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Short Communication

Detection and molecular typing of methicillin-resistant *Staphylococcus aureus* from northeastern part of India

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ABSTRACT

Background: In *Staphylococcus aureus*, methicillin resistance is exhibited by modifications in penicillin-binding protein that minimises the binding affinity to beta-lactam antibiotics. The present study investigated the occurrence of methicillin-resistant *S. aureus* (MRSA) in community-acquired infections, that is, community-acquired MRSA (CA-MRSA) and in-hospital-acquired infections, that is, hospital-acquired MRSA (HA-MRSA) from Northeast India.

Methods: A total of 197 consecutive non-duplicate isolates were collected from Silchar Medical College and Hospital and other private diagnostic laboratories. The isolates were confirmed to be *S. aureus* at our centre. All isolates were subjected to antibiotic susceptibility testing and were screened for methicillin resistance using cefoxitin disc test. All MRSA were subjected to Polymerase Chain Reaction (PCR) assay for detection of *mecA* and *mecC* genes. DNA fingerprinting was performed for determining clonal diversity.

Results: Seventy-one isolates of 127 confirmed *S. aureus* were found to be methicillin resistant by screening test. *mecA* gene was detected in 43 isolates, and none of the isolates were positive for *mecC* gene. Linezolid and teicoplanin showed better activity with susceptibility pattern being 83.6% and 72.44%, respectively, whereas 66.14% were sensitive to vancomycin. Other antibiotic showed low level of activity. Pulsed Field Gel Electrophoresis (PFGE) showed 14 different banding patterns that suggest isolates were of different clonal types.

Conclusion: *mecA* was responsible for methicillin resistance in majority of strains. Polyclonal spread of MRSA infection in the study area indicates its diverse origin and possible lateral transfer. Thus, this study is of clinical interest in terms of selection of proper antimicrobial chemotherapy and infection control management.

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Introduction

Invaded with the new generation antimicrobials, methicillin-resistant *Staphylococcus aureus* (MRSA) has crossed the infection limits and spread the epidemic waves both toward hospital and community settings. It is an important cause of serious infections in both hospitals and community.¹

In Indian context, especially in Northeast India, empirical use of expanded spectrum penicillin and glycopeptides is quite common in the high-risk units of the hospital.² Few studies from the northeastern region of India are available with no data on community-acquired MRSA (CA-MRSA) and hospital-acquired MRSA (HA-MRSA). To add more concern, less data on hospital and community reservoir of MRSA are available from Northeast India. Also, there is no antimicrobial policy in this region to counter the emerging threat of MRSA. Cases of CA-MRSA are reported across the globe including epidemic cases from different corners. Northeastern part of India too has observed a high occurrence rate of CA-MRSA than hospital-acquired cases.³ This study, therefore, focuses in depth comparison within this country on MRSA occurrence and their therapeutic alternatives against infection caused by these pathogens.

Materials and methods

Bacterial isolates and identification

A total of 197 consecutive and non-duplicate isolates were received from a tertiary referral hospital of Northeast India and private diagnostic laboratories, for a period of 9 months from April 2014 to January 2015. *S. aureus* was identified by biochemical testing (catalase, oxidase and coagulase), rapid Latex agglutination slide test (HiStaph™ Latex Test Kit, HiMedia, Mumbai, India) and by *coa* gene Polymerase Chain Reaction (PCR) assay. The reaction condition and primers used were as described previously by Hookey et al⁴ (Table 1).

Phenotypic screening of MRSA

Screening for methicillin resistance was performed using oxacillin (HiMedia) and cefoxitin (HiMedia) disk diffusion test in which *S. aureus* (ATCC 25923) was used as a control. The method adopted was same as used by Lee et al in their study.⁵

Molecular characterisation of methicillin resistance

Polymerase chain reaction assay was performed for characterisation of MRSA.⁶ Two different genes viz *mecA* and *mecC* were targeted (Table 1). The PCR conditions were set with an initial denaturation at 95 °C for 3 min which was followed by denaturation at 95 °C for 25 s, annealing at 50 °C for 40 s and extension at 72 °C for 1 min. This was repeated for 32 cycles with a final extension at 72 °C for 5 min.

Antibiotic susceptibility and minimum inhibitory concentration (MIC) determination

Antibiogram profiling of the isolates were performed by Kirby–Bauer disc diffusion method.⁷ The antibiotics used for disc diffusion method were as listed in Table 2. MIC was determined for oxacillin (HiMedia), vancomycin (Cipla, Goa, India) and teicoplanin (Cipla) by both agar dilution and broth dilution method⁷ (Table 2). All the results were interpreted as per the Clinical Laboratory Standard Institute (CLSI).⁷

Typing of the isolates

Clonality of the methicillin-resistant isolates was assessed by Pulse Field Gel Electrophoresis (PFGE) (Bio-Rad, USA), in which the agarose plug was prepared and casted as per the manufacturer's instruction. The cells in agarose plugs were lysed using lysis buffer and proteinase K followed by washing of agarose plugs. DNA in agarose plugs were digested with *smal* restriction enzyme and the electrophoresis was run for 20 h at 4 V/cm with alternating pulses at a 120° angle in 10–40 s pulse times.

Results

One hundred twenty-seven morphologically screened isolates were confirmed phenotypically to be *S. aureus* by tube and slide coagulase test, latex agglutination test and by *coa* gene PCR.

Among them, 71 isolates showed screening positive for MRSA by oxacillin and cefoxitin disk diffusion method of which 41 were from community and 30 from hospital. The presence of *mecA* was observed in 43 isolates with phenotypically detected multidrug-resistant phenotype (Table 1) (Fig. 1).

In antibiotic susceptibility testing, it was found that the organisms were more susceptible to linezolid (83.46%),

Table 1 – List of primers used for PCR amplification and distribution of *coa* and *mec* genes among test isolates.

Primer pairs	Target	Sequence (5'-3')	Amplified product size (bp)	References	Gene	Total no. of gene present
<i>coa</i> F <i>coa</i> R	<i>coa</i>	ATA GAG ATG CTG GTA CAG G GCT TCC GAT TGT TCG ATG C	673	Hookey et al ⁴	<i>coa</i>	71
<i>mecA</i> F <i>mecA</i> R	<i>mecA</i>	CCTAGTAAAGCTCCGGAA CTAGTCCATTCCGGTCCA	331	Lee et al ⁵	<i>mecA</i>	43
<i>mecC</i> F <i>mecC</i> R	<i>mecC</i>	ACTAGTATCTCGCCTTGG ATCCCGAGTGATTATCCC	102	Becker et al ⁶	<i>mecC</i>	—

'71' represents the no. of isolates containing *coa* gene and '43' represents no. of isolates bearing *mecA* gene.

Table 2 – Antimicrobial susceptibility pattern and minimum inhibitory concentration of MRSA against different antimicrobials.

Antimicrobial susceptibility			Minimum inhibitory concentration organism (<i>Staphylococcus aureus</i> [n = 71])			
Antibiotics used	No. of susceptible isolates (N)	Percentage of susceptibility (%)	Antibiotic concentration (µg/ml)	Antibiotics		
				Oxacillin	Vancomycin	Teicoplanin
Clindamycin	49	38.58	0.5	—	—	—
Pristinamycin	7	0.098	1	—	12	6
Doxycycline	26	36.66	2	—	23	17
Tetracycline	17	23.94	4	—	11	13
Tigecycline	18	25.35	8	7	8	10
Erythromycin	3	0.04	16	13	7	9
Minocycline	30	42.25	32	10	6	4
Linezolid	106	83.46	64	1	—	6
			>64	40	4	6

MRSA, methicillin-resistant *Staphylococcus aureus*.

followed by minocycline (42.25%), clindamycin (38.58%), doxycycline (36.66%), tigecycline (25.35%) and tetracycline (23.94%), whereas pristinamycin (0.098%) and erythromycin (0.042%) showed very low level of sensitivity toward *S. aureus*. While examining the MIC of the resistant isolates teicoplanin (72.44%) came up with good activity followed by vancomycin (66.14%) with below the breakpoint range whereas oxacillin showed no sensitivity against any of the concentration and were found above the breakpoint range (Table 2).

Pulse Field Gel Electrophoresis (PFGE) typing revealed that 14 different clonal types exist in the study area (Fig. 2).

Discussion

Methicillin-Resistant *Staphylococcus Aureus* (MRSA) has long been the study of interest from decades, owing to its unique ability to quickly adapt to new conditions. Day-to-day exposure of β -lactam and other groups of antibiotics has led to increase in multidrug-resistant trait. This study, therefore, played a critical role in analysing and investigating *S. aureus* resistant to methicillin antimicrobials and other groups of antibiotics. This study is important in the context of global and local emerging scenario of this multidrug-resistant pathogen.

Fig. 1 – DNA bands (331 bp) of *mecA* genes in which lanes 1–4 and 6–10 show positive result of the test isolates, whereas lane 5 and lane 11 show negative results with no *mec A* gene expression, and lane 12 has positive control and lane 13 has negative control with lane 14 as *S. aureus* (ATCC 25923) strain.

The present study revealed that the occurrence rate of MRSA infections in the community (33.07%) is found to be higher than the hospital settings (23.62%), which showed similar results to those of Manoharan *et al.*⁸ Whereas various other reports of MRSA infections in Indian hospitals showed higher rate of infection in a hospital setting as compared with our study.²

Endemic nature of MRSA in India has been threatened with hospital-acquired infections. The present study reported that majority of the isolates, that is, 28.34% of MRSA were obtained from the urine sample and 15% were from pus, whereas in previous findings, a high isolation rate ranged from 33% to 83% MRSA from pus sample, and in another study, it was interestingly reported that less than one-third (32.5%) of MRSA urine samples came from hospital inpatient sources.^{9–11}

It was established from the different study that cefoxitin disc diffusion method was more reliable and specific method for accurate detection of MRSA followed by PCR amplification for *mecA* gene. Although cefoxitin is considered to be better than oxacillin for the detection of heteroresistance, in the present study, while using both cefoxitin and oxacillin for screening of MRSA, the former was found to be a more accurate predictor.

Although previous study has been reported² with reduced susceptibility toward vancomycin among MRSA strain, our study came up with a contrasting result as most of the MRSA were susceptible to vancomycin, teicoplanin and linezolid.

Fig. 2 – DNA ladder (1 kb) in lane no. 1 and lane no. 2–15 shows 14 different banding patterns of clonal types.

Conclusion

This study showed that MRSA is predominantly associated with urinary tract infection in this area which is most commonly found associated with CA-MRSA. As CA-MRSA is gradually increasing over HA-MRSA, proper antimicrobial therapy and regular follow-up are necessary for control of their spread over this area. Polyclonal spread of MRSA infection in the study area indicates its diverse origin and possible lateral transfer, role of which may be further investigated.

Conflicts of interest

The authors have none to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.mjafi.2018.08.004>.

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