MOLECULAR EPIDEMIOLOGY OF MRSA ISOLATES IN THE INTENSIVE CARE UNIT DURING A 4-YEAR-PERIOD

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ABSTRACT

Objective: Methicillin resistant Staphylococcus aureus (MRSA) is a common cause of nosocomial infections in hospitalized patients , particularly in those who stay in intensive care unit(ICU). We aimed to compare MRSA strains isolated from ICU in order to detect relatedness between them since accurate epidemiological typing by reproducible and rapid methods is a major step in determining MRSA clones and sources of transmission for immediate infection control.

Methods: From February 1998 to November 2001, 56 MRSA isolates from various clinical specimens from different patients who were hospitalized in ICU of Marmara University Hospital were included in the study. These isolates were identified as MRSA by standard methods. Genotyping analysis was done by AP-PCR(arbitrarily primed polymerase chain reaction).

Results and conclusion: The specimens which the MRSA strains isolated were as follows: 38(67.8%) from respiratory tract(deep tracheal aspirate and sputum), 10(17.8%) from blood, 4(7.1%) from wound infection and abscess, 2(3.6%)from catheters, 1(1.8%) from urine sample and 1(1.8%) from joint fluid. By AP-PCR analysis 28/56 isolates exhibited 11 incidences of clusters throughout the study period. The longest time for a given incidence was 3 months and a given incidence affected 4 patients at most. We conclude that the AP-PCR method can easily be used in order to evaluate genotypic relatedness between MRSA isolates in our institution.

Key Words: Methicilline resistant Staphylococcus aureus, Intensive care unit, Arbitrarily primed polymerase chain reaction

INTRODUCTION

The emergence of multi drug resistant microorganisms is an increasing problem that requires continuous and expensive precautions to control the nosocomial infections. Methicillin resistant *Staphylococcus aureus* (MRSA) emerged as a nosocomial pathogen in the 1960s with outbreaks that were reported in the UK and Europe (1). Today MRSA strains have become a major pathogen of nosocomial infections throughout the world (2). In hospital settings MRSA cause infections particularly in those who stay in intensive care units (ICUs) for extended

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periods of time, have underlying illness and are exposed to high frequency of invasive procedures including mechanical ventilation, catheterization etc. The main mechanism of MRSA transmission is via the hands of hospital staff in contact with patients or contaminated patient materials (1).

In this respect the microbiology laboratory plays an important role in the effort to minimize nosocomial infections and serves as a warning unit by identifying organisms that can be transmitted through the hospital (3). In order to achieve this aim genetic typing methods are widely used to determine whether the organisms are similar or different.

In this paper we analyze the genetic relationship among MRSA strains from ICU patients in a 4-year period with arbitrarily primed polymerase chain reaction (AP-PCR) assay.

MATERIALS AND METHODS

Bacterial isolates:

From February 1998 to November 2001, 56 MRSA isolates recovered from various clinical specimens from different patients who were hospitalized in ICU of Marmara University Hospital, Istanbul, Turkey were included in the study. These isolates were identified as MRSA by standard methods as described previously(4).

DNA extraction:

Bacteria were grown in Mueller Hinton broth medium at 37°C for overnight and 1 ml culture suspension centrifuged at 7500 rpm for 5 min. After removing supernatant, pellet was washed with PBS (pH: 7.4) twice. Bacteria resuspended in 180µl lysis buffer (20 mM Tris-HCl, 2 mM EDTA, 1% Triton X-100, pH: 8 supplemented with 0.2 mg/ml lysostaphin) and incubated for 1 hour at 37°C, then 25µl proteinase K (24 mg/ml) was added to suspension and incubated for 1 hour at 56°C. Digested suspension was added to DNA extraction columns (Nucleospin Tisssue, Macherey Nagel, Germany) and processed by manufacturers instructions. This method allowed purification up to 35µg genomic DNA for PCR amplification. Amount of DNA was detected by gel electrophoresis compared with DNA marker.

PCR Amplification:

For AP-PCR, ERIC-II(5'-AAG TAA GTG ACT GGG GTG AGC-3') primer was used(5). For each sample 5µl MgCl2 (25 mM), 1µl of dNTP mixture, 0.5µl primer, 0.2µl Taq DNA polymerase 5µl of 10x reaction buffer and 5µl target DNA were used and the mixture was made up to 50µl with water. Reactions were carried out in Techne Thermal Reactor (Techne, Cambridge, UK). Amplification was performed under following conditions: 1 cycle at 94°C for 5 min, 30 cycles at 90°C for 30 sec, 50°C for 30 sec, 52°C for 1 min, and 72°C for 1 min; and 1 cycle at 72°C for 8 min. The products were electrophorised on 2% agarose gels contained ethidium bromide in 0.5M TBE (Tris borate EDTA) buffer. Synthetic molecular size marker (1 kb, Gibco,UK) were included in each gel. Gels were run for 3 hours at 90 V and DNA band patterns were visualized with UV light and photographed. Band patterns were evaluated with different persons by visual inspection.

RESULTS

The specimens from which 56 MRSA strains were isolated as follows: 38 from respiratory tract(deep tracheal aspirate and sputum), 10 from blood, 4 from wound infection and abscess, 2 from catheters, 1 from urine sample and 1 from joint fluid.

By AP-PCR analysis 28/56 isolates exhibited 11 incidences of clusters throughout the study period. These incidences are shown in Table I and band patterns for year 2001 is given in figure I. The longest time for a given incidence was 3 months and a given incidence affected 4 patients at most.

Table I: Incidences of clusters due to AP-PCR typing

Patient no.	Isolation date	Specimen	Genotype
1	20.02.1998	sputum	A
2	21.04.1998	abscess	A
3	16.06.1998	DTA*	A
4	18.11.1998	DTA	B
5	31.12.1998	DTA	B
6	14.09.1999	blood	C
7	21.09.1999	DTA	C
8	07.10.1999	DTA	C
9	12.10.1999	DTA	C
10	04.01.2000	catheter	D
11	25.01.2000	DTA	D
12	31.01.2000	DTA	D
13	21.03.2000	DTA	E
14	21.03.2000	catheter	E
15	27.03.2000	DTA	E
16	07.04.2000	DTA	E
17	21.04.2000	DTA	F
18	02.05.2000	DTA	F
19	12.05.2000	swab	G
20	15.05.2000	DTA	G
21	07.08.2000	sputum	H
22	08.08.2000	sputum	H
23	06.12.2000	blood	I
24	11.12.2000	DTA	I
25	04.06.2001	abscess	J
26	22.06.2001	blood	J
27	23.07.2001	DTA	K
28	05.11.2001	aspirate	K

* DTA:deep tracheal aspirate

DISCUSSION

Methicillin resistant Staphylococcus aureus(MRSA) is a common cause of nosocomial infections in hospitalized patients , particularly in those who stay in the intensive care unit(ICU). In ICU, nosocomial infection rates are five to ten times higher than those of general wards and the risk factors for developing MRSA are frequently encountered in this environment(6). According to the results of Nosocomial Infection Surveillance System, 31% of all infections in ICU were nosocomial pneumonia, 83% episodes of pneumonia were associated with mechanical ventilation and 17% of pneumonia was caused by Staphylococcus aureus(7). Respiratory tract was the most commonly affected body site by a 68% of the isolation rate in our study population. We aimed to compare MRSA strains isolated from ICU in order to detect relatedness between them because accurate epidemiological typing by reproducible and rapid method is a major step in determining MRSA clones and sources of transmission. Routine microbiological methods evaluating phenotyping characteristics, such as susceptibility patterns(antibiogram) is not very discriminative because the high resistance rate of the microorganism whereas phage typing is time-consuming and labor intensive. The limitation of phenotyping methods has stimulated the development of DNA-based techniques. Plasmid profile analysis was the first genotyping method used in epidemiological studies of S. aureus(8). Chromosomal DNA has been

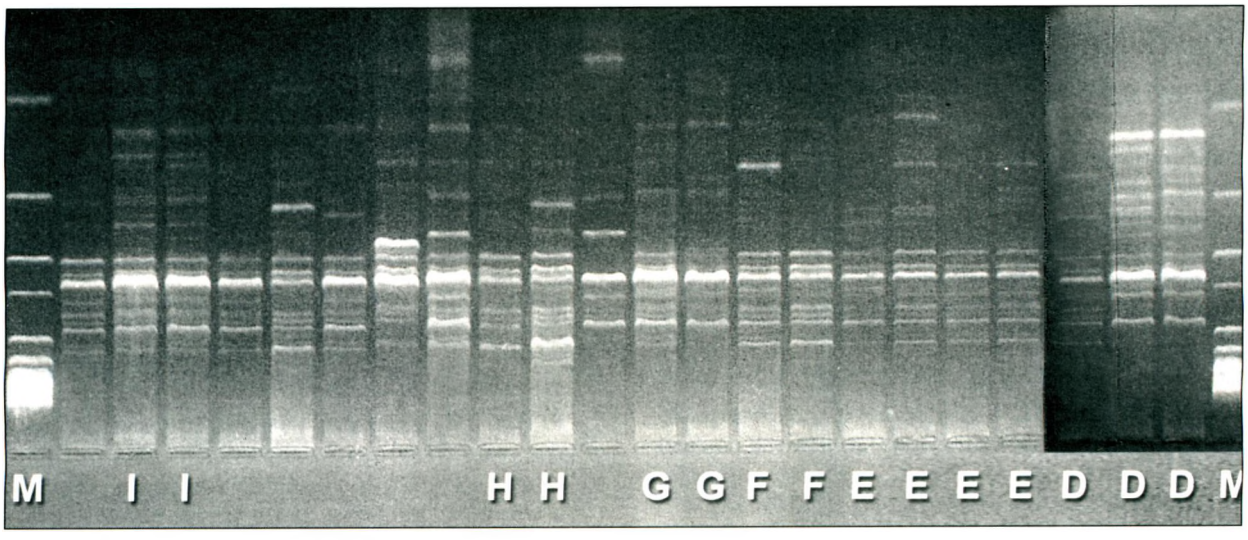

Fig.1: The fingerprinting patterns of strains

analyzed by a variety of techniques, including restriction enzyme analysis, ribotyping, PCR based methods and pulsed-field gel electrophoresis. Randomly amplified polymorphic DNA(RAPD) assays use short primers with an arbitrary sequence to amplify genomic DNA in low stringency PCR(9). These primers randomly hybridize with chromosomal sequences that vary among different strains and that produce different amplicon products. These products can be separated by gel electropheresis to produce fingerprints or pattern characteristics of different epidemiological types. This method was used succesfully in the epidemiological analysis of S. aureus by different authors. Grundmann et al. typed 92 S. aureus isolates (only 2 were MRSA) and identified 14 patterns where the majority of isolates were regarded as epidemiologically unrelated, most of the strains originated from patients who were treated during different periods or in different ICUs with no transfer of carrier patient or staff between the wards(10). In other studies only a single clone responsible from their outbreaks was detected by AP-PCR(11,12). We did not detect such a single clone causing an outbreak. The longest time for a given incidence was 3 months and this affected 4 patients at most. We preferred the AP-PCR for analysis of our isolates mainly because of the low cost and relative simplicity of the method. We conclude that AP-PCR method can easily be used in order to evaluate genotypic relatedness between MRSA isolates in our institution. Comparison of the isolates can be done within a day allowing earlier detection of epidemics. Environmental surveillance cultures and samples from health personnel should also be studied in order to determine the source and immediate infection control measures should be taken.

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