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Analysis of Methicillin Resistant *Staphylococcus aureus* Isolates by Polyacrylamide Gel Electrophoresis in an Intensive Care Unit of İbni-Sina Hospital

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Abstract: This study was designed in order to evaluate the relationship of MRSA isolates obtained from an intensive care unit (ICU) and other wards as well as the epidemiological features and typing methods. Two polyacrylamide gel electrophoretic methods of typing MRSA isolates-sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and native-PAGE (N-PAGE) were evaluated and their antibiotic susceptibility patterns were determined. MRSA isolates were investigated according to the whole cell proteins (WCPs) and filtrate proteins (FPs). No report has been published yet explaining the characterization of staphylococci in SDS-PAGE by use of filtrate protein profiles. One clonal group was identified by SDS-PAGE and N-PAGE, and these two electrophoretic methods were found to be useful for the analysis of strains. Some of these isolates were also used in Belkum's study, the results of which state the presence of only one clone circulating in Turkish hospitals by molecular genotyping analysis. The results of this study done by both SDS-PAGE and N-PAGE have also shown the dissemination of the same clone circulating in Turkish hospitals to the intensive care unit of İbni Sina Hospital, which is in agreement with the results of Belkum, and causes one to consider the usage of these electrophoretic methods in typing systems.

Key Words: Methicillin Resistant *Staphylococcus aureus* (MRSA), N-PAGE, Turkey.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) are significant pathogens which have emerged over the past 30 years and are known to cause both nosocomial and community-acquired infections (1-3). Their possible dissemination in Turkey has also been shown in previous studies (4). Many different methods have been used for typing MRSA isolates. Bacteriophage typing, analysis of cellular protein profiles, analysis of genomic DNA by pulsed field gel electrophoresis and analysis of randomly polymorphic DNA are only some of them (5-8). Pulsed field gel electrophoresis (PFGE) has proven to be highly discriminatory for MRSA isolates, and it has been suggested that it is superior to other genotyping techniques. However, this method is fairly laborious, and the DNA restriction patterns may be difficult to interpret (9). Moreover, interlaboratory standardization of PFGE is still problematic (10). Randomly amplified polymorphic DNA analysis has proven to be a rapid technique that yields epidemiologically valid results. Again, however, its interlaboratory reproducibility needs improvement (8). Thus, the continued need for accurate genotyping systems that can be applied in clinical laboratories is evident. It has been suggested that only the combined application of various typing schemes allows for an accurate analysis of clonal relatedness among MRSA isolates (11). On the other hand, the presence of different band patterns among MRSA isolates has been shown by SDS-PAGE, and its importance in epidemiology has also been implied (6, 12).

The aim of this study was to ascertain the epidemiological pattern and relationship of MRSA isolates obtained from patients of the thoracic surgery ICU and other wards by SDS and N-PAGE after

notification of revealing MRSA from 13 patients, the results of which were extraordinary since no such dissemination had been seen previously in this unit.

Materials and Methods

Hospital: İbni-Sina is a 1200-bed teaching hospital of Ankara University. The Hospital Infection Control Committee (HICC) of this hospital has been performing laboratory-based active surveillance for nosocomial infections (NCIs) since 1992 in twelve clinics where high risk patients are hospitalized. In 1996, the percentage of *Staphylococcus aureus* isolates among all Gram-positive bacteria obtained from these clinics was 48 % and the percentage of MRSA among *S. aureus* isolates was 36 %. The HICC is studying a protocol concerning control of MRSA at present. There is, however, reinforcement of careful handwashing and educational programs throughout the hospital, although in several wards there are not sufficient facilities to implement these programs. The ICU, provided with 8 beds, was the main site of the study and was a part of the thoracic surgery ward that had 46 beds in total. The unit had not has such a MRSA problem. When MRSA was isolated consecutively from 13 patients, all were in the ICU and no other MRSA was isolated from the ward patients simultaneously.

Bacterial isolates: Thirteen MRSA isolates, which were isolated from surgical wounds from January-April 1997, were clinical isolates from ICU patients of the thoracic surgery ward in İbni-Sina Hospital (Ankara). Following these 13 isolates, 29 additional isolates from various wards were identified as MRSA during the same period. These isolates were obtained from surgical wounds, urine, blood, respiratory tract, and a total of 42 strains were taken into account. Colonies were selected on the basis of morphological characteristics, checked by Gram-staining, and tested for catalase and coagulase production. Coagulase production was assayed by the citrate-plasma tube technique (13). Isolates were also identified by growth on mannitol salt agar and by positive DNase test.

Extraction of whole cell proteins (WCPs): The method of Laemmli (14) was used with a few modifications. Following overnight incubation at 35°C in Brain heart infusion (BHI) agar (Difco), one colony was taken and suspended in 3 ml BHI broth. Samples which had been incubated again for 48 h at 35°C were further centrifuged for 3 min at 12100 rpm. The collected cells were washed three times with sterile distilled water and stirred after adding 25 µl SDS sample buffer (0.06 M Tris, 2.5 % glycerol, 0.5 % SDS, 1.25 % β-mercaptoethanol and 0.001 % bromophenol blue). The proteins were denatured in boiling water for 5 min. The supernatant was then centrifuged again for 3 min at 12100 rpm, collected in an eppendorf tube and kept at -50°C until the electrophoresis was carried out.

Extraction of filtrate proteins (FPs): The culture supernatants were passed through a cellulose acetate membrane filter (Sartorius) with a diameter of 0.2 µm and it was stored at -50°C until the electrophoresis was carried out. Methanol-chloroform precipitation was performed by applying some changes to the method recommended by Wessel and Flugge (15). The volume of the sample increased. 400 µl methanol, 200 µl chloroform and 300 µl distilled water were added to the 500 µl sample, it was shaken and centrifuged for 3 min at 10700 rpm. After the centrifugation, before it went into intermediate phase, the supernatant was removed carefully and after adding 300 µl methanol and stirring, it was centrifuged again at 10700 rpm. After the supernatant was removed, the precipitated proteins were air dried and stirred after the addition of 25 µl SDS-sample buffer. Afterwards, proteins were denatured by keeping in boiling water for 5 min.

SDS-PAGE: Denatured proteins were analyzed by SDS-PAGE according to Laemmli (14). This method used a 2 cm layer of 4 % acrylamide stacking gel and a 10 cm layer of 10 % acrylamide separating gel. Sigma wide range marker was used as molecular weight standard in SDS-PAGE. Electrophoresis was performed with a discontinuous buffer system in a BRL gel apparatus model V16-2BRL Gaithersburg MD, USA. The gel was run at a constant current of 35 mA until the bromophenol blue had reached the bottom. Gels were then stained with Coomassie Brilliant Blue R 250 (Sigma).

Native-PAGE for WCPs: After overnight incubation at 35°C in BHI agar, bacterial cells collected from plates were washed three times with distilled water. Proteins were extracted from bacterial cells by suspending cells in pH 6.8 sample buffer (0.06 M Tris, 2.5 % glycerol, 0.001 % (w/v) bromophenol blue) and 4-5 times with freeze and thaw (37°C, -

50°C). After centrifuge for 3 min at 12100 rpm, whole cell proteins were analyzed by Native-PAGE according to Laemmli (14). Denatured proteins were run on a 4 % stacking gel over a 7.5 % acrylamide separating gel. Electrophoresis was carried out and the gel was stained as mentioned above.

Antimicrobial susceptibilities: Antibiotic susceptibilities were investigated by disc diffusion and agar screen methods according to NCCLS quidelines (16). Susceptibility to methicillin by disc diffusion was determined by 1 µg oxacillin (OX1 Difco) discs and an inoculum of 10^8 cfu/ml. The zone of inhibition was interpreted after 24 h of incubation at 35°C. Strains showing a 10 mm or less diameter inhibition with no microcolonies within the halo were defined as MRSA. In the agar screen susceptibility test, a direct colony suspension equivalent to a 0.5 McFarland standard in tryptic soy broth was inoculated onto Mueller-Hinton agar supplemented with 4% NaCl and 6 µg of oxacillin (Sigma) per ml. Plates were read at 24 and 48 h of incubation at 35°C. Any growth was considered a positive test result. In addition to oxacillin, the following antibiotics were tested: amikacin (AN30 Difco), amoxicillin clavulanic acid (AMC30 Difco), ciprofloxacin (CIP5 Oxoid), cephalotin (CR30 Difco), cefuroxime (CXM30 Difco), ceftriaxone (CRO30 Difco), clindamycin (CC2 Difco), erytromycin (E15 Oxoid), gentamicin (GM10 Difco), penicillin (P10 Difco), rifampicin (RD30 Oxoid), teicoplanin (TEC30 Oxoid), trimethoprimsulfamethoxazole (SXT Oxoid) and vancomycin (VA30 BBL).

Results

MRSA isolates in this outbreak were investigated according to SDS-PAGE (WCPs and FPs) and N-PAGE (WCPs) and there was good agreement between the two typing tools. When the band profiles obtained from WCPs were investigated by SDS-PAGE, one pattern was observed in Figure 1. Each profile contained over 50 bands with WCPs. When the band profiles obtained from FPs were investigated, one SDS-PAGE pattern were seen in MRSA isolates. Denatured proteins of BHI broth were run on SDS-PAGE and no protein bands were observed. Each profile contained over 60 bands with FPs. SDS-PAGE analysis of FPs is seen in Figure 2. Band profiles of WCPs with N-PAGE are shown in Figure 3. Identical band pattern was observed in MRSA isolates. Each profile contained over 30 bands with N-PAGE

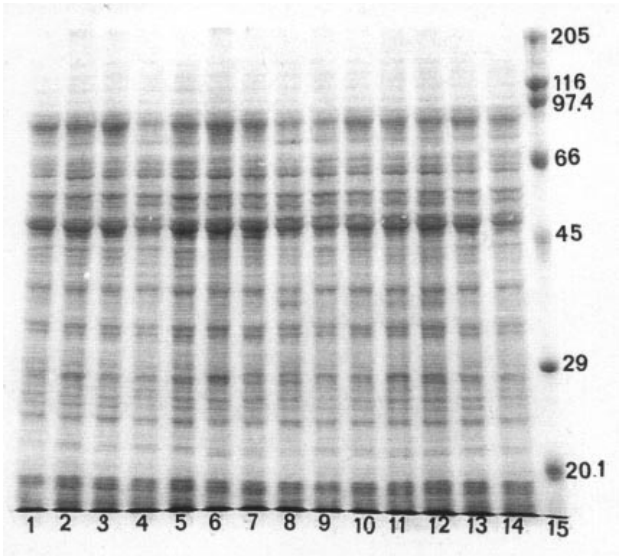

Figure 1. Whole cell protein profiles of MRSA isolates by SDS-PAGE. Line 1-13: MRSA isolates from thoracic surgery ICU; Line 14: MRSA isolate from other wards; Line 15: Molecular weight standard in kD.

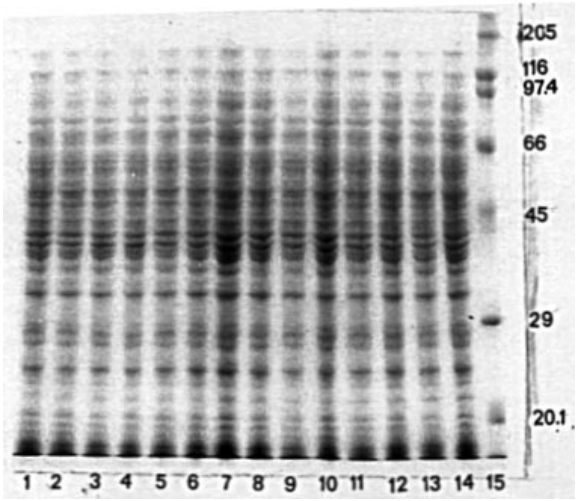

Figure 2. Filtrate protein profiles of MRSA isolates by SDS-PAGE. Line 1-13: MRSA isolates from thoracic surgery ICU; Line 14: MRSA isolate from other wards; Line 15: Molecular weight standard in kD.

(WCPs). All of the isolates showed multiple drug resistance. None of the MRSA isolates tested were susceptible to P, AMC, CIP, CR, CXM, CRO and GM. As shown in the Table 1, other antibiotics exhibited three different susceptibility patterns.

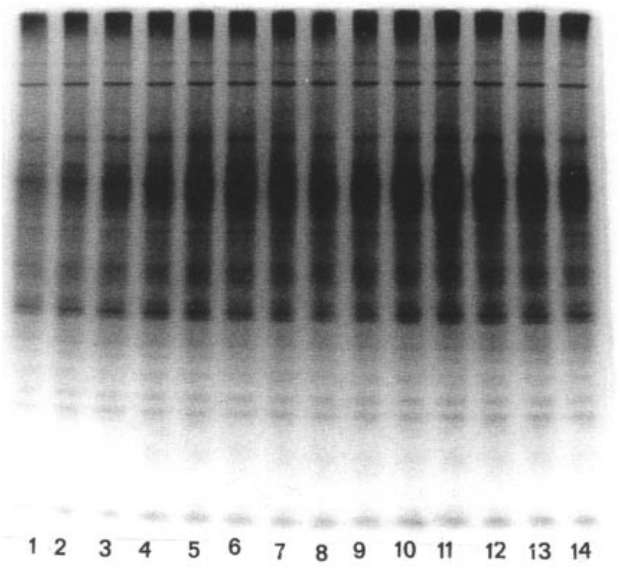

Figure 3. Whole cell protein profiles of MRSA isolates by N-PAGE. Line 1-13: MRSA isolates from thoracic surgery ICU; Line 14: MRSA isolate from other wards.

Discussion

Several methods are used for the identification of MRSA (6,17,18). In the present study, various electrophoretic methods for typing MRSA isolates obtained from the thorasic surgery ICU and other wards were compared. By SDS-PAGE (WCPs and FPs) and N-PAGE (WCPs), all of the 42 MRSA isolates, of which 13 from thorasic surgery ICU and the remaining 29 from other wards, were determined to be identical.

Our results in MRSA isolates by SDS-PAGE with WCPs and those of previous studies clearly show that electrophoretic methods can provide valuable epidemiological information (18,19). It is reported that WCPs can not be used because of the high similarities between their band patterns examined in the differentiation of MRSA strains (6). In order to overcome this problem, MRSA isolates were investigated by SDS-PAGE with the use of FPs and by N-PAGE using WCPs. The problem of band pattern similarities was also overcame when 5-7.5 % gel concentration was used in SDS-PAGE as opposed to 10% gel concentration (unpublished data). It has also been determined that FPs have provided more information both in *S. aureus* strains and in coagulase negative Staphylococci at species level (20). Furthermore, this report was the first study attempting to use N-PAGE with WCPs.

All of the MRSA isolates showed multiple drug resistance. Most patients were found to be treated with one or more antibiotics before isolation of MRSA. Since excessive and inappropriate use of prophylactic and therapeutic antibiotics and/or the lack of any antibiotic policy are the possible reasons for the antimicrobial susceptibility, it was considered normal for patients to show resistance against gentamicin, ciprofloxacin and erythromycin.

One SDS-PAGE and one N-PAGE pattern were observed in this study. It is known that the dissemination of single clone in the mentioned ICU may possibly have arisen from other wards or hospital staff since no such dissemination had occurred before this outbreak. This is in agreement with the results of our previous study pointing out that the MRSA strains circulating among Turkish hospitals probably originated from the same clone (4).

Table 1. Antimicrobial susceptibility patterns of MRSA strains from İbni Sina Hospital.

Antimicrobial susceptibility patterns	MRSA (n)	
	ICU	other wards
VA, TEC, SXT, RD	6	14
VA, TEC, SXT, RD, CC, E	3	6
VA, TEC, SXT, RD, CC, E, AN	4	9

VA: vancomycin
TEC: teicoplanin
SXT: trimethoprim/sulfamethoxazole
RD: rifampicin
CC: clindamycin
E: erytromycin
AN: amikacin

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