COMMUNITY-ACQUIRED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND GENOTYPES AMONG UNIVERSITY STUDENTS IN TURKEY

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Abstract. Nasal carriage of *Staphylococcus aureus* is an important risk factor for nosocomial and community-acquired staphylococcal infections. We investigate the prevalence of community-acquired methicillin-sensitive (CA-MSSA) and -resistant (CA-MRSA), including inducible dormant (ID)-MRSA *S. aureus*, and genotypes of MRSA strains of nasal cultures from 1,108 university students attending Selcuk University, Turkey. Risk factors were based on replies to a questionnaire. *S. aureus* was identified using conventional culture methods and a Stapyloslide® latex test. Antibiotic susceptibility and methicillin resistance were determined by a disk diffusion method, and vancomycin susceptibility was performed using an E-test. Identification of *mecA* and SCC*mec* types were conducted by PCR and genotypes by pulse field gel-electrophoresis (PFGE). Prevalence of *S. aureus* was 17%, with 9% being MRSA. Two isolates were SCC*mec* type III, 11 were SCC*mec* variant IIIA and one SCC*mec* type IV. No ID-MRSA was detected. The majority of the isolates were resistant to penicillin and no strain was resistant to vancomycin. Two MRSA strains were PFGE pulsotype A, 9 pulsotype B, 2 pulsotype C, 1 pulsotype D and 3 pulsotype E. Presence of permanent catheter and use of antibiotics in the previous month were risk factors for MSSA colonization and association with medical facilities were risk factors for MRSA carriers. There is a need for multicenter studies in Turkey to investigate CA- and ID-MRSA prevalence and nosocomial infections.

Keywords: *Staphylococcus aureus*, CA-MRSA, ID-MRSA, nasal colonization, risk factors, Turkey

INTRODUCTION

Staphylococcus aureus is a microorganism with high virulence, which is frequently seen in human infections. Methicillin-resistant *S. aureus* (MRSA) continues to be a significant cause of morbidity and mortality in hospital infections since 1961 when it was first identified (Waldvogel, 2000). While MRSA had been responsible for hospital infections until 1990s, it has been isolated also as a community-acquired bacterium since then (Cheung *et al*, 2008). MRSA pose a major risk factor for the occurrence of both hospital- and community-acquired staphylococcal infections, and is responsible for skin and soft tissue infections as well as fatal systemic infections (Maier *et al*, 2005).

Determining the epidemiology of MRSA carriers and the genotypes are important because the epidemiology of

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community-acquired (CA)-MRSA differs from that of hospital-acquired (HA)-MRSA. The penicillin binding protein 2a, which causes resistance to methicillin is encoded by *mec*A, which is located in a region called the Staphylococcal cassette chromosome *mec* (SCC*mec*) (Vandenesch *et al*, 2003; Deurenberg and Stobberingh, 2008). While CA-MRSA carries SCC*mec* IV and V, HA-MRSA carries SCC*mec* I, II and III (Karahan *et al*, 2008; David and Daum, 2010). In addition, CA-MRSA isolates carrying Panton-Valentine Leukocidin (PVL) gene, which increases the bacterial virulence, cause necrotizing pneumonia and severely progressing skin infections (Gillet *et al*, 2002; Vourli *et al*, 2009). Phenotypically methicillin-susceptible but having *mecA*-positive *S. aureus* isolates, known as inducible dormant methicillin resistance (ID)-MRSA, become methicillin-resistant upon *in vitro* exposure to β-lactams, which lead to the production of protein 2a (Bearman *et al*, 2010). However, detection of ID-MRSA is often not detected during routine susceptibility tests (Kampf *et al*, 2003). Thus it is important to understand the risk factors involved in the spread of MRSA, which include use of antibiotics during the previous 3 months, skin and soft tissue infections, hospitalization during the previous 12 months, HIV infection, underlying disease and increase in hospital visits (Hidron *et al*, 2005).

Pulsed field gel-electrophoresis (PFGE) is an important genetic typing method used in molecular epidemiology to determine genetic differences in microorganisms, and is useful in many areas such as observing the relationship between strains that cause infection and colonization in the same patient, monitoring the spread of microorganisms among patients in the hospital, monitoring the spread of antibiotic-resistant strains within and among hospitals, and in distinguishing infection-producing strains (Onasanya *et al*, 2003).

Currently, as the pathogens of nosocomial infections are becoming resistant to antibiotics, this becomes important in many aspects, such as increased treatment costs and mortality rates and it has been recommended that surveillance studies be carried out continuously to prevent the spread of nosocomial infections caused by HA-MRSA and CA-MRSA (Zer *et al*, 2009). The purpose of this study was to investigate CA- and ID-MRSA prevalence and the clonal relationship of MRSA strains isolated from carriers in Turkey.

MATERIALS AND METHODS

Study location and subjects

A total of 1,108 university students attending Selcuk University, Konya, Turkey were enrolled in the study. The students were informed about the procedure that would be followed and verbal informed consents were obtained. A questionnaire then was administered to the students to determine their demographic characteristics and risk factors. The study was approved by the institutional ethics committee of the Faculty of Medicine, Selcuk University (no. 2011/54).

Bacterial isolates

Nasal samples were taken from both nares with a sterile swab (Cultiplast, Milan, Italy). The samples were inoculated in Mueller Hinton broth and 5% sheep blood agar and incubated for 24 hours at 35°C, and then were sub-cultured on mannitol salt agar for 24-48 hours at 35°C. The white-yellow pigmented colonies in the mannitol salt agar and those producing hemolysis in the sheep blood agar were stained with Gram stain. Catalase-positive

bacteria were identified using *S. aureus* latex agglutination test kit (Staphyloslide Latex Test; BD Diagnostics, Sparks, MD).

Antimicrobial susceptibility test

Susceptibilities of *S. aureus* isolates to penicillin (10 U), cefoxitine (30 μg), erythromycin (15 μg), tetracycline (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), clindamycin (5 μg), trimethoprim-sulfamethoxazole (1.25-23.75 μg), levofloxacin (5 μg), and linezolid (30 μg) were determined using the Kirby-Bauer disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2011). D-zone test was carried out to identify inducible clindamycin resistance according to the guidelines of the CLSI (2011). The vancomycin susceptibility was investigated by an E-test (AB BIODISK, Solna, Sweden) according to manufacturer's instructions. A 0.5 McFarland unit of inoculum was inoculated on Mueller-Hinton agar and a vancomycin E-test strip containing 0.016 to 256 μg/ml vancomycin was placed on top. After 24-hour incubation MICs were determined visually. *S. aureus* ATCC 25923 was used as control strain.

Genotyping

DNA isolation of *S. aureus* isolates was performed with a commercial kit (Qiagen, Hilden, Germany). *S. aureus nuc* (162 bp) (encoding thermonuclease gene) and *mecA* (279 bp) were identified using multiplex PCR with specific primers (Table 1) (Kim *et al* 2001; Oliveira and Lencastre, 2002). The PCR reaction mixture (50 μl) contained 1 μl of DNA, 5 μl of 10X PCR buffer, 25 mM $MgCl_2$, 10 μM dNTPs, 1 μl of each primer (50 pM) and 5 U *Taq* DNA polymerase. PCR was performed on SensoQuest LabCycler (SensoQuest, Göttingen, Germany). Thermocycling conditions were as follows: 94°C for 10 minutes; 35 cycles of 94°C for 90 seconds, 49°C for 90 seconds, and 72°C for 90 seconds; and a final step of 72°C for 10 minutes. *S. aureus* ATCC 43300 was used as control strain. The MRSA SCC*mec* types were identified by multiplex PCR with specific primers (Table 1) according to previously described procedure (Oliveira and Lencastre, 2002). In brief, the PCR reaction mixture (50 μl) contained approximately 5ng of template DNA, 1X PCR buffer, 25 mM $MgCl_2$, 200 μM dNTPs, 400 nM primers CIF2 F2, CIF2 R2, MECI P2, MECI P3, RIF5 F10, RIF5 R13, pUB110 R1, and pT181 R1; 800 nM primers DCS F2, DCS R2, MECA P4, MECA P7, and IS431 P4; 200 nM concentrations of primers KDP F1, KDP R1, RIF4 F3, and RIF4 R9 and 1.25 U *Taq* DNA polymerase. PCR amplifications were performed in SensoQuest LabCycler (SensoQuest, Göttingen, Germany) as follows: 94°C for 4 minutes; 30 cycles of 94°C for 30 seconds, 53°C for 30 seconds, and 72°C for 1 minute; with a final step of 72°C for 4 minutes and standing at 4°C. Amplicons were analyzed by 2% agarose gel-electrophoresis and visualized under UV light following staining with ethideum bromide.

MRSA isolates were genotyped by PFGE method using *SmaI* (10U/μl) (Thermo Scientific, Rockford, IL) in CHEF DR-II system (BioRad Laboratories, Temse, Belgium) at 14°C and 6 V/cm² for 20 hours. The initial switch time was 5.3 seconds, and final switch time 55 seconds. The gel was stained with ethidium bromide and DNA bands were recorded under UV light using Gel logic imaging system (Kodak Rochester, NY). DNA band profiles were analyzed with Gel Compar II software version 5.0 (Applied Maths, Sint-Martens-Latem, Belgium) (Mulvey *et al*, 2001). *S. aureus* ATCC 49775 was used as the standard strain.

Table 1
Primers used in the study.

Primer	Sequence (5´-3´)	Amplicon size (bp)	Target gene
nuc F	GCGATTGATGGTGATACGGTT	279	Internal control
nuc R	AGCCAAGCCTTGACGAACTAAAGC		
MECA P4	TCCAGATTACAACTTCACCAGG	162	*mec*A gene
MECA P7	CCACTTCATATCTTGTAACG		
CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	495	SCC*mec* type I
CIF2 R2	ATTTACCACAAGGACTACCAGC		
KDP F1	AATCATCTGCCATTGGTGATGC	284	SCC*mec* type II
KDP R1	CGAATGAAGTGAAAGAAAGTGG		
MECI P2	ATCAAGACTTGCATTCAGGC	209	SCC*mec* type II, III
MECI P3	GCGGTTTCAATTCACTTGTC		
DCS F2	CATCCTATGATAGCTTGGTC	342	SCC*mec* type I, II, IV
DCS R1	CTAAATCATAGCCATGACCG		
RIF4 F3	GTGATTGTTCGAGATATGTGG	243	SCC*mec* type III
RIF4 R9	CGCTTTATCTGTATCTATCGC		
RIF5 F10	TTCTTAAGTACACGCTGAATCG	414	SCC*mec* type III
RIF5 R13	GTCACAGTAATTCCATCAATGC		
IS431 P4	CAGGTCTCTTCAGATCTACG	381	SCC*mec* type IA
pUB110 R1	GAGCCATAAACACCAATAGCC		
IS431 P4	CAGGTCTCTTCAGATCTACG	303	SCC*mec* type IIIA
pT181 R1	GAAGAATGGGGAAAGCTTCAC		

Statistical analysis

Chi-square and Fisher's exact tests were used for statistical analysis. A p-value < 0.05 is considered statistically significant.

RESULTS

S. aureus was isolated from 192/1,108 (17.3%) of nasal samples, among which 24/106 (23%) were from students of the Faculty of Medicine and 168/1,002 (16.8%) from students of other faculties of Selcuk University. There is no statistical difference in terms of *S. aureus* prevalence between these two groups. There were 175 (91%) MSSA and 17 (9%) MRSA isolates, the latter representing a prevalence of 1.5%, 9.4% and 0.7% of all students, those of the Faculty of Medicine, and students in other faculties, respectively. There is a statistical difference in MRSA prevalence between students staying in hostels and those from the Faculty of Medicine (p = 0.0001). Presence of permanent catheter (p = 0.0001) and use of antibiotics in the previous month (p = 0.041) were risk factors for MSSA colonization, but there was no significant correlation between risk factors and MRSA colonization (Table 2).

Resistance of *S. aureus* isolates to penicillin was 83%, erythromycin 14%, clindamycin 12%, tetracycline 10%, clindamycin 9% (inducible 4%), gentamicin 8%, levofloxacin 8%, linezolid 2%, and trimethoprim-sulfamethoxazole 1%. No isolates were resistant to vancomycin. A comparison of percent antibiotic resistance between MSSA and MRSA isolates

Table 2
Risk factors for MSSA and MRSA nasal colonization.

	Total (N = 1,108) Number (%)	MSSA Number (%)	MRSA Number (%)
Gender			
Female	766 (69)	118 (15.4)	9 (1.2)
Male	342 (31)	57 (16.7)	8 (2.3)
Diabetes mellitus			
Yes	3 (0.2)	2 (66.7)	0 (0)
No	1,105 (99.8)	173 (15.6)	17 (1.5)
Hemodialysis			
Yes	1 (0.1)	1 (100)	0 (0)
No	1,107 (99.9)	174 (15.7)	17 (1.5)
Presence of permanent catheter			
Yes	9 (0.8)	4 (44.4)	0 (0)
No	1,099 (99.8)	171 (15.5)	17 (1.5)
Skin infection			
Yes	100 (9)	16 (16)	1 (1)
No	1,008 (91)	159 (15.8)	16 (1.6)
Liver failure			
Yes	1 (0.1)	1 (100)	0 (0)
No	1,107 (99.9)	175 (15.8)	17 (1.5)
Use of antibiotics in the previous month			
Yes	365 (33)	41 (11.2)	4 (1.1)
No	743 (67)	134 (18)	13 (2.9)
History of surgery			
Yes	199 (18)	41 (20.6)	5 (2.5)
No	909 (82)	134 (14.7)	12 (1.3)
Smoking			
Yes	244 (18)	35 (14.3)	4 (1.6)
No	864 (82)	140 (16.2)	13 (1.5)
Hospitalization			
Yes	226 (20)	39 (17.2)	3 (1.3)
No	882 (80)	136 (15.4)	14 (1.6)
Presence of skin piercing or tattoo			
Yes	37 (3)	5 (13.5)	0 (0)
No	1,071 (97)	170 (15.9)	17 (1.6)
Object sharing			
Yes	83 (7)	12 (14.4)	1 (1.2)
No	1,025 (93)	163 (15.9)	16 (1.6)

showed that all MRSA strains (n = 17) were resistant to penicillin and erythromycin, and > 85% resistant to gentamicin, ciprofloxacin, levofloxacin, clindamycin and tetracycline but all MSSA isolates were susceptible to gentamicin, ciprofloxacin, levofloxacin, 81% resistant to penicillin, and < 10% resistant to erythromycin, clindamycin and tetracycline (Table 3).

The presence of *mec*A was detected in 17/192 (9%) of *S. aureus* isolates and in no ID-MRSA isolates. There were 2 SCC*mec*

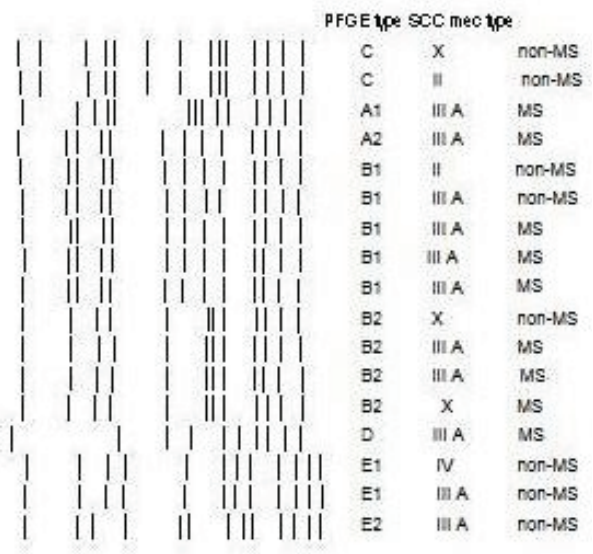

Fig 1–Pulse field gel-electrophoresis (PFGE) profile of 17 MRSA strains. DNA was isolated from MRSA cultured from nasal swabs and subjected to PFGE as described in Materials and Methods. MS, medical students; non-MS, non-medical students.

type II, 11 variant IIIA, 1 type IV and 3 untypable. Of the 10 MRSA strains isolated from the Faculty of Medicine students, 9 were SCC*mec* type IIIA and 1 could not be typed.

Five different (A-E) pulsotypes (clones) were identified using PFGE (Fig 1). Two isolates were pulsotype A (1 A1, 1 A2), 9 pulsotype B (5 B1, 4 B2), 2 pulsotype C, 1 pulsotype D, and 3 pulsotype E (2 E1, 1 E2).

DISCUSSION

The prevalence of nasal MRSA changes from community to community. In previous studies performed in Turkey, *S. aureus* colonization rates are 18%-28% and MRSA 0.07%-1% in healthy preschool and primary school students (Ciftci *et al*, 2007; Kiliç *et al*, 2008; Oguzkaya-Artan *et al*, 2008), prevalence of MSSA and MRSA of 14.1% and 3%, respectively, among students of midwifery in a university (Kirecci *et al*, 2010). We found that there was *S. aureus* and MRSA colonization in 17% and 1.5%, respectively of healthy university students. While there is no statistically significant difference in *S. aureus* prevalence between students from the medical faculty and from other faculties, there was a 10-fold increase in MRSA prevalence between medical and non-medical students. Contact with patients is a likely explanation.

From previous reports, risk factors for *S. aureus* nasal colonization included use of antibiotics, being a healthcare professional or contact with healthcare workers, hospitalization, undergoing dialysis, presence of skin diseases caused by staphylococci, AIDS, crowded environments, impaired skin integrity, common use of objects contaminated with scar drainage, injection, cleaning habits, hygiene and young age in the studies (Creech *et al*, 2005; Halablab *et al*, 2010). In our study, the presence of permanent catheter and use of antibiotics in the previous month were risk factors for MSSA colonization, and association with medical facilities was risk factor for MRSA carriers. On the other hand, a previous study in Turkey reported no significant correlation between risk factors such as hospitalization in the previous year, undergoing surgery, use of antibiotics in the previous six months, skin/soft tissue infection, presence of children under the age of 15 years at home, persons working in healthcare facilities, and living with ≥ 5 people, and it was emphasized that further studies are needed in this regards (Ozguven *et al*, 2008).

The most important factor in the

Table 3
Comparison of antibiotic resistance rates between MSSA and MRSA strains.

Antibiotics	MSSA (n = 175) Number (%)	MRSA (n = 17) Number (%)	p-value
Penicillin	142 (81)	17 (100)	0.049
Erythromycin	10 (6)	17 (100)	0.001
Gentamicin	0	15 (88)	0.001
Linezolid	1 (0.5)	3 (18)	0.001
Ciprofloxacin	0	15 (88)	0.001
Clindamycin	8 (4.5)	16 (94)	0.001
Tetracycline	4 (2)	16 (94)	0.001
Trimethoprim-sulfamethoxazole	0	2 (12)	0.001
Levofloxacin	0	15 (88)	0.001
Vancomycin	0	0	

treatment of MRSA infection is the existence of multiple antibiotic resistances on top of methicillin resistance (Hardy *et al*, 2004). Varying degrees of resistance have been found to macrolides, clindamycin, trimethoprim-sulfamethoxazole and gentamicin in CA-MRSA strains isolated from healthy people, but no vancomycin resistance (Kuehnert *et al*, 2006; Bearman *et al*, 2010). In this study, the highest resistance in MSSA strains was to penicillin (81%). All MRSA strains (n = 17) were resistant to penicillin and erythromycin, and >85% resistant to gentamicin, ciprofloxacin, levofloxacin, tetracycline and clindamycin, but no vancomycin resistance was detected.

Kampf *et al* (2003) investigated ID-MRSA in 447 health professionals in Benjamin Franklin University Hospital, Berlin, Germany and isolated 7 such strains (1.6%) from six physicians and one nurse. Bearman *et al* (2010) detected 1.4% ID-MRSA nasal colonization among 1,000 participants in Richmond, Virginia, USA. Our study, the first in Turkey, detected no ID-MRSA in the 17 MRSA strains (as would be expected).

Various phenotypic and genotypic methods have been described for typing of *S. aureus* isolates (Montesinos *et al*, 2002). In a study using PFGE performed in Tenerife, Canary Islands, Spain, 3 different pulsotypes, namely, A (12 subtypes), B (2 subtypes) and C have been identified in 124 MRSA strains, with pulsotype A mostly (81.6%) being hospital-acquired, among which A1 (64%) is responsible for all epidemics, and pulsotype B (84.6%) found in community-acquired *S. aureus* (Montesinos *et al*, 2002). In a study performed in Erciyes University in Kayseri, Turkey, 10 pulsotypes were found in 80 MRSA strains and 76% of these strains belonged to a single pulsotype (Aygen *et al*, 2004). We detected 5 pulsotypes (A-E) in the 17 MRSA strains. From these 9 B pulsotypes, 6 were isolated from medical students and 3 from students of other faculties. MRSA strains with pulsotype B are thought to be hospital-acquired, because they carry SCC*mec* types II and IIIA. From the questionnaire, the 3 non-medical students had a history of hospitalization.

In summary, the prevalence of MRSA

nasal colonization was higher in students of the Faculty of Medicine than that in students studying in other faculties. Presence of permanent catheter and use of antibiotics in the previous month were risk factors for MSSA colonization, and association with medical facilities were risk factors for MRSA carriers. The majority of MRSA SCC*mec* was type IIIA and belonged to pulsotype B. No ID-MRSA was detected.

Thus, there is a need for multicenter studies to investigate the prevalence of CA-, HA- and ID-MRSA colonization and infections that develop therefrom in the general population of Turkey. Carrying out hospital- and community-based surveillance studies and molecular microbiologic analysis of MRSA isolates will help us understand its epidemiology and evolution.

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