JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2012. Vol. **6**(1), p. 339-343

Molecular Profiling of Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolated from Clinical Samples

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(Received: 12 May 2011; accepted: 17 June 2011)

The present study on molecular profiling was conducted at molecular level to differentiate the bacterial pathogens. The bacterium Staphylococcus aureus was isolated from five different clinical samples and were grouped as SA1, SA2, SA3, SA4 and SA5. They are confirmed as methicillin resistant Staphylococcus aureus based on their biochemical characterization. The molecular weight of plasmid DNA of the entire isolated sample was 19kb whereas the molecular weight 2400 Kb was observed in chromosomal DNA for the isolates. The genetic diversity of the isolates was also checked by restriction digestion analysis with Bam HI, Eco RI and Hind III. The enzyme Hind III with their recognition sequence 5'- AGCTT-3'digests the chromosomal and Plasmid DNA. In addition, the isolated strains were tested for their antibiotic suceptibilty patterns against amphicillin, chloramphenicol, streptomycin, rifampicin, erythromycin and methicillin. The strains were highly resistant to methicillin and susceptible to other tested antibiotics.

Key words: MRSA- Methicillin Resistant *Staphylococcus aureus*, Plasmid DNA, Restriction digestion, Genetic diversity.

Staphylococcus aureus is the most virulent species of Staphylococci which causes a variety of superficial and deep pyogenic infections such as skin infections folliculitis, boils and food poisoning. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for difficult-to-treat infections in humans. It may also be referred to as multidrug resistant *Staphylococcus aureus* or oxacillin resistant *Staphylococcus aureus* (ORSA). These are resistant to a group of β-lactam antibiotics which include the penicillins and the cephalosporins.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major hospital-associated as well as a community-associated pathogen causing a wide range of diseases including endocarditis, osteomyelitis, toxic-shock syndrome, pneumonia, food poisoning and carbuncles (Oliveira *et al.,* 2002 and Chambers 2005). The molecular profiling of bacterial strain is an important clinical tool to investigate hospital outbreaks and to evaluate nosocomial transmission. In addition to tracking outbreaks, genotyping can be used to distinguish between separate episodes of infection and relapse of disease. It also helps in answering an epidemiological query whether the strains causing disease in one geographic area are related to those causing the same disease in other regions (Chung *et al.,* 2004).

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Hence the present study has been focused on molecular typing of MRSA which includes the Plasmid DNA profiling, Chromosomal DNA profiling and Restriction Fragment Length Polymorphism (RFLP).

MATERIALS AND METHODS

Materials Required

Luria Bertini broth, Nutrient broth, Agarose gel, UV transilluminator.

Bacterial Strains

A total of 5 strains of S. aureus used in this study were isolated from the clinical samples obtained from a clinical laboratory at Coimbatore. All the strains were identified by the following biochemical tests and the cultures were stored for further studies.

Characterization of the Isolates

Biochemical characterization of the isolates was done as per the protocol described by Cappuccino *et al.*, (2004).Isolates were inoculated in respective media, incubated and were observed for the results.

Preservation of the Cultures

The characterized cultures were preserved in agar slants and revived once in every three months.

Molecular profiling of the isolates

Preparation of Plasmid DNA

Fresh overnight cultures were inoculated in Luria bertini broth and incubated at 37°C, 120 rpm. Plasmid DNA was isolated as per the modified method of Brimboim and Doly (1979).The samples were then checked on 0.8% agarose gel.

Chromosomal DNA Isolation

Fresh overnight cultures of all the strains were inoculated in 5 ml nutrient broth and were incubated at 37º C and 120 rpm. The chromosomal DNA was isolated and was checked on 0.6% agarose.

Restriction endonuclease analysis of both Plasmid and Chromosomal DNA

The restriction digestion was carried with the set up as mentioned in (Table 1).The sample tubes were then incubated at 37º C in a water bath for 3-4 hours. Approximately 15-20 µl of the digested samples was loaded on 0.8% agarose gel and the results were recorded.

Antibiotic Sensitivity Test (Kirby- Bauer Method)

Fresh overnight cultures of all the strains were inoculated in 5 ml Nutrient broth and were incubated at 37º C, 120rpm. Antibiotic sensitivity test was done by Kirby-Bauer method (1966). The plates were then observed for the zone of inhibition and results were recorded.

Table 1. Restriction Digestion

Restriction digestion Components	BamH I	Eco R I	Hind III
DNA (Chromosomal & Plasmid)	16µl (~1mg)	16µl (~1mg)	16µl (~1mg)
10 X Assay Buffer	2µl (1X)	2µl (1X)	2µl (1X)
Enzyme	2µl (20 U)	2µl (20 U)	2µl (20 U)
Total	20µl	20µl	20µl

Table 2. Characterization of the isolates

Isolated organism	Designation	Gram stain	H2S P Test	I P Test	MR Test	VP Test	CU Test	OT	CT
Staphylococcs aureus	SA 1 SA 2 SA 3 SA 4 SA 5	Gram +ve, Cocci	-ve	-ve	+ve	-ve	-ve	-ve	+ve

RESULTS AND DISCUSSION

Isolation and Characterization of *Staphylococcus aureus* From Clinical Samples

The isolated samples were identified as *Staphylococcus aureus* by biochemical characterization as mentioned by Cappuccino *et al*., 2006 (Table 2) and were designated as SA1, SA2, SA3, SA4 and SA5 for further reference.

Molecular profiling of the isolates

Plasmid DNA Profiling

The Plasmid DNA was isolated and the samples were checked on 0.8% agarose gel. The plasmid DNA profiling has shown that the molecular weight of all the isolates was found to be approximately 19 kb. The following (Table 3) represents the molecular weight of all the isolated plasmid DNA.

Chromosomal DNA Isolation

The Chromosomal DNA was isolated and the samples were checked on 0.8% agarose gel. The molecular weights of all the isolates were found to be approximately 2400 kb as compared with the standard DNA molecular marker. The following (Table 4) represents the molecular weight of the isolated chromosomal DNA.

Restriction endonuclease analysis of both Plasmid and Chromosomal DNA

With the isolated DNA (Plasmid & Chromosomal) restriction endonuclease analysis was carried out. It is found that, both the chromosomal and plasmid DNA was digested with Hind III and it does not show digestion with Bam H I and Eco R I (Table 5a & 5b).

Antibiotic Sensitivity Test

The isolated samples were checked for their susceptibility potential towards different antibiotics. The test results were compared with the standard antibiotic chart.

From this it is inferred that all the strains were resistant to methicillin, additionaly SA2, SA3, SA4 and SA5 shows resistance against amphicillin and strain SA5 was resistant to chloramphenicol. The antibiotic streptomycin showed intermediate action for the strain SA1, SA2, SA3 and SA4.

Rifampicin showed intermediate action for SA3 and SA5. SA2 and SA5 strains showed intermediate action for chloramphenicol.

Additionally, SA2 was intermediate for erythromycin whereas SA5 was intermediate for amphicillin. Except SA1 all the isolated strains was sensitive to erythromycin. SA1, SA3 and SA4 were sensitive to chloramphenicol. SA1, SA5 was sensitive to ampicillin and streptomycin respectively.

With the antibiogram analysis the zone of inhibition was compared with the standards from which it is found that, all the isolates were resistant to methicillin and their antibiotic susceptibility pattern was compared in the following (Table 6a& 6b).

Table 3. Plasmid DNA profiling

Isolated Samples	Molecular Weight (Kb)
SA 1	19.3
SA 2	19.3
SA 3	19.3
SA 4	19.3
SA 5	19.3

Table 4. Chromosomal DNA Profiling

Isolated samples	Molecular weight (Kb)
SA 1	2400
SA 2	2400
SA 3	2400
SA 4	2400
SA 5	2400

Table 5a. Analysis with plasmid DNA

Sample	Restriction endonuclease		
	Hind III	Bam H I	Eco R I
Plasmid DNA	Positive	Negative	Negative

Table 5b. Analysis with Chromosomal DNA

Sample	Restriction endonuclease		
	Hind III	Bam H I	Eco R I
Chromosomal DNA	Positive	Negative	Negative

Table 6a. Antibiotic susceptibility pattern of Methicillin Resistant *S. aureus* (MRSA)

Strain	Diameter of the Zone of inhibition in mm towards the antibiotics					
no	A	C	E	R	S	M
SA1	25	24	20	24	15	-
SA2	2	18	12	25	13	-
SA3	7	20	20	15	18	-
SA4	2	25	23	25	17	-
SA5	11	12	20	15	18	-

Table 6b. Antibiogram analysis

Strain	Antibiotic susceptibility pattern towards the antibiotics					
no	A	C	E	Rf	S	M
SA1	S	S	S	S	I	R
SA2	R	I	I	S	I	R
SA3	R	S	S	I	I	R
SA4	S	S	S	S	I	R
SA5	I	I	S	I	I	R

A-Amphicilin, C-Chloramphenicol, E-Erythromycin, Rf-Rifampicin,
St-Streptomycin, M-Methicillin, S- Sensitive, I-Intermediate, R- Resistant

DISCUSSION

Staphylococcus aureus is the most virulent species moreover evolutions have lead to the development of various strains which are resistant to various antibiotics (Forbes *et al*., 1998).

Hence the present study was undertaken with the advent of molecular tools to differentiate the strains. Kloos & smith, 1980 suggested that antibiotic resistance in s aureus revealed that resistance genes are often located on plasmids so it's necessary to differentiate the strains.

To confirm this, study has made by isolating MRSA strain, extracting their plasmid and chromosomal DNA with 19kb and 2400 kb respectively. The restriction pattern was identified by using enzymes Bam HI, EcoR I and Hind III in which digestion was observed only with Hind III showing recognition sequence 5'-AGCTT-3'.

By this restriction fragment length polymorphism, Tenover *et al*., 1994 has identified around 29 strains. As a result the present study provides an additional method to aid in the characterization of the bacterial strains by using molecular tools.

Hence if homology modeling is done the protein structure was exactly predicted with the above results and it can be further utilized for the drug development studies.

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