Identification of MRSA Strains Using Three Phenotypic as Well as PCR Methods and Determination of Isolated Strains Diversity by RAPD-PCR and Antibiotic Profiling in Tabriz Hospitals

Mojtaba Nikbakht*[1]; Mohammad Reza Nahaei[2]; Mohammad Taghi Akhi[2]; Sahar Nikbakht[3]

1- Valiasr Hospital, Meshkinshahr Health Network, Ardabil University of Medical Sciences; Meshkinshahr, Iran

2-Depertment of Microbiology, School of Medicine,Tabriz University of Medical Sciences; Tabriz, Iran

3- School of Medicine, Ardabil Barnch Islamic Azad University;Ardabil, Iran

mn_skh@yahoo.com

Background & Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are important cause of hospital-acquired infections and their elimination from hospital environment is difficult, because, they are resistant to many other routine use antibiotics as well1. The principle sources and transmission routs of these strains are hospital staff and in-patients. Laboratory diagnosis and typing of these medically important bacteria are important in treatment, eradication and prevention of nosocomial infections. The aims of present study were to determine the prevalence of MRSA strains in Tabriz hospitals using four Methods, on the other hands were to determine of antibiotic profiling and molecular diversity of MRSA isolates by Randomly Amplified Polymorphic DNA (RAPD) – Polymerase Chain Reaction (PCR), finally to study the nasal carriage rate of S.aureus in hospital staff and in-patients.

Methods: During a six-month period in 2004-2005, inpatient and staff of Imam Khomeini and Pediatric hospitals of Tabriz were screened for S. aureus and MRSA strains. During the same period of time clinical specimens submitted to the hospital laboratories were monitored for S. aureus and MRSA strains. All of *S. aureus* and MRSA strains were collected from nasal and clinical specimens by Clinical and Laboratory Standards Institute (CLSI) recommendation. Resistance to methicillin was detected using 30 µg cefxitin and 1 µg oxacillin discs, oxacillin agar screening plate and amplification of mecA gene by PCR Methods3-6. Antibiotic profiling was performed by the disc diffusion Methods, according to the CLSI guidelines against 14 antibiotics. RAPD-PCR has been applied for molecular typing of MRSA isolates by the use of five primers7. Dendrogram was built based on RAPD-PCR results for each primer and for five primers, by cluster analyzing using Ward Methods with SPSS 13 software. Molecular diversity and antibiotic patterns of isolates were compared with each others. Demographic data related to personnel and in-patients were collected by questionnaires and analyzed by SPSS 13 software and Chi-Square tests.

Results: Atotal of 160 *S. aureus* isolates (34.7%) were collected from noses of hospital staff and inpatients. of which 48 isolates (30%) were diagnosed as MRSA. Forty-six S. aureus isolates were collected from clinical speciemens of inpatients, of these, 32 isolates (69.5%) were MRSA ($p<0.001$), presenting more resistance to methicillin in clinical isolates. Examination of mecA genes by PCR Methods in 206 nasal and clinical isolates of S. aureus showed that, while there was a gross correlation between the presence of the gene and the level of bacterial resistance to cefoxitin, oxacillin and methicillin, but 3 mecA positive

isolates could not be distinguished from the mecA negative ones by the susceptibility tests because of their inability to produce PBP2a (cryptical methicillin-resistant *S. aureus* isolates). Accurate isolation and epidemiological typing is of primary importance for the identification of MRSA strains found in a hospital and for enabling sources and routs of transmission to be identified and controlled. In this study 80 isolated MRSA strains (by PCR- 38.8%) fell into 41 antibiotic and 43 RAPD-PCR patterns, respectively that showing well correlation between two typing methods.

Conclusion: This study showed that MRSA isolates with the same RAPD-PCR and antibiotic - resistance patterns belonged to certain wards. However, in some cases, MRSA isolates with the same RAPD-PCR and antibiotic resistance patterns were isolated from different wards, which could be due to the transfer of staff and in-patients between wards inside the hospital. Our study revealed that antibiotic resistance pattern when combined with RAPD-PCR pattern facilitates MRSA typing. The fact that MRSA isolates in our hospitals with identical patterns were isolated from staff and inpatients of certain wards, suggests that the principal rout of MRSA transmission was from patient to patient or from patient to staff and vice versa. Therefore, infected and colonized patients as well as carrier hospital staff could be the main sources of MRSA strains in our hospitals. In result, efficacy hygienic and preventional efforts must be focused on these sources and routs, for prevention of hospital- acquired infections.

Keywords: Methicillin Resistant *Staphylococcus aureus* (MRSA); RAPD-PCR; Hospital-acquired Infection; Antibiotic Resistance Pattern