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Prevalence and Molecular Characterization of MRSA Isolates from Pus and Blood Samples with Special Reference to MecA and ErmA Gene at a Tertiary care in Kanpur

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Abstract: *Background:* Methicillin-resistant staphylococci are now recognized as a major cause of infectious diseases, particularly in hospitals. Molecular epidemiology is important for prevention and control of infection. This study is used to find out the prevalence and gene causing resistance mechanisms for MRSA isolates. *Aim and objectives:* To study the Prevalence and Molecular Characterization of MRSA isolates from Pus and Blood Samples with Special Reference to MecA and ErmA Gene. *Methods:* Our study was a retrospective study which was carried out in the Department of Microbiology and Central Research Lab of RMCH &RC for a period of 2 years 4 months *i.e*, March 2019 to July 2021. The isolates were collected from blood and pus patients of different wards. Phenotypic and Genotypic isolation of MRSA was performed. The bacteria were initially identified by colony morphology, mannitol fermentation, Gram characteristics, catalase test, coagulase test, and DNase activity. In phenotypic methicillin resistance was assessed using the cefoxitin disk diffusion method following the Clinical and Laboratory Standard Institute guidelines (CLSI). The detection of *MecA* and *ErmA* genes was done by isolating DNA following DNA amplification. *Results:* Total 220 isolates were included in our study, out of which 90 were confirmed to be MRSA by CX , OX , E-test and MIC method and remaining 130 were MSSA. From the 90 MRSA isolates, 25 were found to be D test positive, whereas 20 were confirmed cMLSB while the other 25 were noticed to be MS phenotype and 25 were found sensitive phenotypes. All methicillin-resistant staphylococci were tested for their susceptibility against commonly used antibiotics. All MRSA isolates were sensitive to linezolid, Teicoplanin, vancomycin, Gentamycin and Resistance to Cefoxitin and Oxacillin. The presence of MecA gene was detected in all the 90 isolates and the presence of ErmA gene was found in 5 isolates among the MRSA. The presence of MecA and ErmA gene was confirmed by sequencing. The prevalence of MRSA was found to be 40.9% in our study. *Conclusions:* There is an urgent need to develop better measurements among clinical microbiology laboratories for proper detection, identification, and reporting of MRSA isolates with deep knowledge among physicians toward antibiotic and prescription practices for this multi-drug resistant organism.

Keywords: MRSA, MSSA, Antibiotic resistance, CLSI, MecA, ErmA, DNA and PCR.

Introduction

Staphylococcus aureus (SA) is a Gram-positive bacterium, mainly present on the skin, pus and blood [1-2]. SA causes severe diseases and responsible for food borne intoxications [3]. SA has strong pathogenic potential which is concerned to the capacity of acquiring resistance to different antibiotics by generating several virulence factors [4-5]. There are particularly two types of resistances recorded; methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) [6]. The known SA mechanism of the resistance is to modification of aminoglycosides by the inactivation of aminoglycoside modifying enzymes [7-8].

MRSA strains have been recorded for wide pattern of resistance mainly not for the β-lactams but also for aminoglycosides, similarly for other bio-molecules such as

macrolides, lincosamides, and mupirocin [9-10]. For the treatment of SA infections, the macrolide antibiotics are widely used as protein synthesis inhibitor. Several mechanisms of resistance of SA have been known mainly to modification of ribosomal binding site by erm genes (ermA, ermB, and ermC), and active efflux mechanism associated by msr gene [11]. Understandings its diversity have been confirmed by the use of whole genome sequencing and DNA microarray-based analysis [12-13].

In the known SA variants, several antibiotic resistance genes have been identified such as mec A, ermA, ermB, ermC, mupA, msrA, msrB, and tet etc [14-15]. The aim and objectives of the present study was to explore the diversity, prevalence and pathogenicity of *S. aureus* isolates from the blood and pus isolates. In this study, MRSA isolates were collected from human of northern India and were characterized using molecular approaches.

Material and Methods

This was a retro respective study which was carried out in the Department of Microbiology and Central Research Lab of RMCH & RC, Kanpur, Mandhana for a period of 2 years 4 months i.e., March 2019 to July 2021. A Total of 220 samples were included in our study. The isolates were collected from blood and pus of the patients of different wards. Ethical Clearance was taken from the Ethical Committee of RMCH & RC. All the isolates were collected with following sterile conditions and immediately frozen in deep freezer (-20^{0}C) of Remi Lab. Pvt. Ltd.

Inclusion Criteria: Isolates collected from both sexes (males and females) were included in this study.

Exclusion Criteria: The patients those on antibiotic treatment were excluded and diabetic, those have family history of critical disease were also excluded from this study.

Phenotypical Identification of the MRSA: The identification was done by colony morphology, mannitol fermentation, Gram characteristics, catalase test, coagulase test, and DNase activity. The phenotypic methicillin resistance was assessed using the cefoxitin, oxacillin disk diffusion method E-test and MIC in accordance with the Clinical and Laboratory Standard Institute guidelines at our clinical laboratory [16].

Genotypical Identification of the MRSA: The Genomic DNA was extracted using Qiagen kit (Germany) with following standard protocol according to manufacturer's guidelines (Fig.-3). The primers for MecA and ErmA genes were synthesized by Chromous Biotech. Pvt. Ltd. (Bangaluru). The obtained primers were solubilized in TE buffer (1mM, pH-8.0) and working solution of primers were diluted with addition of nuclease free water to make them 10 pm/µl concentration.

The genomic DNA were amplified with PCR (reaction volume 20 µl) by adding 10µl master mix (Takara), 5µl nuclease free water, 1 µl forward and reveres primer each and 3µl DNA as a template. Conditions for PCR was initial denaturation 94 ˚C for 5 min, and then 34 cycle at 94 ˚C for 30 sec for cycle denaturation, 51 ˚C for 45 sec for annealing for *Mec A* gene and 53 ˚C for 1 min for annealing for Erm *A* gene then after extension was performed at 72 ˚C for 1min followed by final extension at 72 ˚C for 7 min. Amplified PCR (BIO-RAD T100 Thermal Cycler, Singapore) product was resolved by using 1% agarose gel electrophoresis containing 1X TAE buffer and stained with ethidium bromide. Primers used in this study were listed in the Table-1.

Table-1: List of primers

Mec A gene	Forward primer	5'- GTTGTAGTTGTCGGGTTTGG-3'	Tm-53.0^{o}C
	Reverse primer	5'- CTTCCACATACCATCTTCTTTAAC -3'	Tm-52.0^{o}C
Erm A gene	Forward primer	5'-TCTAAAAAGCATGTAAAAGAA -3'	Tm-55.0^{o}C
	Reverse primer	5'- TGATTATAATTATTTGATAGCTTC -3'	Tm-54.0^{o}C

Results

Total 220 isolates were included in our study, in which Male (140) were more in number than Females (80). Out of which 55 were Males and 35 were Females among the 90 MRSA isolates, which were confirmed by CX, OX, E-test and MIC method and remaining 130 were MSSA. The maximum number of isolates was in the age group of 41-50 years and least in the age group above 61 years. The maximum number of isolates was from the Blood as compared to the Pus samples and in the OPD ward. From the 90 MRSA isolates, 25 were found to be D test positive, whereas 20 were confirmed cMLSB while the other 25 were noticed to be MS phenotype and 25 were found sensitive phenotypes.

The prevalence of MRSA was found to be 40.9% in our study. The presence of MecA gene was detected in all the 90 isolates and the presence of ErmA gene was found in 5 isolates among the MRSA. The presence of MecA and ErmA gene was confirmed by sequencing.

Table-2: Gender wise Distribution of MRSA and MSSA

S.N.	Gender	Isolates (N=220)	MRSA (N=90)	MSSA (N=130)
1.	Male	140	55	85
2.	Female	80	35	45

Table-3: Age wise distribution of the MRSA isolates

S.N.	Age group (Years)	Male N=140	Female N=80
1.	0-10	15	08
2.	11-20	20	10
3.	21-30	15	15
4.	31-40	20	13
5.	41-50	35	20
6.	51-60	25	05
7.	61-70	10	09

During this samples were collected mainly from the pus and blood with following standard protocols.

Table-4: Sample wise distribution of *S. aureus*

S. N.	Sample collected from	*S. aureus* (N=220)	MRSA (N=90)	MSSA (N=130)
1.	Pus	105 (47.72%)	55	45
2.	Blood	115 (52.27%)	35	85

Fig-1: Sample wise distribution of *S. aureus*

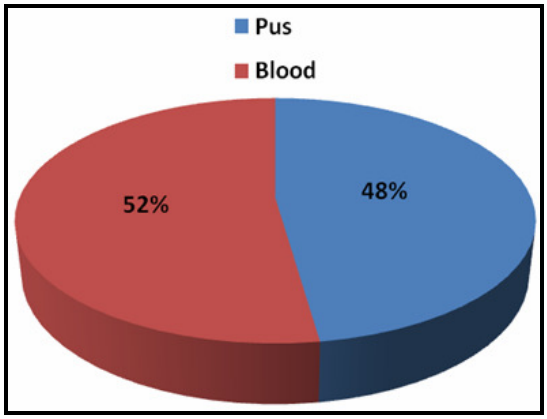
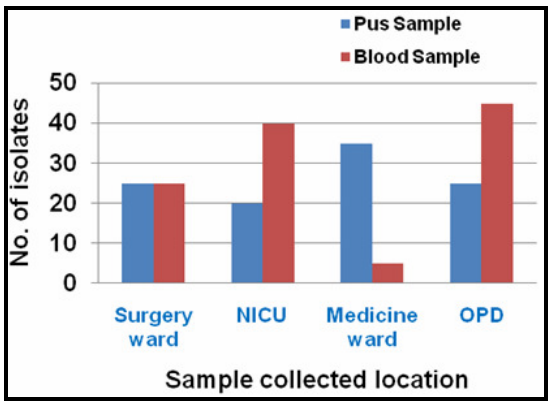


Table-5: Distribution of *S. aureus* from different location

S. N.	Collected location	Isolates collected N=220	Pus N=105	Blood N=115
1.	Surgery ward	50	25	25
2.	NICU	65	20	40
3.	Medicine ward	40	35	05
4.	OPD	65	25	45

Fig-2: Distribution of *S. aureus* from different sites



Out of 90 MRSA isolates, all were found to be Mec A gene positive while 5 were Erm A positive, out of 5 ErmA positive, 3 were belonged from iMLSB and 2 were from cMLSB category. For the study of antibiotic sensitivity pattern disc diffusion test was performed. The obtained resistance and sensitivity zone were mentioned in the following Table-6. All methicillin-resistant staphylococci were tested for their susceptibility against commonly used antibiotics. All MRSA isolates were sensitive to linezolid, Teicoplanin, vancomycin, Gentamycin and Resistance to Cefoxitin and Oxacillin.

Table-6: Antibiotic sensitivity pattern of MRSA (N=90)

S.N.	Antibiotic	Disc potency	Resistance (mm)	Sensitive (mm)
1.	Deoxycycline (D)	30µg	15	75
2.	Erythromycin (ER)	15µg	65	25
3.	Gentamycin (GM)	10µg	10	80
4.	Linezolid	30µg	-	90
5.	Oxacillin (OX)	1µg	90	-
6.	Penicillin (P)	10µg	80	10
7.	Teicoplanin (TEI)	30µg	-	90
8.	Tetracyclin (TE)	30µg	15	75
9.	Vancomicin (VAN)	30µg	-	90
10.	Ampicillin (AMP)	10µg	20	70
11.	Amoxicillin Clavunic acid (AMC)	20/10µg	10	80
12.	Cefoxitin (CX)	30µg	90	-
13.	Chloramphenicol (C)	30µg	25	65
14.	Ciprofloxacin (CIP)	5µg	15	75
15.	Clindamycin (CD)	2µg	25	65
16.	Co-Trimoxozole(COT)	25µg	20	70

Phenotypic Identification of MRSA

Detection of Mec A and Erm A genes: In this study, 90 MRSA isolates were subjected for the molecular analysis. We obtained good quality of DNA from 90 isolates. Gel photographs of the DNA samples are figured below.

Fig-3: Photograph of DNA from *S. aureus* isolates

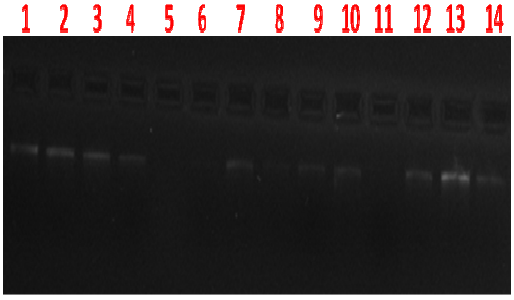


Fig-4: Photograph of amplified Mec A gene in *S. aureus*, the amplified DNA band size was obtained 336 bp, L corresponding to 100bp ladder used, where Lane 15 is the positive control and Lane 17 a Negative control

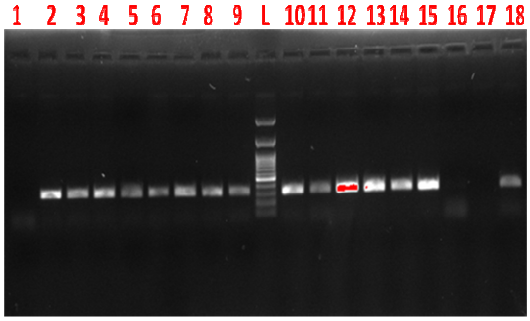
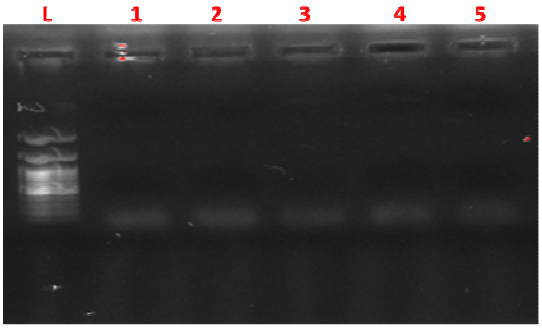


Fig-5: Photograph of amplified Erm A gene in *S. aureus*, the amplified DNA band size was obtained 149bp, L corresponding to 100bp ladder



Graph-1: Obtained gene sequences of *MecA* gene in *S. aureus*

GTTGTAGTTGTCGGGTTTGGTATATATTTTTAT
GCTTCAAAAGATAAAGAAATTAATAATACTAT
TGATGCAATTGAAGATAAAAATTTCAAACAAG
TTTATAAAGATAGCAGTTATATTTCTAAAAGC
GATAATGGTGAAGTAGAAATGACTGAACGTC
CGATAAAAATATATAATAGTTTAGGCGTTAAA
GATATAAACATTCAGGATCGTAAAATAAAAA
AAGTATCTAAAAATAAAAAACGAGTAGATGC
TCAATATAAAATTAAAACAAACTACGGTAACA
TTGATCGCAACGTTCAATTTAATTTTGTTAAAG
AAGATGGTATGTGGAAG

Graph-2: Obtained gene sequences of *ErmA* gene in *S. aureus*

TCTAAAAAGCATGTAAAAGAATTTGCGACCAG
ATTGCAAAATCTGCAACGAGCTTTGGGTTTAC
TCCCCCCGGTGGAGATGGATATAAAAATGCTC
AAAAAAGTACCACCACTATATTTTCCTAAGAA
GCTATCAAATAATTATAATCA

Discussion

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a serious life threatening pathogen in hospitals and among healthy populations. Thus, the characterization of these strains is important for local epidemiology and surveillance studies. Total 220 isolates were included in our study, in which Male (140) were more in number than Females (80) Table-2. Out of which 55 were Males and 35 were Females among the 90 MRSA isolates, which were confirmed by CX, OX, E-test and MIC method and remaining 130 were MSSA (Table No. 2).

From the 90 MRSA isolates, 25 were found to be D test positive, whereas 20 were confirmed cMLSB while the other 25 were noticed to be MS phenotype and 25 were found sensitive phenotypes. This finding is strongly supported by references [17-21] along with the study conducted by Nezhad *et al*., 2017 [22] and also supported. In the Table No.3, we have mentioned Age wise distribution of isolates. The maximum cases were reported in the 41-50 years of age group while the minimum cases were found in the age group of 51-60 (Table-3). This finding is with the agreement of the findings of the studies in Iran [23], Bolivia [24], while this study finding values are higher than Hungary [25] report.

The maximum number of isolates was from the Blood as compared to the Pus samples (Fig.-1) and in the OPD ward (Table-4 and Table-5) (Fig.-2). This finding is fully supported with the Olaniyi *et al*., 2017 [26]. *S. aureus* isolates are principal pathogenic agent of skin and soft tissue infections [27]. In the literature same finding has been reported by Debdas *et al*., 2011 [28] and Jimenez *et al*., 2011 [29] where the maximum number of cases were from the OPD and surgical ward. The prevalence of MRSA was found to be 40.9% in our study. This corresponded to the study done by other authors also where the prevalence was recorded 25-45 % [30].

In our study finding it has been found that maximum sensitivity of antibiotics was from Linezolid, Teicoplanin, Vancomicin and Gentamycin whereas least sensitivity of antibiotics is from Penicillin, and Erythromycin (Table-6). This finding is strongly supported by many coworkers [31-32]. In the literature, the study conducted in Iran [33] has been recorded similar resistance with gentamicin (60%), and kanamycin (57.1%), in the support of our study. In another study, which has been conducted by Lavallee *et al*. [34] in Canada, reported that inducible clindamycin resistance was 64.7% for MRSA isolates. In one more study which was conducted by Fiebelkorn *et al.* [35] has been stated that 34% of 114 erythromycin resistant *S. aureus* isolates demonstrated constitutive resistance with clindamycin and 29% showed inducible resistance.

The presence of MecA gene was detected in all the 90 MRSA isolates (Fig.-4) and the

presence of ErmA gene was found in 5 isolates among the MRSA (Fig.-5). The presence of MecA and ErmA gene was confirmed by sequencing graph (Graph. 1 and Graph 2) with homology with the NCBI data base and found 99% identical.

Conclusion

In this study, *S. aureus* based infections with its risk factors were explored to improve public health and hygiene conditions. This study also indicated about the use of suitable antibiotics to cure infections and proper screening programs should be followed to to avoided. It has also investigated in this study about the selection of suitable antibiotics and put a complete antibiotics resistant pattern for the prescription of rational use in human and animal infections. All the isolates were confirmed by PCR methodology and gene sequencing which is more powerful technique used recently. Overall this study is novel and explored authentic data for the researchers and mankind.

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Conflicts of interest: There are no conflicts of interest.

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