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Protein Profiles and Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Gülhane Military Academy Hospital in Turkey

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Abstract: The present study was undertaken to determine the relationship between Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains isolated from various clinical specimens in Gülhane Military Medical Academy, in Ankara, Turkey. In 1997, at this hospital, the percentage of *S. aureus* among all nosocomial isolates was 20% and the percentage of MRSA among these *S. aureus* isolates was 51%. Both sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Native-Page (N-PAGE) were used for typing MRSA isolates which have common antibiotic susceptibility test results. MRSA isolates were also evaluated according to the whole-cell proteins (WCPs) and filtrate proteins (FPs). Using these methods, we concluded that all of the MRSA strains are from the same clonal group. These results are consistent with the results of our previous study.

Gülhane Askeri Tıp Akademisi'ndeki Metisilin Dirençli *Staphylococcus aureus* (MRSA) Suşlarının Yayılımı ve Protein Profilleri

Özet: Bu çalışmamızda Gülhane askeri Tıp Akademisi'ndeki değişik klinik örneklerden izole edilen MRSA suşları arasındaki ilişki belirlenmiştir. 1997 yılında, bu hastanedeki bütün nosokomiyal izolatlar arasında *S. aureus* oranı %20, bu *S. aureus*'lar arasındaki MRSA (Metisilin dirençli *Staphylococcus aureus*) oranı ise %50 olarak belirlenmiştir. Ortak antibiyotik duyarlılıklarına sahip olan bu MRSA izolatlarının tiplendirimi için hem SDS-PAGE (sodyum dodesil sülfat poliakrilamid jel elktroforez) hem de N-PAGE (nativ poliakrilamid jel elektroforez) kullanılmıştır. Bu metodların neticesinde elde edilen sonuçlar MRSA suşlarının hepsinin aynı klonal gruptan orijinli olabileceğini düşündürmüştür ki elde edilen bu sonuçlar daha önceki çalışmalarımızda elde edilen sonuçlarla uyum içerisindedir (van Belkum A. van Leeuwen W, Werkooyen R, Saçılık SC, Cokmüş C, Verbrugh H: Dissemination of a single clone of methicillin-resistant *Staphylococcus aureus* among Turkish hospitals. Journal of Clinical Microbiology (1997) 35: 978-981).

Introduction

Methicillin-resistant staphylococci are significant pathogens which have emerged over the past 30 years and are known to cause both nosocomial and community-acquired infections (1-3). MRSA is the term used to refer to the strains of *Staphylococcus aureus* which possess intrinsic resistance to methicillin and all beta lactam antibiotics. These strains are also resistant to macrolides, lincosamides, aminoglycosides, quinolones and rifamycins. The glycopeptide antibiotics seem to be the only agents active against MRSA. Besides being multiple resistant to common anti-staphylococcal agents, some MRSA strains spread more readily than others once introduced into hospitals and are often difficult to eradicate once established (4). Transmission of MRSA occurs primarily from colonized or infected patients to other patients or staff, or vice versa (5-7). Examination of the number and rate of newly affected patients and their epidemiological association may suggest the presence of cross-transmission. If sufficient means for isolate discrimination exist, the demonstration of strain identity among patients with positive cultures can be convincing evidence to support the epidemiological hypothesis. There are few studies about comparison typing methods using a large number of epidemiologically related and unrelated isolates. Because of this, no single typing system can yet be considered the preferred method. Different typing methods may be appropriate for different indications and purposes (8, 9).

In this study our aim was to characterize whole cell and filtrate proteins of MRSA isolates from Gülhane Military Medical Academy (GMMA) which had common antibiotic susceptibility test results.

Materials and Methods

Hospital. Gülhane Military Medical Academy is a teaching hospital with 2250 beds. The Hospital Infection Control Committee (HICC) has been performing a laboratory-based active surveillance study for nosocomial infections. In 1997, the percentage of *S. aureus* among all nosocomial isolates in this hospital was 20% and of MRSA among *S. aureus* isolates was 51%.

Bacterial isolates. Thirty-seven nosocomial MRSA isolates from surgical wounds were studied. The isolates were identified as *S. aureus* by growth on mannitol salt agar, and by positive coagulase and DNase tests. *S. aureus* ATCC 25923 and 43300 were used as controls.

Extraction of WCPs. Clinical specimens were inoculated to brain heart infusion (BHI) agar (Difco) (supplemented with 5% sheep blood). After overnight incubation at 35°C, a single colony was taken and transferred to 3 ml BHI broth and after reincubation for 48 hours at 35°C, centrifuged for 3 minutes at 12100 rpm. The supernatants were stored for extraction of FPs. Collected cells were washed 3 times with sterile distilled water. Washed cells were stirred after adding 25 µl SDS sample buffer (0.06 M Tris, 2.5 % glycerol, 0.5 % SDS, 1.25 % ß-mercaptoethanol and bromophenol blue

0.001% (w/v)) and the proteins were denaturated in boiling water for 5 min. They were centrifuged again for 3 min at 12100 rpm, collected in a microfuge tube and stored at -50°C until the electrophoresis process was carried out.

Extraction of Filtrate Proteins (FPs). Stored supernatants were passed through a cellulose acetate membrane filter (Sartorious) with a diameter of 0.2 µm and stored at -50°C until the electrophoresis had been carried out. Methanol-chloroform precipitation method, recommended by Wessel and Flugge , was performed with some modifications (10). Over the 500 µl filtered supernatants, 400 µl methanol, 200 µl chloroform and 300 µl distilled water were added. They were shaken and centrifuged for 3 min at 10700 rpm. After centrifugation, without touching the intermediate phase, the supernatant was removed carefully. Then 300 µl methanol was added, stirred and centrifuged again at 10700 rpm. Following the removal of the supernatant, precipitated proteins were dried by the air current and stirred after the addition of 20 µl SDS sample buffer. Finally, proteins were denaturated by keeping in boiling water for 5 min. Denaturated proteins were separated by 10 % SDS-PAGE according to the recommendations of Laemmli (11) and gels were stained with Coomassie Brilliant Blue R 250 (Sigma).

Native-PAGE. Following overnight incubation at 35°C in BHI agar, bacterial cells were washed 3 times with distilled water. After suspending the bacterial cells in sample buffer (pH 6.8) (0.06 M Tris, 2.5 % glycerol, bromophenol blue 0.001 % (w/v)), proteins were extracted by multiple freezing and thawing (37, -70°C) and centrifuged again for 3 min at 12100 rpm. Whole cell proteins were analyzed by Native-PAGE according to Laemmli (11). Proteins were loaded to wells in a 4 % stacking gel over a 7.5 % acrylamide separating gel. Electrophoresis was performed with a discontinuous buffer system in a BRL gel apparatus (model V16-2 BRL Gaithersburg, MD, USA). The gel was run at a constant current of 30 mA until the bromophenol blue marker reached the bottom. Whole cell protein gels were stained with Coomassie Brillant Blue.

Antimicrobial susceptibilities. Strains which were identified as MRSA were stored at –70°C in Protect TM Bacterial Preserver Vials (Key Scientific Products, TX, USA) until ready for susceptibility testing. Oxacillin, penicillin, erythromycin, vancomycin, ofloxacin, and gentamicin were tested according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) Document M100-S8 (12).

Results

All MRSA isolates were shown to be susceptible to vancomycin and, resistant to penicillin, erythromycin, ofloxacin, and gentamicin. The results of the MRSA isolates collected from GMMA which were analyzed using SDS-PAGE (WCPs and FPs) and N-PAGE (WCPs) methods and the results of both techniques, were in good agreement. When WCPs were analyzed by SDS-PAGE, only one profile was observed (Figure 1). As can be seen in Figure 1, the first 4 lanes are methicillin-resistant *Staphylococcus aureus* (MRSA), the fifth lane is methicillin-sensitive *Staphylococcus aureus* (MSSA), and the sixth lane is methicillin-resistant *Staphylococcus epidermidis* (MRSE) isolates.

As far as the band profiles in SDS-PAGE of FPs are concerned, the common band patterns are seen in MRSA isolates lanes. We did not observe any band when we analyzed the solubilized proteins obtained from BHI broth, by running on SDS-PAGE. SDS-PAGE results of FPs are shown in Figure 2. Band profiles of WCPs obtained using N-PAGE are shown in Figure 3. As can be seen in this figure, identical band patterns can be observed again in MRSA isolates. Again, the characteristic band pattern differences can be seen between MRSA loaded in the first four lanes; MSSA loaded in the fifth lane and MRSE in the last lane.

Discussion

S. aureus has remained a major cause of nosocomial morbidity and mortality, and as an additional clinical problem, MRSA emerged at an alarming rate in the 1980s, and

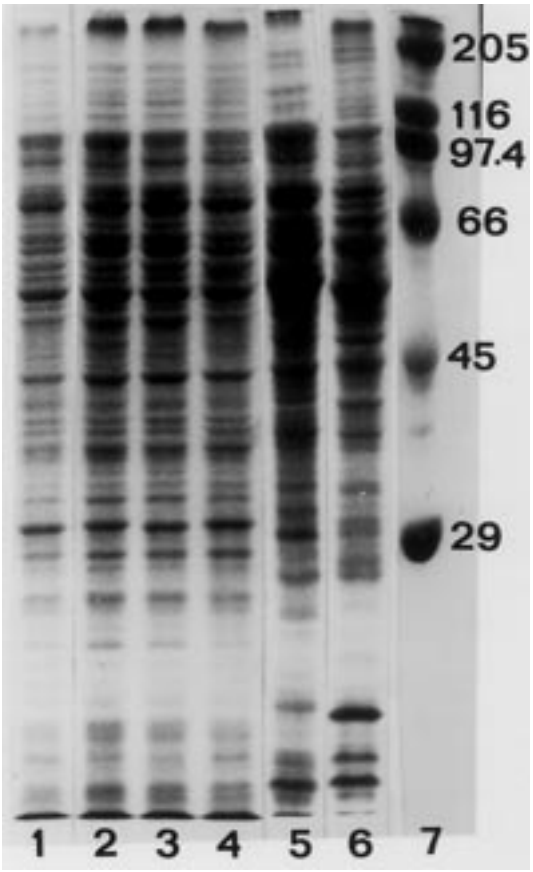

Figure 1. Whole cell protein profiles of Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates by SDS-PAGE. Line 1-4, MRSA isolates; Line 5, methicillin-sensitive *S. aureus*; Line 6, methicillin-resistant *S. epidermidis*; Molecular size standard is in line 7.

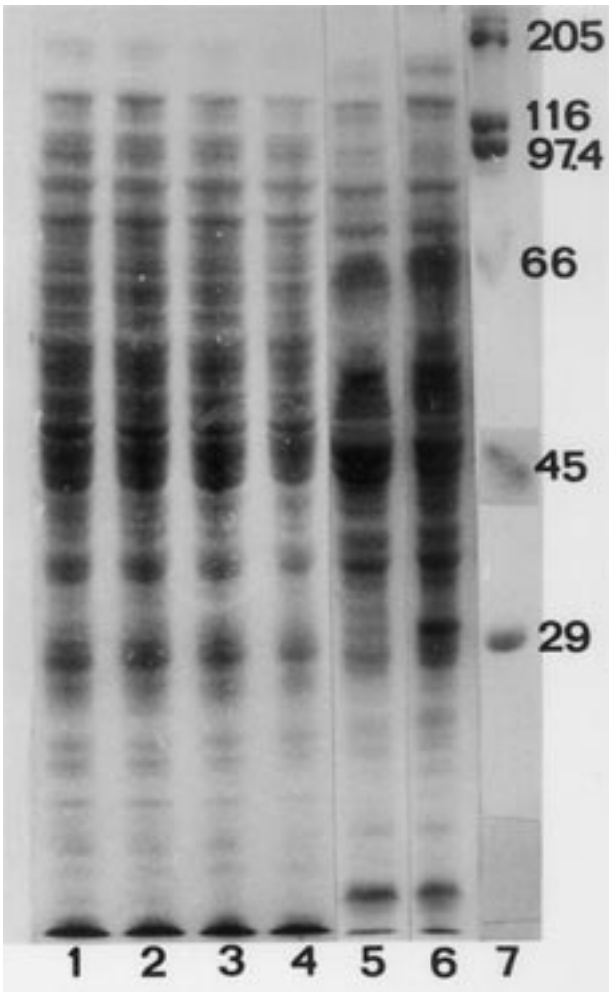

Figure 2. Filtrate protein profiles of MRSA isolates by SDS-PAGE. Line 1-4, MRSA isolates; Line 5, methicillin-sensitive *S. aureus*; Line 6, methicillin-resistant *S. epidermidis*; Molecular size standard is in line 7.

analysis or dissemination of MRSA isolates has been a research focus for the past decade (13, 14). However, epidemiologic surveillance studies require reliable techniques capable of differentiating independent strains from clonally related strains and also molecular pheno- and genotyping techniques have been optimized for the purpose of studying the spread of MRSA. Several methods are used for the typing of MRSA (15-20) but still there is not a single typing system that can be considered a preferred method (7-9, 21). Pulsed-field gel electrophoresis (PFGE) has proven to be highly discriminatory for MRSA isolates. However, this method is fairly laborious and the DNA restriction patterns may be difficult to interpret (22), also interlaboratory standardization of PFGE is still problematic (23). Randomly amplified polymorphic DNA analysis has

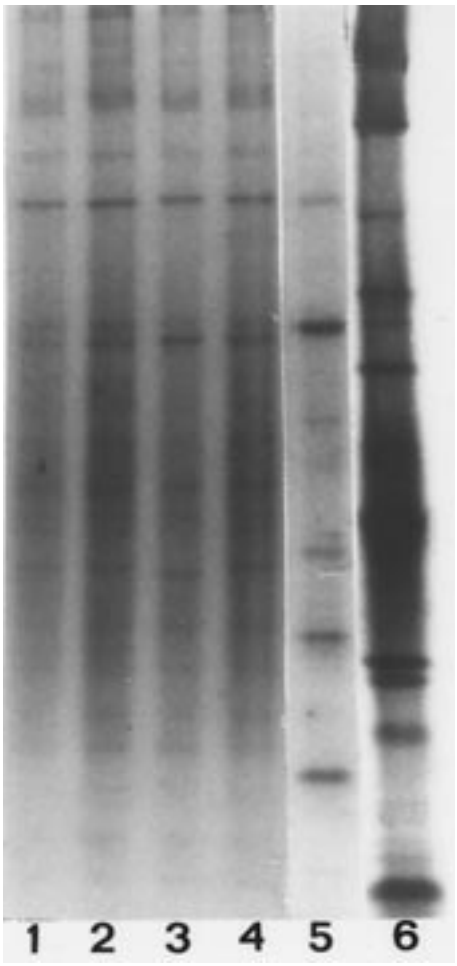

Figure 3. Whole cell protein profiles of MRSA isolates by N-PAGE. Line 1-4, MRSA isolates; Line 5, methicillin-sensitive *S. aureus*; Line 6, methicillin-resistant *S. epidermidis*.

proven to be a rapid technique that yields epidemiologically valid results. However, its reproducibility needs improvement (21).

On the other hand, it is reported that different band patterns can be observed in MRSA isolates by SDS-PAGE. Our MRSA results obtained by SDS-PAGE of WCPs and those of previous studies clearly show that electrophoretic methods can provide valuable epidemiological information (16, 20). It is reported that WCP analysis may not be preferred because of the high similarities of their band patterns, especially when used for differentiation of MRSA strains (15). However, we overcame the problems faced with band pattern similarities when 5-7.5 % gel concentration was used in SDS-PAGE as

compared with 10 % gel concentration used in other published data (unpublished data). To support the results of SDS-PAGE, MRSA isolates were analyzed again by SDS-PAGE, but this time using FPs and by Native -PAGE using WCPs of MRSA isolates. No report has been published yet to explain the characterization of MRSA by filtrate protein profiles obtained from both SDS and Native-PAGE. This report is the first study known so far which attempts to use the WCPs in Native-PAGE.

Consequently, in this study we compared the various electrophoretic methods for MRSA typing to each other. As an addition to pulsed-field gel electrophoresis used in previous study (19), in this study we showed by SDS-PAGE and Native-PAGE that a major MRSA clone circulates in GMMA in Ankara. After analyzing the isolates from GMMA and comparing them with the ones already obtained from other hospitals in Ankara and Bursa, we may conclude that the same major clone of MRSA was also seen in GMMA. The result of this study is in agreement with the results of our previous study and the same major clone of MRSA seems to circulate among GMMA hospital.

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