Print ISSN: 2321-6379
Online ISSN: 2321-595X
DOI: 10.17354/ijss/2017/50

Original Article

Methicillin-resistant *S. aureus* in Eastern India: Some Molecular Epidemiological Perspectives

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Abstract

Background: *Staphylococcus aureus* is the most important nosocomial pathogen causing skin and soft tissue infection. Methicillin-resistant *S. aureus* (MRSA) is now established in both community-acquired and hospital-acquired infections. The defining feature of MRSA is the staphylococcal cassette chromosome *mec* (SCC*mec*), which is a mobile genetic element, carrying the central determinant for broad-spectrum beta-lactam antibiotic resistance encoded by the *mecA* gen, whereas the presence of panton-valentine leukocidin (PVL) gives more virulence to the MRSA.

Aim: To assess molecular epidemiology of MRSA in Eastern India.

Design: Descriptive cross-sectional study.

Materials and Methods: In this study, from 940 samples in a tertiary care hospital in rural India, over 6 months, 20 MRSA isolates were identified. These isolates were typed to study the diversity in the structures of SCC*mec* elements and *ccr* types. Statistical analysis was performed by SPSS software.

Results: Isolates carrying SSC*mec* Type IV were found to be dominant (60%), whereas a small proportion was SSC*mec* Type V (10%). Some composite strains were found. PVL gene was found to be associated with community-associated MRSA (CA-MRSA) genotypes.

Conclusion: Genotypical blurring of CA-MRSA and hospital-acquired MRSA was seen. Composite SCC*mec* strains found showing need for new nomenclature methods.

Key words: Epidemiological typing, Methicillin-resistant *Staphylococcus aureus*, Staphylococcal cassette chromosome

INTRODUCTION

The resistance of *Staphylococcus aureus* to beta-lactam antibiotics is associated with the expression of penicillin binding protein 2a. This protein is encoded by the *mecA* gene, which is situated on a mobile genetic element, staphylococcal cassette chromosome *mec* (SCC*mec*). Five different SCC*mec* types have been identified in methicillin-resistant *S. aureus* (MRSA) strains. SCC*mec* Types I, II, and III are mainly found in hospital-acquired MRSA (HA-MRSA), whereas SCC*mec* Types IV and V are mainly associated with community-acquired MRSA (CA-MRSA).[1]

During 1990s epidemic MRSA-15 (EMRSA-15) emerged in the hospital settings as per the reports from the UK. Later, this classical EMRSA-15 has been isolated from community settings as well and characterized as SCC*mec* Type IV CA-MRSA. The majority of the CA-MRSA causing infections in Indian subcontinent have been identified as SCC*mec* IV. A variant of EMRSA-15 which produces panton-valentine leukocidin (PVL) toxin has been isolated

Access this article online

www.ijss-sn.com

Month of Submission : 12-2016
Month of Peer Review : 01-2017
Month of Acceptance : 01-2017
Month of Publishing : 02-2017

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and characterized by pulsed-field gel electrophoresis method from the Indian population both in hospital and community settings.[2]

SCC*mec* contains the mec complex (*mecA* and its regulators) and the *ccr* gene complex, which encodes site-specific recombinases, responsible for the mobility of SCC*mec*. Several different *ccr* genes have been identified: *ccr*A1 and *ccr*B1 in SCC*mec* Type I, *ccr*A2 and *ccr*B2 in SCC*mec* Types II and IV, *ccr*A3 and *ccr*B3 in SCC*mec* Type III, *ccr*A4 and *ccr*B4 in SCC*mec* Type IV, and *ccr*C in SCC*mec* Type V.[3]

Epidemiological typing of the infectious agent can be considered as one of the important weapons in the armamentarium of the infection control specialists as this pin points the epidemiological clone of MRSA prevalent in the community. Unfortunately, there is not much-published data available about the epidemiological type of MRSA from this part of India. Hence, the objective of this study was to characterize the MRSA strains.

Objective

The objective of this study was to assess the epidemiological type of MRSA among the isolates from clinical samples in a tertiary care hospital in rural Eastern India.

MATERIALS AND METHODS

All clinically isolated *S. aureus* over the 6 months study period were tested for methicillin-resistance according to CLSI guidelines.[4] Staphylococci were identified morphologically and biochemically by standard laboratory procedures. *S. aureus* was identified by tube coagulase, along with mannitol fermentation on mannitol salt agar (Himedia). Methicillin-resistance was confirmed using a cefoxitin disk on Mueller-Hinton agar (Oxoid).

Isolation of Genomic DNA

The DNA was isolated from all the isolates growing in nutrient broth at 37°C overnight, using Genei bacterial DNA isolation kit as per manufacturer's instruction and was confirmed by visual examination of ethidium bromide agarose gel.

16S Ribotyping

Partial amplification of 16SrDNA was done using a universal primer set 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). All samples were amplified under the same conditions: Denaturation at 94°C for 30 s, annealing at 50°C for 40 s, and extension at 72°C for 90 s with 35 cycles of amplification. After separation in a 1% agarose gel and retrieval with a Qiagen Gel Extraction kit, amplicons were subjected to Sanger sequencing in Applied Biosystem sequencer machine using primer 27F. Sequencing reaction was performed using BigDye Terminator Cycle Sequencing Kit following the manufacturer's protocol.

Phylogenetic Analysis

Assessment of PVL and *mecA* gene: Polymerase chain reaction (PCR) was performed with the primers designed to detect the *lukS-PV* and *lukF-PV* genes, which encode for PVL toxin based on the method described by Lina *et al.* with some modifications[5] and the *mecA* gene, which confers methicillin-resistance to the MRSA strain.

SSCmec Typing

All the MRSA isolates were typed by SCC*mec* typing for SCC*mec* IV and V and presence or absence of PVL gene. The SSC*mec* typing was performed by PCR analysis using the primers set described by Govindan *et al.* (Table 1). In this study, only the most common and prevalent CA-MRSA types in India, i.e., SSC*mec* Type IV and Type V were included. Total 3 primer sets were used to amplify the necessary genes for SSC*mec* Type IV and Type V screening. SSC*mec* Types IV isolates were next subjected to screening for the presence of IS 1272 element for reconfirmation. Frequency distribution of various types of MRSA such as PVL positives, SCC Type IV and V were analyzed using SPSS.

RESULTS

About 940 clinical specimens in the period December 2014 to June 2015 were taken in this study, out of which 122 were identified as *S. aureus*. Among the122 *S. aureus* isolated, 20 were MRSA by phenotypic testing. Out of the 20 MRSA isolates 12 were HA-MRSA and 8 were CA-MRSA by phenotypic criteria. Molecular results: All the 20 isolates were found to be positive for *mecA* gene (Figure 1) as internal control. The isolates in this study were predominantly of SCC*mecA* Type IV (12/20) having the *ccr*B2 (Figure 2) and *dcs* region (Figure 3) in their genetic makeup, which were further checked for presence of IS-element, and were confirmed by presence of band at 1.4 KB region. Two isolates were of Type V, showing the *ccr*C gene (Figure 4), and 6 isolates carried *ccr*C gene and also the *ccr*B2, without *dcs* region. PVL was present in three isolates among all (Figure 5), which were community acquired SCC*mec* A Type IV category (Table 2).

DISCUSSION

The prevalence of MRSA in India[6-8] in various studies in last 5 years ranges from 48% to 53%. The prevalence during our study period of 6 months was 17% approximately. This may be because the study was conducted in a

Table 1: Primers used in the study

Gene	Forward primer	Backward primer	References
MecA	5'-ACTGCTATCCACCCTCAAAC-3'	5'-CTGGTGAAGTTGTAATCTGG-3'	Govindan *et al.*[2]
*ccr*B2	5'-AGTTTCTCAGAATTCGAACG-3'	5'-CCGATATAGAAAGGGTTAGC-3'	
*ccr*C	5'-GTACTCGTTACAATGTTTGG-3'	5'-ATAATGGCTTCATGCTTACC-3'	
Dcs	5'-CATCCTATGATAGCTTGGTC-3'	5'-CTAAATCATAGCCATGACCG-3'	
PVL	5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3'	5'-GCATCAASTGTATTGGA-3'	
IS	5'-AACGCCACTCATAACATATGG-3'	5'-TATACCAAACCCGACAAC-3'	

PVL: Panton-valentine leukocidin

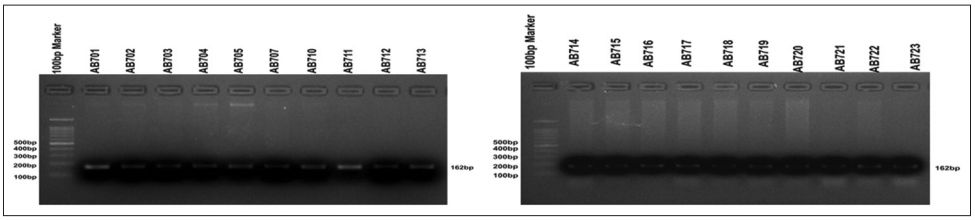

Figure 1: Detection of *mecA* gene in methicillin-resistant *Staphylococcus aureus* isolates

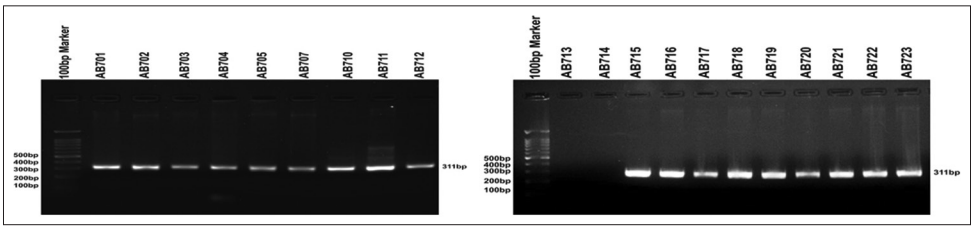

Figure 2: Detection of *ccr*B genes in methicillin-resistant *Staphylococcus aureus* isolates

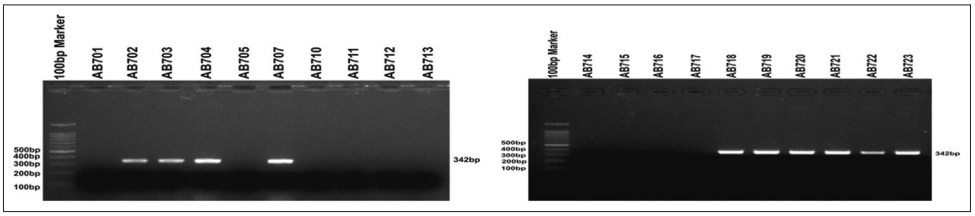

Figure 3: Detection of *dcs* gene in methicillin-resistant *Staphylococcus aureus* isolates

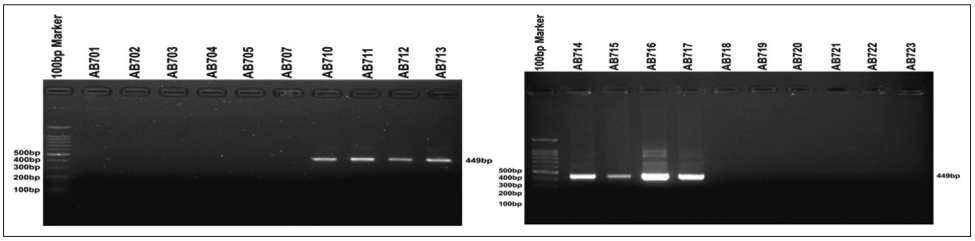

Figure 4: Detection of *ccr*C genes in methicillin-resistant *Staphylococcus aureus* isolates

hospital serving a rural population with limited access to cosmopolitan cities. Furthermore, this may not reflect the true prevalence as the study was conducted on samples collected over 6 months only. The majority of the MRSA were found to be SCC*mec* Type IV (60%). Two others were SCC*mec* Type V (10%), but 6 were composed of an SCC with *ccr*C as well as an SCC*mec* with a class B2 mec gene complex (30%). Such composites of two or more scc elements carrying two *ccr* gene complexes have been identified in other studies and have posed nomenclature problems (such as *S. aureus* ZH47).[9]

All strains were evenly distributed among the phenotypically identified HA-MRSA and CA-MRSA which once again shows that that this distinction is now blurred and not useful. The previous research shows that genotypically

Table 2: Break up of genes detected in the MRSA isolates

Number of isolates (*n*=23)	SCC*mec* type	*ccr* gene complex		*MecA* gene complex present	PVL gene present
		*ccr*B2	*ccr*C		
12	IV	+	-	+	3
2	V	-	+	+	0
6	Novel (*Staphylococcus aureus* strain ZH47)	+	+	+	0

PVL: Panton-valentine leukocidin, MRSA: Methicillin-resistant *Staphylococcus aureus*

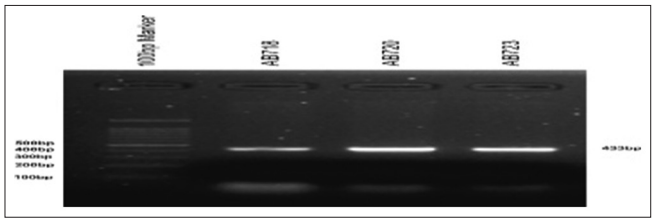

Figure 5: Detection of panton-valentine leukocidin gene in methicillin-resistant *Staphylococcus aureus* isolates

the original CA-MRSA were seen to be SCC Type IV and V. Hence, the MRSA in our study can be assumed to be derived from the community strains as all are contain genes which are present in Type IV or Type V or both. Hence, the phenotypically defined hospital-acquired strains have their origins in the community, and have evolved, maybe via horizontal gene transfer of virulence factors.[10] The importance of hand hygiene once again is to be emphasized for prevention of such evolution of CA-MRSA to more invasive strains. The more virulent EMRSA derived from CA-MRSA[11,12] has already been found in some studies in India. PVL was identified in only three strains of SCC*mec* Type IV only. PVL has been shown as acquired by EMRSA strains in other studies. None of the PVL gene possessing isolates in our study were from pneumonia patients, So PVL being a necrotizing toxin in staphylococcal pneumonia caused by CA-MRSA could not be verified.[13]

ACKNOWLEDGMENTS

Authors thank Dr. Jayanta Bikash Dey, Professor and Head, Department of Microbiology, BSMC, Bankura, West Bengal for allowing the work to be done in the department.

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How to cite this article: Bhattacharyya C, Dey R, Roy T, Ghosh A, Jana H. Methicillin-resistant *Staphylococcus aureus* in Eastern India: Some Molecular Epidemiological Perspectives. Int J Sci Stud 2017;4(11):66-69.

Source of Support: Nil, **Conflict of Interest:** None declared.