"Molecular Characterization of erm A Gene in MRSA isolates at a Tertiary care centre in Kanpur"

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Abstract:

Background: Methicillin-resistant Staphylococcus aureus (MRSA) is an emerging pathogen that is difficult to treat due to the multiresistance of the bacteria upon infection. Molecular epidemiology is important for prevention and control of infection. The present study is undertaken to find out the prevalence and gene causing resistance mechanisms for MRSA isolates.

Objectives: Molecular Characterization of ermA Gene in MRSA isolates at a Tertiary care centre in Kanpur.

Methods: Our study was a cross sectional study which was carried out in the Department of Microbiology and Central Research Lab of RMCH &RC for a period of 1 year i.e , February 2021 to January 2022. The bacteria were initially identified by colony morphology; mannitol fermentation, Gram characteristics, catalase test, coagulase test, and DNase activity the antimicrobial susceptibility to vancomycin, linezolid, ciprofloxacin, clindamycin, erythromycin, chloramphenicol, fusidic acid, gentamicin, quinupristin-dalfopristin, rifampicin, sulfamethoxazole/trimethoprim, and tetracycline were measured in accordance with the Clinical and Laboratory Standard Institute guidelines at our laboratory. Resistance mechanisms for the detection of ermA gene was then analyzed by DNA extraction using Qiagen DNA kit followed by polymerase chain reaction.

Results: All the MRSA isolates were sensitive to linezolid, Teicoplanin, vancomycin, Gentamycin and Resistance to Cefoxitin and Oxacillin, following the CLSI guidelines standards. A total of 180 isolates was included in our study, out of which 80 were confirmed to be MRSA by CX, OX and E-test methods. . The prevalence of MRSA was found to be 44.4 % in our study. Remaining 100 were MSSA. From the 80 MRSA isolates, 21 were found to be D test positive, whereas 16 were confirmed cMLSB while the other 15 were noticed to be MS phenotype and 15 were found sensitive phenotypes .Molecular characterization for the detection of drug resistance gene ermA gene was carried out in which 12 isolates carried ermA gene.

Conclusions: High levels of resistance to second-line antimicrobials threaten the treatment of nosocomial respiratory infections due to methicillin-resistant S. aureus with decreased susceptibility to linezolid and vancomycin. Hence, there is a need for continuous monitoring and implementation of better control strategies for the control of Antibiotic resistance.

Keywords: ermA, Methicillin-resistant Staphylococcus aureus , Antibiotic resistance, DNA and PCR

Introduction

Staphylococcus aureus (S. aureus) is an important human pathogen that is transmitted in both hospitals and the community. MRSA is a major challenge to hospitals all over the world due to the emergence and spread of isolates with decreased susceptibilities to several antibiotics classes including methicillin and other members of ß-lactam family [1] SA has strong pathogenic potential which is concerned to the capacity of acquiring resistance to different antibiotics by generating several virulence factors [2,3]. 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 [4].A wide range of resistance mechanisms have been described for S. aureus including PBP alterations (β-lactam agents), cell wall structure modifications (glycopeptides), point mutations in the quinolone resistance-determining regions of GyrA and GrlA (quinolones), inactivating enzymes (aminoglycosides) ribosome alterations (macrolides, lincosamides, oxazolidones and tetracyclines), efflux pumps (tetracyclines, macrolides, quinolones) or spontaneus mutations in the gene) [5-6]

Recently, innovation of different and precise molecular techniques has played a big role in the detection of ermA gene, including DNA hybridization and polymerase chain reaction (PCR) [7].

The present study was undertaken to study the Prevalence and Molecular Characterization withspecial reference to ermA Gene in MRSA isolates at a Tertiary care centre in Kanpur.

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Material and Methods

The study was carried out in the Department of Microbiology and Central Research Lab of RMCH & RC, Kanpur, Mandhana for a period of 1 year February 2021 to January2022.The isolates were collected Ethical Clearance was taken from the Ethical Committee of RMCH & RC.

A total of 180 Staphylococcus spp. Were isolated from clinical samples The bacteria were initially identified by colony morphology, mannitol fermentation, Gram characteristics, catalase test, coagulase test, and DNase activity The antimicrobial susceptibility to vancomycin, linezolid, ciprofloxacin, clindamycin, erythromycin, chloramphenicol, fusidic acid, gentamicin, quinupristin-dalfopristin, rifampicin, sulfamethoxazole/trimethoprim, and tetracycline were measured method in accordance with the Clinical and Laboratory Standard Institute guidelines at our clinical laboratory [8].

Phenotypical Identification of the MRSA

The phenotypic Methicillin resistance was assessed using the cefoxitin, Oxacillin disk diffusion method and Etest method in accordance with the Clinical and Laboratory Standard Institute guidelines at our clinical laboratory. [8]

Genotypical Identification of the MRSA

The Genomic DNA was extracted using Qiagen kit (Germany) with following standard protocol according to manufacturer's guidelines. The primer for 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 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 [9].

Table 1 primer used for ermA gene

erm A gene	**Forward primer**	5'-TCTAAAAAGCATGTAAAAGAA -3' Tm-55.0^{o}C
	Reverse primer	5'-TGATTATAATTATTTGATAGCTTC -3' Tm-54.0^{o}C

Results

All the MRSA isolates were sensitive to linezolid, Teicoplanin, vancomycin, Gentamycin and Resistance to Cefoxitin and Oxacillin, following the CLSI guidelines standards. A total of 180 isolates was included in our study, out of which 80 were confirmed to be MRSA by CX, OX and Etest methods. . The prevalence of MRSA was found to be 44.4 % in our study. Remaining 100 were MSSA. From the 80 MRSA isolates, 21 were found to be D test positive, whereas 16 were confirmed cMLSB while the other 15 were noticed to be MS phenotype and 15 were found sensitive phenotypesMolecular characterization for the detection of drug resistance gene ermA gene was carried out in which 12 isolates carried ermA gene.

Table-2 Distribution of S.aureus

S.NO	No. of isolates
MRSA	80
MSSA	180

Table-3 Gender wise Distribution of MRSA and MSSA

S.N.	Gender	Isolates N=180	MRSA N=80	MSSA N=100
1.	**Male**	**106**	**47**	**62**
2.	**Female**	**74**	**33**	**38**

Table-4 Age wise distribution of the MRSA isolates

S.N.	Age group (Years)	Male N=47	Female N=33
1.	0-10	1	0
2.	11-20	3	2
3.	21-30	4	4
4.	31-40	13	13
5.	41-50	15	10
6.	51-60	5	2
7.	61-70	4	1
8.	≤ 80	2	1

Out of 180 isolates of S.aureus 80 were MRSA and 100 were MSSA in which 47 (47%) were Males and 33 (41.25%) were Females.

The maximum cases were reported in the 41-50 years of age group followed by 31-40 years while the minimum cases were found in the age group in children and above 60 years of age.

Table-5 Sample wise distribution of S. aureus

S.N.	Sample collected from	MRSA N=80
1.	Pus	22
2.	Blood	17
3.	Urine	8
4.	Sputum	14
5.	Throat swab	6
6.	Body fluids	13

Table-6 Distribution of *S. aureus* from different location

S.N	collected location	Pus	Blood	Urine	Sputum	Throat Swab	Body Fluids
1.	Surgery ward	6	5	3	2	2	4
2.	NICU	1	0	0	0	0	0
3.	Medicine ward	12	5	2	7	3	5
4.	OPD	3	7	3	5	1	4

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

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

Genotypic Identification of MRSA

Detection of erm A gene: In this study,a total of 80 MRSA isolates were subjected for the molecular analysis. The DNA extraction was done using the Qiagen DNA extraction kit and got the DNA from 80 isolates. Gel photographs of the DNA samples are figured below.

Figure 1: Image of DNA Extracted from MRSA isolates

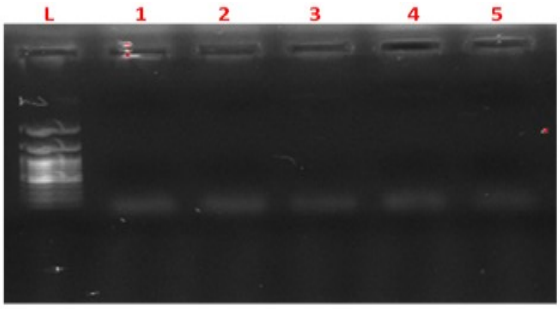

Figure 2 Image of amplified erm a gene in MRSA isolates, the amplified DNA band size was obtained 149bp, L corresponding to 100bp ladder

TCTAAAAAGCATGTAAAAGAATTTGCGACCAGATTGCAAAATCTGCAACGAGCTTT
GGGTTTACTCCCCCCGGTGGAGATGGATATAAAAATGCTCAAAAAAGTACCACCAC
TATATTTTCCTAAGAAGCTATCAAATAATTATAATCA

Graph 1: Obtained gene sequences of ermA gene in MRSA isolates

Discussion

The drug resistance Staphylococcus aureus (MRSA) is a serious life threatening pathogen in hospitals andin healthy populations. Thus, the characterization of these strains is important for local epidemiology and surveillance studies. From the Table No.2, 3 ,4 5 and 6 we found out that out of 180 isolates of S.aureus 80 were MRSA and 100 were MSSA in which 47 (47%) were Males and 33 (41.25%) were Females. The maximum cases were reported in the 41-50 years of age group followed by 31-40 years while the minimum cases were found in the age group in children and above 60 years of age. This finding is with the agreement with the finding of other authors [10,11]. The maximum numbers of isolates were from the Blood and Pus samples. This study was in support with the other study performed by the other author where the rate of pus and blood isolates were more Puthiya Purayil Preeja et al., [12]. The prevalence of MRSA in our study was 44.4% which was parallel to the studies by author [13] [14] where the prevalence was found to be 41% and 40 % and in contrast with the study by Maj Puneet Bhatt et al., where the prevalenc e was only 20% [15]. From the 80 MRSA isolates, 21 were found to be D test positive, whereas 16 were confirmed cMLSB while the other 15 were noticed to be MS phenotype and 15 were found sensitive This finding is strongly supported with the study conducted by Nezhad et al., 2017 [16] [17] . All the MRSA isolates were sensitive to linezolid, Teicoplanin, vancomycin, Gentamycin and Resistance to Cefoxitin and Oxacillin, following the CLSI guidelines standards. This was in support with the study by [15] [18]. The Molecular characterization for the detection of drug resistance gene ermA gene was carried out among the MRSA isolates in which 12 isolates carried ermA gene. This study was in support with the other study where there was 4 ermA gene isolated in among 9 MRSA isolates [18] and also parallel to the study by Saderi et al. [19].

Conclusion

The implementation of strict aseptic techniques in hospitals to prevent the colonization of the hospital environment by resistant strains, the identification and treatment of carriers, and the screening of hospital staff and facilities are some of the key measures that can mitigate the spread of MRSA. All the resistance isolates were confirmed by PCR methodology and gene sequencing which is more powerful technique used recently. Hence, there is a need for continuous monitoring and implementation of better control strategies for the control of Antibiotic resistance.

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