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Phenotypic and Molecular Characterization of Methicillin-Resistant Staphylococcus Aureus Isolated from Tertiary Care Hospitals

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ABSTRACT
Methicillin-resistant Staphylococcus aureus (MRSA) is a common pathogen capable of producing various clinical illnesses. It was initially reported in hospitals (hospital-associated or HA-MRSA) but was later also observed in communities (community-associated or CA-MRSA). Molecular characterisation of MRSA can help predict its spread and treatment. The study aimed to determine the morphological and antibiotic-resistant profiles of S. aureus isolates and the determination of responsible genes in Hospital and community settings. 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 and 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. Molecular methods such as Rt-PCR are useful in detecting HA-MRSA and CA-MRSA strains from the clinical isolates. Molecular typing techniques such as SCCmec typing are useful in identifying the strain types circulating in the healthcare setting.

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is a common pathogen capable of producing a wide variety of clinical illnesses[1]. The first report of methicillin resistance in Staphylococcus aureus (S. aureus) was published in 1961[1]. Methicillin resistance has also increased among coagulase-negative Staphylococci (CoNS)[1].

Methicillin-resistant Staphylococcus aureus (MRSA), which is frequently responsible for nosocomial infections, was initially reported in hospitals (hospital-associated or HA-MRSA) but was later also observed in communities (community-associated or CA-MRSA). Indiscriminate use of antibiotics has led to the emergence of MRSA resistant to multiple antibiotics leaving vancomycin and clindamycin as a last resort for treating β-lactam-resistant S. aureus infections. However, vancomycin-resistant and vancomycin-intermediate S. aureus (VRSA and VISA) have been reported globally, including from different parts of India and resistance to clindamycin has also been observed[2].

The HA-MRSA and CA-MRSA have been reported to have distinctive phenotypic features and molecular epidemiology. HA-MRSA is typically multi-drug resistant and has SCCmec Type I, II and III, while CA-MRSA are susceptible to non-β-lactam antibiotics, harbor SCCmec Types IV or V and tend to express the Panton-Valentine leucocidin[3]. However, recent studies have shown penetration of CA-MRSA into hospital settings and vice versa and occurrence of HA-MRSA with SCCmec type IV or V and CA-MRSA with SCCmec type I, II or III have been reported worldwide, including from India[4,5].

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[2]. However, more epidemiological information is required for accurate characterization of the prevalent MRSA clones and their resistance patterns for appropriate prognostication and therapy as well as for devising hospital protocols[2].

MATERIALS AND METHODS

The study was conducted in Index Medical College, Hospital and Research Centre, Indore, M.P. 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 and slide) and DNase test[6].

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[7] and Streptococcus pneumonia[8]. 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 for the detection of mecA gene.

RESULTS AND DISCUSSIONS

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 was from the male patient's 31-40 age group (8), and females were from the 31-40 age group. MRSA was more prevalent in pus (27%, n=92).

All MRSA isolates confirmed by phenotypic and genotypic methods were further categorized into HA-MRSA and CA-MRSA based on the definition by the Centers for Disease Control and Prevention (CDC). Based on the CDC definition, the 45 confirmed MRSA isolates were categorized into 18(40%) HA-MRSA and 27 (60%)CA-MRSA. [Table 1]

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 2]

Genotypic confirmation of MRSA by the mecA gene: out of 45 MRSA strains, 40 strains possess the mecA gene, and 5 strains were negative. Where 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[3].

When these 40 MRSA strains with the mecA gene were subjected to detection of the PVL gene, it was positive

Table 1: Categorization of MRSA isolates into HA-MRSA and CA-MRSA.

Mrsa	Category	Number
	HA-MRSA	18
	CA-MRSA	27

Table 2: 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

Table 3: SCCmec typing

Mrsa	SCCmec	SCC mec II	SCCmec III	SCCmec IV	Not detected
(n=45)	4	7	16	13	5

Table 4: Interrelationship of genotypes of MRSA (n=45)

mecA gene detection		PVL gene detection		SCCmec typing			
		positive	Negative	I	II	III	IV
Positive	40	29	11	4	7	16	13
Negative	5	2	3	0	0	0	5

in 29. The remaining 4 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 4].

Dissemination of MRSA from hospitals to the community and vice versa and emergence of β-lactam-resistant strains is a cause of significant concern worldwide. However, there have been very few reports from India on molecular profiling of MRSA isolates collected from human patients in the hospital or community settings.

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%). This is consistent with the study done in Yemen[9] and Kerala[10]. In contrast to our study, other studies from Iran and Nigeria reported a high rate of isolation from blood (29%)[11] and urine (76%)[12], respectively. A Kenyatta study observed a high isolation rate from pus (68%)[13]. 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 our study, the prevalence rate of MRSA was 30%, which was higher than the survey done by Oberoi[14], who reported a prevalence of 28.86% in their study in northern India, which was lower than ours. Silvana[15]. Their survey in Brazil documented a prevalence of 37.7% of S. aureus in ICU patients. A study from Southeast Nigeria revealed a prevalence of 60.4%[16].

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].

In the present study, comparing two phenotypic methods proved that the cefoxitin (30µg) disc diffusion method is better than the oxacillin (1µg) disc diffusion method in screening MRSA strains.

In our study, the isolation rate of HA-MRSA and CA-MRSA was 40% and 60%, respectively. In discordance with our study, Nagaraju[17] reported a low prevalence of 11.8% CA-MRSA in their study[18]. documented almost equal prevalence of HA-MRSA (54%) and CA-MRSA (52%) in their study. A study from Raichur, Karnataka, reported 75% and 25% prevalence of HA-MRSA and CA-MRSA, respectively[19]. D'souza *et al*. reported 54% CA-MRSA[20]. Available reports demonstrated variations in the prevalence of HA and CA MRSA in different places at different times. The prevalence of CA-MRSA infections in males was noticed in many studies[21,22]. Naimi *et al.* found the prevalence of CA and HA MRSA was 12% and 85%, respectively, in their study in the USA[23].

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%). The remaining, though negative, might have carried other methicillin resistance genes like mec B and mecC genes[24], or there could be a loss or mutation in the gene or hyperproduction of β-lactamase, production of normal PBP with altered binding capacity and other unidentified factors[25]. Though cefoxitin is a better inducer of the mecA gene, in our study, there is a discrepancy between the phenotypic resistance to cefoxitin and the presence of the mecA gene in PCR. However, few other studies reported almost no disagreement between phenotypic cefoxitin disc diffusion test and molecular mecA gene

detection[26,27].

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[28]. In this study, Kaur[29] from Belgaum, South India, and D'Souza[20] 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. MRSA strains with the PVL gene get transmitted from draining wounds[13].

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[30]. 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[28] and 5(11.1%) were negative for SCCmec.

This study revealed that community strains are being introduced into hospitals[30]. This contrasts with the study from Mumbai, which showed that among the MRSA, 25% of the isolates were SCCmec III, 34% were SCCmec IV and 41% were SCCmec V[31]. Goolam *et al.* observed 53% of SCCmec type I and 47% of SCCmec type IV among MRSA[32].

A study from Iran by Javid[34] 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 strains, which were also negative for the mecA gene, 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-center 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 the detection of HA-MRSA and CA-MRSA strains from the clinical isolates.

Molecular typing techniques such as SCCmec typing are useful in identifying the strain types circulating in the health-care setting. It is observed that there is an inflow of CA-MRSA strain into hospitals, causing hospital-acquired infections and blurring the line between CA-MRSA and HA-MRSA.

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