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Original Article

Microbiology Section

Screening for *mecA* and *mecC* Gene Carriage among Clinical Isolates of Methicillin Resistant *Staphylococcus aureus* at a Tertiary Care Hospital: A Cross-sectional Study

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ABSTRACT

Introduction: *Staphylococcus aureus* (*S. aureus*) has emerged as one of the most important human pathogen, and has been a leading cause of hospital and community acquired infections. Methicillin-Resistant *Staphylococcus aureus* (MRSA) carrying the *mecA* gene is resistant to the majority of β-lactam antibiotics. In 2007, a new *S. aureus* strain harboring *mecA* gene homologue, *mecC*, was found in England which posed diagnostic problems. Accurate and rapid detection of MRSA is required for effective treatment.

Aim: To screen for *mecA* and *mecC* genes among methicillin resistant isolates of *S. aureus* using conventional Polymerase Chain Reaction (PCR) and to associate their presence with Kirby-Bauer disc diffusion and automated Vitek 2 methods.

Materials and Methods: This cross-sectional observational study was conducted in the Department of Microbiology, Victoria hospital, Bangalore Medical College and Research Institute, Bengaluru, Karnataka, India, from July to October 2019. A total of 60 non duplicate *S. aureus* samples were obtained from various clinical samples during the study period. Isolates were subjected to antibiotic susceptibility by cefoxitin disc diffusion and automated Vitek-2. Isolates were screened for *mecA* and *mecC* gene carriage using conventional PCR. Descriptive statistics were used for the comparison of data, and appropriate statistical charts were used to present the data.

Results: Among 60 *S. aureus* isolates, 41 (68.33%) were considered MRSA by conventional Disc Diffusion Method (DDM), and 48 (80%) were considered MRSA by automated Vitek-2. By conventional PCR, only 34 (56.67%) isolates carried the *mecA* gene, and none of the clinical isolates possessed the *mecC* gene.

Conclusion: An overall MRSA prevalence of 56.67% was observed by PCR in present study. The *mecC* gene was not detected in any of the *S. aureus* isolates. The study indicates the presence of *mecA* and *mecC* negative phenotypically identified MRSA isolates. Rather than absolute dependence on the *mecA* gene as the defining standard in determining MRSA, alternative mechanisms of resistance-presence of *mecC*, mecB genes, hyperproduction of β-lactamase; can potentially be a knowledge trove for researchers to delve into.

Keywords: Antibiotic resistance, Cefoxitin disc diffusion, Polymerase chain reaction, Vitek-2

INTRODUCTION

S. aureus has emerged as one of the most important human pathogens, and has been a leading cause of hospital and community acquired infections. It is associated with a variety of infections including septicaemia, pneumonia, wound sepsis, septic arthritis, osteomyelitis and postsurgical toxic shock syndrome with substantial rates of morbidity and mortality. Its impact has been enhanced by the development of MRSA that is resistant to virtually all β-lactam antibiotics [1,2]. According to the Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, overall prevalence of MRSA is about 40% [3].

The majority of research in this field suggests that resistance in MRSA is conferred by the acquisition of a mobile genetic element, the *Staphylococcal* Cassette Chromosome (SCC*mec*), carrying the *mecA* gene, which encodes an altered Penicillin Binding Protein (PBP)-PBP2a/PBP2′- that has reduced affinity for β-lactam antibiotics. As a result, cell wall biosynthesis in MRSA strains continues even in the presence of otherwise inhibitory levels of β-lactam antibiotics [1,2].

Molecular amplification of the *mecA* gene is recognised as the gold standard to detect MRSA in the community as these genes are highly conserved among staphylococcal species [4].

In 2007, a new *S. aureus* strain harboring *mecA* gene homologue, *mecC*, was found as an isolate from a bulk tank milk sample in Southwest England, which conferred beta lactam resistance with 70% sequence similarity to *mecA* gene [5]. Subsequently, *mecC* MRSA have been isolated from 14 different domestic and wild animal species and in a range of infections in humans. These are predominantly skin and soft-tissue infections, but include severe bone infections, nosocomial pneumonia and fatal bacteraemia [1].

Previous studies [6-8] have shown that *S. aureus* carrying *mecC* gene were identified as MRSA by Vitek 2 automated system but misidentified as *mecA*-negative, and therefore methicillin sensitive, by routine PCR and commercial slide agglutination assays for *mecA*-encoded PBP2a. Thus, the presence of the *mecC* gene is determined only by molecular methods using specific primers for *mecC* [6-8]. The report of *mec*C gene has posed diagnostic problems with the potential to be misdiagnosed as methicillin-sensitive *S. aureus*, with important potential consequences for individual patients and for the surveillance of MRSA [2].

This study seeks to screen for *mecA* and *mecC* genes among methicillin resistant isolates of *S. aureus* using conventional PCR and to associate their presence with Kirby-Bauer disc diffusion and automated Vitek-2 methods.

MATERIALS AND METHODS

This cross-sectional observational study was conducted in the Department of Microbiology, Victoria hospital, Bangalore Medical College and Research Institute, Bengaluru, Karnataka, India, from July to October 2019. Institutional Ethical Clearance (IEC) was obtained (No:BMCRI/PS/82/2019-20).

Sample size calculation: Was done using the formula with

n=z2*p*(1-p)/e2;

n=required sample size; p=prevalence of study (40%); e=margin of error, 12%; z=1.96 at 95% CI.

A total of 60 *S. aureus* strains isolated from various clinical samples such as pus, blood etc., were used in the study. Clinical specimens were as follows: Pus- 47 (78.33%), Blood- 8 (13.33%), Others- Corneal swab 1 (1.67%), Cerebrospinal Fluid (CSF)- 1 (1.67%), Urine- 1 (1.67%), Lacrimal sac abscess- 1 (1.67%), Nasal swab- 1 (1.67%). Confirmation of strains was done using standard tests like catalase, slide and tube coagulase, and growth on Mannitol salt agar [9]. Antibiotic susceptibility testing was carried out using cefoxitin disc diffusion test [9] and Automated Vitek-2 methods [10].

Cefoxitin disc diffusion test: All 60 *S. aureus* isolates were subjected to a cefoxitin disc diffusion test using a 30 µg disc. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture was done on Muller Hinton Agar (MHA) plates. Plates were incubated at 37°C for 18 hours and zone diameters were measured [9]. Zone size was interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines 2019 criteria. Isolates with an inhibition zone diameter of ≤21 mm were reported as MRSA and ≥22 mm were considered Methicillin-Susceptible *S. aureus* (MSSA) [11].

Automated Method: Antibiotic susceptibility to oxacillin and cefoxitin among clinical isolates of *S. aureus* was carried out using Vitek-2- AST-P628 susceptibility cards (bioMerieux). Suspensions of cultures were made and loaded into the test cards according to manufacturer's instructions [12,13]. As per CLSI 2019 isolates with cefoxitin screen positive and oxacillin MIC ≥4 µg/mL were considered as MRSA and isolates with cefoxitin screen negative and oxacillin MIC ≤2 µg/mL were regarded as MSSA [11].

DNA Extraction by boiling lysis method: A fresh 24 hour bacterial culture on chocolate agar was taken. Only 5-6 bacterial colonies were picked using a straight wire and were suspended in 50 µL nuclease free water. They were then boiled at 99°C for 10 minutes and centrifuged at 13000 rpm speed for one minute and 5 µL of the supernatant was used as template DNA.

Detection of *mecA* and *mecC* genes using conventional PCR:

The primer sequences and product length for *mecA* and *mecC* are as follows:

mecA-- Forward 5'- TCCAGATTACAACTTCACCAGG-3' and

Reverse- 5'-CCACTTCATATCTTGTAACG-3' (162 bp) [8];

mecC- Forward -5'-GAAAAAAAGGCTTAGAACGCCTC-3' and

Reverse-5'-GAAGATCTTTTCCGTTTTCAGC-3' (138 bp) [8]

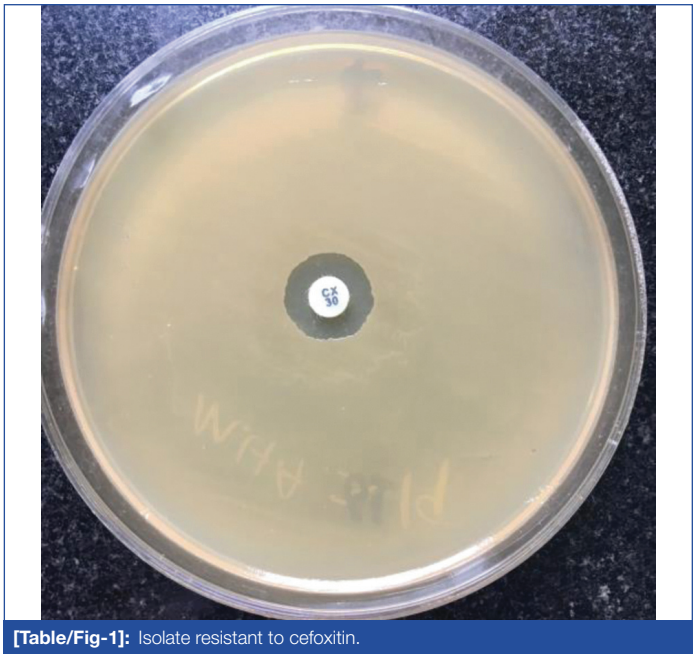
Reaction conditions for both the genes: Amplification was performed with the following program: 15 minutes at 94°C, followed by 30 cycles of 30 seconds at 94°C, one minute at 59°C, and one minute at 72°C, with a final 10 minutes elongation step at 72°C [8]. PCR products (5 µL) were analysed using 2% agarose gel electrophoresis with ethidium bromide dye under ultraviolet transilluminator.

STATISTICAL ANALYSIS

Descriptive statistics was used for the comparison of data and appropriate statistical charts were used to present the data.

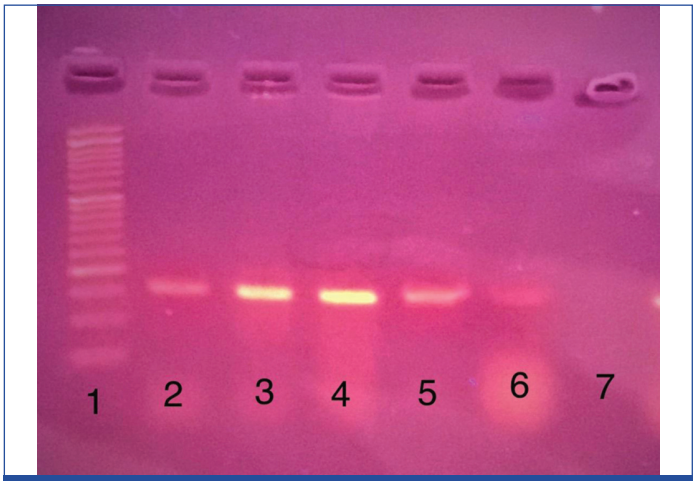
RESULTS

A total of 60 *S. aureus* strains were isolated from various clinical specimens. By conventional DDM, 41 isolates (68.33%) were regarded as MRSA and 19 (31.67%) of them were regarded as MSSA [Table/Fig-1]. By automated Vitek-2 method, 48 (80%) isolates were regarded as MRSA and 12 (20%) isolates were regarded as MSSA. Conventional PCR is considered as the gold standard method of detection of MRSA [Table/Fig-2,3]. Among 60 clinical isolates of *S. aureus* 34 (56.67%) were positive for *mecA* gene, remaining 26 (43.33%) isolates were *mecA* negative.



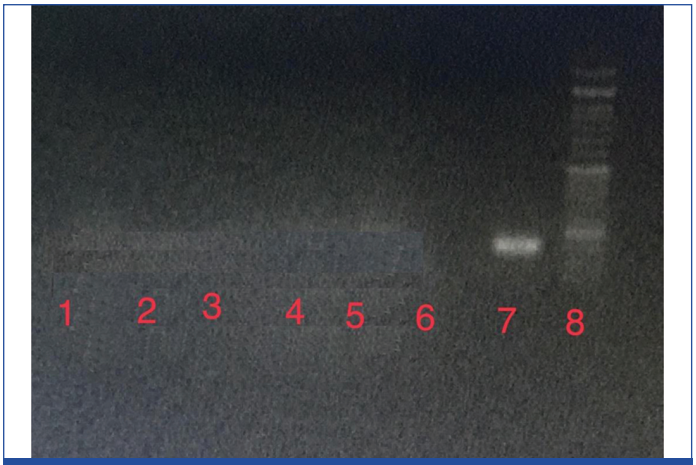
[Table/Fig-1]: Isolate resistant to cefoxitin.

[Table/Fig-2]: (From left to right) Lane 1-50 bp ladder; Lane 2,3,4- *mecA* positive isolates; Lane 5,6- *mecA* negative isolates; Lane7- negative control; Lane 8- positive control.



[Table/Fig-3]: Gel electrophoresis- (From left to right) Lane 1-50 bp ladder; Lane 2,3,4,5- *mecA* positive isolates; Lane 6- positive control; Lane 7- negative control.

Out of 41 isolates identified as MRSA by DDM, only 33 (80.48%) of them possessed *mecA* gene. Also, one isolate, phenotypically identified as MSSA possessed *mecA* gene. Out of 48 isolates identified as MRSA by automated Vitek 2 only 34 (70.88%) of them possessed the *mecA* gene. Among 60 clinical isolates of *S. aureus,* none of them possessed the *mecC* gene which includes the category of *mecA* negative phenotypically identified MRSA isolates [Table/Fig-4]. Comparative data and diagnostic performances of different methods in detection of MRSA have been shown in [Table/Fig-5,6].



[Table/Fig-4]: (From left to right) Lane 1,2,3,4,5- *mecC* negative isolates; Lane 6- negative control; Lane 7- positive control; Lane 8- 50 bp (basepair) ladder.

Method used for detection	MRSA n (%)	MSSA n (%)
Cefoxitin disc diffusion	41 (68.33)	19 (31.67)
Vitek 2	48 (80)	12 (20)
Conventional PCR	34 (56.67)	26 (43.33)

[Table/Fig-5]: Comparison of different methods in detection of MRSA.

	Cefoxitin disc diffusion		Vitek 2	
mecA PCR	Resistant	Sensitive	Resistant	Sensitive
mecA positive (34)	33	1	34	0
mecA negative (26)	8	18	14	12
Total=60	41	19	48	12

[Table/Fig-6]: Results of the phenotypic methods and correlation with *mecA*.

DISCUSSION

The MRSA is a major pathogen associated with severe nosocomial infections and due to its multidrug resistance, the treatment options are limited. Accurate and early detection of methicillin resistance is of immense importance in the prognosis of infections caused by *S. aureus*.

Identification of the *mecA* gene is the major evidence (gold standard) for the detection of MRSA isolate. This statement was approved by many researchers all over the world: in India, the USA, England, Japan, Spain *etc*., [4]. Phenotypic methods of detection of MRSA like conventional disc diffusion and automated Vitek-2 methods have been compared to the gold standard *mecA* gene detection by PCR in many studies. A study by Anand KB et al., all the 32 isolates detected as MRSA by cefoxitin disc diffusion possessed the *mecA* gene [9]. A study by Thampi DK et al., in a tertiary care centre highlighted that both the methods gave results in agreement with PCR [12]. Another study by Madhavan A et al., reported two false negative isolates by disc diffusion, whereas they were found to be MRSA by Vitek 2 [13]. Both the methods reported high concordance with PCR. Most of the studies around the world have reported disc diffusion by cefoxitin as an accurate surrogate marker and automated Vitek 2 method as a time saving approach for early detection [12,13].

However, in present study, only 33 of 41 (80.48%) isolates and 34 of 48 (70.88%) identified as MRSA by cefoxitin disc diffusion and automated Vitek-2 methods respectively tested positive for *mecA* gene. This indicates the presence of *mecA* negative phenotypically identified MRSA isolates. One isolate identified as MSSA by cefoxitin disc diffusion possessed *mecA* gene, whereas, it was reported as MRSA by Vitek-2. The false negative result can be explained by the fact that *mecA* positive strains differ in their level of resistance, i.e., resistance is usually heterogeneous with only a few cells (one in 10^4/mm^3 or 10^6/mm^3) expressing the phenotype [13]. Also expression of drug resistance depends on the other conditions like growth media, temperature, osmolarity [14]. Other studies like in Sudan [4], Turkey [15], Egypt [16] have not demonstrated the *mecC* gene.

A study in Iraq, demonstrated the presence of 22 (30%) out of 27 *mecA* negative isolates. Many studies around the world have also highlighted *mecA* negative phenotypically identified MRSA isolates as mentioned in [Table/Fig-7] [4,15-19]. A study by Pal S et al., in Uttarakand, out of 196 isolates determined as MRSA by cefoxitin disc diffusion only 164 showed the presence of *mecA* gene. 32/196 (16.3%) tested negative for *mecA* [19]. Few studies have demonstrated the *mecC* gene amongst such isolates. A study by Bali N et al., in Kashmir demonstrated the presence of *mecC* gene in India for the first time with 3.9% of 102 MRSA isolates having the *mecC* gene [20]. A study by Khan AA et al., in Pakistan also demonstrated the *mecC* gene in 3% of MRSA isolates and both the genes in one MRSA isolate [21]. Also, a previous study in Nigeria reported the complete absence of *mecA* genes and five major SCCmec types in randomly selected 36 phenotypically identified MRSA [22].

Study	Number of MRSA isolates identified by cefoxitin disc diffusion	Number of *mecA* positive MRSA isolates	Number of *mecA* negative MRSA isolates	*mecC* gene detection in these isolates
Elhassan MM et al., [4]	123	111	12 (9.8%)	Absent
Cikman A et al., [15]	494	315	179 (36.23%)	Absent
Rania AA et al., [16]	110 MRSA+40 MR-CoNS	144	6 (4%)	Absent
Dhungel S et al., [17]	34	32	2 (5.8%)	Not tested
Degaim ZD et al., [18]	71	44	27 (38.02%)	Present in 22 (30%)
Pal S et al., [19]	196	164	32 (16.3%)	Not tested
Present study	41	33	8 (19.51%)	Absent

[Table/Fig-7]: *mecA* negative phenotypically identified MRSA isolates [4,15-19].

The absence of detection of *mecA* and *mecC* genes among phenotypically identified MRSA highlights the presence of other factors contributing to the resistance. Gene instability and primer design could be proposed as one of the reasons [22,23]. Olayinka BO et al., reported the loss of mecA gene during storage at - 80° celsiuslayinka. The study included collecting isolates over a period of time, preliminary characterisation, storing and subculturing over a considerable length of time before final transportation for molecular characterisation [22]. It may be related to borderline (low level)-methicillin resistant strains of *S. aureus* (BORSA) that lack the *mec*A gene but identified as MRSA by phenotypic methods. This happens generally due to hyper production of Type A β-lactamase [22]. Heterogenous methicillin resistant strains with thicker and rougher cell walls and hence more Penicillin-Binding Protein 2 (PBP2) could produce methicillin resistance without the *mec*A gene [24]. Another new discovery includes the *mecB* gene. The *mec*B gene has been found as a part of plasmid, distantly related to a macrococcal plasmid which provides MRSA. Routine phenotypic methods for susceptibility testing cannot distinguish between methicillin resistance determinants thus, *mec*B encoded methicillin resistance can remain undiscovered [25]. These findings provide clear evidence of mechanisms other than *mec*A and *mecC* genes responsible for beta-lactam, resistance of MRSA and molecular methods alone are not enough for confirmed characterisation of MRSA isolates [5].

Limitation(s)

This study, however, is subject to several limitations. Authors were unable to further research into the rationale behind *mec*A and *mecC*

negative phenotypically identified MRSA strains due to time and resource constraints.

CONCLUSION(S)

Present study showed an overall MRSA prevalence of 56.67% by PCR. Phenotypic methods of MRSA detection are good alternatives to molecular methods in resource restrained setup. The *mecC* gene was not detected in any of the *S. aureus* isolates. However, authors believe that this study will encourage further research into unanswered questions concerning the origin of the *mec* homolog and the epidemiology of Staphylococcal isolates harboring *mecC* in India. It is crucial for diagnostic laboratories to understand, that absolute dependence on the *mecA* gene as the defining standard in determining resistance of *S. aureus,* has become the subject of distrust by many researchers. Alternative mechanisms of resistance have to be further explored and taken into consideration while testing.

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