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Molecular typing of methicillin-resistant Staphylococcus aureus isolated from bloodstream infections in a university hospital

Aim: Bloodstream infections due to methicillin-resistant Staphyococcus aureus (MRSA) strains are one of the major problems in many hospitals. Molecular typing provides very useful information about the origin and the spreading ways of strains. The aim of the present study is to assess the clonal relationship amongst MRSA strains isolated from bloodstream infections of patients in Turgut Özal Medical Center, İnönü University in Turkey.

Materials and methods: A total of 37 consecutive MRSA strains were identified from the blood cultures from January to December 2003. Methicillin resistance was confirmed with amplification of the mecA gene by polymerase chain reaction (PCR). Clonal relatedness of the strains was investigated by arbitrarily primed polymerase chain reaction (AP-PCR) and pulsed-field gel electrophoresis (PFGE).

Results: Of the 37 mecA positive S. aureus strains identified in a 1-year period, 29 (78.3%) were from intensive care units (ICUs) and the remaining 6 from other wards. The MRSA strains were resistant to most clinically useful anti-staphylococcal agents. AP-PCR and PFGE typing methods indicated that 67.6% and 60.7% of the typed strains were clonally related, respectively. Clonally related strains were not restricted in a specific clinic and period.

Conclusion: Our findings indicated that MRSA bloodstream infections in our hospital were not originated from any predominant clone and AP-PCR typing can be used to screen clonal relatedness of these strains. The present data showed that there was no predominant MRSA clone in our hospital. However, because of the high rates of MRSA and clonally related strains, the infection control practices were reconsidered and more strict rules were proposed to the infection control committee to eliminate the spread of these strains between wards in our hospital.

Key words: Methicillin-resistant Staphyococcus aureus, bloodstream infections, AP-PCR, PFGE

Bir üniversite hastanesinde kan dolaşımı enfeksiyonlarında izole edilen metisilin-dirençli Staphylococcus aureus izolatlarının moleküler tiplendirilmesi

Amaç: Metisilin-dirençli Staphyococcus aureus (MRSA) suşlarının neden olduğu kan dolaşımı enfeksiyonları pekçok hastanedeki major problemlerden biridir. Moleküler tiplendirme suşların kaynağı ve yayılımı ile ilgili çok önemli bilgiler sağlamaktadır. Bu çalışmanın amacı İnönü Üniversitesi Turgut Özal Tıp Merkezi'ndeki kan dolaşımı enfeksiyonlarından izole edilen MRSA suşlarının klonal benzerliğini araştırmaktır.

Yöntem ve gereç: Ocak-Aralık 2003 tarihleri arasında kan kültürlerinden izole edilen 37 MRSA suşu incelenmiştir. Metisilin direnci, polimeraz zincir reaksiyonu (PCR) yöntemiyle suşların mec A geni çoğaltılarak doğrulanmıştır. Suşların klonal benzerliği arbitrarily primed polymerase chain reaction (AP-PCR) ve pulsed-field gel electrophoresis (PFGE) yöntemleriyle araştırılmıştır.

Bulgular: Bir yıllık periyodda 37 MecA pozitif S. aureus suşu tanımlandı. Bunların 29 (% 78,3)'u yoğun bakım ünitelerinden, 6'sı çeşitli servislerden izole edilmiştir. MRSA suşları klinikte kullanılan birçok antistafilokok antibiyotiğe dirençliydi. AP-PCR ve PFGE tipleme metodları suşların sırasıyla % 67,6 ve % 60,7 oranında klonal olarak ilişkili olduğunu göstermiştir. Klonal olarak benzer suşlar belli bir klinik ya da belli bir periyodda yığılma göstermemiştir.

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Sonuç: Hastanemizde kan dolaşımı enfeksiyonu etkeni olan MRSA suşları arasında dominant bir klon olmadığı ve AP-PCR tipleme yönteminin suşların klonal ilişkisini saptamada bir tarama metodu olarak kullanılabileceği sonucuna varılmıştır. Çalışmanın sonuçları hastanemizde predominant herhangi bir MRSA klonunun olmadığını göstermektedir. Fakat yüksek MRSA izolasyonu ve klonal ilişkili suşların çokluğu nedeniğle; infeksiyon control önlemleri yeniden gözden geçirildi ve suşların hastanemizdeki klinikler arasında yayılımını engelemek için infeksiyon control komitesine daha sıkı tedbirler önerildi.

Anahtar sözcükler: Metisilin dirençli Staphyococcus aureus, kan dolaşım enfeksiyonları, AP-PCR, PFGE

Introduction

Severe infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) are serious problem for health care institutions. All β-lactams and β-lactam/β-lactamase inhibitor combinations and carbapenems are clinically ineffective against methicillin resistant staphylococcal infections. Methicillin resistance primarily results from the production of an altered penicillin binding protein (PBP2a), which is usually a direct consequence of the acquisition of another penicillin-binding protein (PBP 2a) gene, namely *mecA* (1,2). Today there are new clones showing limited resistance to antibiotics and even old multiple resistant clones, including aminoglycosides, macrolides, clindamycin, and tetracycline, has become less resistant thus limiting available therapeutic options (3).

The rate of MRSA considerably varies amongst different geographic areas. Relatively high proportions of MRSA have been reported from hospitals in central and southern Europe while such strains seem to be much less frequent in northern European countries (4). In another review, the prevalence of MRSA was recorded as 20% in Europe and 33%-55% in the United States (5).

International epidemiologic surveillance requires reliable techniques that can differentiate sporadic strains from clonally related ones. Many genotyping techniques have been developed for studying the clonal relationship among MRSA (6,7). AP-PCR has been widely used for genetic analysis and monitoring of nosocomial spreading of staphylococci because it is one of the easy and rapid typing methods (6,8-10). However, PFGE has been accepted as a "gold standard" for confirmation relatedness among strains (11). Data about clonal relation among the strains isolated from severe infections can help to improve more effective control precautions to prevent spread of these resistant strains from patients to patients in a hospital. The aim of the present study was to assess clonal relatedness among the blood stream isolates from inpatients in Turgut Özal Medical Center, İnönü University in Turkey.

Methods

Data recording

MRSA infection: Patients with clinical signs and symptoms of an infection and a positive MRSA sample from a corresponding culture. The decision, whether it was a MRSA infection, was made by the treating physician in case of doubt.

Nosocomial MRSA cases: As a uniform approach the cases were considered as nosocomial when the positive clinical specimen or screening culture was taken more than 48 h after admission to the hospital and no previous positive MRSA result was available. All MRSA and methicillin sensitive *S. aureus* (MSSA) strains in this study were the strains of proved nosocomial bloodstream infections.

Nosocomial MRSA incidence density was defined as nosocomial MRSA cases per 1000 patient days.

MRSA strains: A total of 37 consecutive MRSA strains were identified amongst the 109 *S. aureus* strains isolated from 5360 blood cultures from January to December 2003 in a teaching hospital with a 690-bed capacity and 9 intensive care units (ICUs). ICUs have 72-bed capacity. One hundred and nine *S. aureus* strains were isolated from blood cultures of 109 patients. Repeated isolation of the same strains in one patient was not included in the study. Epidemiologic data of all patients with MRSA sepsis were collected prospectively (Table).

Table. Comparison of epidemiologic and genotyping data.

Strains #	Service[a]	Diagnosis at admission[b]	Major invasive procedures[c]	Lenght of time since admission to infection	Isolation date	AP-PCR results	PFGE[d]	White blood cell/mm^3	Temperature (°C)
1	Urology	Bladder Ca, Ileus	Urin.bladder cat., colostomy	3	13.04.03	A	1	14900	39
2	Re ICU	FUO	Pacing in cardiology	7	09.06.03	A	1	11300	38
3	Ns ICU	Fulminant hepatitis, hearth insufficiency (ex)	Tracheostomy	36	18.07.03	A	-	18200	38
4	Re ICU	COPD	IV cat.	32	04.06.03	a1 (subtype)	1a (pr)	15000	38
5	Ts	Traffic accident	Thorax tube	90	06.07.03	B	2	16000	37.5
6	Re ICU	CRF	Dialysis	10	08.06.03	B	2	10000	38
7	Nephrology	CRF	IV cat.	3	18.12.03	C	-	13000	38
8	Im ICU	SAH, Postoperative aneurysma	IV cat.	20	24.12.03	C	-	14000	37.5
9	Im ICU	Meningitis	IV cat.	3	26.12.03	C	-	15000	37
10	Im ICU	Traumatic SAH	IV cat.	5	08.09.03	D	3	13500	37
11	Gs ICU	Stomach Ca, Fever	Gastrostomia	30	22.09.03	D	3	2800	38
12	Nephrology	Traffic accident.	Central cat.	5	09.08.03	e	4	14000	37
13	Re ICU	Multiple trauma	Abd.tube, Sigmoid resection.IV cat	7	26.09.03	e1 (subtype)	5	23500	39
14	Re ICU	Intracranial Tm	Tracheostomy Thorax tube	47	01.09.03	F	6	14200	38
15	Ca ICU	Cardiac arrest, Encephalopathy	Mech.ventilation	18	05.09.03	f1 (subtype)	-	24400	38
16	Re ICU	Traffic accident Colon Ca	Colonoscopy, multiple abrasion	90	10.08.03	g	7a (cr)	15000	39
17	Re ICU	SAH	Tracheostomy Mech. ventilation	135	10.07.03	g1(subtype)	7b (cr)	16700	38
18	Re ICU	COPD, DM	Tracheostomy	30	15.06.03	g2 (subtype)	-	19200	39
19	Gs ICU	Postoperative ileus (sepsis, ex)	Intubation	8	08.09.03	H	8	14800	38.5

Table. (Continued).

Strains #	Service[a]	Diagnosis at admission[b]	Major invasive procedures[c]	Lenght of time since admission to infection	Isolation date	AP-PCR results	PFGE[d]	White blood cell/mm^3	Temperature (°C)
20	Hematology	DIC(ex)	Mech. ventilation	11	19.09.03	H	8	10500	38
21	Gs ICU	Mesenteric ischemia	Trachea tube, IV cat.	80	30.12.03	I	9a (cr)	20000	39
22	Ns ICU	SAH	IV cat.	65	30.12.03	I	9b (cr)	22000	39
23	Ns ICU	SAH	Intubation, Ventricular drainage, Tracheostomy	42	18.12.03	i1 (subtype)	10	13000	38
24	Re ICU	Sigmoid Ca, Sepsis (ex)	Abdominal cat.	7	28.08.03	i2 (subtype)	10	7000	38
25	Gs ICU	Intra abdominal abscess	IV cat	68	20.10.03	i3 (subtype)	-	11000	39
26	Re ICU	Staphylococcal Pneumonia	IV cat.Bronchoscopy	90	28.10.03	J	11	20000	37.5
27	Re ICU	Gun shut wound	IV cat.	13	12.09.03	K	_	15000	39
28	Re ICU	Multiple trauma	IV cat, NGT	25	10.01.03	L	12	18000	39
29	Re ICU	Traffic accident, Brain edema	Tracheostomy NGT	17	18.07.03	M	-	23500	38
30	Re ICU	Brain edema, SAH	Tracheostomy	46	09.09.03	N	8a (pr)	11500	38
31	Hematology	AML	IV cat.	34	11.06.03	O	13	30000	38
32	ORL	Rhinocerebral Mucormucosis (ex)	IV cat.	7	26.09.03	P	14	20000	39
33	Re ICU	Hypophysis adenoma	IV cat.	37	02.10.03	R	15	17000	38.5
34	Ns ICU	Hypophysis adenoma	Central Venous cat, percutaneous gastrostomy	47	28.09.03	S	16	9300	36
35	Pediatrics	Meningitides	IV cat	7	02.09.03	T	17	15600	38
36	Re ICU	SAH	Tracheostomy, NGT, decubitus	16	25.08.03	U	18	10500	38
37	Cv ICU	Aortic dissection	TPN	30	02.08.03	V	8b (cr)	18000	38.5

[a]ICU: Intensive care unit, Re: Reanimation, Gs: General surgery, Cv: Cardiovascular surgery, Ns: Neurosurgery, Im: Internal medicine, Ts: Thoracic Surgery, Orl: Otorhynolaringology; Ca: Cardiology

[b]Ca: Cancer;FUO: Fever of unknown origin; COPD: Chronic obstructive pulmonary disease; CRF: Chronic Renal Failure, SAH: Subarachnoid Hemorrhage, Tm: Tumor , DM: Diabetes mellitus; DIC: Disseminated intravascular coagulation; AML: Acute Myelogenous Leukemia,

[c]Cat: Catheter ;IV cat: Intravenouse catheter, Abd: Abdominal, Mech: Mechanical ;NGT: Nasogastric Tube, TPN: Total Parenteral Nutrition

[d]Pb: Probably related, cr: Closely related

All the strains were considered as nosocomial infections based on the criteria, such as patient's hospitalization time, fever, white blood cell count, and response to antibiotic treatment. Further research of colonization from the patients or their surroundings was not performed. Only the strains proven to cause sepsis were included in the study. Indwelling catheter cultures were not included in the study. Epidemiologic studies of MSSA strains were not performed.

Species identification was carried out using Gram staining, catalase test, and the tube coagulase test. Methicillin resistance was firstly searched using 1 μg oxacillin disks (Oxoid) on Mueller-Hinton agar following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (12). Minimal inhibitory concentrations of oxacillin were determined with the broth microdilution method (13). All oxacillin resistance strains were screened for *mecA* gene by the PCR method described previously (14).

Susceptibility of the strains to other antibiotics: The standardized disk-diffusion method was performed on Mueller-Hinton agar, according to the Kirby-Bauer method, and following the criteria of CLSI (12). *S. aureus* ATCC 25923 was used as standard strain. The antibiotic disks (Oxoid) used were ciprofloxacin, erythromycin, gentamicin, rifampicin, tetracycline, trimethoprim/sulphamethoxazole, clindamycin, vancomycin, and teicoplanin.

Molecular typing of the MRSA strains:

AP-PCR typing: We used AP-PCR genotyping procedure optimized previously (15,16). Briefly, bacterial cells were lysed by proteinase K (500 μg/ml) and 0.5% laroxyl sarcosine in TE buffer [(10mM Tris-HCl, 1mM EDTA (pH 8.0)]. Then phenol-chloroform extraction and ethanol precipitation were performed. AP-PCR reaction mixture (50 μl) contained 100 ng of genomic DNA, 100 pmol of M13 primer (5'-GAG GGT GGC GGT TCT-3'), 2.5 unit of *Taq* DNA polymerase (Promega Corporation, USA), 200 μmol deoxynucleoside triphosphate mix, 10 mM Tris-HCl (pH: 8.0), 50 mM KCL, 2.5 mM $MgCl_2$. Amplification was performed with a Thermal Cycler (MJ Research Inc. PTC-200, Peltier Thermal Cycler Massachusetts, USA) according to the following conditions: 2 cycles, each consisting of 5 min at 94 °C, 5 min at 40 °C, and 5 min at 72 °C; 40 cycles, each consisting of 1 min at 94 °C, 1 min at 40 °C, and 2 min at 72 °C. Amplification products were electrophoresed on 1.5% agarose gel with ethidium bromide and were visualised under UV illumination. To provide optimal experimental conditions, all DNA samples were amplified using the same amplification master mix in the same thermal cycling program and electrophoresis conditions were similar for all strains. The band patterns of the strains were analyzed with GelCompar software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium). Dice coefficient was used to calculate similarities between the pairs of isolates [(number of the shared bands × 200)/total number of DNA bands in the 2 strains]. According to the similarity coefficient, strains were classified as the same type having a similarity coefficient of ≥ 90%, subtype having a similarity coefficient between 70% and 90%, and a different type having a similarity coefficient < 70% .

PFGE: PFGE typing was performed on 27 strains that were available stocks. Isolation and deproteinisation of the genomic DNA were carried out following the protocol of Maslow et al. (17), with a modification; lysostaphin (5 U/mL) was used instead of lysozyme. Genomic DNA in the plugs was restricted by 30 U of *Sma*I (Promega Corporation, USA) for 24 h at 25 °C in a water bath. DNA fragments were separated on 1% agarose gels run in 0.5× Tris-borate-EDTA buffer using a CHEF-DR II system (Bio-Rad Laboratories, Nazareth, Belgium). The electrophoresis conditions were 14 °C at 6 V/cm for 24 h. The initial and final switch times were 5 sec and 40 sec, respectively. The gel was stained with ethidium bromide and photographed under UV light. According to the interpretative criteria of Tenover et al. (18) the isolates were classified as indistinguishable (cluster), closely related, possibly related, or different.

Results

A total of 37 *mecA* positive *S. aureus* strains were identified in a 1-year period (37 isolates from 37 different patients). The ratio of MRSA in all *S. aureus* strains isolated from bloodstream infection in this period was 34% (37/109). It was respectively 63% (29 MRSA in 46 *S. aureus* strains) in ICUs and 11.8% (8

MRSA in 68 *S. aureus* strains) in the other wards. Forty three percent of all the MRSA strains (16/37) were isolated from the reanimation ICUs (Figure1). All MRSA blood cultures were positive at least in both aerobic bottles, except only 1 patient gave positive signal in 1 bottle. The distribution of strains in services were as follows: 29 strains (78.3%) from ICUs, 2 (5.4%) from Nephrology, 2 (5.4%) from Hematology, 1 (2.7%) from Urology, 1 (2.7%) from Otorhinolaryngology, 1 (2.7%) from Thoracic Surgery, and 1 (2.7%) from Pediatrics wards (Figure1).

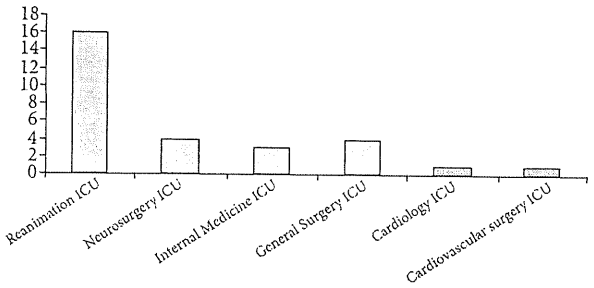


Figure 1. Distribution of MRSA in Intensive Care Units.

The MRSA incidence density was 0.43 MRSA cases per 1000 patient days, being the lowest in the department of Pediatrics (0.2 cases per 1000 patient days) and the highest in the department of Nephrology (5.5 cases per 1000 patient days). The mean MRSA incidence density was 0.7 in ICUs, 0.8 in Pediatric Surgery, 0.6 in Hematology, 0.5 in Chest Diseases, 0.4 in Otorhinolaryngology, and 0.3 in Urology. Epidemic curve of the MRSA and MSSA strains showed a strong increase in September and December (Figure 2). Distribution of these strains in the other months is presented in Figure 3.

All 37 *mecA*-positive isolates had oxacillin's MIC values that ranged from 4 to 64 mg/L. Twenty-seven (72.9%) of the isolates showed high-level resistance to oxacillin (MIC 16-64 mg/L). Resistance to oxacillin was most commonly accompanied by resistance to erythromycin, gentamicin, and ciprofloxacin. All MRSA isolates exhibited multi-drug resistant phenotypes that included the most clinically useful anti-staphylococcal agents. The resistance rates of

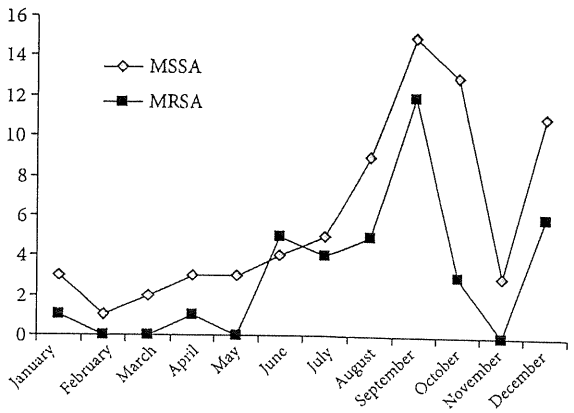


Figure 2. Epidemic curves of MRSA and MSSA strains.

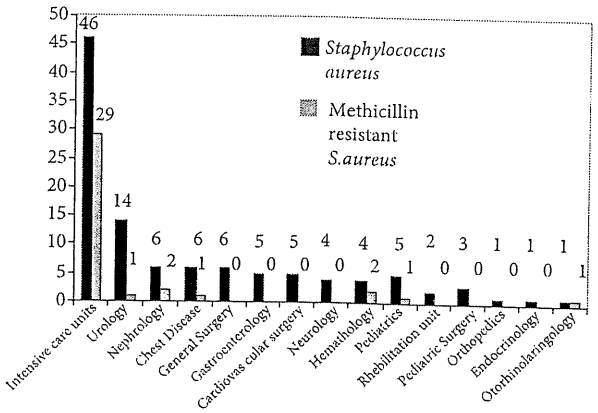


Figure 3. Distribution of MSSA and MRSA in all services.

MRSA strains were 97.2% to erythromycin, 89.1% to rifampicin, 78.3% to ciprofloxacin, 81.0% to gentamicin, 59.4% to tetracycline, 51.3% to clindamycin, and 35.1% to trimethoprim/sulphamethoxazole. There was no isolate resistant to glycopeptides.

All of the 37 MRSA strains were genotyped with AP-PCR and 28 of these strains were also typed with PFGE. AP-PCR and PFGE typing methods yielded 21 and 18 different genotypes, respectively. The rates of the isolates having clonal relation were found in 67.6% (25/37strains) and 60.7% (17/28 strains) by AP-PCR and PFGE typing, respectively. AP-PCR showed that 14 strains had a similarity coefficient equal to or greater than 90% and clustered into 6 groups (range of

groups was 2 to 3 strains), 11 strains were in 5 subgroups and 12 strains had unique profile (Figure 4). According to the PFGE protocol; 10 strains were classified in 5 clusters (range of cluster was 2 strains), 5 strains were closely related, 2 were possibly related, and 10 strains yielded a unique pattern. Of the 14 strains with the same AP-PCR profile, 8 were indistinguishable, 2 were closely related in PFGE, and 9 were not studied using PFGE. More detailed information regarding the comparison of these typing methods is provided in the Table.

Epidemiological data of the strains having a clonally related pattern with both PFGE and AP-PCR has shown that these strains were not restricted in a specific unit or ward. As can be seen in the Table, the strains 1 and 2 in the same AP-PCR profile (type a) and PFGE type 1 groups were isolated from Urology and Reanimation ICU, the strains 5 and 6 in the same AP-PCR (type b) and PFGE (type 2) groups were from Thoracic surgery and Reanimation ICU, respectively. A similar pattern was observed for the other 4 clustered strains. Considering the patients' isolates within the same services, there were 16 MRSA isolates in the Reanimation ICU; 11 had different genotype, 5 had subtype profile, and no strain showing an identical typing profile. According to the isolation date, the interval between the isolation periods of the strains that have identical genotypes varied from the same day (strains 21 and 22) to about 3-month apart (strains 1 and 3) (Table).

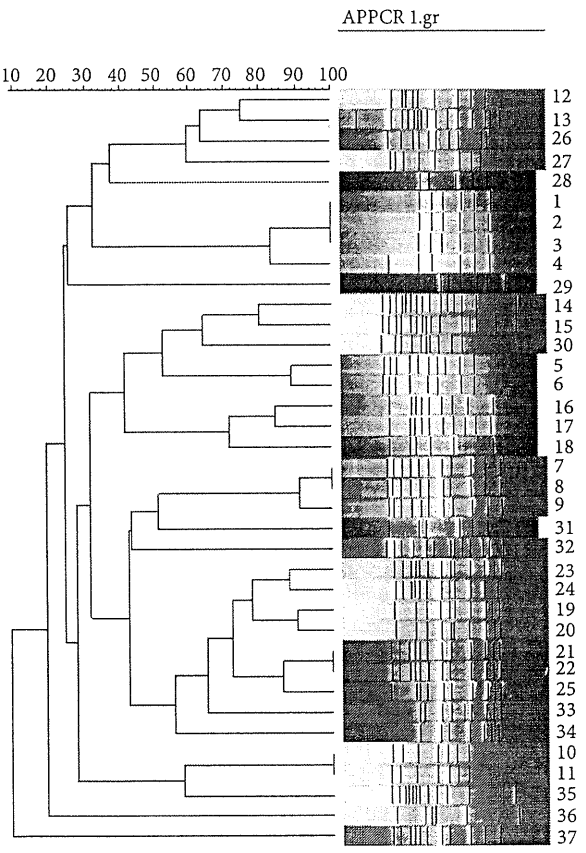


Figure 4. Dendrogram of the AP-PCR typing results. Fourteen strains (1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 19, 20, 21, 22) showing a similarity coefficient equal or greater than 90% were clustered in 6 groups, 11 strains were in 5 subgroups, and 12 strains had a unique profile.

Discussion

The rates of bloodstream infections caused by *S. aureus* have increased in recent years (19,20). Also, methicillin resistance in *S. aureus* isolates is a growing problem. MRSA strains have become the most prevalent and important antimicrobial resistant pathogen causing serious nosocomial infections throughout the world (20,21). In addition to previous studies reporting that MRSA bacteremia causes significant increases in the duration of hospitalization (19,20). Similarly, our study shows that the MRSA isolates cause infections in patients who are hospitalized for long periods of time and have invasive procedures. MRSA infections are also diagnosed in patients in intensive care units (20). In this study, MRSA was mostly observed in patients in intensive care units and it accounted for approximately one third (34%) of the *S. aureus* sepsis observed in 2003. In another study in our hospital, we determined 47 nosocomial sepsis with MRSA (43.5%) in a total of 108 *S. aureus* blood stream infections in 2004 (22). The rates of MRSA in sepsis varied from 24.8% to 42% in other hospitals from different countries (23,24).

The number of MRSA and MSSA strains was determined as 12 and 15, respectively, in September. In addition, 4 MRSA strains were determined from the ReICUs. We recognized that before sampling the blood for culture, 5 patients with 5 MRSA strains were transferred from the ReICUs to these services. As a result, ReICUs are responsible for 9 MRSA strains. However, only 3 isolates (10-11, 14-15, and 19-20)

were identical. Ten of the 15 MSSA strains were isolated from the ReICUs. Therefore, ReICUs are supposed to be responsible for the increase in the number of MRSA and MSSA cases in September. Since MRSA strains are mostly non-identical, it can be concluded as a polyclonal MRSA outbreak.

Regarding the nosocomial MRSA incidence density, our MRSA cases per 1000 patient days is comparable with the literatures (25). Four different university hospitals reported that nosocomial MRSA incidence density varied between 0.53 and 0.96 MRSA cases per 1000 patient days. We found 0.7 MRSA cases for ICUs and 0.43 MRSA cases per 1000 patient days in general.

MRSA isolates are usually resistant to various antimicrobial agents. Our MRSA isolates are resistant to more than one drug class. These results reflect heavy antibiotic use and confirm, as the general trend, that MRSA strains have a highly multi-antibiotic resistant state (26,27,2). A variety of epidemiological markers were used for typing MRSA. These include phenotypic methods (antibiotic resistance profile typing, phage typing) and DNA based methods (pulsed field gel electrophoresis, randomly amplified polymorphic DNA analysis) (6,7).

PFGE is a molecular method that is widely used in many hospitals and reference laboratories for typing MRSA, but it is time consuming and expensive [6]. A good correlation was observed in the current study between the AP-PCR and PFGE typing results. Three of the 10 strains have unique PFGE profile and were classified in subtypes by AP-PCR, the remaining 8 strains were also the same as AP-PCR. These strains were clustered by PFGE and were not found different in AP-PCR. From these results it can be concluded that AP-PCR can be preferable for screening clonal relatedness among the MRSA strains because of its rapidity, simplicity, and cost-effectiveness, as indicated previously (28), PFGE should be used to confirm the AP-PCR results that show clonal relation.

We demonstrated that 6 patients, hospitalized during the same time period in the same clinic, were infected by the same or the subtype strain (strain no:2-4, 8-9, 22-23). Other patients were hospitalized in different times or in different clinics. These findings have suggested that some strains were circulating and infecting factors for the patients with underlying disease or the patients with major invasive procedures particularly. The detection of the same strains in different clinics and at different times can be explained with a suspicious factor, such as the circulating carriers. These carriers appear to be devices that are shared by the patients, such as mechanical ventilator, or hands of hospital staff. Acquisition of the same MRSA strains in different clinics in a relatively close time interval can arise from reduced numbers of trained nurses and hygiene failures predominantly involving hand-touch sites. It has been reported that patient acquisitions increased 7 times more during periods of nurse understaffing (29).

Although there was a peak of MRSA sepsis at the 8^{th} and 9^{th} months, only the 19^{th} and 20^{th} strains were identical. During this period, the strains were mostly isolated in ICUs and General Surgery Clinic. In the peak period, these strains from Reanimation ICUs are mostly non-identical, on the other hand, the 3 of 4 strains from General Surgery ICUs are identical as AP-PCR. We expected that both MRSA and MSSA increased during relatively the same time interval due to temporary hygiene failure or nurse understaffing.

Investigations of the MRSA clones and resistance patterns are particularly useful in patient management and prevention from horizontal transmission. In a recent study performed on MRSA strains collected from multi-centers in Turkey indicated a single predominant clone in different regions of the country (30). Another study performed in a university hospital in Turkey also found a predominant MRSA clone (31). In contrast to these data, our data showed that there was no predominant MRSA clone in our hospital. However, because of the high rates of MRSA and clonally related strains, the infection control practices should be reconsidered and more strict rules were advised to the infection control committee for elimination of the spread of these strains among the wards in our hospital.

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