Abstract

Aim: This study was to determine the virulence encoding genes, and the antibiotic resistance patterns of the Staphylococcus aureus isolates, which were isolated from the nasal samples of chest clinic patients.

Materials and Methods: The nasal samples of the in-patients (431) and out-patients (1857) in Kayseri Training and Research Hospital’s Chest Clinic, Kayseri, Turkey, were cultured on CHROMagar (Biolife, Italiana) S. aureus, and subcultured on sheep blood agar for the isolation of S. aureus. Disc diffusion method was used for antimicrobial susceptibility testing. The occurrence of the staphylococcal virulence encoding genes (enterotoxins [sea, seb, sec, see, seg, seh, sei, sej], fibronectin-binding proteins A, B [fnbA, fnbB], toxic shock syndrome toxin-1 [tst]) were detected by polymerase chain reaction.

Results: Forty-five of the 55 (81.8%) S. aureus isolates from inpatients, and 319 (90.6%) isolates from tested 352 out-patient’s isolates were suspected to all the antibiotics tested. methicillin-resistant S. aureus (MRSA) was detected in 1.2% of S. aureus isolates. Rifampin, trimethoprim-sulfamethoxazole, clindamycin, erythromycin, gentamicin resistance rates were 1.2%, 1.7%, 2.0%, 8.8%, and 1.2%, respectively. The isolates were susceptible to teicoplanin and vancomycin. The genes most frequently found were tst (92.7%), seg (85.8%), sea (83.6%), fnbA (70.9%). There was no statistical significance detected between MRSA and mecA-negative S. aureus isolates in encoding genes distribution (P > 0.05).

Conclusion: Our results show that virulence factor encoding genes were prevalent in patients with S. aureus carriage, whereas antibiotic resistance was low. These virulence determinants may increase the risk for subsequent invasive infections in carriers.

Key words: Carriage, Staphylococcus aureus, virulence factors

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Introduction

Staphylococcus aureus is a dangerous pathogen that causes a variety of severe diseases. Nasal colonization is the cause of recurrent staphylococcal infections. The host tissue colonization by S. aureus is an important factor in disease pathogenesis. S. aureus expresses fibronectin-binding protein A (fnbA), which mediates the adhesion to...
fibrinogen, elastin and fibronectin. In addition, S. aureus isolates associated with invasive diseases, including endocarditis, primary septic arthritis and/or osteomyelitis were more likely to have both genes fnbA and fnbB. The pathogenity of S. aureus results from its ability to produce specific toxins and hydrolytic enzymes. The virulence of S. aureus is defined by a large repertoire of virulence factors, among which secreted toxins play a preeminent role. [1,2] Staphylococcal enterotoxins (SEs), toxic shock syndrome toxin-1 (tsst-1), and exfoliative toxins produced by some S. aureus isolates causes staphylococcal food poisoning, staphylococcal toxic shock syndrome (tss), staphylococcal scalded skin syndrome. [3-5] The arginine catabolic element (ACME) prevalent, especially in methicillin-resistant S. aureus (MRSA) isolates of sequence type 8 and staphylococcal cassette mec type IVa (USA300) and evidence suggest that ACME enhances the ability of ST8-MRSA-IVa to grow and survive on its host. ACME has been identified in a small number of isolates belonging to other MRSA clones but is widespread among coagulase-negative staphylococci. [6] A mobile genetic element-encoded gene, sasX has been attributed to the acquisition of virulence determinants by horizontal gene transfer, and the researchers demonstrate that sasX has a key role in MRSA colonization and pathogenesis, substantially enhancing nasal colonization, lung disease and abscess formation and promoting mechanisms of immune evasion. [7]

Antimicrobial resistance of bacteria is a growing global problem. In low-resource countries, where extensive empirical use of antibiotics is common, the extent and the impact of this phenomenon tend to be even larger than in industrialized countries. The increase of MRSA in hospital settings and in the community is worrisome. Increasing resistance to antibiotics and the growing prevalence of MRSA can be connected to antibiotic overuse in primary care and requires to be addressed promptly. [5,6,8]

In this study, we detected: (i) The occurrence of the virulence genes: tsst-1, SEs (sea, seb, sec, see, seh, sei, sej, seg) and fnbA and fnbB. (ii) The antibiotic resistance patterns of the inpatients and outpatients in a chest clinic who colonized with S. aureus.

**Materials and Methods**

This study is a part of a Erciyes University Scientific Research Project called “Determination of S. aureus nasal carriage, investigation of risk factors, and virulence traits among out-patients and in-patients in Kayseri Training and Research Hospital’s Chest Clinic” (no: 4674). In this study, data about the virulence factors and antibiotic resistance patterns of S. aureus isolates obtained from in-patients and out-patients are given. This study was conducted in January from 1 to 31 2014.

The nasal swabs were taken from 1857 out-patients and 431 in-patients. The aim of this study was explained to all of the volunteers, and they signed a written consent form afterwards.

Nasal swabs were obtained from the anterior nares of the volunteers by rotating a sterile swab of Stuart agar gel transport medium. The microbiological study was realized in Erciyes University Halil Bayraktar Health Services Vocational College’s Microbiology Laboratory (Kayseri, Turkey). The swabs were immediately streaked on CHROMagar S. aureus (Biolife, Italiana). After incubation for 24 h at 37°C suspected violent colonies were isolated and subcultured on to 5% sheep blood agar plates. After incubation for 24 h at 37°C suspected colonies were confirmed to be S. aureus by standard biochemical techniques and conventional methods (colony morphology, Gram stain, catalase activity, tube coagulase test). All S. aureus isolates were tested for antibiotic resistance. Test for methicillin-resistance was performed by the Kirby–Bauer disc diffusion method, using cefoxitin disc on Muller Hinton agar with 24 h incubation at 35.8°C. We also used CHROMagar MRSA for detecting of MRSA. The tested antimicrobial agents were: Cefoxitin (30 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (TMP-SMX, 1.25/23.75 µg), erythromycin (15 µg), clindamycin (2 µg), teicoplanin (30 µg), vancomycin (30 µg), rifampin (30 µg) (all Bioanalyse, Turkey). Erythromycin-induced clindamycin resistance was detected by disk approximation test (D-test).

Results were interpreted according to the criteria of Clinical and Laboratory Standards Institute 2007 guidelines. [9] The reference strain S. aureus ATCC 29213 was used as internal quality control.

All S. aureus isolates from inpatients (55 isolates) and 51 isolates from out-patients, which were taken by systematic sampling were studied for virulence factors determination. The S. aureus isolates from out-patients were ranked by their number and the samples were selected by systematic sampling for virulence factors determination (352/50 = 7, taken in 7th). The determination of species specific parts of the genes was performed with oligonucleotide primers, listed in Table 1. DNA of the isolates was prepared with PureLink™ Genomic DNA Kits (Invitrogen, Carlsbad, USA) according to the instructions of the manufacturer with the use of lysozyme (20 mg/ml, Sigma) in the cell lysis step. The polymerase chain reaction (PCR) reaction mixture consists of PCR master mix (Thermo Scientific, ×2, Califronia, USA), forward primer (0.4 um), reverse primer (0.4 um), template DNA (~30 ng) and nuclease-free water (thermo scientific). The DNA amplification was carried out with the following cycling conditions: Initial denaturation (95°C, 3 min), 35 cycles of denaturation (95°C, 30 sec), annealing (49–51°C, 30 sec), extension (72°C, 1 min) and final extension (72°C, 5 min). Amplicons obtained by PCR amplification were
run in 2% agarose gel at 70 V for 90–120 minutes and visualized in ChemiDoc MP Imaging System (Biorad, USA). 100 bp DNA marker was used (abm, Canada). The presence of the following genes were studied: Tst, SEs (sea, seb, sec, see, seh, sei, sej, seg) and fnbA and fnbB listed in Table 1.

This study was planned and performed in accordance with the Helsinki declaration and was approved by the Ethics Committee of the Erciyes University Medical Faculty. Work permit from the Kayseri Training and Research Hospital's Scientific Committee was also taken.

The data are shown in numbers and frequencies. The evaluation of the difference in categorical variants made by Chi-squared Fisher’s exact test. A P < 0.05 indicated a significant difference statistically.

**Results**

In this study, 2283 patients were tested for nasal S. aureus carriage. The 352 outpatients (n = 1852, 19.0%), and the 55 in-patients (n = 431, 12.8%) were positive for carriage. The demographic characteristics of the patients were shown in Table 2. All the S. aureus isolates were tested for antibiotic resistance (n = 407). In Table 3, the antibiotic resistance patterns of these isolates were shown.

**Inpatients**

Only erythromycin resistance in three isolates; cefoxitin, clindamycin and erythromycin resistant one isolate; rifampin, cefoxitin, clindamycin, erythromycin and gentamycin resistant one isolate; rifampin, cefoxitin, clindamycin, erythromycin, gentamycin two isolates; rifampin, clindamycin, erythromycin and gentamycin one isolate; clindamycin, erythromycin resistance found in one isolate and two isolates were positive for D-test. The resistance rates for the following antibiotics were: Rifampin, cefoxitin, trimethoprim-sulfamethoxazole, clindamycin, erythromycin, gentamicin, vancomycin, teicoplanin, 9.1%, 9.1%, 0, 9.1%, 18.2%, 9.1%, 3.6%, 0, 0, respectively.

**In outpatients**

Only trimethoprim-sulfamethoxazole resistance in 7 isolates; erythromycin resistant in 24 isolates; clindamycin, erythromycin resistant one isolate; trimethoprim-sulfamethoxazole, erythromycin and D-test positivity in one isolate and there 22 D-test positivity. The resistance rates for the following antibiotics were: Rifampin, cefoxitin, trimethoprim-sulfamethoxazole, clindamycin, erythromycin, gentamicin, vancomycin, teicoplanin, 0, 0, 2%, 0.9%, 7.4%, 0, 0, 0 respectively.

Of all the isolates (n = 407) the antibiotic resistance rates were rifampin, cefoxitin, trimethoprim-sulfamethoxazole, clindamycin, erythromycin, gentamicin, vancomycin, teicoplanin; 1.2%, 1.2%, 1.7%, 2%, 8.8%, 1.2%, 0, 0 respectively [Figure 1].

| Table 1: Oligonucleotide primers used for the amplification of the toxin gens and fnb A, fnb B |
|-----------------|---------------------------------|-----------------|-----------------|
| **Target gen**  | **Oligonucleotide sequence 5'-3'** | **Size of amplified product (bp)** | **Reference**    |
| Sea             | GGTTATCAATGTCGGGGTGG             | 102              | Mehrotra et al. [10] |
| seb             | CGGCACCTTTTTTCTCTCCGG            | 164              | Mehrotra et al. [10] |
| sec             | GATGATGGGTTAATGAGGC              | 451              | Mehrotra et al. [10] |
| see             | AGATGAAAGTATTGATGTATGG           | 278              | Mehrotra et al. [10] |
| seg             | CTGGTCCACCTTTGGAAGGG             | 327              | Monday and Bohach [11] |
| seh             | CTGGTCCACCTTTGGAAGGG             | 360              | Monday and Bohach [11] |
| sei             | CTGGTCCACCTTTGGAAGGG             | 465              | Monday and Bohach [11] |
| seh             | CTGGTCCACCTTTGGAAGGG             | 142              | Casagrande Proietti et al. [12] |
| fnbA            | GGGAGATCAAGAAGACAA               | 1279             | Yapor and Öğuz [13] |
| fnbB            | CCAACTGATCCGTCCTGTTG             | 812              | Yapor and Öğuz [13] |
| tst             | ACGCTGATGTCCTTACATCC             | 326              | Mehrotra et al. [10] |

fnbA=Fibronectin-binding proteins A; fnbB=Fibronectin-binding proteins B; tst=Toxic shock syndrome toxin-1
There was no statistically significant difference detected between MRSA and mecA-negative S. aureus (MSSA) isolates in encoding genes distribution (P > 0.05).

All the 106 isolates had multiple virulence genes (100%; min: 2, max: 8). Two virulence genes in one isolate (0.9%), three virulence genes in four isolates (3.8%) and follows as: Four in seven isolates (6.6%), five in 19 (17.9%), six in 37 (34.9%), seven in 28 (26.4%), eight in 10 isolates (9.4%).
Discussion

In adults, S. aureus demonstrates a niche preference for the anterior nares.\[14\] The nasal carriage of S. aureus represents a risk factor for subsequent invasive infection of patients and also interpatient transmission. For both clinicians and antibiotic policy makers, it is important to identify populations at risk for infections with resistant microorganisms and to better understand the epidemiology of antibiotic resistance.\[15\] In our study, the MRSA carriage rate was 1.2% in S. aureus isolates, all of them were from inpatients. There were a statistically significant differences between outpatients and inpatients (<0.001). There were also statistically difference between resistance patterns of rifampin, clindamycin, erythromycin and gentamicin in outpatients and inpatients (<0.001, 0.002, 0.018, <0.001 respectively). A large number of studies have been done all over the world. Scribel et al.\[16\] from Brasil found no MRSA from the patients at a primary healthcare unit. Sfeir et al.\[17\] found 1.5% cefoxitin, gentamicin; 21.4% erythromycin; 16.8% clindamycin resistance and all strains found susceptible to vancomycin and rifampin in 1526 outpatients. Choi et al.\[18\] found 2.5% gentamicin, erythromycin, clindamycin; 1.2% rifampicin; 1.2% oxacillin resistance in health adults. Netsyetaye\[a\] et al.\[19\] found erythromycin-induced clindamycin resistance 33.3% in MRSA isolates, and 5.6% in MSSA isolates. They found erythromycin-induced clindamycin resistance rate significantly higher among MRSA isolates than MSSA isolates (P < 0.05). From Turkey Ctk et al.\[20\] found 25.7% MRSA carriage in in patients and 27.6% from out patients. Their group were smaller than ours. In patients 191, outpatients 123 patients. They found no statistically significant difference in MRSA carriage in these groups (P > 0.05). In our study it is pleasing to see the antibiotic resistance was lower than the studies stated. Even Versporten et al.\[21\] validated 2011 total national whole scale antibiotic-use data of six southern and eastern European countries and regions. According to European Surveillance of Antimicrobial Consumption project Turkey had the highest antibiotic use in Europe, and they also mentioned that Turkish government already published a Rational Drug Use National Action Plan 2013–2017. The study showed that in our country the most used antibiotics were β-lactam antibacterial, penicillins, and the second group was cephalosporins. Especially, the combinations of penicillins, including β-lactamase inhibitors is the most used drugs. In this study, we used the antibiotic discs which were used in tertiary care referral center’s antibiogram schema for S. aureus isolates.

Our patient group was specific, patients who have upper or lower respiratory tract diseases welcomed in this clinic. MRSA was found only in patients, outpatients had no MRSA, especially erythromycin, and clindamycin resistance was important. D-test positivity was 6.1%. Netsyetayeva

![Figure 1: Percentage of isolates to various antibiotics](image1)

![Figure 2: Agarose gel electrophoresis of polymerase chain reaction (PCR) products of Staphylococcus aureus isolates. Genomic regions containing the target genes (Seg, Set) were amplified by PCR and PCR products of 327 bp and 465 bp were obtained. Amplified DNA fragments were run in 2% agarose gel and visualized. Presence of PCR products was confirmed. Each number represents a S. aureus isolate. Arrows represent the band sizes of the 100 bp DNA marker. M = DNA Marker, bp = base pair](image2)

![Figure 3: Agarose gel electrophoresis of polymerase chain reaction (PCR) products of S. aureus isolates. Genomic regions containing the target genes (See, fnbA, fnbB) were amplified by PCR and PCR products of 209 bp, 1279 bp and 812 bp were obtained. Amplified DNA fragments were run in 2% agarose gel and visualized. Presence of PCR products was confirmed. Each number represents a S. aureus isolate. Arrows represent the band sizes of the 100 bp DNA marker. M = DNA Marker, bp = base pair](image3)
et al.[8] found 33.3% D-test positivity. Whenever clindamycin is intended to be used for S. aureus infections, D-test should be performed.

According to the results of the present study all the 106 isolates had multiple virulence genes (100%; min: 2, max: 8). The genes most frequently found were tst (92.7%), seg (85.8%), sei (83.6%), fnbA (70.9%). Demir et al.[13] showed that 79 isolates (65.8%) were positive for one or more virulence genes. Tekeli et al.[21] showed virulence genes in 86% isolates of S. aureus from blood cultures of patients in Ankara, Turkey. These different results may be due to geographical differences or different ecological origins of the isolates. Demir et al.[13] found 14.2% harbored isolates in S. aureus isolates from Mustafa Kemal University Hospital, Turkey. Nashev et al.[4] found only two positivity for tst in 44 S. aureus isolates. Lozano et al.[6] found tst in 28.3% carriers of S. aureus from Spain. Nashev et al.[10] from nasal carriers found that seg and sei is the most prevalent whereas Schaumburg et al.[12] found sea (34.4%), seg (33.1%), sei (33.1%) the most prevalent genes in carriers. Yapar et al.[13] studied 50 S. aureus isolates from child carriers and found fnbA (28%), and fnbB (10%). Nashev et al.[4] were reported as 100% for fnbA, 40% for fnbB in 16 healthy carriers. Nestyeyaeva et al.[5] detected fnbA in 59% of S. aureus isolates of healthy Ukrainian adults. Zmantar et al.[21] focusing on isolates from soft tissue infections the rate of fnbA was reported as 76.1%.

There was no statistically significant difference between MRSA and MSSA isolates among gene distribution in our study. Clindamycin resistance was 14% in sei negative isolates, in sei positives the resistance rate was 1.6% (Fisher’s Chi-square, P = 0.017). Clindamycin resistance was 12.1% in sei negative isolates, in sei positives the resistance was not observed (Fisher’s Chi-square, P = 0.015). Erythromycin resistance was 22.4% in sei negative isolates while in sei positives the rate was 4.2% (Fisher’s Chi-square, P = 0.010). Yilmaz et al.[24] showed that the isolates harbored with sea had multiple antibiotic resistances, especially MRSA (85.7%).

In our study we found that most of the S. aureus isolates were susceptible to all antimicrobial agents. However, high prevalence of virulence factor encoding genes were detected. To gain knowledge of S. aureus isolates circulating among carriers as well as the capacity of these isolates to produce virulence factor encoding genes, and antibiotic susceptibility patterns in order to understand the complex evolution of S. aureus and MRSA infections.

**Conclusion**

It is important for primary care physicians to know the antibiotic susceptibility patterns of S. aureus isolates, which are circulating among carriers in their region as it shows differences among regions and also virulence factors which the isolates posed for the empirical therapy.

**References**

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