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# Risk factors for acquisition of methicillin-resistant *Staphylococcus aureus* and clonal spread of the isolates in a medical intensive care unit*

**Aim:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is still the commonest pathogen in hospital-acquired infections with high morbidity and mortality. MRSA colonization usually precedes infection and dissemination of the microorganism. The aim of this study was to determine risk factors for the colonization and infection with MRSA in a medical intensive care unit (MICU) and to show the genetic relation of strains.

**Materials and methods:** This study was conducted prospectively between 1 December 2004 and 31 January 2006 in MICU. Patients (>16 years) admitted to the MICU were screened for MRSA on admission (in the first 48 hours), at the end of the first week, and at the ICU discharge using anterior nares, axilla, and groin swabs. Risk factors for colonization and infection of MRSA were evaluated. Strains isolated from colonized patients were evaluated for genetic relation.

**Results:** During the study period, 259 patients were evaluated for the risk factors of MRSA acquisition. The colonization rate was 18.5%, and 64.6% of the patients were colonized in the first week. In multiple logistic regression analysis, only the length of stay in MICU and mechanical ventilation were significant risk factors for colonization. MRSA infection occurred in 15 (31%) of 48 colonized patients during MICU stay. MRSA colonization and tracheostomy were significant risk factors for MRSA infection. A genetic relation was found in 48 isolates from colonized patients in MICU and 23 isolates from colonized patients on admission. Overall, 4 clones (clone A, B, C, and D) were determined from colonized patients on admission; clone A (37%), clone B (32%), clone C (21%), and clone D (10%). Moreover, patients colonized during MICU stay had similar clones (clone A-30%, clone B-57%, and clone C-13%) with these patients.

**Conclusion:** This study shows a high colonization rate and dissemination of MRSA in a developing country with inadequate infrastructure (lack of nurse, lack of isolation rooms, and heavy workload).

**Key words:** *Staphylococcus aureus*, intensive care unit, colonization, infection control

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## Bir dahiliye yoğun bakım ünitesinde metisiline dirençli *Staphylococcus aureus* kazanımı için risk faktörleri ve izolatların klonal yayılımı

**Amaç:** Metisiline dirençli *Staphylococcus aureus* (MDSA) hastane kaynaklı enfeksiyonlarda en sık görülen ve yüksek morbidite ve mortaliteye sahip patojenlerden biridir. MDSA kolonizasyonu, genellikle enfeksiyona ve mikroorganizmanın yayılımına öncülük eder. Bu çalışmanın amacı bir dahiliye yoğun bakım ünitesi (DYBÜ)'nde MDSA kolonizasyonu ve enfeksiyonu için risk faktörlerini tespit etmek ve suşların genetik yakınlığını göstermekti.

**Yöntem ve gereç:** Bu çalışma prospektif olarak 1 Aralık 2004 ve 31 Ocak 2006 tarihleri arasında yapıldı. DYBÜ'ye yatan hastalar (>16 yaş) ön burun kanatları, aksilla ve kasık sürüntü örnekleri ile MDSA varlığı açısından yatışta (ilk 48 saat), birinci hafta ve YBÜ'den çıkarken araştırıldı. MDSA kolonizasyonu ve enfeksiyonu için risk faktörleri analiz edildi. Kolonize hastalardan izole edilen suşların genetik yakınlığı araştırıldı.

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**Bulgular:** Çalışma döneminde, 259 hasta MDSA kazanımı için risk faktörleri açısından değerlendirildi. Kolonizasyon oranı % 18.5 idi ve hastaların % 64.6'sı ilk haftada kolonize oldu. Çok değişkenli lojistik regresyon analizinde, sadece DYBÜ'de yatış süresi ve mekanik ventilasyon kolonizasyon için önemli risk faktörleri olarak bulundu. DYBÜ'sinde yatış sırasında kolonize olan 48 hastanın 15 (% 31)'inde MDSA enfeksiyonu gelişti. MDSA kolonizasyonu ve trakeostomi MDSA enfeksiyonu için önemli risk faktörleri olarak bulundu. DYBÜ'de kolonize olan hastalardan izole edilen 48 izolat ve yatışta kolonize hastalardan izole edilen 23 izolat arasında genetik ilişki bulundu. Yatışta kolonize olan hastalardan toplam olarak dört klon (klon A, B, C, D) tespit edildi; klon A (% 37), klon B (% 32), klon C (% 21) ve klon D (% 10). Ayrıca DYBÜ'sinde kolonize hastalardan izole edilen suşlarda benzer klonlara sahipti (klon A-% 30, klon B-% 57, klon C-% 13).

**Sonuç:** Bu çalışma yetersiz alt yapıya sahip (kısıtlı hemşire, izolasyon odası yokluğu, ağır iş yükü) gelişmekte olan bir ülkede, MDSA ile yüksek kolonizasyon oranını ve yayılımını göstermektedir.

**Anahtar sözcükler:** *Staphylococcus aureus*, yoğun bakım ünitesi, kolonizasyon, enfeksiyon kontrolü

## Introduction

Nosocomial infections constitute an important health-care problem in ICU patients with high mortality, morbidity, and high cost. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading cause of nosocomial infections, and they are usually associated with poor outcomes in ICUs (1). Furthermore, ICU-acquired infections are a serious problem in Turkey and the incidence was reported as 56 in 1000-patient days (2). In a one-day point prevalence study, a total of 56 ICUs from 22 university and teaching hospitals participated and 49% of patients had one or more ICU-acquired infections (3). Pneumonia and bacteremia are the most frequent nosocomial infections in Turkish ICUs and MRSA is one of the important cause of these infections with high mortality (3,4). MRSA infection is generally preceded by colonization and patients usually acquire MRSA during their hospital stay. Furthermore, colonization plays a major role in the dissemination of this organism (5).

Erciyes University Hospital is a 1300-bed tertiary-referral teaching hospital with 9 ICUs and approximately 250-875 patients admitted to each ICU annually. In the ICUs, the bed-to-bed space is very close (<1 m) and there is insufficient number of healthcare-workers (HCWs). Despite the efforts for infection control, nosocomial infection rates are still high (20/1000 patient days) in ICUs (surveillance report of hospital) and these infections are usually due to multi-drug resistant pathogens. *S.aureus* infections constitute 13% of ICU-acquired infections with excess morbidity and mortality and 90%-95% of *S. aureus* isolates are resistant to methicillin (6-8). Probably, patients get colonized during their stay in our ICUs, and cross-transmission occurs from patient to patient because of inadequate infrastructure. This study was planned to determine the risk factors for the colonization and infection with MRSA in our medical ICU (MICU) and examine the genetic relation of the strains to show the dissemination of isolates.

## Patients and methods

### MICU design and infection control measures

This study was conducted prospectively between 1 December 2004 and 31 January 2006 in our MICU. There are 9 beds in the MICU and approximately 750 patients are admitted per year. Patient-based surveillance is performed actively and daily by an infection control nurse. Infection control physician visits the MICU twice a week and there are reminder posters about infection control measures and hand hygiene on the walls. Also there is a routine infection control education program for all health-care personnel twice a year. Standard infection control measures are used for all patients. Due to MRSA endemicity, all patients in ICUs have a daily bath with chlorhexidine. However, there is no isolation room for colonized patients and HCWs try to apply other contact measures (hand hygiene, use of gowns and gloves, etc.) for MRSA colonized or infected patients. Patient/nurse ratio is 4/1 in a shift. There are 3 washbasins for 9 beds and alcohol-based solutions are placed at the bedsides of all patients.

**Patients**

Patients (>16 years) admitted to the MICU were screened for MRSA on admission (in the first 48 hours), at the end of first week, and at ICU discharge using anterior nares, axilla, and groin swabs. Patients who stayed shorter than 48 h and already had MRSA colonization or infection were not evaluated for risk factors. Patients colonized only with MRSA were included into the study. Furthermore, HCWs who were close contact with these patients were screened for nasal colonization at the beginning and end of their ward rotation.

Data collected for risk factors included age, sex, APACHE II score, SOFA score, underlying diseases (diabetes mellitus, hypertension, cardiac disease, chronic obstructive lung disease, chronic renal failure, cirrhosis, cerebrovascular disease, active neoplastic disease, trauma, neutropenia, and immunosuppressive therapy), length of stay (LOS) in MICU before colonization, previous antibiotic use, length of antibiotic therapy before colonization, catheterization (peripheral, central venous, arterial, Swann-Ganz, and urinary), administration of total parenteral nutrition (TPN), intubation, mechanical ventilation, application of tracheostomy, presence of nasogastric tube, presence of prosthesis, prior surgery, presence of an open wound, and decubitis ulcer.

This study was approved by the local ethical committee of Erciyes University (Approval date:04 May 2004; Number:132).

**Microbiological studies**

**Microorganism identification**

MRSA isolates were preliminarily identified at the Bacteriology Laboratory of Erciyes University Hospital. Swabs were processed using selective mannitol salt agar with 6 mg/L of oxacillin for 24 h at 37 °C. Methicillin-resistant colonies were subcultured on 5% sheep blood agar for 18 h at 37 °C and underwent a rapid slide agglutination test for coagulase and protein A (Staphaurex; Plus).

All catalase positive gram positive isolates from clinical samples were tested for methicillin resistance by oxacillin (1 μg, Oxoid, UK) disk diffusion test. The rapid slide agglutination test for coagulase and protein A (Staphaurex; Plus) were performed for species identification. The staphylococci identified as MRSA were stored at –80 °C in microbanks until analysis.

**Molecular typing**

Strains isolated from colonized patients (colonized on admission and colonized in MICU) were evaluated for genetic relations. Genotyping analysis was performed by pulsed-field gel electrophoresis (PFGE) at the Molecular Laboratory of Infectious Diseases Unit, Hacettepe University, Ankara, Turkey.

First, isolates were subcultured to blood agar to ensure viability and purity. MRSA DNA embedded in agarose blocks was prepared as described by Lencastre et al. (9). The DNA fixed in the agarose disk was incubated for 20 h in 45 μL of restriction buffer and Sma I (20 U). The reaction was stopped with 5 μL of loading buffer. Gels with 1.1% agarose (Genexis Spech bach, Germany) were prepared in 0.5 × TBE buffer (50 mM boric acid, 0.2mM EDTA). PFGE was carried out by General Navigator, Pharmacia (Uppsala, Sweden). The running conditions were performed for 22 h at 14 °C at 150 V. Pulse times were 20 s for 15 h, 35 s for 7 h, 50 s for 15 h, and 90 s for the last 3 h. PFGE patterns were assigned to the same clone and clonal variant. Strains that differed by up to (and including) 6 bands were considered to belong to different clonal variants; strains that differed by more than 6 bands were considered to belong to different clones. Clones and clonal variants were designated by letters and by numbers in suffix, respectively (10).

**Definitions**

**MRSA colonization:** If any of the following cultures, anterior nares, axilla, or groin, was positive for MRSA , the patient was accepted as colonized. If the admission culture was positive for MRSA, it was labeled as "colonized on admission". On the other hand, if the admission culture was negative for MRSA and any of the following cultures, anterior nares, axilla or groin, was positive for MRSA during ICU stay, it was defined as "colonized during MICU stay".

**MRSA infection:** The diagnosis of infections was based on the criteria of the Centers for Disease Control and Prevention (CDC) (11). If the patient had the infection signs and symptoms with the yield of MRSA in culture (blood, sputum, urine, operation site, etc.), MRSA infection diagnosis was placed. For the diagnosis of ventilator associated pneumonia (VAP), quantitative cultures (the cut off value was $>10^5$) of endotracheal aspirates were used.

### Statistical analysis

Data were given as mean ± SD or median (minimum-maximum) and a P value of <0.05 was accepted as significant. Continuous variables were compared using Student's t test or the Mann-Whitney U test, when appropriate. Chi-square test was used for categorical variables. Univariate and multiple logistic regression analysis (forward stepwise) was performed for significant risk factors of MRSA acquisition. Variable with $P < 0.10$ value in the univariate logistic regression analysis included in the multiple logistic regression analysis. All analyses were carried out using SPSS 13.0.

## Results

During the study period, 408 patients were admitted to the MICU and admission swabs were taken from all patients in the first 48 h. A total of 126 patients stayed in the MICU less than 48 hours and 23 (5.6%) patients were already colonized on admission. All these colonized patients had been transferred from other wards of the hospital.

Overall 259 patients were evaluated for risk factors of MRSA acquisition. The median age was 57.2 years with a range of 16-95 years. One hundred thirty six (52.5%) of the patients were male, whereas 123 (47.5%) were female. The median length of stay in the MICU was 10.1 days (range 3 to 111 days) and the median length of stay in the hospital was 18.3 days (range 3 to 113 days). Forty seven percent of 259 patients were transferred from other parts of the hospital to MICU.

While 137 patients stayed in the MICU for less than 7 days, 122 patients stayed longer. MRSA isolated from 28 (27.5%) of 102 swabs taken at the first-week and 20 (16.3%) (3 of these patients colonized in the first week) of 123 discharge swabs. Overall, 48 (18.5%) patients got colonized with MRSA during MICU stay and 64.6% of the patients colonized at the first week. The anterior nares were the most frequent site of MRSA colonization; 42 (87.5%) of the patients had only nasal colonization, whereas 1 patient had axilla and groin colonization, 3 patients had only axilla and 2 had only groin colonization.

MRSA infection occurred in 15 (31%) of 48 colonized patients. In 4 patients, MRSA infection developed unless colonization was determined. The majority of MRSA infections were VAP (12 of 19 patients), the others were bacteremia (6 of 19 patients) and soft-tissue infection (1 of 19 patients). The median time for the development of MRSA infection was 15.3 days (range 4 to 53 days) after admission.

Nasal carriage of MRSA was not detected in HCWs during the study period.

### Risk factors for MRSA colonization

In the univariate analysis of the study, underlying diseases were not found as significant risk factors for acquisition of MRSA ($P > 0.05$) (Table 1). The significant risk factors were LOS in the MICU before colonization, previous use of antibiotic, length of antibiotic therapy before colonization, administration of total parenteral nutrition, intubation, mechanical ventilation, and application of tracheostomy (Table 2).

Table 1. Evaluation of underlying diseases for MRSA colonization in the univariate analysis.

| Risk factors | MRSA colonized patients (n = 48) | MRSA non-colonized patients (n = 211) | P value* |
|---|---|---|---|
| Hypertension | 16 (33.3% ) | 82 (38.9%) | 0.584 |
| Diabetes mellitus | 12 (25%) | 56 (26.5%) | 0.970 |
| Cardiac disease | 8 (16.7%) | 36 (17.1%) | 1.000 |
| Chronic obstructive lung disease | 8 (16.7%) | 37 (17.5%) | 1.000 |
| Chronic renal failure | 8 (16.7%) | 27 (12.8% ) | 0.635 |
| Cirrhosis | 3 (6.3%) | 40 (19%) | 0.055 |
| Cerebrovascular disease | 14 (29.2%) | 42 (19.9%) | 0.225 |
| Active neoplastic disease | 5 (10.4%) | 33 (15.6%) | 0.486 |
| Neutropenia | 0 (0.0% ) | 8 (3.8 %) | 0.358 |

Table 2. Risk factors for MRSA colonization in the univariate analysis.[&]

| Risk factors | MRSA colonized patients (n = 48) | MRSA non-colonized patients (n = 211) | P value* |
|---|---|---|---|
| Age med (min - max) | 64 (16 – 86) | 64 (16 – 95) | 0.502 |
| Male (n = 136) | 30 (62.5%) | 106 (50.2%) | 0.169 |
| Female (n = 123) | 18 (37.5%) | 105 (49.8 %) | 0.169 |
| APACHE-II (mean ± SD) | 23.27 ± 8.09 | 22.94 ± 8.38 | 0.802 |
| SOFA (mean ± SD) | 7.13 ± 3.05 | 6.95 ± 3.81 | 0.730 |
| LOS in the MICU before colonization med (min -max) | 15 (4 – 111) | 5 (3 – 51 ) | 0.001 |
| Previous antibiotic use | 45 (93.8%) | 149 (70.6 %) | 0.002 |
| Length of antibiotic therapy med (min - max) | 7 (1 – 26) | 5 (1 – 14 ) | 0.023 |
| Peripheral venous catheter | 33 (68.8%) | 124 (58.8 %) | 0.265 |
| Central venous catheter | 37 (77.1 %) | 129 (61.1%) | 0.056 |
| Arterial catheter | 6 (12.5 %) | 21 (10%) | 0.795 |
| Swan-Ganz catheter | 0 (0%) | 2 (0.9 %) | 1.000 |
| Urinary catheter | 46 (95.8%) | 186 (88.2%) | 0.190 |
| TPN | 38 (79.2%) | 124 (58.8%) | 0.013 |
| Intubation | 44 (91.7%) | 102 (48.3%) | 0.001 |
| Mechanical ventilation | 43 (89.6 %) | 100 (47.4%) | 0.001 |
| Tracheostomy | 18 (37.5 %) | 13 ( 6.2%) | 0.001 |
| Prosthesis | 2 (4.2 %) | 11 (5.2%) | 1.000 |
| Operation | 6 (12.5 %) | 34 (16.1%) | 0.686 |
| Trauma | 6 (12.5 %) | 12 (5.7%) | 0.114 |
| Open wound | 4 (8.3 %) | 20 (9.5%) | 1.000 |
| Decubitus ulcer | 4 ( 8.3%) | 13 (6.2%) | 0.529 |
| Immunosuppressive therapy | 7 (14.6%) | 24 (11.4%) | 0.710 |

[&] Mann-Whitney U test was performed for median values and Student's t-test was performed for mean ± SD values
*P < 0.05 was accepted significant
LOS: Length of stay; TPN: Total parenteral nutrition

In the univariate logistic regression analysis, LOS in the MICU before colonization, previous use of antibiotic, length of antibiotic therapy before colonization, central venous catheterization, administration of total parenteral nutrition, intubation, mechanical ventilation, and application of tracheostomy were found to be significant risk factors. However, in the multiple logistic regression analysis, only LOS in MICU before colonization and mechanical ventilation were significant risk factors for colonization (Table 3).

**Risk factors for MRSA infection**

In univariate analysis of the study, underlying diseases were not found as significant risk factors for MRSA infection (P > 0.05) (Table 4). Significant risk factors in univariate analysis were LOS in MICU before colonization, previous use of antibiotic,

Table 3. Risk factors for MRSA colonization in the multiple logistic regression analysis (forward stepwise).

| Risk factors | Odds ratio | 95% CI | P value* |
|---|---|---|---|
| LOS in the MICU before colonization | 1.083 | 1.040 - 1.129 | 0.001 |
| Mechanical ventilation | 4.186 | 1.359 - 12.895 | 0.013 |

*P < 0.05 was accepted as significant

Table 4. Evaluation of underlying diseases for MRSA infection in the univariate analysis.

| Risk factors | MRSA colonized patients (n = 19) | MRSA non-colonized patients (n = 240) | P value* |
|---|---|---|---|
| Hypertension | 7 (36.8 %) | 91 (37.9%) | 1.000 |
| Diabetes mellitus | 2 (10.5%) | 66 (27.5% ) | 0.173 |
| Cardiac disease | 3 (15.8%) | 41 (17.1%) | 1.000 |
| Chronic obstructive lung disease | 2 (10.5%) | 43 (17.9%) | 0.543 |
| Chronic renal failure | 3 (15.8%) | 32 (13.3%) | 0.729 |
| Cirrhosis | 2 (10.5%) | 41 (17.1%) | 0.748 |
| Cerebrovascular disease | 6 (31.6 %) | 50 (20.8%) | 0.260 |
| Active neoplastic disease | 3 (15.8%) | 35 (14.6%) | 0.747 |
| Neutropenia | 0 (0.0% ) | 8 (3.3%) | 1.000 |

administration of total parenteral nutrition, intubation, mechanical ventilation, application of tracheostomy, and MRSA colonization (Table 5).

In the univariate logistic regression analysis, LOS in the MICU before colonization, MRSA colonization, and application of tracheostomy were found as significant risk factors. However, in the multiple logistic regression analysis only MRSA colonization and application of tracheostomy were significant risk factors for infection (Table 6).

**Genetic relation**

A genetic relation was found in 48 isolates from colonized patients in MICU and 23 isolates from colonized patients on admission. Overall, 4 clones (clone A-D) were determined from colonized patients on admission; clone A (37%), clone B (32%), clone C (21%), and clone D (10%). Patients colonized during MICU stay had the same clones with the patients colonized on admission (clone A-30%, clone B-57%, clone C-13%).

## Discussion

MRSA infections have an important effect on patient morbidity, mortality, and incremental hospital costs. Moreover, the changing epidemiology of MRSA by its movement from hospitals to the community poses a public threat. The emergence of vancomycin resistant *S. aureus* and *Enterococcus* spp. is another major reason for the control of MRSA because vancomycin use and MRSA infection are important risk factors for vancomycin resistant *S. aureus* and *Enterococcus* spp. (12,13). Consequently, control of MRSA is an important issue in many hospitals.

Colonized and infected patients are the major reservoirs and are responsible for the colonization of their environment and the other patients. In previous reports, the prevalence of MRSA colonization in ICUs has been reported as 4%-52% (14-18). Furthermore, the colonization rate was high (18.5%) in our MICU and 64.6% of the patients colonized in the first week. The anterior nares were the most common

Table 5. Risk factors for MRSA infection in the univariate analysis.[&]

| Risk factors | MRSA colonized patients (n = 19) | MRSA non-colonized patients (n = 240) | P value* |
|---|---|---|---|
| Age med (min - max) | 71 (19-86) | 63 (16-95) | 0.806 |
| Male ( n = 136) | 12 (63.2%) | 124 (51.7%) | 0.467 |
| Female (n = 123) | 7 (36.8%) | 116 (48.3 %) | 0.467 |
| APACHE-II (mean ± SD) | 22.63 ± 6.52 | 23.03±8.45 | 0.899 |
| SOFA (mean ± SD) | 7 ± 2.57 | 7 ± 3.75 | 0.737 |
| LOS in the MICU before colonization (colonization or infection?) med (min -max) | 21 (8 – 111) | 6 (3 – 89) | 0.001 |
| Previous antibiotic use | 19 (100%) | 175 (72.9 %) | 0.005 |
| Length of antibiotic therapy med (min - max) | 6 (2 – 14) | 7 (1 – 26) | 0.773 |
| Peripheral venous catheter | 14 ( 73.7%) | 143 (59.6 %) | 0.334 |
| Central venous catheter | 16 (84.2 %) | 150 (62.5 %) | 0.099 |
| Arterial catheter | 3 (15.8%) | 24 (10 %) | 0.430 |
| Swan-Ganz catheter | 0 (0 %) | 2 (0.8 %) | 1.000 |
| Nasogastric tube | 7 (36.8 %) | 125 (52.1%) | 0.298 |
| Urinary catheter | 18 (94.7%) | 214 (89.2%) | 0.703 |
| TPN | 17 (89.5%) | 145 (60.4%) | 0.023 |
| Intubation | 19 (100%) | 127 (52.9%) | 0.001 |
| Mechanical ventilation | 19 (100 %) | 124 (51.7%) | 0.001 |
| Tracheostomy | 12 (63.2 %) | 19 (7.9%) | 0.001 |
| Prosthesis | 0 (0%) | 13 (5.4%) | 0.607 |
| Operation | 1 (5.3%) | 39 (16.3%) | 0.324 |
| Trauma | 1 (5.3%) | 17 (7.1%) | 1.000 |
| Open wound | 1 (5.3 %) | 23 (9.6%) | 1.000 |
| Decubitus ulcer | 3 (15.8%) | 14 (5.8%) | 0.118 |
| Immunosuppressive therapy | 2 (10.5%) | 29 (12.1%) | 1.000 |
| MRSA colonization | 15 (78.9 %) | 33 (13.8 %) | 0.001 |

[&] Mann-Whitney U test was performed for median values and Student's t-test was performed for mean ± SD values
*P < 0.05 was accepted as significant,
LOS: Length of stay; TPN: Total parenteral nutrition

Table 6. Risk factors for MRSA colonization in the multiple logistic regression analysis (forward stepwise).

| Risk factors | "Odds ratio" | % 95 CI | P value* |
|---|---|---|---|
| MRSA colonization | 14.080 | 3.896 – 50.884 | 0.001 |
| Tracheostomy | 8.245 | 2.454 – 27.703 | 0.001 |

*P < 0.05 was accepted as significant.

colonization site (87.5%) in our study, which is in line with the literature. The low concentrations of systemically administered antibiotics in the nares allow the persistence of the microorganism. The daily bath with chlorhexidine in our ICUs may explain the low colonization rate of axilla and groin. Nasal decolonization with mupirocin in ICUs is an unresolved question and there is poor evidence for its use. On the other hand, widespread and prolonged use of mupirocin in these settings has been associated with the development of low- and high-level resistance (19). HCWs can be carrier of MRSA. However, we did not detect any HCW nasal carriage in our study, which is parallel with previous studies reporting low rates (19,20). This may be explained by transient or short term carriage. There is no evidence to support routine staff screening in endemic settings. Also nasal decolonization of HCW in endemic settings is not recommended due to recolonization (19).

Identifying the risk factors for MRSA colonization and infection will help to focus on infection-control measures at high-risk groups for MRSA colonization and infection. Despite our efforts to include all risk factors for MRSA colonization or infection, we still could not include all the risk factors (e.g. steroid use, transfer from another unit) and this was a limitation of the study. The length of stay in the hospital and ICU, serious underlying diseases, previous antibiotic therapy, use of invasive devices, and trauma were cited among the major risk factors for the colonization and infection of MRSA in previous studies (14,18,20,21). However, none of the underlying diseases was found to be a risk factor for colonization and infection with MRSA in our study. The length of stay in MICU before colonization, previous use of antibiotic, length of antibiotic therapy before colonization, administration of total parenteral nutrition, intubation, mechanical ventilation, and application of tracheostomy were found as risk factors for colonization in univariate analysis; however, only the length of stay in MICU before colonization and mechanical ventilation were found to be the most significant risk factors in the multiple logistic regression analysis. Probably, the high contact rate of HCWs and performing more invasive procedures increase colonization rates in ventilated patients. Therefore, HCWs should pay more attention to infection control measures in ventilated patients and in those who stay long in ICUs. On the other hand, only MRSA colonization and tracheostomy were found to be significant risk factors for the MRSA infection. In the literature, a 10-fold increase in the rate of infection in colonized patients has been reported (22), and in our study MRSA infection occurred in 31% of colonized patients with a 14-fold increase. The most common MRSA infection was VAP in this study. Also MRSA is an important cause of VAP with high mortality in many ICUs (23-25). Although we did not calculate the attributable mortality for MRSA infections, the prognosis of MRSA infections was previously found poorer than methicillin-susceptible *S. aureus* infections (25). Despite appropriate glycopeptide therapy, there is an increased attributable mortality for MRSA pneumonia (26). Also, MRSA bacteremia was associated with significantly higher mortality rate compared to MSSA bacteremia. In a study from Turkey, investigators showed that methicillin resistance was one of the independent risk factors for high mortality rate (27).

Previous antibiotic use and length of antibiotic therapy were not found as important risk factors for colonization and infection in multiple logistic regression analysis in this study. However, higher antibiotic consumption is usually related to higher rates of antibiotic resistant microorganisms as well as MRSA in previous studies (14,16). Reduction of antibiotic selection pressure may have a beneficial effect on MRSA colonization. In addition, rational antibiotic therapy is an important issue to reduce antibiotic costs in countries with limited-resources.

Colonized patients are the main reservoir for the spread of MRSA from patient to patient, and HCWs' hands are the most common way of the spread. In our study, 5.6% of patients were already colonized on admission. All these patients had been transferred from other parts of the hospital and there were only 4 clones (clones A, B, C, and D). Moreover, strains from patients colonized during MICU stay were identical to these strains (clones A,B, and C). These results show the spread of isolates from patient-to-patient in the hospital and ICU from colonized patients. However, in this study we did not provide an estimation of colonization pressure during our study

period; previous reports show the impact of colonization pressure and high workload on patient acquisition of MRSA (28,29). An effective infection control program is needed to prevent and control MRSA colonization and infection in the ICU. Active surveillance of newly admitted patients for MRSA and rapid and effective isolation and treatment ("search and destroy policy") may help control the spread of MRSA (30,31). Furthermore, active surveillance cultures and contact/droplet precautions for control of MRSA was found cost-effective (32). The lack of isolation facilities is a common problem in developing countries. However, the success of single-room isolation is controversial in endemic settings and there are detrimental effects on patient care in ICUs, especially in understaffed centers. The isolation rooms may improve the compliance of HCWs to contact precautions (19,33,34).

The major mode of transmission from patient-to-patient is through contaminated hands of HCWs, so the hand hygiene compliance of HCWs and standard precautions are important infection control measures to control MRSA in endemic settings. In many studies, compliance of hand hygiene is poor (<%50) and many factors influence the compliance. In developing countries understaffing, high workload, poorly accessible sinks, and expensive alcohol-based hand disinfectants are the major barriers for the hand hygiene compliance, and so adequate compliance is practically impossible (35). However, we did not observe the hand hygiene compliance of HCWs; the dissemination of MRSA in MICU showed the low compliance despite the alcohol-based products placed at the bedsides and 3 washbasins for 9 beds. Probably understaffing (patient:nurse ratio was 4:1) and heavy workload are important reasons for low compliance and healthcare associated infections in our MICU. Also in many studies from Turkey, compliance with hand washing was low (12%-34 %) and related to low staff-to-patient ratio, excessive use of gloves, and deficiencies in the infrastructure of ICU (36-41). Hugonnet et al. (42) showed that 26.7% of all infections acquired in the ICU could be prevented providing that around the clock nurse-to-patient ratio is maintained more than 2.2:1. Moreover, in a recent study, investigators examined the infection control practices in the hospitals that had low and high MRSA rates in the Mediterranean, including Turkey. They found that there was no significant correlation between the infection control set-up, hand hygiene facilities, and antibiotic stewardship practices between the hospitals. However, hospitals reporting frequent episodes of overcrowding, particularly involving several departments, and having on-going difficulties in providing isolation beds had significantly higher MRSA proportions. It was reported that the mere presence of infection control teams, suitable hand hygiene facilities, as well as antibiotic stewardship initiatives does not necessarily guarantee the adequate control of multiresistant infections such as MRSA (43).

This study shows the clonal spread of MRSA in an ICU and supports the results of previously published reports. Despite the efforts to improve hand hygiene, such as reminder posters, bedside alcohol-based products, and staff education, the problem emerges because of understaffing and high workload in developing countries.

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