

## Methicillin Resistance and its Clinical Correlation at a Tertiary Care Centre

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### ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is responsible for an increasing number of serious hospital and community acquired infections. Methicillin resistance has become an important problem in the community. Thus it is very essential to study the clinical correlation to know the significance. This study reports the prevalence of MRSA isolated at a tertiary care centre, Hyderabad and the clinical correlation. A total of 110 consecutive *S. aureus* isolates were obtained from clinical samples during the study period of Jan 2013-May 2014. All the strains were tested phenotypically by conventional methods and genotypically by Uniplex-PCR targeting *S. aureus nuc* gene, Pantone-valentine leucocidin (PVL) toxin gene and *mec A* resistance genes. Of these 82 (74.5%) of the total number of the isolates were recovered from pus/ exudates (skin and wound infections) followed by blood 21 (19%) and sputum (lower respiratory tract infection) 7 (6.3%). 53(48%) isolates of MRSA caused infections. All the 110 strains previously identified phenotypically as *S.aureus* with bacteriological examination were positive for amplification of 460 base pair fragments specific for *nuc* gene of *S.aureus*. Moreover, 48% of *S. aureus* were positive for amplification of 300 base pair fragments specific for *mec A* gene, while only 47% of MRSA were positive for amplification of 976 base pair fragments specific for PVL gene. Methicillin resistance was 48 %

#### Keywords

MRSA, PVL gene, *Staphylococcus aureus*, Uniplex PCR.

#### Article Info

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### Introduction

*Staphylococcus aureus* is the most commonly isolated human bacterial pathogen and is an important cause of skin and soft tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis [1]. Methicillin-resistant *S. aureus* (MRSA) isolates are resistant to all available penicillins and other  $\beta$ -lactam antimicrobial drugs. They were once confined largely to hospitals, other health care environments, and patients frequenting these facilities.

Since the mid-1990s, however, there has been an explosion in the number of MRSA infections reported for populations lacking risk factors for exposure to the health care system [2, 3, 4]

The purpose of this study is to detail the prevalence of MRSA isolated from clinical specimens and to know the clinical spectrum of infectious syndromes associated with them, which ranges from a soft tissue infection to severe, overwhelming blood stream infection

and also highlight the antibiotic sensitivity pattern.

## **Materials and Methods**

### **Bacterial isolates**

A total of 110 isolates of *Staphylococcus aureus* were collected from Gandhi hospital, Secunderabd, Telangana, during the period of Jan 2013-May 2014. All the strains were tested phenotypically by conventional methods and genotypically by PCR for direct detection of *S. aureus* specific *nuc* gene and *mec A* gene for methicillin resistance. Of these 82 (74.5%) of the total number of the isolates were recovered from skin and wound infections followed by blood 21 (19%) and lower respiratory tract infection 7 (6.3%). 53 (48%) isolates of MRSA caused infections.

All the isolates were identified according to colonial and microscopical morphology, catalase, slide and tube coagulase, OF-Glucose fermentation, mannitol fermentation and DNase production.

### **Antimicrobial Susceptibility Test**

#### **Detection of cefoxitin resistance by phenotypic method**

##### **Cefoxitin disk diffusion method**

The cefoxitin disc (30µg) discs were used. The cefoxitin disc diffusion test were performed using the routine disc diffusion procedure, and the results were evaluated according to the interpretive criteria of CLSI, 2013.

#### **Antimicrobial susceptibility test to non-beta-lactam drugs**

The antimicrobial susceptibility test was performed using the disks which include Ampicillin (10µg), Amoxyclav (20/10µg),

PenicillinG (10U), Cefazolin (30µg), Ciprofloxacin (5µg), Erythromycin (15µg), Clindamycin (2µg), Tetracycline (30µg), Cotrimoxazole (1.25/23.75µg), Linezolid (30µg), Tigecycline and Vancomycin (30µg) as adopting the Kirby-Bauer disk diffusion method using Muller-Hinton agar and antibiotic disks(HIMEDIA) and results interpreted according to the recommendations of CLSI. *S. aureus* ATCC25923 was the control strain used. Based on the antibiotic susceptibility pattern 53 isolates were identified as MRSA and the rest as MSSA. D test was performed to detect Inducible Clindamycin Resistance.

### **Extraction of DNA from bacterial isolates**

The bacterial DNA extractions were performed using protocol supplied by Hi Yield Genomic Mini Kit.

### **UNIPLEX PCR done separately for detection of *nuc* gene, *mec A* gene, PVL genes**

The reaction mixture consisted of 0.5 µl of extracted DNA template of the bacterial isolates, 2 µl of RasTaq10x assay buffer, 1 µl each of DNTP'S, 0.5 µl each of RasTaq DNA Polymerase and 0.5 µl each of Primers specific (both forward and reverse). The volume of the reaction mixture was completed to 20 µl using 15.5 µl of RNase free water. The above components were added to a thin walled 200 µl micro centrifuge tube on ice gently mixed by vortexing and then briefly centrifuge to collect the components at the bottom of the tube. The tubes were placed in Bio-Rad Thermal cycler. The thermal cycler was adjusted as follows:

#### **For *nuc* PCR and *mecA* PCR**

Denaturation of 95°C for 5 min, followed by 30 cycles of denaturation of 94°C for 30sec, annealing of 55°C for 30 sec and extension of

72°C for 1 min, and final extension of 72°C for 10 min, and the PCR products were stored in the thermal cycler at 4 °C until they were collected.

### **Detection of Panton-Valentine leukocidin (PVL) genes**

The PVL genes (*lukS-PV* and *lukF-PV*) were detected by PCR. A methicillin susceptible *S. aureus* (*nuc+pvl* +ATCC 25923) isolate served as a positive control.

### **For PVL PCR**

Denaturation of 95°C for 5 min, followed by 30 cycles of denaturation of 94°C for 30sec, annealing of 65°C for 30 sec and extension of 72°C for 1 min, and final extension of 72°C for 10 min. the hold at 4 °C.

### **Analysis of the reaction products**

The amplicons of all the PCR reactions were visualized using a Ultraviolet light box following electrophoresis on a 1.5% agarose gel for *nuc* PCR, *mecA* PCR & PVL PCR and 1.8% agarose gel for SCC *mec* typing which contained 5 µl of 20µg /ml of ethidium bromide, stock solution. A molecular weight marker of a 100 bp ladder was included as a reference to specify lanes on each electrophoresis gel. The list of primers used for *nuc* PCR, *mec* A PCR and PVL PCR are shown in Table 1.

### **Results and Discussion**

A total of 110 consecutive *S. aureus* isolates were obtained from clinical samples.

### **Genotypic confirmation of *S. aureus***

All the isolates of *S. aureus* were confirmed genotypically by presence of *nuc* gene. *nuc* PCR specific product size of 460bp was

observed in all the isolates shown in (Image 1).

Of these 82 (74.5%) of the total number of the isolates were recovered from skin and wound infections followed by blood 21 (19%) and lower respiratory tract infection 7 (6.3%). 53(48%) isolates of MRSA caused infections.

40/82(48%) of skin and soft tissue infections and 13/30(43%) of the invasive infections were resistant to methicillin. Skin and soft tissue infections are the most common source of *S. aureus* infections (MRSA and MSSA).

MRSA isolated were common in the age group of 19-34 years and MSSA in the elderly group. MSSA were commonly isolated from the elderly group.

PNW-Post Natal Wards, NICU-Neonatal Intensive Care Unit, PICU-paediatric Intensive Care Unit. FCWD-Female Cardiac Ward, OP-Out-Patient Department, MMW-Male Medical wards. ORTHO-orthopedic wards, DVL-Department of Venereology and Leprosy, SSI-Surgical site infections, BSI-Blood Stream Infections,

Predominantly the isolates appear to occur from Postnatal Wound infections and Out-patient department followed by surgical wards.

Hospital associated infections are more among MRSA and Community acquired isolates among MSSA.

### **Antimicrobial susceptibility tests**

53(48%) of MRSA and 57 (51%) of MSSA were identified by AST. All the MSSA isolates 100% were resistant to penicillin and ampicillin, amoxycalv (90%), cefazolin (94%) and cephalexin (95%) The resistance for other antibiotics in MSSA include 26% for

tetracyclines, cotrimoxazole (66%), Erythromycin (57%) and ciprofloxacin (43%). (Image 4).

The MRSA isolates were 100% sensitive to linezolid, tigecycline and vancomycin. The resistance pattern of other antibiotics were ciprofloxacin (73%), tetracycline (13%), cotrimoxazole (56%), Erythromycin (77%) and levofloxacin (23%).

According to the definition of Niami *et al.*, [6], 38 were identified as HA and 15 were identified as CA-MRSA.

PVL PCR: PVL toxin production was different in MRSA and MSSA isolates. PVL toxin gene was present in 20 isolates (37%) of MRSA (Image 3.) of which HA were 28(6%) and CA were 14(26%). In MSSA PVL toxin production was 25 (35%) only.

**Table.1** List of primers used

PRIMER	SEQUENCE(5'-3')	Remark	Product size
<i>NucF</i>	GTGCTGGCATATGTATGGCAATTGT	Thermo-nuclease gene	460 bp
<i>NucR2</i>	TCTTTGACCTTTGTCAAACCTCGA		
<i>MecAF</i>	TGGCTATCGTGTCACAATCG	Methicillin resistance	300bp
<i>MecAR</i>	CTGGAACCTTGTTGAGCAGAG		
<i>Pvl F</i>	GGCGCTGAGGTAGTCAAAAG	Panton-Valentin eleukocidin gene specific	976 bp
<i>PvlR</i>	TCGGAATCTGATGTTGCAGT		

**Table.2** Based on the site of infection

Site of infection	MRSA		MSSA	
	No of isolates (n=53)	Percentage	No of isolates (n=57)	Percentage
Skin and wound infections (82)	40	75%	42	73.68%
BSI (21)	9	16%	12	21%
LRTI (7)	4	7.54%	03	5.26%

**Table.3** Age wise distribution of MRSA isolates

Age	MRSA		MSSA	
	No. of isolates (n=53)	Percentage	No of isolates (n=57)	Percentage
<1year	8	15%	4	7%
1-18years	6	11.3%	15	26%
19-34years	28	52%	18	31%
>35 years	11	20.7%	20	35%

**Table.4** Distribution of isolates according to the wards

Ward	MRSA		MSSA	
	No. of isolates (n=53)	Percentage	No. of isolates(n=57)	Percentage
PNW (Post LSCS,SSI and Episiotomy)	12	22%	20	35%
NICU(BSI)	6	11%	04	7
SURGICAL(SSI)	08	15%	6	10
BURNS	04	7.5%	01	1.7%
SKIN/DVL(SSI)	02	3.7%	1	1.7%
FCWD(BSI)	1	1.80%	-	-
OP(SSI)	13	24%	16	28%
MMW(BSI)	02	3.7%	1	1.7%
GYN(SSI)	1	1.8%	2	3.7%
ORTHO(SSI)	03	5.6%	2	3.7%
PICU(BSI)	01	1.8%	1	1.7%

**Table.5** Distribution of isolates CA vs HA

Type	MRSA (n=53)	MSSA (n=57)
CA (n=38)	15(39%)	23(61%)
HA (n=72)	38(52 %)	34(47%)

**Table.6** Prevalence of MRSA in community and Hospital

Total <i>S. aureus</i>	MRSA	CA-MRSA	HA-MRSA	MSSA
<b>110</b>	<b>53</b>	<b>15(28%)</b>	<b>38(52%)</b>	<b>57</b>

**Table.7** Showing PVL positivity and source of infection

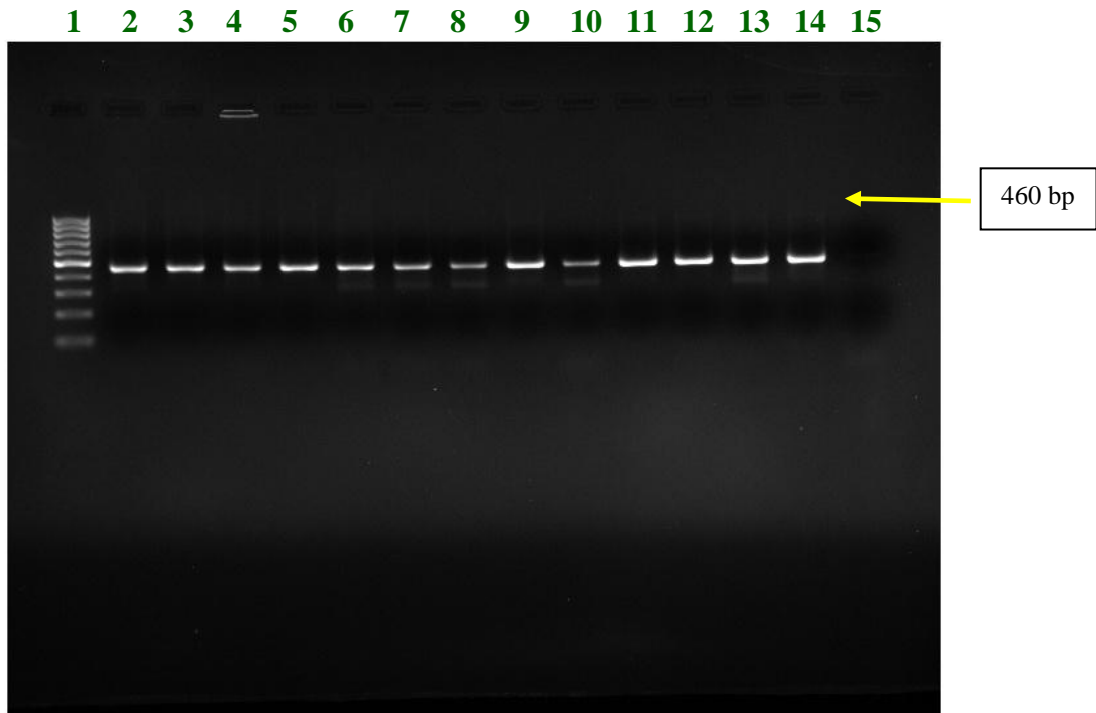
Source	PVL positivity	
	MRSA	MSSA
Skin and soft tissue infection	13(24%)	17(29%)
Blood	5(13%)	06(10%)
LRTI	2(3.7%)	2(3.5%)

Total 20

**Table.8** Showing PVL- and the isolates

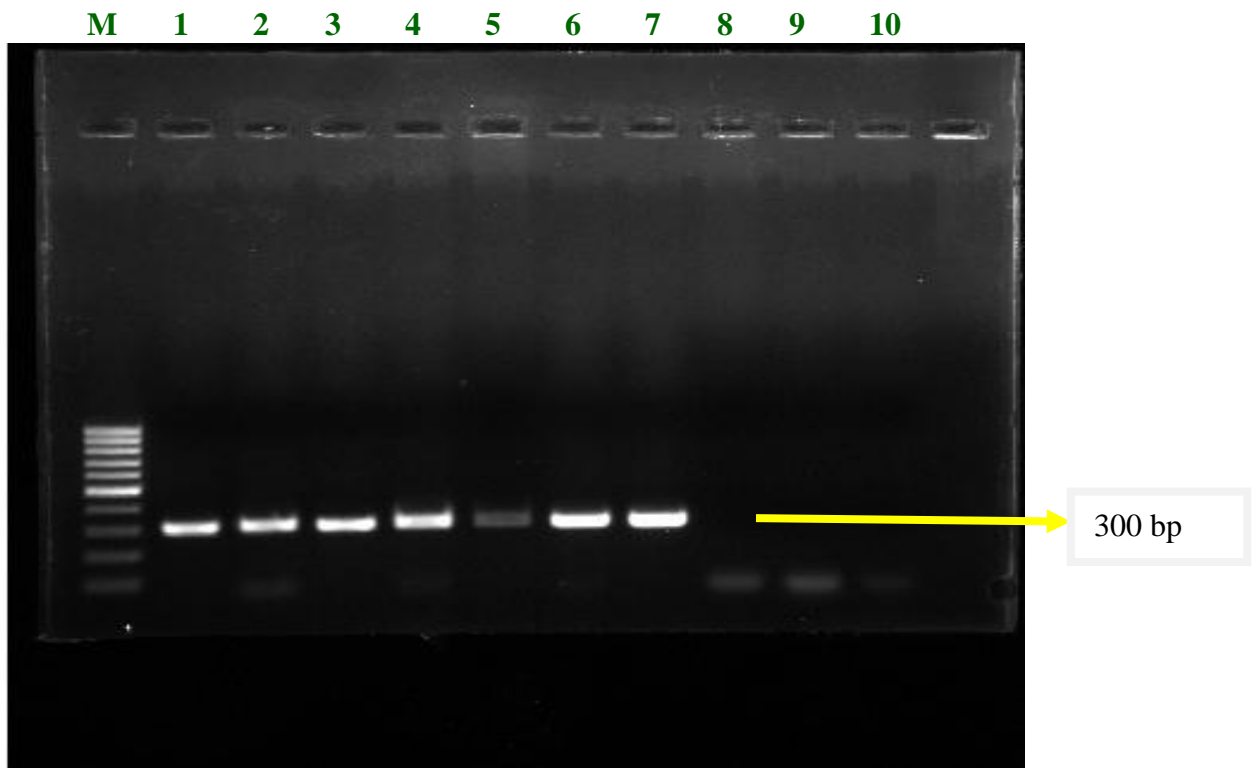
Isolates	No. of Isolates	PVL positivity
<i>Staphylococcus aureus</i> (total)	110	45(40%)
MSSA	57	25(43%)
MRSA	53	20(37%)
CA-MRSA	15	14(26%)
HA-MRSA	38	6(11%)

Image.1 nuc PCR



Lane 1-100bp ladder; Lane 2-12 positive for *nuc* gene-460bp; Lane13, 14-ATCC 25923; Lane 15- Negative control.

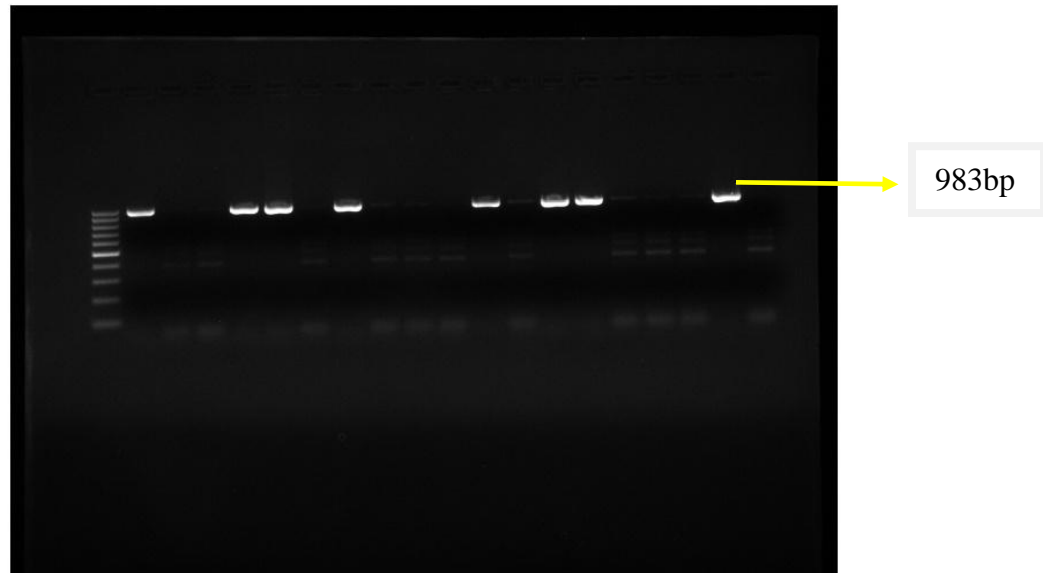
Image.2 mec A PCR



Lane 1 -100bp ladder; Lane 2-7 *mec A* positive -300bp ; Lane 8-positive control ; Lane 9,10- Negative control.

### Image.3 PVL PCR

M 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20



Lane1-100bp ladder ; Lane2; -*pvl* positive(983bp), Lane 3, 4-*pvl* negative ; Lane5, 6- *pvl* positive ; Lane 7- negative; Lane 8- *pvl* positive ; Lane 9,10,11-*pvl* negative, Lane12-*pvl* positive ; Lane 13- negative ; Lane 14,15- positive ; Lane 16,17,18- negative ; Lane 19 – ATCC25923, Lane 20-Negative control.

The most common source of PVL toxin production was seen in Skin and soft tissue infection. MRSA

PVL toxin production was more in CA-MRSA compared to HA-MRSA. (Figure).

#### **mecA PCR**

All the MRSA isolates were *mecA* positive with a PCR specific product size of 300bp suggesting the genes for methicillin resistance and MSSA isolates were *mecA* negative.

Methicillin resistant *Staphylococcus aureus* (MRSA), is an important pathogen responsible for many nosocomial infections. Over the past few decades it has emerged in the community as well, and it is currently considered a threat to public health.

The present study was undertaken to elucidate the prevalence and molecular characterization of MRSA.

The prevalence was 53% which was similar to other studies in India -Dar JA *et al.*, [7] in Uttar Pradesh was 54.8% and Hanumathappa *et al.*, [8] in Davangere as 43%. The most common source of MRSA infections is skin and soft tissue infections (45%). Most common age group being 18-34 years. This correlated with a study Hsin Huang *et al.*, [9] and females being most common susceptible group.

The distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) is worldwide, but the frequency varies among different countries. Rates of MRSA in the subcontinent countries of Pakistan and India have been shown to be high when compared with rates in northern Europe, Arakere *et al.*, [10]. Rates in India are comparable, with resistance in clinical isolates in two studies being 51.6 and 54.9 %, respectively (Anupurba *et al.*, 2003 [11]. The study demonstrates that a high proportion (69%) of MRSA patients identified in our institution

had CA-MRSA infections.

Skin and soft tissue infections were the most common sites. This finding was in concordance with many studies done in Shimla by Neha Sharma *et al.*, [12] and South Africa by Adebayo Shittu *et al.*, [13].

In this study, all the clinical MRSA strains (100%) were resistant to penicillin, ampicillin, gentamicin and amoxycylav. A similar result was noted for gentamicin and ampicillin in South India, Saravanan M, *et al.*, [14].

MRSA has emerged as the most common identifiable cause of skin and soft-tissue infections across the country. Although more than 80% of the patients with skin and soft-tissue infections associated with MRSA in this study received empirical therapy, the isolate was resistant in majority of the patients. This finding suggests a need to reconsider empirical antimicrobial choice for skin.

All the strains which appeared methicillin resistant phenotypically were positive for amplification of 300bp fragments specific for mec A gene, while only 20(47%) showed positive amplification of 976bp fragments specific for *lukS/F-PV* genes. The majority of the PVL genes were recorded in skin and soft tissue infections, especially from necrotizing skin infections (Zhang K Mc.Clure *et al.*, [15]). PVL was also found in 2(4.6%) of the 7 strains recovered from respiratory tract infections (Bronchopneumonia). The obtained results confirm the conclusion of Bocchini *et al.*, [16], they mentioned that PVL is a cytolytic toxin associated with *S. aureus* skin infections and necrotizing pneumonia.

The prevalence of MRSA in the present study was 48%. MRSA isolates identified by conventional phenotypic methods correlated well with the genotypic confirmation by the

presence of mec A gene. The MRSA isolates obtained from clinical specimens were predominantly from skin and soft tissue infections and also from blood stream infections which were resistant to most of the antibiotics. Thus there is prior need to reconsider antimicrobial while treating MRSA isolated from the most common skin infections.

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