"Phenotypic and Genotypic Characterization of MRSA in a Tertiary Care Hospital at Kanpur"

R. Sujatha*, Deepak S[1], Nashra A[2], Arunagiri D.[3]

Abstract:

Background: Methicillin resistant Staphylococcus aureus is a global health challenge nowadays creating problem in antibiotic therapy. This study was aimed to study phenotypic and genotypic characterization of MRSA in order to formulate antibiotic policy for control of MRSA at a tertiary care hospital of Kanpur.

Material and Methods: Clinical samples were processed from hospitalized patients in Rama Medical College Hospital & Research Centre Mandhana, Kanpur. Out of 140 S. aureus isolates 55 were found to be MRSA by disk diffusion method and E- test strip method. Bacterial DNA was extracted and PCR for the detection of Mec A was performed.

Results: Out of 140 S. aureus isolates, the Prevalence of MRSA was 55 (39.2%). The molecular characterization was performed where the DNA from the test isolates was extracted which was further processed for the PCR for the detection of the Mec A gene. All 55 MRSA samples detected the presence of Mec A gene, which was further confirmed by sequencing.

Conclusion: The present study reveals the prevalence of MRSA from the Kanpur region and indicates the magnitude of antibiotic resistance in and around the study area.

Keywords: MRSA, E-test, DNA, PCR.

Introduction

Staphylococcus aureus (S. aureus) is an opportunistic bacterium, which most frequently causes hospital and community-acquired infections [1]. The bacterium is responsible for a wide range of infections, including endocarditic, folliculate, food toxicity, chronic osteomyelitis, pneumonia, septic arthritis, skin and deep tissue infections, etc [2].

The frequency of methicillin-resistant S. aureus (MRSA) isolates is increasing, [3, and 4] and this issue can lead to severe therapeutic dilemmas and exacerbate the control of infections in hospitals [5]. MRSA, which was first reported in the 1960s [6], has become endemic in hospitals and health-care settings worldwide. The term methicillin-resistance is a classic term that implies resistance to all beta-lactam antibiotics, except for recently introduced anti-MRSA cephalosporin's, such as ceftobiprole. The mec A gene, which encodes for a modified penicillin-binding protein, PBP2a, with decreased beta-lactams affinity, [7] is responsible for methicillin-resistance among bacteria, including MRSA. The gene is located on a mobile genetic element defined as staphylococcal cassette chromosome mec (SCC mec) [80. Till now, 13 types of SCCmec elements have been characterized (I-XIII) that each type has its own specific characteristics. Among them, Types I, II and III of SCC mec are the most prevalent types of SCC mec among hospital-acquired MRSA (HA-MRSA), whereas Types IV and V are the most seen types found in the community-acquired MRSA (CA-MRSA) strains [9]. For efficient epidemiological surveillance and control of health-care-associated MRSA infections, it is of great importance to determine the antibiotics susceptibility pattern as well as to study it's the molecular characterization of bacteria.

*Prof and Head, Department of Microbiology, Rama Medical College Hospital and Research Center Kanpur (India)
[1]Tutor, Department of Microbiology, Rama Medical College Hospital and Research Center Kanpur (India)
[2]Research Assistant, Central research lab, Rama Medical College Hospital and Research Center Kanpur (India)
[3]Professor & HOD, Dept of Endodontic, MDC, Kanpur(India)

Material and Methods

It was a cross sectional study which was carried out in the Department of Microbiology and Central Research Laboratory, Rama Medical College Hospital and Research Centre RMCHRC, Mandhana, Kanpur for a period of 1 year February 2018 to February 2019. The ethical clearance was taken from the Ethical committee of Rama Medical College.

A total of 140 non repeated pus, wound swab, blood, nasal swab, throat swab, urine, clinical samples received from the patients for bacteriological culture were included in our study.

Isolation and Identification of *Staphylococcus aureus*

The specimens were inoculated on blood agar and mannitol salt agar (HiMedia laboratories private limited, India) and incubated aerobically at 37°C for 48 hours. The strains of *Staphylococcus aureus* were identified on the basis of colony morphology, Gram's stain, and different biochemical tests [10].

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was performed by modified Kirby-Bauer disc diffusion technique using

Mueller-Hinton agar (HiMedia laboratories private limited, India) following Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. Penicillin(10µg)-P,Cefoxitin-E-test(0.25-250µg)-CX,Cephalexin(30µg)-CEP, Erythromycin(5µg)-E,Tetracycline(10µg)-TE,Ciprofloxacin(5µg)-CIP,Levofloxacin(5µg)-LE,Linezolid(30µg)-LZ,Vancomycin(30µg)-VA,Teicoplanin(30µg)-TEI, Gentamycin(10µg)-GEN, Amikacin(30µg)-AK, Chloramphenicol(30µg)-C, Cefoxitin (30µg)-CX, Oxacillin (1mcg)-OX, Vancomycin- Etest (0.16-256 µg)-VA, Clindamycin (2 mcg)-CD, Co-Trimoxazole (1.25/23.75 mcg)-COT, mupirocin (5 mcg), Doxycyclin (30 mcg).

Phenotypic Detection Method for MRSA

All isolates of *S.aureus* were tested by Oxacillin disc diffusion and Cefoxitin disc diffusion method. A standard strain of ATCC 29213 (Mec A) negative and a PCR-positive control strain (ATCC 49476) (MecA-positive) were used as controls for all methods.

Identification of MRSA through Oxacillin disc diffusion method

All the strains were tested with the 1mg oxacillin discs (Hi-media) on Mueller-Hinton agar plates. A bacterial suspension adjusted to 0.5 McFarland was used for each of the strain. The zone of inhibition was determined after 24 h incubation at 35°C. Zone size was interpreted according to CLSI (2008) [12] criteria: susceptibility >=13mm; intermediate, 11-12mm; and resistance <=10mm

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Identification of MRSA Cefoxitin disc diffusion method

All strain was tested with 30mg cefoxitin discs (Hi-Media) on Mueller-Hinton agar plates. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used. The zone of inhibition was determined after 16-18h incubation at 37 °C. Zone size was interpreted according to CLSI [12] criteria: susceptibility >=22mm; resistance <=21mm.

Identification of Staphylococci strains

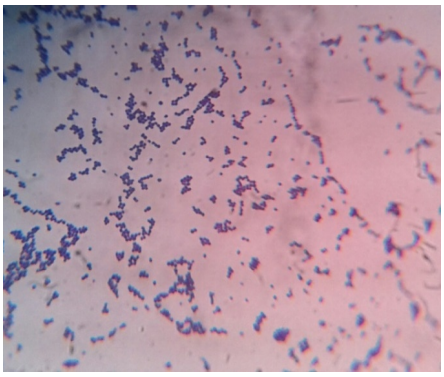

Figure 1: Gram staining of S. aureus

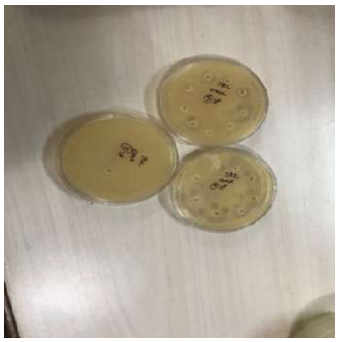

Figure 2: AST showing positive result for MRSA

Figure 3: E-Test showing positive result for MRSA

Genotypic Detection of MEC A Gene By PCR

Detection of MEC A gene by Polymerase Chain Reaction:

Bacterial DNA was extracted by **QIAGEN DNA KIT** by following manufactures guidelines. *S. aureus* previously extracted DNA was used for the amplification of mec A gene. A volume of 20 µl PCR reaction mixture consisting of 10 µl master mix, 1µl of each forward and reverse primers gene specific for the target gene, 3 µl of DNA template, and the volume was made up by adding nuclease free water. A 336-bp fragment of the *mec* A gene was obtained. The forward and revered primers GTTGTAGTTGTCGGGTTTGG and CTTCCACATACCATCTTCTTTAAC were used respectively. The mixture was briefly centrifuged and the tubes were transferred into PCR machine which has been programmed with the following conditions. The initial denaturation step for 7 minutes at 94°C followed by 94°C for 30 sec, 47°C for 40 sec, 72°C for 45 sec, 72°C for 7 min. The total cycles were 37. The PCR programming was very similar as followed by Jonas *et al*., 2002. The PCR products was electrophoreses, stained with 10 µM ethidium bromide, bromophenol blue visualized by using UV Tran illuminator [13].

The positive cases have shown PCR product of 336 bp.

Table No. 1: Phenotypic Identification of MRSA with the use of different test

Microscopic observation	Gram's test	Catalase test	Coagulase test		Unease test	Cefoxitin (cx) and Oxacillin (ox)	DNA ase Test
Cocci form (For all 140 cases)	+	+	Slide +	Tube +	+	+	+

Table No. 2: Genotypic Identification of MRSA

Type of Organisms	Mec A gene (MRSA)
MRSA	+

Results

Clinical samples were isolated from hospitalized patients in RMCH & RC Mandhana, Kanpur. Out of 140 *S.aureus* isolates 55 were found MRSA by disk diffusion method and E-test strip method and 85 were MSSA. The molecular characterization of the test isolates detects the presence of Mec A gene, which was confirmed by sequencing, The PCR product of the isolates that carried the Mec A gene have been sent to Chromous Biotech Pvt. Ltd., Bengaluru for gene sequencing. No 39, 3rd Floors, Metropolis Business Park, Industrial Area, 1st Main, Yelahanka New Town, Bengaluru - 560064. The gene sequences were confirmed by homology of NCBI blast.

Table No 3: Distribution of *S. aureus* isolates in cases groups

Type of Isolates	No. of Isolates	Percentage (%)
MSSA	85	60.7%
MRSA	55	39.2%

Table No 4: Identification of Staphylococcal strains with the use of different microbiological test

Organism	Disc diffusion test	E-test
MSSA (S. aureus)	85	-
MRSA	55 (CX, OX)	40
Total	140	

Table No 5: Table showing Identification of Mec a gene in S. aureus

S. No.	Characteristics	Total no of samples	No. of samples in which *Mec A* gene obtained	95 % CI	P-Value
1	MRSA	55	55	100	0.001
	Total	55	55	100	0.001

Molecular Work

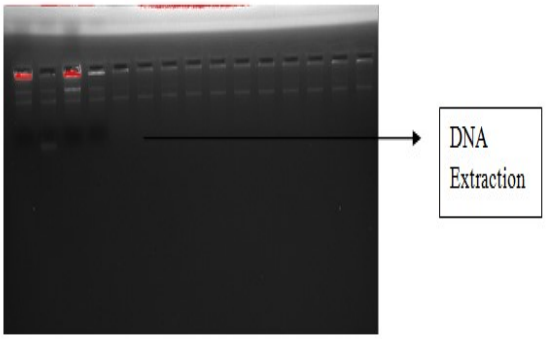

Figure 4: Isolated DNA from Bacterial culture of *S. aureus*

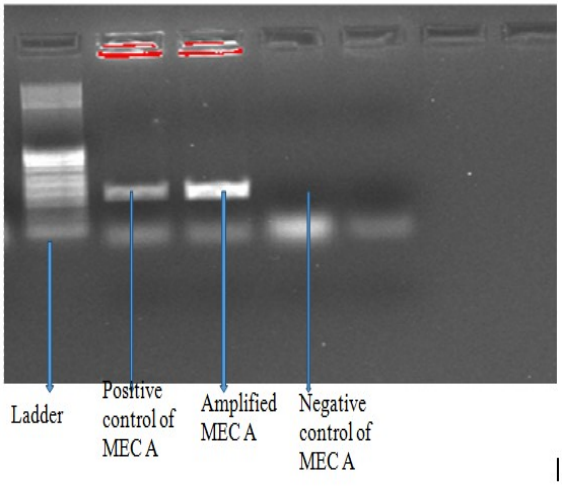

Figure 5: Amplified DNA with PCR for Mec A gene of *S. aureus* in this fig. Lane 1 is DNA ladder; Lane 2 is positive control of Mec A gene; Lane 3 is Amplified Mec A gene; Lane 4 is Negative control for Mec A gene

Discussion

In a study it was found that out of 300 patient, 140 (46.67%) were found to be nasal carriage for *S. aureus*, among which MRSA was found to be 23 (7.67%) [14], which was similar to our study discussed above.

Table No 6: Prevalence of S. aureus in different studies

Sr. no.	Author	Strains of S. aureus	Prevalence of MRSA	Place	Year
1	S Anupurba et al.,[15]	MRSA	54.85%	India	2003
2	J. Ojulong et al.,[16]	MRSA	31.50%	Uganda	2008
3	Arora S et al.,[17]	MRSA	46%	India	2010
4	Sangeeta Joshi et al.,[18]	MRSA	41%	India	2013
5	S. Deyno et al.,[19]	MRSA	47%	Ethiopia	2017
6	**Present study**	**MRSA**	**39.20%**	**RMCH&RC, Kanpur, India**	**Feb-2018-2019**

In our study the Prevalence of MRSA was 39.2% which was similar to the other study. Whereas, in our study Mec A gene was isolated in all the 55 MRSA isolates which was similar to the study by G E Bigarndi etal.,[20] in which out of Eighty-three isolates of *S. aureus* for which MICs of methicillin of 4-16 mg/L had previously been recorded were tested for the presence of the mecA gene with a DNA probe and a PCR assay. There were other studies also done where the presence of Mec A gene in the MRSA sample was confirmed by PCR [21].

Conclusion

In conclusion, our study indicates that the PCR assay is an easy and reliable tool for detection of MRSA and also confirms the presence of Mec A gene from patients and carrier individuals Furthermore, this method may reduce misuse of antimicrobial classes that are more expensive and toxic, thus contributing to the selection of antibiotic-resistant Staphylococcus spp.

On the other hand, with respect to the emergence of multidrug resistant MRSA strains, rapid identification and timely treatment of their infections help to reduce the mortality and avoid the spread of these organisms.

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