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Absence of *mecA* gene in methicillin resistant *Staphylococcus aureus*: A tertiary care center's perspective

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ABSTRACT

Background:*Staphylococcus aureus* is among the most critical pathogens because of both the diversity and the severity of infections it can cause. The continuous upsurge in the drug resistance among Staphylococci has been a significant concern. Methicillin-resistant *S.aureus* (MRSA) is a notorious nosocomial pathogen and its role has dramatically increased over the past few years. The emergence and spread of such MRSA strains pose serious therapeutic challenges for clinicians. Understanding the prevalence, antibiotic resistance patterns and information on accurate and reliable detection methods of MRSA strains is necessary for appropriate antibiotic treatment and effective infection control.**Methods:**The study was conducted over a period of eighteen months (January 2014 to June 2015) and a total of 497 consecutive, non-duplicate strains of *S.aureus* were collected from various clinical specimens. All the *S.aureus* isolates were subjected to antimicrobial susceptibility testing on Muller-Hinton agar with 2% NaCl. Methicillin resistance was evaluated using the cefoxitin disc diffusion (CDD) test, PBP2a latex agglutination test and PCR for detection of *mecA* gene.**Results:** Out of total 497 *S.aureus* isolates, on the basis of the CDD test, 196 (39.4%) strains were identified as MRSA and 301 (60.6%) strains as MSSA. The presence of *mecA* gene product PBP2a was determined by latex agglutination test for all the 196 MRSA isolates (on the basis of CDD test) and the test results reflected that 173/196 (88.3%) strains were positive. However, 23/196 (11.7%) strains tested negative. Further all 196 *S.aureus* isolates were subjected to *mecA* gene PCR and 164/196 (83.7%) strains showed the presence of *mecA* gene. However, 32/196 (16.3%) strains tested negative for the same.**Conclusion:**Drug resistance in *S.aureus* is of considerable importance in clinical practice. Our study findings underline the mechanisms other than the presence of *mecA* gene responsible for beta-lactam resistance among MRSA strains. It is essential for diagnostic laboratories to understand these alternative mechanisms and to take them into consideration while testing clinical samples.

Keywords:Antibiotic resistance, Cefoxitin, MRSA, Penicillin-binding proteins.

INTRODUCTION

Staphylococcus aureus is among the most important pathogens because of both the diversity and the severity of infections it causes which may range from minor skin and soft tissue infections (SSTIs) to life-threatening conditions such as endocarditis, pneumonia and septicemia.[1,2] The continuous upsurge in the drug resistance among Staphylococci has been a major concern. Methicillin-resistant *S.aureus* (MRSA), first described in 1961 is a notorious nosocomial pathogen and its role has dramatically increased over the past few years.[3,4] MRSA strains show distinct microbiological, clinical and therapeutic features compared to their methicillin-sensitive (MSSA) counterparts.[5] Today the whole world contends with MRSA as amajor public health problem with many strains being multidrug-resistant (MDR) causing significant morbidity, mortality and elevated health care costs.[6,7] The emergence and spread of such MRSA strains pose serious therapeutic challenges for clinicians.[8]

Usually *S.aureus* produces 4 penicillin-binding proteins (PBPs), enzymes that are anchored on the cytoplasmic membrane, the functions of which are assembly and regulation of the latter stages of the cell wall biosynthesis.[9] These 4 PBPs are susceptible to modification by beta-lactam antibiotics, thus inhibiting bacterial cell wall synthesis, which eventually leads to bacterial death. However, MRSA strains possess an altered penicillin-binding protein, PBP2a, encoded by *mecA* gene carried on a mobile DNA element, the staphylococcal cassette chromosome mec (SCCmec).[10] PBP2a is an inducible 76-78 kDa PBP, which in MRSA strains substitutes the other PBPs and because of its low affinity for all beta-lactam antibiotics, it enables the organism to assemble the cell wall even in the presence of the drug thus rendering the organism resistant.[9-12]

Detection of *mecA* gene by PCR or the discovery of PBP2a in a latex agglutination assay can be used to

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confirm the diagnosis of MRSA.[13] The *mecA* genes are highly conserved among staphylococcal species and hence their detection is considered as a benchmark for the determination of MRSA strains.
Understanding the prevalence, antibiotic resistance patterns and information on accurate and reliable detection methods of MRSA strains is necessary for appropriate antibiotic treatment and effective infection control. Considering these, the current study was undertaken to evaluate the usefulness of *mecA* gene amplification and its reliability in the identification of MRSA strains.

MATERIALS AND METHODS

This descriptive observational study was conducted over the period of eighteen months (January 2014 to June 2015) in the Department of Microbiology, Veer Chandra Singh Garhwali Government Medical Sciences & Research Institute, Srinagar Garhwal, Uttarakhand and the study protocol was approved by Institutional Ethics committee.
Collection of isolates: Various clinical specimens (pus, wound swab, ear swab, blood, urine and other body fluids) received for routine laboratory diagnosis were processed as per standard microbiological procedures.[14,15] On the basis of colony morphology and gram staining, the suspected staphylococcal isolates were collected. Further, based on the catalase test and, slide and tube coagulase test, a total of 497 consecutive, non-duplicate strains of *S.aureus* were collected.
Antimicrobial susceptibility testing: All the *S.aureus* isolates were tested for antibiotic susceptibility on Muller-Hinton agar supplemented with 2% NaCl using the Kirby-Bauer disc diffusion method. The procedures were carried out and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[16] *S.aureus* ATCC 25923 and ATCC 29213 were used as control strains for MSSA and MRSA, respectively. All the dehydrated media and antimicrobial discs were procured from Himedia Laboratories Pvt. Ltd, Mumbai, India

Determination of Methicillin susceptibility:
Methicillin resistance was evaluated using three methods,

1) Cefoxitin (30µg) disc diffusion (CDD) test as recommended by CLSI
2) PBP2a latex agglutination test as per manufacturer's instructions (MRSA-Screen test, Denka Seiken Co. Ltd, Japan)
3) PCR for detection of *mecA* gene
PCR amplification of *mecA* gene: Bacterial DNA was extracted from all pure S.aureus isolates by a rapid boiling extraction method.[17] All *S.aureus* isolates were screened for the resistance by *mecA* gene PCR using the published primers, *mecA* MR3 (5'-AAA ATC GAT GGT AAA GGT TGG C-3') and *mecA* MR4 (5'-AGT TCT GCA GTA CCG GAT TTT GC -3').[18] Table 1 shows the details of the oligonucleotide primers used. The 25µl amplification reaction mixture [Table 2] was prepared and a Thermocycler (Veriti Thermal Cycler, Applied Biosystems) was used to process the samples through 30 cycles with an initial denaturation at 95°C for 5 min; cyclic denaturation at 94°C for 30 secs; annealing at 55°C for 30 secs and extension at 72°C for 1 min and a final extension at 72°C for 5 min. The amplification product was analyzed by electrophoresis on 2% agarose gel containing 1 µg/ml of ethidium bromide. Electrophoresis was conducted at 100v for 35 min using 5x TBE running buffer (4.84g/L Tris, 0.37g/L EDTA, pH 8.0). Detection involved visualization of the amplicon size of 533bp [Figure 1] under UV trans-illuminator. A 100bp DNA ladder was included in each run as DNA molecular weight standards.

RESULTS

Of the total 497 *S.aureus* isolated, on the basis of CDD test, 196 (39.4 %) strains were identified as MRSA and 301 (60.6 %) strains as MSSA. The sample source and their categorization based on CDD test are depicted in Table 3.

Table 1: Oligonucleotide primers used for *mecA* PCR amplification for identifying MRSA

Primer *mecA*	Primer sequence (5' – 3')	Amplicon size (bp)	Nucleotide position
MR3	AAA ATC GAT GGT AAA GGT TGG C	533	1282 - 1303
MR4	AGT TCT GCA GTA CCG GAT TTT GC		1814 - 1793

Table 2: Preparation of *mecA* gene PCR Reaction mixture

S. No.	Reaction Components	Stock Conc.	Final Conc.	Vol. per 25 µl
1	PCR grade water	-	-	18.9
2	Buffer, 1.5mM MgCl2	10X	1X	2.5
3	dNTP mix	5 mM	200 mM	1.0
4	MR3 (Sigma Aldrich)	100 µM	0.80 µM	0.2
5	MR4 (Sigma Aldrich)	100 µM	0.80 µM	0.2
6	Taq DNA polymerase	5 Unit/µl	1 Unit	0.2
7	Template DNA	-	-	2.0
	Total	-	-	25

Table 3: Sample source and their categorization on the basis of cefoxitin disc diffusion test

Sample source	Total No. of samples	MSSA	MRSA
Pus	161	95	66
Wound Swab	84	49	35
Blood	69	39	30
Ear swab	74	51	23
Urine	86	49	37
Others*	23	18	05
Total	497	301	196

MRSA: Methicillin-resistant *Staphylococcus aureus*; MSSA: Methicillin sensitive *Staphylococcus aureus*; Others*: Pleural fluid, Knee aspirate, Semen, Vaginal discharge

Table 4: Comparison of the results of CDD test, PBP2a latex agglutination test and *mecA* gene PCR (n=196)

MRSA on the basis of CDD test		PBP2a Latex agglutination test		*mecA* gene PCR	
		Positive	Negative	Positive	Negative
Pus	66	58	08	55	11
Wound swab	35	31	04	30	05
Blood	30	27	03	27	03
Ear swab	23	20	03	18	05
Urine	37	32	05	29	08
Others*	05	05	Nil	05	Nil
Total	196	173	23	164	32

CDD: Cefoxitin disc diffusion test; MRSA: Methicillin-resistant *Staphylococcus aureus*; Others*: Cerebrospinal fluid, Pleural fluid, Knee aspirate, Semen, Vaginal discharge

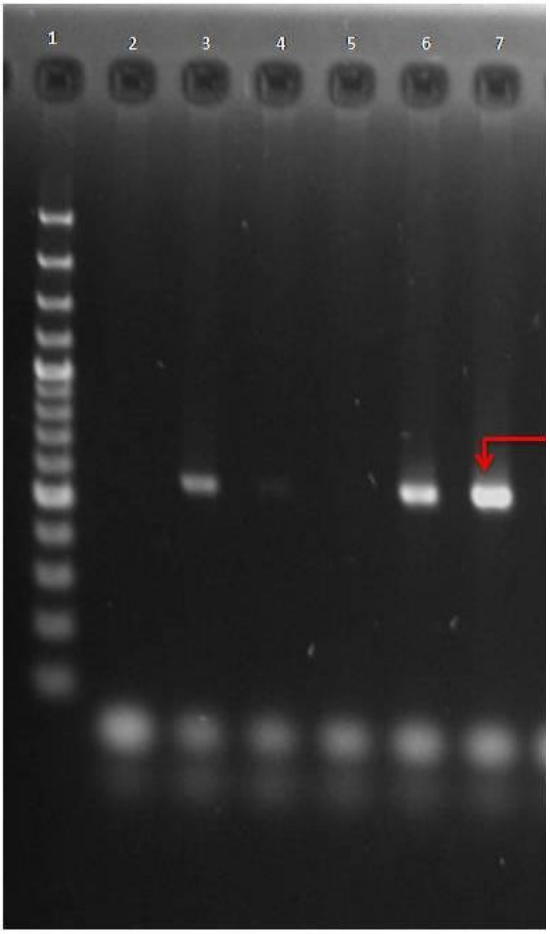

Figure 1: Polymerase chain reaction for mecAgene. Lane 1: 100 bp DNA ladder; Lane 2: Negative control; Lane 3, 4 and 6: Positive clinical sample; Lane 5: Negative clinical sample; Lane 7: Positive Control

The presence of *mecA* gene product PBP2a was determined by the latex agglutination test for all the 196 MRSA isolates (on the basis of CDD test) and the test results reflected that 173/196 (88.3 %) strains were positive for PBP2a latex agglutination test. However, 23/196 (11.7%) strains tested negative. Further, all 196 *S.aureus* isolates were subjected to *mecA* gene PCR and 164/196 (83.7 %) strains showed the presence of *mecA* gene. However, 32/196 (16.3%) strains tested negative for the same. Table 4 depicts the comparison between CDD test, PBP2a latex agglutination test and *mecA* gene PCR. All the MSSA isolates (301) tested negative for *mecA* gene PCR.

DISCUSSION

MRSA is traditionally considered to be a nosocomial pathogen, however it is evident that the epidemiology of MRSA infections is rapidly changing.[19] Consequently, as the microbiology and epidemiology of MRSA has evolved, the traditional definitions have broken down, arguing in favor of molecular typing of the strains. Identification of MRSA by *mecA* typing is an essential diagnostic as well as an epidemiological tool providing insight of the antibiotic resistance in staphylococci. Generally the CDD test is the most widely used phenotypic method and *mecA* gene PCR the most commonly used molecular method for identification of MRSA.

In the present study, our aim was to evaluate the usefulness of *mecA* gene PCR and its reliability in the identification of MRSA isolates. One of the significant finding in our study was the high percentage of MRSA strains (39.4 %) based on CDD test. A considerable discordance was seen between CDD test, PBP2a latex agglutination test and *mecA* typing. Out of the total 196 MRSA isolates based on CDD, 23 isolates (11.8 %) were found negative by PBP2a latex agglutination test and 32 isolates (16.3%) by *mecA* gene PCR. This result caused a reduction in the sensitivity and specificity of the

PCR assay, however it might be explained by some other mechanisms rather than the absence of *mecA* gene.

The absence of *mecA* gene within resistant staphylococcal isolates has been reported previously.[20-22] One of the probable reasons for the lack of *mecA* gene within resistant staphylococcal isolates is the hyper-production of beta-lactamase enzymes. Such strains often test negative for *mecA* gene as well as PBP2a, and are termed as borderline (low level) methicillin-resistant strains of *S.aureus* (BORSA). Olayinka *et al*.,[23] reported the complete absence of 5 major SCCmec types and *mecA* genes as well as the gene product PBP2a in isolates, which were phenotypically identified as MRSA, suggesting a probability of the hyper-production of beta-lactamase.

Recently some MRSA strains have been reported from both animals and humans, which possess a different form of *mecA* gene homolog termed as *mecC* (previously $mecA_{LGA251}$).[24-27] The PBP encoded by *mecC* has specific alterations in various amino acids present in penicillin-binding protein cascade (PBP1, 2, 3) and hence it differs from the PBP encoded by *mecA*. Such modified PBPs have altered binding capacity and are termed as MODSA. Due to the degree of nucleotide divergence between *mecC* and *mecA*, these *mecC* positive MRSA isolates (*mecC*-MRSA) test negative for PCR assays and latex agglutination tests that detect *mecA* gene and PBP2a, respectively and hence may be misclassified as MSSA, potentially leading to treatment failure.[28]

CONCLUSION

This study confirms the presence of MRSA in our healthcare setting (HCS) and the findings of our study point towards the mechanisms other than the presence of *mecA* gene responsible for beta-lactam resistance among MRSA. It is crucial for diagnostic laboratories to understand these alternative mechanisms and to take them into consideration while testing clinical samples. Also, it is prudent that phenotypic methods, when coupled with molecular techniques, can be helpful in the accurate characterization of the MRSA isolates. Drug resistance in *S.aureus* is of considerable importance in clinical practice. Since the antimicrobial sensitivity pattern varies from one HCS to another, the knowledge on the epidemiology of MRSA will underpin effective prevention and control strategies, including the rational use of antibiotics.

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