Abstract

*Staphylococcus aureus* is a pathogen of major concern because of its ability to cause a diverse array of diseases ranging from minor infections to life threatening septicemia and its ability to adapt to adverse environmental conditions. The first description of penicillinase-producing strains of *S. aureus* was published in 1944 (Kirby WMM, 1944). Within a few years, most hospital isolates were resistant to penicillin (Barber M, 1948). Within two years of introduction of methicillin into therapy, *S. aureus* strains resistant to methicillin were detected. The increase in frequency of MRSA as the causative agent of nosocomial infection and the possibility of emergence of resistance to vancomycin demands quick and trustworthy characterization of isolates and an investigation of clonal spreading within hospitals. This would help to generate enough information to permit the implementation of appropriate measures for the control of these infections.

In India the prevalence and spread of MRSA has been recognized late which led to its emergence as a real threat to community and hospital settings. Prevalence of MRSA has widely been reported in several parts of India where MRSA has become a major nosocomial pathogen and few states have even reported the emergence of VISA and VRSA strains. Studies on MRSA have been conducted in all most all the Southern states except Kerala where no such reports are available so far. So we have undertaken this study to determine the antimicrobial susceptibility pattern and the molecular characteristics of MRSA obtained in Kerala.

A collection of 104 methicillin resistant *Staphylococcus aureus* strains were obtained from pus samples randomly from hospitalised patients and out patients from various hospitals in Kerala during the period from Jan 2006 to Jun 2009 and were processed using standard microbiological methods. Antimicrobial susceptibility testing was performed in Mueller Hinton agar plate according to CLSI standards. The central resistance determinants viz. *mecA*, *femA* and *femB* genes were isolated and sequence analysed. Epidemiological typing of the isolates was carried out using phage typing. Molecular typing by Cassette chromosome typing and pulsed field gel electrophoresis
was carried out using standard procedures. Microsatellite marker studies were conducted to analyse the clonal diversity of the strains.

The results of the present study indicated that all the isolates were multidrug resistant and were 100% resistant to ampicillin and oxacillin. All the strains were found to be HA-MRSA (hospital associated MRSA) and carried type III cassette chromosome. The sequencing and comparison of mecA gene revealed that the sequences showed 98% sequence homology to mecA gene of reference strain MRSA252 from Genbank database. Strains were classified into 4 different PFGE types. PFGE revealed that the MDR strains were genetically very homogenous and the majority showed the dominant profile A (46%); others showed very closely related profiles A1 (27%), A2 (16%) or A3 (11%). Phage typing classified the isolates into 13 phage types and non typable strains. Majority of the strains (53%) were non typable using the standard set of phages. In HVR-PCR the strains showed bands corresponding to four different amplicon sizes and slight variations were observed in the number of repeats while the amplification of non coding region between uvrA and hprK revealed uniform bands of 800bp and highly similar sequences.

Molecular typing studies revealed that MRSA strains analysed in this study could be considered as closely related and most probably derived from a distinct clone. This clone was probably endemic given that they were collected at different times and different locations. The degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to region and vancomycin is the only antibiotic found to give uniform sensitivity so far. But this study also reports the emergence of VISA strains in Kerala which is an alarming result. When antimicrobials including vancomycin are considered for treatment, in vitro susceptibility testing of every isolate of MRSA must be tested in the clinical laboratories.