Detection of *mecA* and panton-valentine leukocidin genes in methicillin resistant *staphylococcus aureus* isolated from various clinical samples in a tertiary care hospital

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Abstract

Context: *Staphylococcus aureus* is a Gram positive bacterium which has an impending ability to cause an array of infection ranging from skin lesions to life threatening systemic illness. In the past few decades there is a drastic rise in Methicillin resistant Staphylococcus aureus (MRSA). The acquisition of Staphylococcal cassette chromosome mec (SCC mec) carrying mecA gene leads to Methicillin resistance among Staphylococcus aureus.

Aim: The aim of this study is to identify the prevalence of MRSA and identify the SCC mecA and Panton-Valentine Leukocidin (PVL) gene by Polymerase chain reaction (PCR) among Staphylococcus aureus among various clinical isolates in our hospital. Material and **Materials and Methods:** A prospective cross-sectional study was conducted in a tertiary care hospital at Puducherry. The study was conducted over a period of 1year from January 2014 to December 2014. The isolates were obtained from various clinical samples such as pus, aspirate, urine, blood and sputum. PCR amplification of mecA and pvl genes was performed to check their prevalence among the isolates collected from a tertiary care hospital. PCR reaction mixtures (10µl) contained 1µlTaq PCR buffer (10X) with 15mM MgCl2, 0.1 µl (0.5U) of Taq polymerase (New England Biolabs, US), 0.25mM of dNTP mix (Thermo Scientific, US), 0.25pM each of forward and reverse primers (Eurofins, Bangalore, India) and 10ng of genomic DNA template.

Results: Around 228 samples of Staphylococcus aureus isolated from various clinical samples were analyzed for methicillin sensitivity. Out of these samples around 49 (21.49%) samples were MRSA positive isolates. Out of 49 mecA harboring isolates pvl gene was amplified in 26 (53%) isolates.

Conclusion: MRSA being a dreadful pathogen has minimal treatment options. PVL gene with added virulence further worsens the clinical outcome among infected patients. Hence the knowledge of its prevalence adds an insight among the infection control practitioners to adhere effective prevention protocol.

Keywords: SCC, Pvl, CA-MRSA.

Introduction

Staphylococcus aureus is a Gram positive bacterium which has an impending ability to cause an array of infection ranging from skin lesions to life threatening systemic illness.[1] The various factors such as adhesion, enzymes, toxins and antibiotic resistance mechanisms mark their treatment cumbersome. In the past few decades there is a drastic rise in Methicillin resistant *Staphylococcus aureus* (MRSA).[2] Furthermore the menace of multiple drug resistance is distressing.[3] The acquisition of Staphylococcal cassette chromosome*mec* (SCC *mec*) carrying *mec*A gene leads to Methicillin resistance among *Staphylococcus aureus*. Moreover, they are resistant to all Beta-lactam antibiotics as the Penicillin binding protein (PBP2a) is altered.[4] MRSA can be either hospital acquired (HA) or community acquired (CA). There are 11 types of SCC*mec* detected in *Staphylococcus aureus*. HA –MRSA carry SCCmectypes I-III whereas CA-MRSA carry SCC*mec*types IV or V.[5] The genotyping methods include multilocus sequence typing (MLST), pulse field gel electrophoresis (PFGE) and SCCmec typing. These methods help in identifying the prevalence of MRSA and aid in clinical therapy and infection control. Panton-Valentine Leukocidin (*pvl*) is a cytotoxin which damages the tissue causing necrosis.[6] The SCC *mec* in combination with *pvl* genes increase the resistance and virulence of MRSA. In addition, the misuse of antibiotics causes a selective pressure for developing resistant strains, which already harbors the *pvl* gene. The aim of this study is to identify the prevalence of MRSA and identify the SCC *mec*A and *pvl* gene by Polymerase chain reaction (PCR) among *Staphylococcus aureus* among various clinical isolates in our hospital.

Materials and Methods

Study Design

A prospective cross-sectional study was conducted in a tertiary care hospital at Puducherry. The study was conducted over a period of 1year from January 2014 to December 2014.The study was approved by the ethical committee. An informed consent was obtained from all patients who participated in the study. The clinical relevance and severity of MRSA was explained to all patients.

Sample Collection

The isolates were obtained from various clinical samples such as pus, aspirate, urine, blood and sputum. The sample size was considered with consecutive sampling method during the study period. Around 500 clinical samples were screened. All consecutive isolates identified as *Staphylococcus aureus* from various clinical samples during the study period were included, whereas repeated isolates from the same patient or patient with known MRSA infection were excluded. All the clinical samples were processed by standard bacteriological procedure. The

isolates which were Gram positive cocci in clusters, catalase positive, coagulase positive, urease produced and mannitol fermented were identified as *Staphylococcus aureus*. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method. We followed standard protocol as per the CLSI guidelines.[7] Anti-staphylococcal antibiotics such as penicillin (10U) ciprofloxacin (5µg), tetracycline(30µg), erythromycin (15µg), gentamicin (10µg), cotrimoxazole (1.25+23.75µg), clindamycin (2µg), teicoplanin (30µg), and linezolid(30µg) were used (Himedia, Mumbai, India). Vancomycin and teicoplanin were tested by Epsilometer strips (E -strips) as shown in Fig. 1. All MRSA were identified by using cefoxitin (30µg) disc as surrogate marker. A zone diameter of ≥ 22mm was considered as sensitive and zone diameter≤ 21 mm was considered resistant. Repeated isolation of MRSA from same patient was excluded. *Staphylococcus aureus* ATCC strain 25923 was used as control for antibiotic sensitivity testing.

Amplification of *mecA* and *pvl* genes

PCR amplification of *mecA* and *pvl* genes was performed to check their prevalence among the isolates collected from a tertiary care hospital. PCR reaction mixtures (10µl) contained 1µl*Taq* PCR buffer (10X) with 15mM $MgCl_2$, 0.1 µl (0.5U) of *Taq* polymerase (New England Biolabs, US), 0.25mM of dNTP mix (Thermo Scientific, US), 0.25pM each of forward and reverse primers (Table 1) (Eurofins, Bangalore, India) and 10ng of genomic DNA template. PCR amplifications were performed using Veriti thermocycler (Applied Biosystems, USA) with the following reaction conditions: Initial denaturation step of 5min at 94°C, was followed by 30 cycles. Each cycle constituted 30s at 94°C, 45s at 55°C and 1 min at 72°C, and a final extension step of 7 min at 72°C. The PCR amplified products from all the strains were analyzed by agarose gel electrophoresis (1% agarose) and the results were documented. (Fig. 2,3)

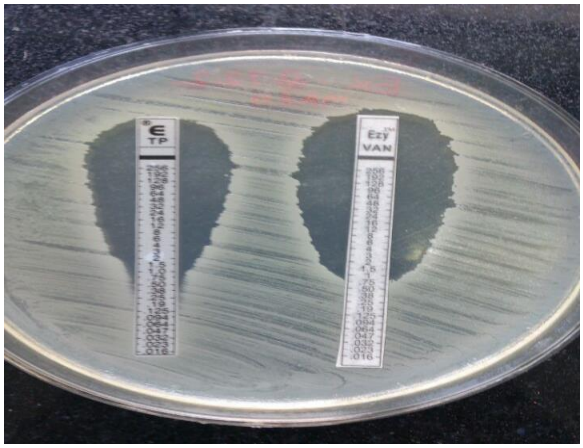

Fig. 1: Vancomycin and teicoplanin MIC testing by E – strips

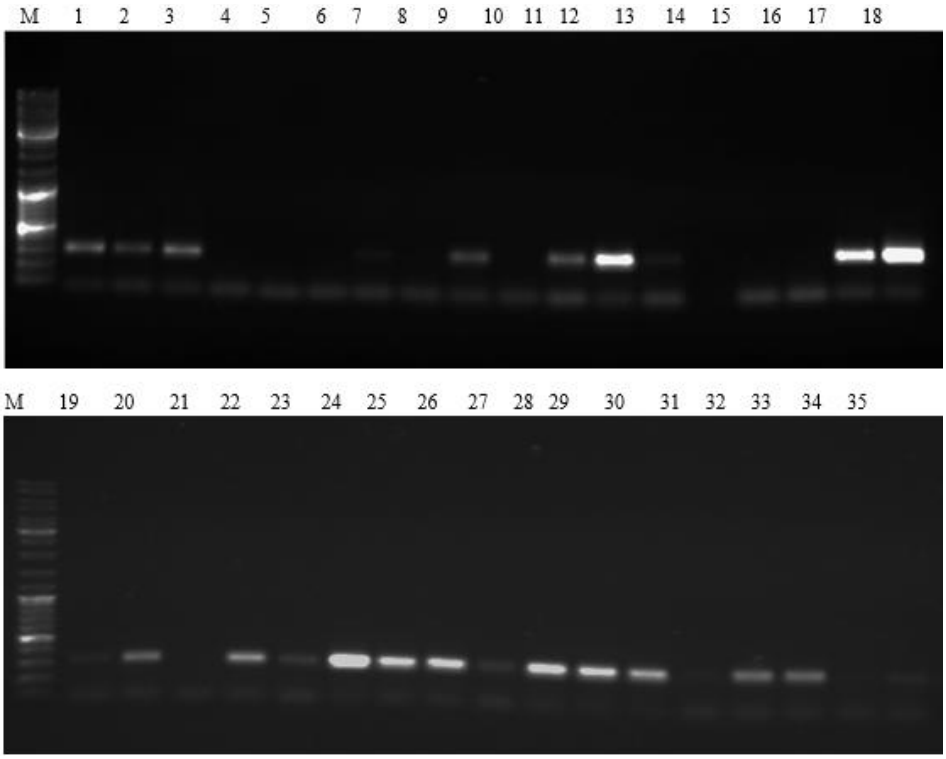

Fig. 2:
Lanes M – 10 Kb DNA ladder (Gene'O'ruler, Thermo scientific, USA)
Lanes 1,2,3,9,11,12,13,17,18,19,20,22,23,24,25,26,27,28,29,30,32,33,34,35 – shows *mecA* amplicons

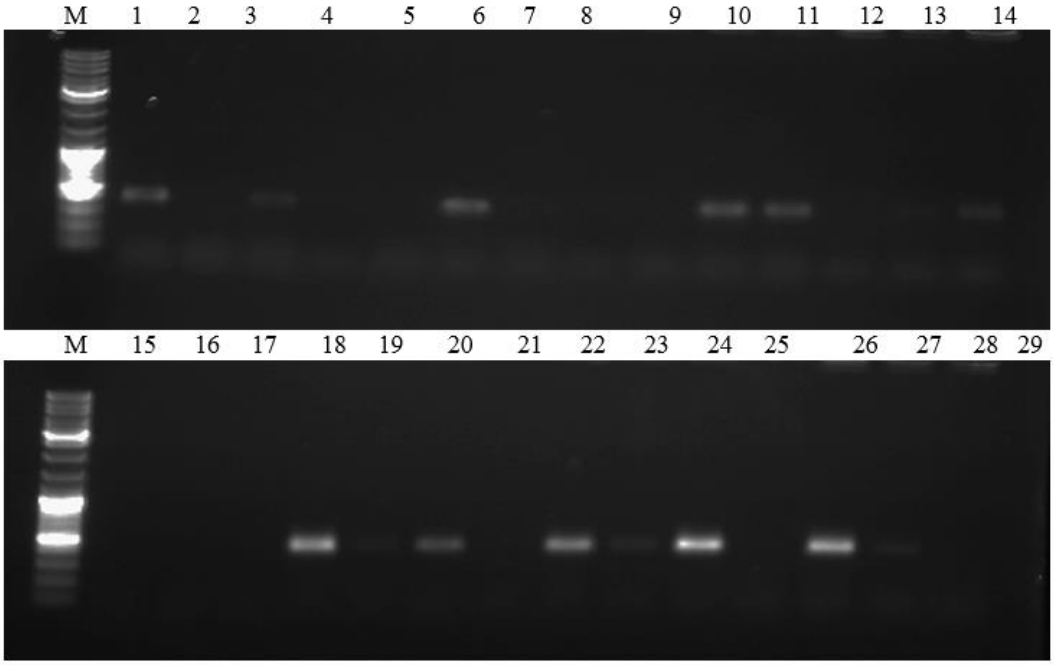

Fig. 3:
Lanes M – 10 Kb DNA ladder (Gene'O'ruler, Thermo scientific, USA)
Lanes 1,3,6,10,11,14,18,19,20,22,23,24,26 – shows *pvl* amplicons

Results

Around 228 isolates of *Staphylococcus aureus* isolated from various clinical samples were analyzed for methicillin sensitivity. Out of these samples around 49 (21.49%) samples were MRSA positive isolates. The age wise distribution of MRSA is shown in Table 2. The gender wise distribution of MRSA cases was almost equal with 25 (51.02%) isolates in male and 24 (48.97%) isolates in female.

The MRSA isolates were obtained from various clinical departments. Table 3, 4 shows the distribution of isolates from departments and samples. Most of the isolates were obtained from pus (87.7%) followed by aspirate (8.1%) and sputum (2.04%). Around 17.5% of isolates were resistant to ciprofloxacin, followed by cotrimoxazole (14.4%) and gentamicin (14.4%). All MRSA isolates were found to have *mecA* gene. Out of 49 *mecA* harboring isolates *pvl* gene was amplified in 26 (53%) isolates. Among MRSA isolates 17.5% of isolates were resistant to ciprofloxacin and 14% of isolates were resistant to cotrimoxazole and gentamicin as shown in Table 5. Vancomycin and teicoplanin tested by E strip were sensitive among all the MRSA isolates. Vancomycin and teicoplanin showed MIC less than 2µg/ml and 8µg/ml respectively.

Table 1: Primers used for amplification of *mecA* and *pvl* among the clinical isolates *S. aureus*

Gene	Forward (5'-3')	Reverse(5'-3')
mecA	CATTTTGAGTTCTGCACTACC	GCAATACAATCGCACATACATTAATAG
pvl	ATCATTAGGTAAAATGTCTGGACATGATCCA	GCATCAASTGTATTGGATAGCAAAAGC

Table 2: Age wise distribution of MRSA

Age in years	MSSA	MRSA Positive cases	Percentage
1-15	05	1	2.04%
16-25	37	09	18.3%
26-45	85	24	48.9%
46-75	49	16	32.6%
>75	03	0	0
Total	179	49	

Table 3: Specialty wise distribution

Department	MRSA positive cases	Percentage
Orthopedics	3	6.1%
General Surgery	35	71.4%
Obstetrics & Gynecology	6	12.2%
ENT	1	2.04%
General Medicine	3	6.1%
Urology	1	2.04%
Total	49	

Table 4: Percentage of MRSA from samples

Samples	MRSA positive cases	Percentage
Aspirate	4	8.1%
Sputum	1	2.04%
Tissue	1	2.04%
Pus	43	87.7%
Total	49	

Table 5: Resistance pattern of MRSA

Antibiotics	Resistance	Percentage
Penicillin	49	100%
Gentamicin (10µg)	32	14.05%
Erythromycin (15µg)	23	10.08%
Clindamycin (2µg)	5	2.1%
Tetracycline (30µg)	12	5.2%
Ciprofloxacin (5µg)	40	17.5%
Cotrimoxzole (1.25+23.75µg)	33	14.4%
Vancomycin (30µg)	0	
Teicoplanin	0	
Linezolid (30µg)	0	

Discussion

Staphylococcus aureus causes a wide array of infections owing to the repository of virulence factors. The infections include soft tissue infections, blood stream infections, respiratory infections and urinary tract infections.[8] Methicillin being a semi-synthetic penicillin was invented to evade the growing resistance, however resistance against β lactamase enzyme started developing 2 years after the drug discovery.[9] Since then it is a great burden to the patients and heath care providers. It is a major scourge in treatment and management of cases as it also leads to multiple drug resistance. In addition to β lactam group it shows resistance to fluroquinolones, tetracyclines, aminoglycosides and lincosamide group of antibiotics.[10,11] Hence it is necessary to conduct periodic studies on the prevalence and the molecular pattern of MRSA in each health care setting.

In this present study around 21.49% of MRSA was isolated from a total screen of 228 samples. The gender wise distribution of MRSA cases was almost equal with 25 (51.02%) isolates in male and 24 (48.97%) isolates in female. There was no significant correlation with gender and MRSA acquisition as most other studies.[12]

Majority of the isolates were reported from pus samples (87.7%). Our study corroborates with a similar study conducted in South India which isolated 80% of cases from postoperative wound infections.[13] Comparatively higher number of MRSA are being reported from surgical units compared to the medical units.[14] Improper infection control practices and patients with asymptomatic colonization are a potential source of infection. Every hospital should follow effective screening of health care workers and infection control practices to prevent the acquisition of MRSA in the hospital environment.

All MRSA isolates were found to have *mecA* gene by PCR. Out of 49 *mecA* harboring isolates *pvl* gene was amplified in 26 (53%) isolates. PVL being a gama toxin made up of F and S subunits targets the leukocytes and causes damage and necrosis.[15] *pvl* gene is predominantly present in CA-MRSA harboring the SCC mec types IV & V compared to HA- MRSA.[8] In the present study around 53% of isolates were positive for *pvl* gene by PCR. The results corroborates with a similar conducted in South India where 57.8% of isolates were *pvl* gene positive. However the report varies based on the geographic location and type of sample analyzed. Various studies from USA, Thailand and Iran have reported 36%, 49% and 23% respectively.[15-17] The limitation of the study could be the limited sample size and restriction to *mec* A and *pvl* gene screening alone. Further multicentric studies involving various genotyping methods such as MLST, PFGE and sequence analysis would enlighten the knowledge and help in managing the morbidity and mortality among patients.

Conclusion

MRSA being a dreadful pathogen has minimal treatment options. *pvl* gene with added virulence further worsens the clinical outcome among infected patients. Hence the knowledge of its prevalence adds an insight among the infection control practitioners to adhere effective prevention protocol.

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Conflict of Interest: None.

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