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ORIGINAL RESEARCH

To determine the Molecular characterization of methicillin-resistant Staphylococcus aureus from different clinical samples using a real-time PCR technique in a tertiary care Hospital.

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Abstract
The study's findings on the molecular characterization of MRSA from different clinical samples using real-time PCR techniques in a tertiary care Hospital are significant. The study aimed to determine the Molecular characterization of methicillin-resistant Staphylococcus aureus, antibiotic-resistant profiles of *MRSA* isolates and the determination of responsible genes in Hospital & community settings. Out of a total of 150 staphylococcus aureus isolates, 45 were MRSA. Where 18 were from hospitals, and 27 were from the community. The phenotypic and genotypic methods were used to categorize HA-MRSA and CA-MRSA.
Out of 45 strains processed for *SCC mec* typing, 16(35.6%) had *SCC mec type* III, 13 (28.9%) had *SCC* IV type, 7(15.6%) had SCC mec III type, 4(8.9%) and 5 (11.1%) had not been detected. Out of 45 MRSA isolates, 40 MRSA strains were *mecA* gene & were subjected to detection of the PVL gene; it was positive in 29. In this study, 16(35.6%) had *SCC mec type* III, 13 (28.9%) had *SCC* IV type, 7(15.6%) had SCC mec III type, 4(8.9%) and 5 (11.1%) had not been detected.
Conclusion: Molecular methods such as Rt-PCR are useful in detecting HA-MRSA and CA-MRSA strains from the clinical isolates.
Keywords: mec A gene, PVL gene, SCC mec gene

INTRODUCTION

Infections caused by Staphylococcus aureus remain a major global healthcare problem. Multidrug-resistant Staphylococcus aureus strains are a prevention report, and MRSA causes 5% of all HAIs. MRSA is usually spread by direct contact with an infected wound or via contaminated hands. [1]
Methicillin-resistant Staphylococcus aureus (MRSA) is a common pathogen capable of producing various clinical illnesses. The first report of methicillin resistance in Staphylococcus aureus (S. aureus) was published in 1961. Methicillin resistance has also increased among coagulase-negative Staphylococci (CoNS). [2]
Molecular epidemiology studies of MRSA are limited in India; studies have shown a predominance of ST239 among the isolates from New Delhi, ST772 from Karnataka, and ST22 from Mumbai.[3] However, more epidemiological information is required for accurate characterization of the prevalent MRSA clones and their resistance patterns for appropriate prognostication and therapy and for devising hospital protocols [3].

MATERIALS & METHODS

The study was conducted in Index Medical College, Hospital & Research Centre, Indore, M.P. This observational study was undertaken From ……..to … after getting approval from the ethical committee. A Total of 150 *S. aureus* isolates from different clinical samples were subjected to MRSA screening using conventional microbiological methods. The clinical Specimens included pus, sputum, genital specimen (high vaginal swab, semen, and urethral discharge), urine, devices (urinary catheter, cup catheter, etc.), blood, and body fluids.
The standard microbiological methods were followed in this study during culture and antibiotic sensitivity tests following universal precautions. All isolates were identified by conventional methods, including colony morphology, Gram staining, catalase test, coagulase test (tube & slide), and DNase test.[4]
Antibiotic susceptibility testing was performed by using the disc diffusion method and as per the Clinical and Laboratory Standards Institute guidelines for the following antibiotics: amikacin (30 µg); ciprofloxacin (5 µg); co-trimoxazole (25 µg); vancomycin (30 µg); linezolid (30 µg)
All the confirmed S. aureus strains were subsequently tested for methicillin resistance based on the Kirby-Bauer disk diffusion method using oxacillin discs (1 µg) obtained from Hi-Media Laboratories Pvt. Ltd. The isolates were considered methicillin-resistant if the zone of inhibition was 12 mm or less.

DNA extraction from each *S. aureus* isolates deoxyribonucleic acid (DNA) extraction was performed by modifying the straightforward crude extraction techniques that had previously been reported for Salmonella enterica [5] and Streptococcus pneumonia [6].
The existence of the mecA gene was detected by Real-time polymerase chain reaction (RT-PCR) assay. Primers mecA F1 -AAA ATC GAT GGT AAA GGT TGG C and mecA B1 -AGT TCT GCA GTA CCG GAT TTG C were used to detect the mec A gene.

RESULT
A Total of 150 *S. aureus* isolates from different clinical samples were subjected to MRSA screening using conventional microbiological methods. The clinical Specimens included pus, sputum, genital specimen (high vaginal swab, semen, and urethral discharge), urine, devices (urinary catheter, cup catheter etc.), blood, and body fluids.

Out of 150 Staph. aureus isolates, males were 85(57%), and females were 65(43%). [Table 1]

Table 1: Gender and age distribution among the study subjects.

Gender	Number	%
Male	85	57
Female	65	43

All MRSA isolates confirmed by phenotypic and genotypic methods were further categorized into HA-MRSA and CA-MRSA based on the Centers for Disease Control and Prevention (CDC) definition. Based on the CDC definition, the 45 confirmed MRSA isolates were categorized into 18(40%) HA-MRSA and 27 (60%) CA-MRSA. [Table 2]

Table 2: Categorization of MRSA isolates into HA-MRSA and CA-MRSA.

	Category	Number
MRSA	HA-MRSA	18
	CA-MRSA	27

Out of 150 S. aureus isolates, the highest number of S. aureus were isolated from pus samples 92(61.3%), followed by urine 25(16.7%), blood 13(8.7%), ear swab 11(7.3%) and catheter tip culture 9 (6%) and isolates 45 were MRSA, the maximum isolation of MRSA was from pus 25(27%), followed by Urine 9(36%), Blood 5(38%), ear swab 3(33%), and tip culture 3(33%)—[Table 3]

Table 3: Prevalence of MRSA isolates from different clinical samples

clinical samples	Number of S. aureus (n=150)	MRSA (n=45)	MRSA %
Pus	92	25	27%
Urine	25	9	36%
Blood	13	5	38%
Ear Swab	11	3	27%
Tip culture	9	3	33%
Total	150	45	30%

Of 150 staphylococcus aureus isolates, 45(30%) were MRSA stains. The incidence rate of male MRSA is 30/45 (66.7%), and female MRSA is 15/45 (33.3%). Most MRSA were from the male patient's 31-40 age group (8), and females were from the 31-40 age group (5).- [Table 4]

Table 4: Age & sex-wise distribution of different isolates.

Age group (In year)	MRSA		
	Male	Female	Total

10-20	0	0	0
21-30	7	3	10
31-40	8	5	13
41-50	5	3	8
51-60	6	3	9
61-70	3	1	4
71-80	1	0	1
Total	30	15	45

The antibiotic sensitivity pattern amongst the MRSA isolates shows that 100% were sensitive to vancomycin and linezolid, 86.7% were sensitive to Co-trimoxazole, 84.4% were sensitive to clindamycin, 82.2% were sensitive to gentamycin, 75.6% were sensitive to erythromycin, and 64.4% were sensitive to ciprofloxacin. Similarly, no resistance was seen with vancomycin and linezolid. - [Table 5]

Table 5: antibiotics sensitive pattern of MRSA (n=45)				
antibiotics	sensitive	Percentage	Resistant	Percentage
Clindamycin	38	84.4%	7	15.6%
Ciprofloxacin	29	64.4%	16	35.6%
Co-trimoxazole	39	86.7%	6	13.3%
Erythromycin	34	75.6%	11	24.4%
Gentamycin	37	82.2%	8	17.8%
Vancomycin	45	100%	0	0
Linezolid	45	100%	0	0

Genotypic confirmation of MRSA by the mec A gene*:* out of 45 MRSA strains, 40 possess the mecA gene, and 5 were negative. Where out of 45strains processed for *SCC mec* typing, 16(35.6%) had *SCC mec type* III, 13 (28.9%) had *SCC* IV type, 7(15.6%) had SCC mec III type, 4(8.9%) and 5 (11.1%) had not been detected. [Table 6].

Table 6: SCC mec typing					
MRSA (n=45)	SCC mec	SCC mec II	SCC mec III	SCC mec IV	Not detected
	4	7	16	13	5

When these 40 MRSA strains with *the mecA gene were subjected to detection of the* PVL gene, it was positive in 29. The remaining four strains were negative for the mecA gene; when subjected to PVL gene detection, PVL was positive in 2 and negative in 3. Thus, the overall positive status for the PVL gene was 31 out of 45 strains. Similarly, 40 mecA genes were subjected to SCCmec typing: 16 were SCCmec III, 13 were SCCmec IV type, 7 were SCCmec II type, and 4 were SCCmec type I. Interestingly, all 5 MRSA strains harmful to mecA were found to be non-typable under SCCmec typing. – [Table 7].

Table 7: Interrelationship of genotypes of MRSA (n=45)							
Mec A gene detection		**PVL gene detection**		**SCC mec typing**			
		positive	**Negative**	**I**	**II**	**III**	**IV**
Positive	40	29	11	4	7	16	13
Negative	5	2	3	0	0	0	5

DISCUSSION

The hospital environment is crucial in spreading pathogenic organisms such as MRSA. MRSA can be transferred from person to person or from person to frequently touched objects in the hospital environment and vice versa.

In our study, out of 45 MRSA isolates, the maximum isolation of MRSA was from pus 25 (27%), followed by urine 9 (36%), blood 5 (38%), ear swab 3 (33%), and tip culture 3 (33%). A study conducted by Kenyatta observed a high isolation rate from pus (68%) [7]. An increased isolation rate of S. aureus from pus may be due to the exposure of wounds or skin breaches, making them more prone to invasion of S. aureus infections. In many cases, poor hygiene is a predisposing factor.

In the present study, 45(30%) of 150 staphylococcus aureus isolates were MRSA stains. The incidence rate of male MRSA is 30/45 (66.7%), and female MRSA is 15/45 (33.3%). Most MRSA were from the male patient's 31-40 age group (8), and females were from the 31-40 age group (5).

Another significant finding of this study showed that all MRSA isolates were significantly less sensitive to antibiotics than MSSA. However, all S. aureus isolates were sensitive to vancomycin and linezolid. The sensitivity pattern of MRSA strains with other antibiotics was 86.7% were sensitive to Co-trimoxazole, 84.4% were sensitive to clindamycin, 82.2% were sensitive to gentamycin, 75.6% were sensitive to erythromycin, and 64.4% were sensitive to ciprofloxacin.

Like many other studies from India [8,9] and Iran [10], all the isolates, irrespective of their methicillin sensitivity or resistance status, were sensitive to linezolid (100%), teicoplanin (100%) and vancomycin (100%). Our study adds to the existing facts that glycopeptides (vancomycin and teicoplanin) and linezolid appear to be the most beneficial options available for treating MRSA infections.

Out of 45 strains of MRSA analyzed for the presence of the mecA gene, it was found to be positive in 40 strains (88.9%), and among the 45 strains processed for the PVL gene, 31 (68.9%) were positive, highly represented among community-acquired MRSA strains. PVL is a leucocyte-destroying cytotoxin responsible for severe necrotizing pneumonia and skin and soft tissue infections [11]. In this study, Kaur et al. [12] from Belgaum, South India, and D'Souza et al. [13] from Mumbai reported 85% and 64% positivity for the PVL gene among MRSA, respectively. The higher prevalence of the PVL gene in these studies might be due to the misuse, overuse, and abuse of antibiotics, indicating the progress of resistant strains along with this PVL gene.[14]

SCCmec typing is one of the molecular techniques used to correlate the relationship of MRSA strains with their source -community or hospital-acquired. It has been reported that SCCmec V and IV are associated with community-acquired strains [15]. Similarly, in this study, out of the 45 strains processed for *SCC mec* typing, 16(35.6%) had *SCC mec type* III, 13 (28.9%) had *SCC* IV type, 7(15.6%) had SCC mec III type, 4(8.9%) and 5 (11.1%) which are prevalent among CA-MRSA strains [16] and 5(11.1%) were negative for SCCmec.

A study from Iran by Javid et al. [17] documented the prevalence of SCCmec as follows: SCCmec types were type III (48.31%), type V (19.1%), type I (16.85%), and type IV (3.37%).

Five of the 45 MRSA strainswerenegative for the mecA gene and were found to be nontypable under SCCmec typing. Due to technical constraints, further SCCmec typing beyond SCCmec V was not attempted.

The study's limitations are its single-centre design and the non-availability of whole genome sequencing. The sample size for the genotypic characterization was limited to 45. Analysis of SCCmec was limited to SCCmec I-V types only. Phage typing could not be completed for this study's MRSA strains.

CONCLUSION

Molecular methods such as Rt-PCR are useful in detecting HA-MRSA and CA-MRSA strains from clinical isolates. Molecular typing techniques such as SCCmec typing are useful in identifying the strain types circulating in the healthcare setting. An inflow of CA-MRSA strains into hospitals is observed, causing hospital-acquired infections and blurring the line between CA-MRSA and HA-MRSA.

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