

Multiplex PCR based detection of *mecA*, *mecC* and PVL gene in analysis of prevalence, circulation, transmission of MSSA/MRSA strains in a tertiary care hospital

S. H. Shifa Meharaj¹, S. Jayanthi^{2*}, D. Danisvijay³, A. Sujhithra⁴, J. Perumal⁵

¹Assiatnt Professor, ²Professor, ^{3,4}Research Scholar, ⁵Senior Laboratory Technician, Dept. of Microbiology, Chettinad Hospital & Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu, India

Abstract

Introduction: *Staphylococcus aureus* a Gram positive cocci causes major and minor infections in humans. It is the most important pathogen causing Hospital acquired and community-acquired (CA) infections. The commonest being the Methicillin resistant *Staphylococcus aureus* (MRSA) which is due to the insertion of SCC *mec* into methicillin-susceptible *S. aureus* (MSSA) lineages. Though, a number of toxins are involved in the pathogenesis of its infections, Panton Valentine leukocidin (PVL) is considered the common virulence factor for community acquired MRSA. Our study aimed to determine the prevalence of *mecA* and *mecC* gene and the presence of PVL toxin genes (*lukS-PV* and *lukF-PV*) in *S. aureus* isolates.

Materials and Methods: The study was carried out in Department of Microbiology at Chettinad Hospital & Research Institute, a tertiary care center for 3 months. A total of 109 clinical isolates of *S. aureus* were subjected for the study (Wards and Outpatient departments). The identification and antimicrobial sensitivity testing were done by standard microbiological techniques. Multiplex PCR was used to detect *mecA*, *mecC* and PVL genes.

Results: Out of 109 *Staphylococcus aureus* isolates, 24(22%) were MRSA and 85(78%) were Methicillin sensitive. Among MRSA strains, 13(54.1%) were positive for *mecA*, 8(33.3%) were positive for PVL, 3 (12.5%) were found positive for *mecC*. Among the 85(78%) MSSA isolates, 3(3.5%) were positive *mecA* gene and only 1(1.1%) isolate had *mecC* gene. The isolates from hospital environment were negative for both *mecA* and *mecC* but was positive for PVL genes.

Conclusion: Our study showed high prevalence among community acquired MRSA isolates.

Keywords: Community acquired staphylococcus aureus, MRSA, *mecA*, *mecC*, PVL gene, Multiplex PCR.

Introduction

Staphylococcus aureus (*S. aureus*) a Gram positive cocci, is usually seen as normal commensal in the human body. About 20% - 30% of normal population stay colonized by *S. aureus*, commonly on outer skin surfaces and the upper respiratory tract, particularly the anterior nares¹. It causes minor skin and soft tissue infections like boils and abscess, life-threatening bacteremia, pneumonia, osteomyelitis, endocarditis, sepsis and toxic shock syndrome. Both Hospital-acquired (HA) and community-acquired infection (CA)² caused by *S. aureus* infections are increasing nowadays.

S. aureus acquires resistance to methicillin known as Methicillin-resistant *S. aureus* (MRSA) which was due to the presence of an additional penicillin-binding protein (PBP2a), which may be obtained from other species. Multiple drug resistance is also common among them due to the use of various antibiotics. MRSA differs from methicillin-sensitive *S. aureus* (MSSA) by the presence of *mecA* gene that encodes the Penicillin binding protein (PBP).¹

Some *S. aureus* strains though they have *mecA* gene, they are methicillin-sensitive and are referred to as pre-MRSA³. Selective pressure and over use of the antibiotic can lead to mutations or deletions of *mecA* region giving

rise to constitutive PBP2a expression which may go for homogeneous or heterogeneous methicillin resistance depending on the population structure of strain. They have decreased susceptibility to several antibiotic classes of β -lactam family. HA-MRSA infects the patients who are under treatment for prolonged periods in a health care facility. Whereas, CA-MRSA and methicillin-susceptible *S. aureus* (MSSA) infect healthy persons without any risk factors.⁴

Panton-Valentine Leucocidin (PVL) is a powerful cytotoxin gene connected to CA-MRSA strains.⁵ Recently *mecC* associated MRSA infections were isolated in small numbers from humans and other species in several countries. This *mecC* can be misdiagnosed as methicillin-sensitive *S. aureus* because its methicillin resistance is not easily detectable by conventional tests of MRSA.⁶ *mecA* specific PCR does not routinely detect the new *mecA* homologue which is designated as *mecC*.

In our country, data regarding the epidemiology and prevalence of *mecC* associated methicillin-resistant *Staphylococcus aureus* (MRSA) and its transmission among the human population are inadequate. Therefore, our study is concerned about the human and bovine methicillin resistant *S. aureus* isolates carrying a new *mecA* gene homologue, *mecA_{LGA251}* known as *mecC*⁶ is also included.

*Corresponding Author: S. Jayanthi, Dept. of Microbiology, Chettinad Hospital & Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu, India

Email: jayanthitan@gmail.com

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The present study was aimed to determine the prevalence and the presence of *mecA*, *mecC*, PVL among *S.aureus* clinical isolates in human population.

Aim and Objective

Our aim of the study is to determine the prevalence of MSSA and MRSA isolates as well as to differentiate the hospital acquired from community acquired strains both from patient and environmental samples.

Materials and methods:

Our current study was conducted in Chettinad Hospital and Research Institute and was approved by the Institutional Human ethics committee. This study was carried out for a period of three months March, April and May. Samples like wound swab, pus, blood, urine, sputum, body fluids, ear swab, vaginal swab and eye swab were collected from various departments and processed.

Samples were processed on appropriate media and incubated aerobically at 37°C for 18- 24 hours. Culture identification of *Staphylococcus aureus* were based on tube coagulase test, mannitol fermentation⁷ and antimicrobial susceptibility pattern were done using standard protocol⁷

Isolated *Staphylococcus* species were also cultured on Mannitol salt agar (Hi media) and DNase agar. Both were incubated at 37°C for 24 to 48 hours. *S.aureus* isolates produced yellow coloured colonies on Mannitol salt agar. (The colonies on DNase agar were flooded with 1N Hydrochloric acid. Vacuum pipette was used to remove the excess acid and the reaction was observed. A positive DNase reaction was indicated by the clear zones around the bacterial colonies⁸.

Antimicrobial susceptibility testing was performed for *Staphylococcus aureus* isolates which includes eleven antibiotics, penicillin G (1µg), cefoxitin (30µg), erythromycin (30µg), clindamycin (30µg), cotrimoxazole (1.25/23.75 µg), ciprofloxacin (5µg), gentamicin (10µg), tetracyclines (30µg), vancomycin (30µg), teicoplanin and linezolid (30 µg) according to standard guidelines⁹ (Fig. 1).

Molecular analysis¹⁰

The individual bacterial colonies were picked and suspended in 100 µl of milli-Q water. Initially, the suspensions were boiled for 15 min followed by rapid cooling to -20°C for 5 min. The bacterial cell debris suspensions were centrifuged for 10 min at 15,000 rpm and the supernatant collected, was used as a template DNA. The DNA concentration was measured by using UV spectrophotometry. Table 1 shows the PCR primers used to detect the target gene After the phenotypic confirmation of Methicillin resistant *Staphylococcus aureus* (MRSA), strains were taken for genotypic identification of *mecA*, *mecC* and PVL gene by using multiplex PCR amplification method.



Fig. 1: Antibiotic susceptibility testing

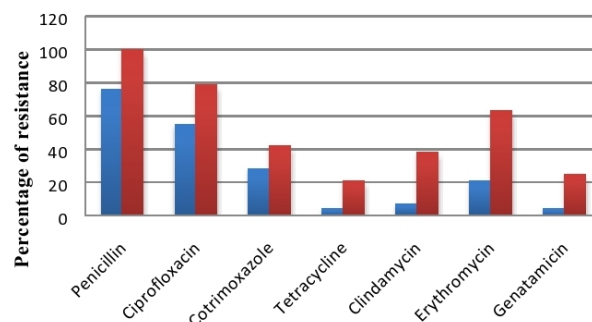


Fig. 2: Resistance pattern of *S.aureus*

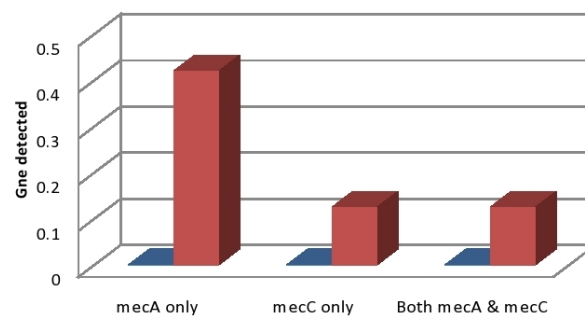


Fig. 3: Detection of *mecA* and *mecC* From patient sample

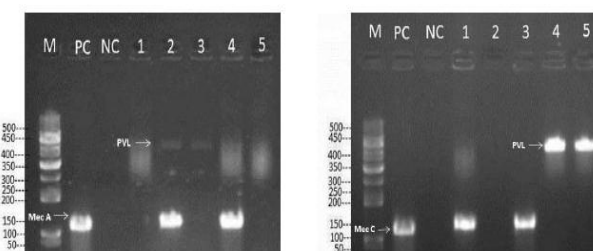


Fig. 4: Gel document of *mecA*, *mecC*, and PVL

Table 1: Primers used

Gene	Primer sequence	Base pairs	Reference
MecA	F:5'-TCCAGATTACAACCTTCACCAGG-3'	162bp	Dogan et al ²⁴
	R:5'-CCACTTCATATCTTGTAACG-3'		
MecC	F:5'-GAAAAAAAGGCTTAGAACGCCTC-3'	138bp	Dogan et al ²⁴
	R:5'-GAAGATCTTTCCGTTTTTCAGC-3'		
Luk-PVL	F:5'ATCATTAGGTAA AATGTCTGGACATGA TCC-3'A	433bp	Dharm et al ¹⁰
	R:5'GCA TCA AGT GTA TTG GAT AGC AAA AGC-3'		

PCR Mixture

1. Primer -2µl
 2. Template - 5µl
 3. Master Mix - 12.5µl
 4. Nuclease free solution - 5.5µl
- Total Volume -25µl

PCR Amplification procedure

1µl of the stored DNA was added to the PCR mixture. Initial Denaturation: 94°C for 5 minutes, Denaturation: 94°C for 35 seconds, Primer annealing: 54°C for 1 minute, Heat stable extension: 72°C for 35 seconds, Final Extension: 72°C for 7 minutes, Total number of cycles = 32, Holding temperature: - 4°C, DNA fragments were visualized as band pattern after adding the dye ethidium bromide.

Environmental swabs

Screening swabs were randomly collected near the MRSA positive ward patients from the frequently handled surfaces of the environment like the injection trolleys, cot railings, and patient bed side table. Swabs were streaked on appropriate media and incubated aerobically at 37°C for 16-18 hrs. Identification of the organisms and the antibiotic susceptibility pattern were done following the standard protocol.⁷ Environmental swabs were also processed simultaneously along with the pathogenic samples.

Results

The prevalence of *Staphylococcus aureus* in a tertiary care hospital were found to be n=109 out of the total 3706 samples (3.4%) in three months. *S.aureus* were isolated from sputum (n=3), blood (n=6), urine (n=13), pus (n=14), wound swab (n=63), high vaginal swab (n=1), nasal swab (n=1), ear swab (n=4), throat swab (n-1) etc. Among them 24 were MRSA-positive isolates. The proportion of MRSA infections among all *S. aureus* isolates was 22% during the study period. The gender wise distribution of *S.aureus* isolates (MSSA and MRSA) were 55,30 and 16,8 respectively.

All the 109 *S.aureus* isolates were positive for catalase test. Catalase test was coupled with a selective and differential solid screening medium using mannitol salt agar to isolate *Staphylococcus aureus* isolates. *Staphylococcus aureus* can be identified by using mannitol salt agar (MSA) in which it was able to tolerate the high salt content and ferment mannitol for growth. All these isolates were able to grow on MSA producing yellow coloured colonies. *Staphylococcus aureus* isolates were confirmed by DNase agar test and tube coagulase test(TCT). Among these

98(89%) isolates were found to be positive for DNase - positive while the remaining 11(10%) were DNase-negative. All the 109 isolates were coagulase positive and confirmed as *S.aureus*.

Among these total *S.aureus*, 85(78%) isolates were identified as MSSA by disc diffusion method. The antimicrobial susceptibility pattern among the MSSA isolates showed 76% resistance to Penicillin, 55% to Ciprofloxacin followed by 28% to Cotrimoxazole, 21% to Erythromycin, 7% Clindamycin, 4% to Tetracycline and Gentamicin. Nearly 8% of these isolates were positive for D test (Fig. 2)

Further confirmation was done by Polymerase chain reaction. These strains were found to be negative for both *mecA* and *mecC*. Of the total 109 isolates only 24(22%) were identified to be MRSA by disk diffusion method. The resistance pattern in the MRSA isolates showed 100% resistance to Penicillin and Cefoxitin followed by 79%, 63% resistance to Ciprofloxacin, Erythromycin respectively. Nearly 38% of these isolates showed D test positive. All strains were susceptible to Vancomycin, Teicoplanin and Linezolid. The resistance pattern of *S.aureus* isolates were given in (Fig. 2).

The genes *mecA*, *mecC* and PVL were screened among the *S.aureus* isolates by conventional PCR. All the 85 cefoxitin susceptible isolates were negative for *mecC* and *mecA*. All the MRSA strains n=24(22%) were tested for the presence of *mecA* and *mecC*, 10 out of 24 isolates were positive for *mecA* only (42%), 3 out of 24 (12.5%) were positive for both *mecA* and *mecC* (Fig. 3)The primers used in this study are shown in the table1. The amplicon images are shown in Fig. 4.

PVL virulent marker gene was used to differentiate between Community acquired MRSA and Hospital acquired MRSA. All 13 isolates which were positive for *mecA* were found to be positive for PVL gene also indicating it could be due to community acquired MRSA. Gel documentation of PVL gene by multiplex PCR is shown in Figure 4.

Among the 24 MRSA isolates, 10 were from OPD and 14 were from the Wards. Hence hospital environment samples were screened for seven MRSA positive (by disc diffusion) patients. *S.aureus* was isolated from various surfaces like patient cot, pillow, side railings, floor, toilet door handles and window.

Sensitivity to different antibiotics were tested, among which two isolates showed resistance to penicillin, cefoxitin and ciprofloxacin and cotrimoxazole, three were resistant to penicillin, ciprofloxacin and gentamicin and two were resistant to penicillin, cotrimoxazole and tetracycline. The

two cefoxitin resistant isolates were screened for *mecA* gene which was found to be negative but were positive for PVL.

Discussion

In the present study among the 3706 samples, 109(3.4%) were *S.aureus* isolates. Among them 71(65%) were from males and 38(35%) were isolated from females. Our study was in concordance with Garoy et al., study in 2019 in the gender distribution of *S.aureus* isolates with 58.5% male and 41.5% female.¹¹

The prevalence of *Staphylococcus aureus* in our study was low n=109 (3.7%) in three months when compared to the other studies done by Ahamed et al,¹² Garoy et al.,¹¹ the above studies were done for five years.

In our study major source of *S.aureus* samples were from wound swabs (58%) followed by pus (12.8%), urine (12%) and blood (5.5%). Anupurba et al,¹³ documented that the maximum yield of isolates were high in exudate samples which is in concordance to our study. Another study by Rajadurai pandiet al,¹⁴ showed maximum *S.aureus* isolates were from the throat swabs which is not in concordance with our study.

In our study, antimicrobial susceptibility pattern of MSSA showed 76% resistance to Penicillin, 55% to Ciprofloxacin followed by the other drugs. All MRSA isolates were resistant to penicillin and 79% to Ciprofloxacin. Kattee et al,¹⁵ showed higher resistance to penicillin G followed by ampicillin and gentamicin which was again similar to our study. The susceptibility to Vancomycin, Teicoplanin and Linezolid of all strains were in concordance to Hussain et al.¹⁶ and Ahmed et al.¹²

Among the 109 isolates, 22% were found to be MRSA and similar documentation was done by Ahamed et al,¹² 2018. In our study, sample source of MRSA isolates from wound swabs, pus and blood specimen were 54%, 25% and 12.5% whereas Anupurba et al,¹³ has documented 72% MRSA isolates from pus samples.

The yield of MRSA isolates from OPD patients were 42% which is similar in our study whereas Tiwari et al,¹⁷ documented 67% and 33% MRSA from ward and OPD respectively. In this study MRSA patients were 42% from OPD'S and 37% from wards and 21% from ICU's. In the study by Ray et al,¹⁸ isolation rates for MRSA from outpatients, ward- inpatients and ICU were 28, 42 and 43 per cent which is dissimilar with our study.

According to the study by Stegger et al,¹⁹ in the screening of *mecA* and *mecC* 20 MSSA isolates were both *mecA/mecC* negative, 38 MRSA isolates were *mecA*-positive and *mecC*-negative. Our study showed, both *mecA* and *mecC* gene were negative among the MSSA isolates and 13 MRSA isolates were positive for *mecA* (42%) among which 3 were positive for *mecC* alone which is in concordance to Stegger et al,¹⁹

The MRSA with *mecA* positive (42%) isolates were found to be similar to the study by Amini et al.,²⁰ Unnerstad et al.,²¹ studied retrospectively for the presence of *mecC* gene among 58 MRSA (*mecA* positive) isolates and found

four to be positive for *mecC* and more likely similar to our study.

According to the study by Roy et al,²² of the total 69 isolates, 52(75.3%) and 17(24.6%) were MSSA and MRSA respectively. Presence and absence of *mecA* gene by PCR among the MSSA and MRSA were 49(71.01%) and 20(28.9%) respectively. The PVL gene were detected in 19 out of 49 MSSA (38.7%) and 3 out of 20 MRSA (15%) which is not similar to our study. The PVL genes were detected in all 13(54.1%) *mecA* positive isolates. In the MSSA isolates none of the above genes were detected.

Swabs were collected near the seven MRSA ward patients for screening of multi drug resistant strains, *mecA* and PVL. Cefoxitin resistance was observed only in two isolates (28%) by disk diffusion. These two isolates were negative for *mecA* but PVL positive by PCR. In a study by Carolene et al,²³ 20% were MRSA with PVL gene and 8% showed *mecA* and PVL gene and found to be similar to our study.

Limitation

PVL typing, *mecA* and *mecC* with demographic profile in a large population can clearly distinguish Hospital acquired from Community acquired MRSA with a statistical correlation.

Source of Funding

None.

Conflict of Interest

None.

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