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**MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT
STAPHYLOCOCCUS AUREUS (MRSA) ISOLATES FROM DIFFERENT REGIONS
OF HIMACHAL PRADESH**

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ABSTRACT

The emergence of methicillin resistant *S. aureus* (MRSA) is a severe epidemiological problem. Nowadays nosocomial infection is a major problem in the world including India. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are resistant against several antibiotics and reflect intrinsic resistance to β -lactam antibiotics. MRSA have a particular characteristic to spread in hospitals rapidly and are present in most of the countries. The present study was planned to investigate the prevalence of MRSA and their rate of resistance to different anti-staphylococcal antibiotics. A total of 80 samples (collected from different sources) were screened for MRSA and Antibiotic susceptibility test was performed on each sample, out of which 58 found *S. aureus*. Out of 58 isolated strain of *S. aureus*, 24 (41.37%) were found to be methicillin resistant. Almost all MRSA strains were resistant to methicillin followed by oxacillin. About 60%-70% MRSA strains were resistant to tetracycline, ciprofloxacin, cotrimoxazole, cefotaxime erythromycin. The determination of prevalence and antibiotic sensitivity pattern of MRSA will be used as first line treatment. The results of PCR revealed that 6 out of 24 isolates were carrying *mecA* gene with amplicon size of 310 bp. In this study no correlation was obtained between phenotypic and genotypic characteristics of *S. aureus*. This study showed that all

MRSA isolates, which are multidrug-resistant microorganism and the principal nosocomial pathogen, were significantly less sensitive to other antibiotics.

Keywords: *S. aureus*, MRSA, PCR, *mecA* gene, Antibiotic susceptibility

INTRODUCTION

Staphylococcus aureus is a versatile pathogen, which colonizes among 20%–50% of the human population [1]. It is responsible for a broad spectrum disease ranging from nosocomial infections, septicaemia, wound sepsis, pneumonia, post-surgical infections, septic abortion, skin pustules, osteomyelitis and to serious infections such as bacteraemia, endocarditis, renal abscess, gastroenteritis meningitis, toxic shock syndrome [2]. Up to 50% of *S. aureus* infections are caused by methicillin-resistant *S. aureus* strains (MRSA) [3]. Methicillin was first introduced in the 1960s to treat *S. aureus* infections. Shortly after its introduction, strains of MRSA began to appear [4]. MRSA is a group of *S. aureus* strains resistant to all member of the beta lactam group (penicillin, methicillin, oxacillin etc.) of antibiotics. *S. aureus* is one of the most prominent causes of nosocomial and community-acquired bacterial infections. The emergence of community acquired MRSA drastically changed the picture by increasing the risk of MRSA infections [5]. Methicillin resistance is encoded by the *mecA* gene which is present on a mobile genetic element and is

known as staphylococcal cassette chromosome (SCC) [3]. Besides the *mecA* gene, the SCC*mec* element contains regulatory genes, an insertion sequence element (*IS431mec*) and a unique cassette of recombinases genes (*ccr*) responsible for the integration and excision of SCC*mec* [6]. Until now eleven types of SCC*mec* are identified. Type I – III SCC*mec* (size 37-64kb) cassettes are associated with hospital acquired MRSA, whereas Type IV-VIII SCC*mec* (size 24 kb) cassettes code the presence of community acquired MRSA. MRSA has been a major cause of nosocomial infections since the early 1960s [4] and since 1997 another type of MRSA, producing Pentone Valentine Leucocidin (PVL) has emerged in the community. It is associated with surgical infections and with necrotizing pneumonia, in children and adolescents. Recently, numerous studies have reported the emergence of CA-MRSA (2002) within the hospital setting and have become public health threat [7]. In present the effective antibiotics available to treat MRSA infections are the glycolipid antibiotics like vancomycin and teicoplanin. Our study was

carried out to determine the prevalence of MRSA infection in wounds and there in *vitro* susceptibility pattern to various antimicrobial agents. Genotype of resistant strains was also studied. The study therefore holds epidemiological significance as there has been lack of data on the status of MDR in *S. aureus* particularly from this state.

MATERIAL AND METHODS

IRB/ EC Approval

The experimental protocol was approved by institutional ethical committee of Shoolini University, Solan, India under Registration Number: SUIEC/13/36.

Chemicals and reagents

Chemicals and reagents of analytical grade of Hi Media and Genaxy were used.

Collection and processing

80 samples were procured from various regions of Himachal Pradesh-India and were mixed in 40% glycerol stock solution. Collected samples were streaked on nutrient agar plates and were incubated for 24 hrs at 37°C.

Phenotypic characterization

Identification of *S. aureus* isolates was based on its growth and colonial morphology on nutrient agar and fermentation on Mannitol salt agar. Other tests performed were gram staining (Gram positive cocci in clusters) and biochemical tests including catalase,

coagulase, MR test, VP test, Alkaline Phosphatase, Urease, Arginine utilization, Mannitol, Sucrose, Lactose, Arabinose, Raffinose, Trehalose and Maltose. All the biochemical tests except catalase, coagulase and MR4 were done by using KB004 HiStaph™ Identification kit.

Drug sensitivity assay:-

Antibiotic susceptibility test

In vitro sensitivity of *S. aureus* strains (58) to eight antibiotics Oxa (Oxacillin), Met (methicilin), Ery (Erythromycin), Tet (Tetracycline), Cot (Cotrimoxazole), Cip (Ciprofloxacin), clin (Clindamycin) and Cef (Cefotaxime) was determined using Bauer-Kirby disc diffusion assay modified by Clinical Laboratory Standards Institute guidelines.⁵ All tests were performed on Mueller-Hinton agar supplemented with 4% NaCl.

Genotypic characterization

DNA isolation

DNA from cultured bacteria was isolated by using protocol from Sambrook *et al.*, 1989 [8]. 24 isolates showing high resistance towards both Methicillin and Oxacillin were chosen for further genotypic characterization as MRSA by PCR. Bacterial culture was grown in nutrient broth for overnight (12-14hr). Overnight grown bacterial culture was centrifuged at 12000 rpm for 2 min to pellet

down the cells. Supernatant was discarded without disturbing the cell pellet and extraction was carried out following the protocol.

PCR assay

The following oligonucleotides were used in PCR amplification: primers for *mec*

F:-GTAGAAATGACTGAACGTCGATAA and R:- CCAATTCACATTGTTTCGGTCTAA

which amplified a 310-bp fragment of the *mecA* gene . PCR amplification was done according to Ciftci *et al.*, 2009 [9] with little modifications. The cycle followed for amplification was as: 94°C for 4 min of initial denaturation; 30 cycles of 94°C for 45 s, 55°C for 30 s and 72°C for 90 s; and a final extension at 72°C for 10 min. Amplicons were loaded onto 1% Agarose Gel containing 1µg/ml ethidium bromide.

RESULTS

Phenotypic characterization

Traditional analyses of 80 samples were carried out using bacterial isolation and biochemical identification. These samples were collected from various regions of Himachal Pradesh-India. Results revealed the presence of Gram positive, non-spore forming cocci, arranged in form of grapes or in irregular clusters. The colonies were circular, smooth and glistening.

Biochemically; they were catalase, coagulase positive and mannitol fermenter which proved to be *S. aureus*. Other biochemical tests were done by using KB004 HiStaph™ Identification kit and isolates were positive for mannitol, VP, MR, Alkaline Phosphatase, sucrose, lactose, trehalose, maltose, weakly positive for urease, arginine utilization and negative for ONPG, arabinose and raffinose. On the basis of phenotypic characteristics, 58 isolates found *S. aureus* positive.

Antibiotic susceptibility test

Antibiotic sensitivity test was carried out using eight antibiotic disks (HiMedia). Antibiotic sensitivity test against 58 *S. aureus* isolated strains, showed resistance against Oxacillin (75%), Methicillin (75%), Erythromycin (65), Tetracycline (70%), Cotrimoxazole (60%), Ciprofloxacin (50%), Clindamycin (5%) and Cefotaxime (45%) (Figure-1).

Genotypic characterization

24 isolates showed high resistance towards both oxacilline and methicillin and therefore were chosen for further genotypic characterization as MRSA by PCR. Of these 24, 7 isolates showed amplicon of 310 bp, indicating the presence of *mecA* gene (Figure-2).

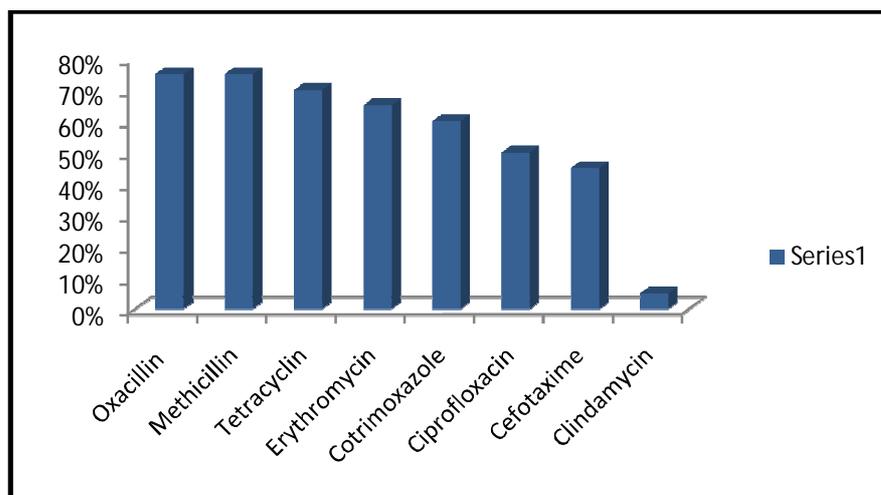


Fig.1:- Resistant Pattern of *S. aureus* against various antibiotics.

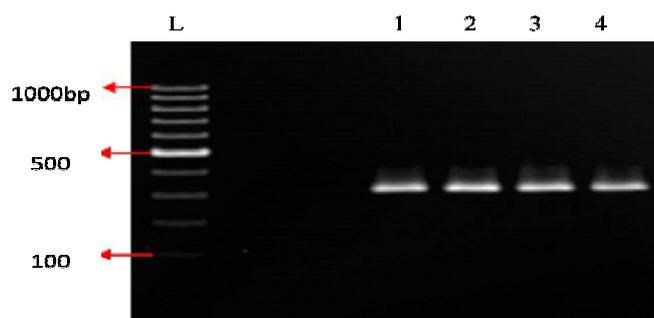


Fig 2:- Amplification products of *S. aureus mecA* gene by PCR. Lane 1: 100bp ladder lane 1, 2, 3, 4 were *mecA* positive samples.

DISCUSSION

Staphylococcus aureus is a highly adaptive and versatile gram-positive bacterium. Methicillin resistant *Staphylococcus aureus* (MRSA) strains are usually resistant to several antibiotics and also inherent resistance to β -lactam antibiotics. *S. aureus* is one of the most prominent causes of nosocomial and community-acquired bacterial infections worldwide. On the bases of gram staining and biochemical test all

isolates were identified as *S. aureus*. In the present study both phenotypic and genotypic characterizations of the given isolates were performed to know the phenotype prevalent in this region (Himachal Pradesh) and to choose the appropriate antibiotic treatment. On resistance profiling 24 isolates out of 58 were found to be MRSA as they were showing resistance to 3 or more than 3 antibiotics and 75% were resistant to oxacillin & Methicillin. These results point to

the fact that the given isolates were methicillin resistance *S. aureus* (MRSA) and the worst feature of MRSA has been drug resistance to many of the antibiotics. This fact has been supported by the findings of the present study that higher level of resistance was also observed against tetracycline (70%), erythromycin (65%) and cotrimoxazole (60%). Ciprofloxacin & cefotaxime showed intermediate level of resistance (50% & 45%) whereas some strains were found sensitive to clindamycin (95%). Other studies have also shown sensitivity of *S. aureus* towards clindamycin [10, 11] but present study has shown remarkably good activity of clindamycin against *S. aureus*, compared to previous studies. Therefore, for the treatment of *Staphylococcus aureus* particularly in methicillin-resistant *S. aureus* (MRSA) infections, clindamycin can still be used as second line antibiotic. Our study shows high incidence of MRSA and the prevalence rate is found to be 75% which is much higher than most of the reports from other places of India [12, 13, 14]. This may be due to random use of antibiotics and lack of proper awareness. This study also showed that all MRSA isolates were significantly less sensitive to other antibiotics and is a multidrug-resistant microorganism and the major nosocomial pathogen. On resistance

profiling isolates were detected phenotypically as MRSA. 75% resistance was observed for methicillin & therefore the resistant isolates were subjected to PCR. This study aimed to examine the distribution of *mecA* in the *S. aureus* population [15]. Methicillin resistance is encoded by the *mecA* gene present on a mobile genetic element, staphylococcal cassette chromosome (SCC) [3]. Genotypic characterization of 24 MRSA isolated was done for the presence of *mecA* gene by PCR. The results revealed only 6 isolates carrying *mecA* gene with amplicon size 310 bp. The fact that methicillin resistance is heterogeneous in nature is supported by the studies of many authors [9, 16, 17].

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