnology, PR China) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines<sup>11</sup>. Antibiotic discs (Becton Dickinson, USA) were placed on Mueller-Hinton agar plates, incubated at 37°C for 24 h, and the diameter of each zone was measured in millimeters. The following antibiotic discs were used: penicillin (10 U), erythromycin (15  $\mu$ g), oxacillin (1  $\mu$ g), tetracycline (30  $\mu$ g), gentamicin (10  $\mu$ g), amoxicillin-clavulanic acid (30  $\mu$ g), clindamycin (2  $\mu$ g), vancomycin (30  $\mu$ g), trimethoprim-sulphamethoxazole (25  $\mu$ g), and ciprofloxacin (5  $\mu$ g).

Oxacillin disc diffusion test - Oxacillin disc susceptibility testing was performed on all isolates of *S. aureus* according to CLSI recommendations using a 1  $\mu$ g oxacillin disc. Oxacillin disc (Becton Dickinson Microbiology Systems, USA) were placed on Mueller-Hinton agar plates without NaCl supplementation. Subsequently, the plates were incubated 24 h at 37°C. The zone size was interpreted according to the CLSI criteria<sup>11</sup>. *S. aureus* ATCC 29213 and *S. aureus* ATCC (43300) were chosen as the negative and positive control strains, respectively.

*Determination of minimum inhibitory concentrations (MIC)*: MIC values of antibiotics were determined by the broth microdilution test. All isolates were subcultured on blood agar and incubated for 24 h at 37 °C. Then, two-fold serial dilutions of each antibiotic were made in Mueller-Hinton broth to achieve a concentration range from 0 to 256  $\mu$ g/ml. After incubation at 37° for 24 h, the MIC was defined as the lowest concentration

of antibiotics that produced no growth. The broth microdilution tests were performed according to the CLSI guidelines<sup>11</sup>.

**DNA isolation:** For nucleic acid isolation from staphylococcal isolates, the frozen samples were thawed rapidly, and were cultivated in brain-heart infusion broth (Merck, Germany) at 37°C with shaking overnight. Total DNA was isolated from 5 ml of a broth culture grown overnight<sup>12</sup>. After incubation, bacterial cells were harvested by centrifugation at 3000 × *g* for 10 min, the cell pellet was re-suspended in phosphate-buffered saline with 100 µg of lysostaphin (Sigma, USA) per ml, and incubated at 37°C for 30 min. The phenol/chloroform extraction method was used for nucleic acid extraction and DNA was precipitated in 1 ml 70 per cent ethanol. The DNA precipitate was dissolved in 50 µl of TE buffer [10 mM Tris chloride-1 mM EDTA (pH 8.0)], and stored at -20°C until processing.

The oligonucleotide primers<sup>3</sup> for the *mecA*, *femA*, 16S rDNA, *aac(6')/aph(2'')*, *aph(3')-IIIa* and *ant(4')-Ia* genes were selected and the primers for *erm(A)*, *erm(B)*, *erm(C)*, *tet(K)*, *tet(M)*, *clf A*, *msrA* and *blaZ*, genes were selected based on earlier studies<sup>13,14</sup> (Table I).

**PCR method for *aac(6')/aph(2'')*, *aph(3')-IIIa* and *ant(4')-Ia*:** The PCR amplification was performed in a 25 µl reaction mixture [2.5 ml of 10x reaction buffer without MgCl<sub>2</sub> (Promega Corp., USA); 200 µM of each deoxynucleoside triphosphate (AB Gene, UK), 2 mM MgCl<sub>2</sub>; 2.5 pmol of each primer (*aac(6')/aph(2'')*, *aph(3')-IIIa* and *ant(4')-Ia*] and approximately 10 ng of template DNA, and brought up to a 25 µl final volume with distilled water. In order to reduce the formation of nonspecific extension products, a “hot-start” protocol was used<sup>12</sup>. PCR reactions were hot started for 5 min at 95°C and placed on ice, and 1 U of Taq polymerase (Fermentas, USA) was added. Reaction mixtures were subjected to 30 PCR cycles (95°C for 2 min, 1 min at 54°C and 1 min at 72 °C). A final elongation step at 72°C for 7 min was applied in a thermal cycler (Bioder/Thermal Blocks xp Cycler, Tokyo, Japan).

**PCR method for 16S rDNA, *mecA* and *femA*:** Multiplex PCR conditions for 16S rDNA, *mecA* and *femA* included the same constituents as the procedure of aminoglycoside resistance genes except for the MgCl<sub>2</sub> concentration (3.0 mM) and the primers, which were used at 20 pmol of primers for 16S rDNA, *mecA* and *femA*.

**Table I.** The primer sequences and predicted sizes used in the multiplex PCRs

Gene	Oligonucleotide sequence (5'-3')	Size of amplified product (bp)
<i>mecA</i> <sup>3</sup>	5'-CCTAGTAAAGCTCCGGAA-3' 5'-CTAGTCCATTCGGTCCA-3'	314
16S rDNA <sup>3</sup>	5'-CAG CTC GTG TCG TGA GAT GT-3' 5'-AAT CAT TTG TCC CAC CTT CG-3'	420
<i>femA</i> <sup>3</sup>	5' AAAAAAGCACATAACAAGCG 3' 5' GATAAAGAAGAAAACCGCAG 3'	132
<i>aac(6')/aph(2'')</i> <sup>3</sup>	5'-GAAGTACGCAGAAGAGA-3' 5'-ACATGGCAAGCTCTAGGA-3'	491
<i>aph(3')-IIIa</i> <sup>3</sup>	5'-AAATACCGCTGCGTA-3' 5'-CATACTCTCCGAGCAA-3'	242
<i>ant(4')-Ia</i> <sup>3</sup>	5'-AATCGGTAGAAGCCAA-3' 5'-GCACCTGCCATTGCTA-3'	135
<i>erm(A)</i> <sup>13</sup>	5'-AAG CGG TAA ACC CCT ATG A-3' 5'-TTC GCA AAT CCC TTC TCA AC-3'	190
<i>erm(B)</i> <sup>13</sup>	5'-CTATCTGATTGTTGAAGAAGGATT-3' 5'-GTTTACTCTGGTTTAGGATGAAA-3'	142
<i>erm(C)</i> <sup>13</sup>	5'-AAT CGT CAA TTC CTG CAT GT-3' 5'- TAA TCG TGG AAT ACG GGT TTG-3'	299
<i>tet(K)</i> <sup>13</sup>	5'-GTA GCG ACA ATA GGT AAT AGT-3' 5'-GTA GTG ACA ATA AAC CTC CTA-3'	360
<i>tet(M)</i> <sup>13</sup>	5'-AGT GGA GCG ATT ACA GAA-3' 5'-CAT ATG TCC TGG CGT GTC TA-3'	158
<i>msrA</i> <sup>14</sup>	5'-TCCAATCATTGCACAAAATC-3' 5'-AATTCCCTCTATTTGGTGGT-3'	163
<i>blaZ</i> <sup>14</sup>	5'-ACTTCAACACCTGCTGCTTTC-3' 5'-TGACCACTTTTATCAGCAACC-3'	173

Superscript numerals denote referene numbers

DNA amplification was carried out in a thermal cycler and reactions were hot started for 5 min at 94°C and placed on ice, and 1 U of Taq polymerase (Fermentas, USA) was added. Reaction mixtures were subjected to 35 PCR cycles (94°C for 2 min, 2 min at 55°C, and 1 min at 72°C). A final elongation step at 72°C for 7 min was also applied.

**PCR method for *erm(A)*, *erm(B)*, *erm(C)*, *tet(K)*, *tet(M)*, *msrA*, *blaZ*:** The PCR amplification was carried out in a total volume of 25 µl. PCR amplification was achieved as follows: 5 µl of genomic DNA (approximately 50 ng) sample was added to 20 µl of PCR mixture (20 mmol/l Tris-HCl, pH 8.4; 50 mmol/l KCl, 10 mmol/l MgCl<sub>2</sub>, and 200 µmol/l each of deoxynucleoside triphosphates (dNTPs), 0.6 µmol/l each primers and 1 U Taq DNA polymerase. The amplification process was started with an initial denaturation step (95 °C, 3 min). Each cycle consisted of three steps (denaturation, annealing, and extension). Each PCR reaction consisted of 30 cycles of amplification (denaturation at 95°C for

**Table II.** The MIC values and the resistance patterns of staphylococci by the disc diffusion method

Antibiotics	MIC range ( $\mu\text{g/ml}$ )	<i>S. aureus</i> (n=139)	CoNS (n=159)	Total (n=298)
		Number (%)	Number (%)	Number (%)
Methicillin	0.125-64	23 (16.5)	30 (18.9)	53 (17.8)
Gentamicin	0.5-16	53 (38.1)	41 (25.8)	94 (31.5)
Tetracycline	0.5-32	57 (41.0)	49 (30.8)	106 (35.6)
Erythromycin	0.5-4	84 (60.4)	61 (38.4)	145 (48.7)
Penicillin	0.125-0.5	129 (92.8)	133 (86.6)	262 (87.9)
Amoxicillin-clavulanic acid	4-16	32 (23.0)	54 (34.0)	86 (28.9)
Clindamycin	0.5-4	53 (38.1)	87 (54.7)	140 (47.0)
Trimethoprim-sulphamethoxazole	1-4	31 (22.3)	65 (40.9)	96 (32.2)
Ciprofloxacin	1-4	57 (41.0)	99 (62.3)	156 (52.3)
Vancomycin	0.25-1	0 (0)	0 (0)	0 (0)

30 sec, annealing at 54 °C for 30 sec, and DNA chain extension at 72°C for 30 sec). A final extension cycle was performed at 72°C for 4 min. The resistance genes were studied in five different groups: group 1 (*mecA* and *femA*); group 2 [(*aac(6')*/*aph(2'')*), *aph(3')-IIIa*, *ant(4')-Ia*]; group 3 (*16S rDNA*, *ermA*, *ermC*); group 4 (*msrA*, *16S rDNA*, *ermC*); and group 5 (*erm B*).

After amplification of the resistance genes, 10  $\mu\text{l}$  of the PCR products were mixed with 3  $\mu\text{l}$  of loading buffer (10 %, w/v, Ficoll 400; 10 mmol/l Tris-HCl, pH 7.5; 50 mmol/l EDTA; 0.25% bromophenol blue) and then loaded onto a 2 per cent agarose gel and electrophoresis was performed in Tris-borate-EDTA buffer containing 0.5 g of ethidium bromide per ml. The PCR products were analyzed in a 2 per cent (w/v) agarose gel in 1 x TAE buffer (40 mmol/l Tris-acetate, 1 mmol/l EDTA). Ethidium bromide (0.5  $\mu\text{g/ml}$  TAE)-stained DNA amplicons were visualized using a gel imaging system (Wealtec, Dolphin-View, USA).

**Statistical analysis:** All data were evaluated by  $\chi^2$  test using Statistical Package for Social Sciences (SPSS1 for Windows V. 11.5, Chicago, USA) software.

### Results

A total of 16.5 per cent (23/139) of the coagulase positive staphylococcal isolates showed resistance to methicillin and 18.9 per cent (30/159) coagulase negative isolates showed methicillin resistance, phenotypically. The highest drug resistance was obtained against penicillin in both *S. aureus* 92.8 per cent and the CoNS 86.6 per cent. Overall, antibiotics resistance rates for gentamicin, tetracycline and erythromycin were 31.5, 35.6, 48.7 and 87.9 per cent, respectively (Table II). The staphylococcal isolates showed moderate resistance to

amoxicillin-clavulanic acid (28.9%), and trimethoprim-sulphamethoxazole (32.2%). Both CoNS and *S. aureus* strains were vancomycin susceptible (Table II).

The susceptibility results determined by conventional methods were compared with the results of the multiplex PCR assay (Table III). All *S. aureus* isolates (positive for coagulase test) carried the *femA* gene. Methicillin resistance rate among 139 *S. aureus* isolates was 16.5 and 25.9 per cent (36/139) of *S. aureus* isolates carried *mecA* gene. Methicillin resistance rate in CoNS was 18.9 and 29.6 per cent of CoNS carried *mecA* gene. Thirty isolates which were phenotypically methicillin-susceptible, were found to carry the *mecA* gene. While a total of 139 isolates were identified as *S. aureus* by phenotypic methods,

**Table III.** The rates of antibiotic resistance genes in staphylococci by multiplex PCR method

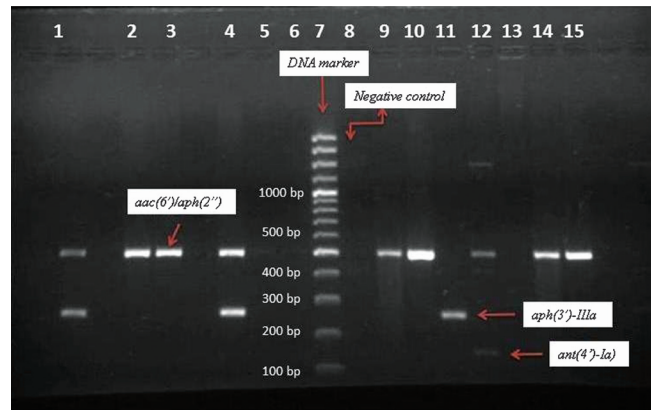
Genes	<i>S. aureus</i> (n=139)	CoNS (n=159)	Total (n=298)
	Number (%)	Number (%)	Number (%)
<i>mecA</i>	36 (25.9)	47 (29.6)	83 (27.9)
16S rDNA	139 (100)	159 (100)	298 (100)
<i>femA</i>	139 (100)	12 (7.5)	151 (50.7)
<i>aac(6')</i> / <i>aph(2'')</i>	29 (20.9)	26 (16.4)	55 (18.5)
<i>aph(3')-IIIa</i>	18 (12.9)	16 (10.1)	34 (11.4)
<i>ant(4')-Ia</i>	13 (9.4)	9 (5.7)	22 (7.4)
<i>erm(A)</i>	46 (33.1)	18 (11.3)	64 (21.5)
<i>erm(B)</i>	8 (5.8)	5 (3.1)	13 (4.4)
<i>erm(C)</i>	30 (21.6)	37 (23.3)	67 (22.5)
<i>tet(K)</i>	19 (13.7)	24 (15.1)	43 (14.4)
<i>tet(M)</i>	40 (28.8)	38 (23.9)	78 (26.2)
<i>msrA</i>	13 (9.4)	8 (5.0)	21 (7.0)
<i>blaZ</i>	130 (93.5)	138 (86.8)	268 (89.9)

the presence of the *femA* gene was determined in 151 (Table III).

Among AME genes, *aac(6')/aph(2'')* was detected in 18.5 per cent, *aph(3')-IIIa* and *ant(4')-Ia* were found in 11.4 and 7.4 per cent of the isolates, respectively. All 94 isolates identified as gentamicin resistant phenotypically contained at least one of the gentamicin resistance genes [*aac(6')/aph(2'')*, *aph(3')-IIIa*, *ant(4')-Ia*] (Fig. 1). A total of 17 gentamicin-susceptible isolates were found positive in terms of one or more resistance genes [*aac(6')/aph(2'')*, *aph(3')-IIIa*, *ant(4')-Ia*] by multiplex PCR (Table IV).

A total of 145 isolates were resistant to erythromycin, and contained at least one of the erythromycin resistance genes (*ermA*, *ermB*, *ermC* and *msrA*). The *ermA* and *ermC* genes were presenting 77; *ermB* could be detected in 13 isolates (Fig. 2). Eleven isolates carried both *ermA* and *ermB*. A total of 21 isolates had *msrA* gene (Table V).

A total of 106 staphylococcal isolates were found resistant to tetracycline, phenotypically, 121 isolates were resistant to tetracycline and carried either *tetK* or *tetM* or both resistance genes (Table VI). With regards to *blaZ* gene, the majority of tested staphylococci possessed the *blaZ* gene. The 130 (93.5%) *S. aureus* isolates carrying the *blaZ* gene expressed the phenotype, and the presence of the *blaZ* gene was found as 86.8 per cent (138/159) among the coagulase negative staphylococci (Table VII).



**Fig. 1.** Ethidium bromide-stained multiplex PCR products after gel electrophoresis for the *aac(6')/aph(2'')* (491 bp), *aph(3')-IIIa* (242 bp) and *ant(4')-Ia* (135 bp) genes. DNA molecular size marker (100 bp). Lanes: 1-4, 9, 10, 12, 14 and 15: *aac(6')/aph(2'')*; Lane 7: DNA marker (molecular size standard); Lane 8: Negative control; Lane 1 and 4: *aac(6')/aph(2'')* and *aph(3')-IIIa*; Lane 11: *aph(3')-IIIa*; Lane 12: *aac(6')/aph(2'')* and *ant(4')-Ia*.

### Discussion

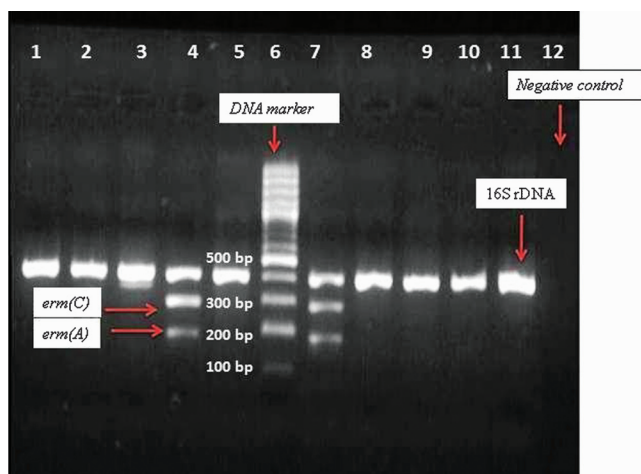
In the present study, the results of antibiotic susceptibility by disk diffusion method were compared with gene analysis results in staphylococcal isolates. The phenotypic expression of antimicrobial resistance has been reported to be influenced by various factors<sup>15</sup>. The antimicrobial susceptibility test was performed for all isolates of *S. aureus* and CoNS by disc diffusion and broth microdilution test. Antibiotic susceptibility test results showed a high level of resistance among the staphylococcal isolates to most of the commonly used antimicrobials. In our study, all staphylococcal isolates

**Table IV.** Relationship between gentamicin resistance and the presence of three resistance genes (*aac(6')/aph(2'')*, *aph(3')-IIIa*, *ant(4')-Ia*)

Staphylococcal isolates	Gentamicin resistance by phenotypic method	The distribution of gentamicin resistance genes			Number of PCR negative isolates
		<i>aac(6')/aph(2'')</i>	<i>aph(3')-IIIa</i>	<i>ant(4')-Ia</i>	
<i>S. aureus</i>	53 (resistant)	25	17	11	0
<i>S. aureus</i>	86 (susceptible)	4	1	2	79
CoNS	41 (resistant)	22	13	6	0
CoNS	118 (susceptible)	4	3	3	108
	Total	55	34	22	187

**Table V.** Relationship between erythromycin resistance and the presence of four resistance genes (*ermA*, *ermB*, *ermC*, or *msrA*)

Staphylococcal isolates	Erythromycin resistance by phenotypic method	The distribution of erythromycin resistance genes				Number of PCR negative isolates
		<i>ermA</i>	<i>ermB</i>	<i>ermC</i>	<i>msrA</i>	
<i>S. aureus</i>	84 (Resistant)	44	8	24	8	0
<i>S. aureus</i>	55 (susceptible)	2	0	6	5	42
CoNS	61 (Resistant)	18	5	30	8	0
CoNS	98 (susceptible)	0	0	7	0	91
	Total	64	13	67	21	133



**Fig. 2.** Ethidium bromide-stained multiplex PCR products after gel electrophoresis for the *16S rDNA* (420 bp), *erm(A)* (190 bp) and *erm(C)* (299 bp) genes. DNA molecular size marker (100 bp). Lanes: 1-5, and 7-11; *16S rDNA*; Lane 4 and 7: *16S rDNA*, *erm(A)* and *erm(C)*; Lane 6: DNA marker (molecular size standard); Lane 12: Negative control.

**Table VI.** Relationship between tetracycline resistance and the presence of the *tetK* and *tetM* genes

Staphylococcal isolates	Tetracycline resistance by phenotypic method	The distribution of tetracycline resistance genes		Number of PCR negative isolates
		<i>tetK</i>	<i>tetM</i>	
<i>S. aureus</i>	57 (resistant)	21	36	0
<i>S. aureus</i>	82 (susceptible)	3	4	75
CoNS	49 (resistant)	16	33	0
CoNS	110 (susceptible)	3	5	102
	Total	43	78	177

**Table VII.** Relationship between penicillin resistance and the presence of the *blaZ* gene

Staphylococcal isolates	Penicillin resistance by phenotypic method	Number of <i>blaZ</i> positive isolates	Number of <i>blaZ</i> positive isolates
<i>S. aureus</i>	129 (resistant)	129	0
<i>S. aureus</i>	10 (susceptible)	1	9
CoNS	133 (resistant)	133	0
CoNS	26 (susceptible)	5	21
	Total	268	30

were found as positive by PCR for *16S rDNA* gene and, all *S. aureus* isolates were positive to the species specific *femA* gene.

Methicillin resistance in staphylococci has been reported to be associated with the presence of penicillin-

binding proteins, PBP2' (PBP2a), encoded by the *mecA* gene. The *mecA* gene may be heterogeneously expressed and, therefore, all methicillin-resistant staphylococcal strains may not be detectable with phenotypical methods<sup>2-5</sup>. However, the detection of *mecA* gene by PCR techniques is considered the gold standard method. Also, phenotypic methods require at least 24 h for evaluation of the results<sup>2,3</sup>.

Methicillin resistance was observed in 17.8 per cent isolates when tested by methicillin disk diffusion method, whereas 27.9 per cent isolates had *mecA* gene. Phenotypically methicillin susceptible 30 isolates also carried the *mecA* gene. Comparison of conventional method and multiplex PCR assay did not show a good agreement. Results about resistance to methicillin found in this study were lower than those reported from other parts of Turkey<sup>16,17</sup>, but were in accordance with those in similar studies from Europe (25%) but were lower than those in USA<sup>18,19</sup>.

Our results showed that the *blaZ* gene was widely spread among both *S. aureus* and CoNS. In various studies from our country, penicillin resistance has been reported between 61 and 100 per cent<sup>20,21</sup>. The resistance rate in various countries of the world ranged from 50 to >91 per cent<sup>22,23</sup>.

The staphylococcal isolates were analyzed for the presence of the aminoglycoside-modifying enzyme genes *aac(6')/aph(2'')*, *aph(3')-IIIa*, and *ant(4')-Ia* by multiplex PCR. The presence of at least one of the AmE genes was seen in 111 of 298 isolates. A total 62.8 per cent isolates negative for AME genes were resistant to gentamicin. In accordance with previous studies<sup>3,5</sup>, the *aac(6')/aph(2'')* gene was the most prevalent AME gene in staphylococci. In our study, *aac(6')-aph(2'')* gene was the most frequently found gene among MRSA isolated. This result was similar to the studies carried out in Europe<sup>4</sup>. Ida *et al*<sup>6</sup> from Japan reported that *aac(6')/aph(2'')* gene in MRSA strains was encountered less frequently among clinical isolates<sup>6</sup>.

Erythromycin resistance in staphylococci is encoded by *erm* genes<sup>24</sup>. All isolates found to be resistant to erythromycin by phenotypic methods contained at least 1 erythromycin resistance gene. While resistance to erythromycin in staphylococci was found as 48.7 per cent by phenotypic methods, this ratio was 55.4 per cent by multiplex PCR assay. *erm A*, *erm B* and *msrA* genes in *S. aureus* isolates were found at higher rates than in CoNS, and among the CoNS, *ermC* gene was

the more prevalent. In 34 isolates, neither *ermA* nor *ermC* gene was detected, although these were detected as resistant. Erythromycin resistance might be due to the presence of *msrA* or *ermB* gene as previously described in CoNS<sup>24</sup>.

Lim *et al.*<sup>25</sup> reported that the *ermA* gene was more prevalent than the other erythromycin resistance genes in *S. aureus* isolates, and *ermC* gene was found mostly in CoNS. Similarly, in a study performed by Martineau *et al.*<sup>14</sup>, the *ermC* gene has been reported to be more prevalent in CoNS. However, *ermA* has been reported to be the more common gene in CoNS in another study<sup>26</sup>. Our results were consistent with that of Lim *et al.*<sup>25</sup> and Martineau *et al.*<sup>14</sup>, but were contradictory to that of Thakker-varia *et al.*<sup>26</sup>.

In the present study, the phenotypic resistance to tetracycline in *S. aureus* and CoNS was observed as 41 and 30.8 per cent, respectively. Whereas 42.4 per cent *S. aureus* isolates carried the *tetK* and *tetM* genes, 39 per cent of CoNS were found to carry these genes. Tetracycline resistance genes *tetK* and *tetM* were found positive by PCR method in 15 isolates which were phenotypically sensitive to tetracycline.

Identification and determination of the susceptibility to antibiotics of staphylococci by conventional methods requires a minimum of two-days period, whereas the detection of antibiotic resistance genes by PCR assay can be done within a few hours. Rapid and reliable methods for antibiotic susceptibility are important to institute appropriate therapy. Multiplex PCR can be used for confirmation of the results obtained by conventional phenotypic methods when needed.

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