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Circulation of SCC*mec* type V Methicillin resistant staphylococcus aureus containing the Panton Valentine Leukocidin genes in hospitalized patients in Tamil Nadu

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ABSTRACT

The hospital epidemiology of Methicillin-Resistant *Staphylococcus aureus* (MRSA) has changed in the past few years due to the infiltration of community associated MRSA (CA-MRSA) strains into health care settings. A total of 100 clinical isolates of *Staphylococcus aureus* strains were isolated from various clinical samples, identified by standard biochemical tests. Antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method and Vancomycin screen agar (6µg/ml) was used to detect Vancomycin resistance. Patient data were collected and all the isolates were found to be hospital acquired MRSA (HA-MRSA) strains based on CDC definition. To detect *mec* A gene, *fem* A gene and *pvl* gene; triplex PCR was used. SCC*mec* typing was done by a multiplex PCR. Four different SCC*mec* types were detected, of which 42% of all isolates belonged to SCC*mec* type I (n=22) and III (13), whereas SCC*mec* type II was completely absent, while 39% MRSA (n=33) carried SCC*mec* type V. Thirty isolates (36%) carried the genes for PVL of which (67%) were strongly associated with SCC*mec* type V, (10%) SCC*mec* type I, (10%) SCC*mec* type III, (3%) SCC*mec* type IV and (17 %) were Non-Typeable. The high proportion of HA -MRSA strains carrying SCC*mec* types V, together with the considerable occurrence of PVL positive MRSA strains suggest strong infiltration of CA-MRSA into the hospital set up. The presence of PVL among HA-MRSA requires further research to confirm whether PVL can be considered as a surrogate marker for CA-MRSA.

INTRODUCTION

Methicillin Resistant *Staphylococcus aureus* represents a challenge for virtually all health care institutions and guidelines have been promulgated regarding how to manage and control the spread of MRSA within the health care institution [1,2]. MRSA, one of the most common nosocomial pathogens, usually carries genetic element that confers resistance to broad range of antibiotics [3,4]. Methicillin resistance in Staphylococci is based on the expression of a modified penicillin binding protein (PBP), PBP 2a, which is encoded by the *mec* A gene. This gene is located on the Staphylococcal cassette chromosome *mec* (SCC*mec*), a mobile genetic element integrated in the chromosome [5, 6] which also carries gene for specific recombinases *(ccr*) necessary for its integration and excision [7]. The *ccr* gene complex is composed of *ccr* genes and surrounding open reading frames (ORFs), and *mec* gene complex composed of the *mec* A gene, regulatory genes, and insertion sequences upstream or downstream of *mec* A. Several *mec* and *ccr* allotypes have been found among SCC*mec* elements, which has led to following classification [11, 12, 22]; type I SCC*mec*, carrying class B *mec* and type 1 *ccr*, type II SCC*mec*, with class A *mec* and type 2 *ccr*, type III SCC*mec*, with class A *mec* and type 3 *ccr*, type IV SCC*mec* , with class B SCC*mec* and type 2 *ccr*, and type V SCC*mec*, class C2 *mec* and type5 *ccr*. Previous studies showed that health care associated MRSA (HA-MRSA) infections are generally caused by multi drug resistant strains harboring SCC*mec* types I,II, III, but rarely SCC*mec* type IV [8,9]. On the other hand, community associated (CA-MRSA) strains carry SCC*mec* type IV and V, or VII are commonly susceptible to the majority of non-Beta lactam antibiotics; frequently produce the Panton-Valentine leukocidin (PVL); and differ in their Pulse Field Gel Electrophoresis (PFGE) pattern [9].

Recent studies have reported that the hospital epidemiology of Methicillin Resistant *Staphylococcus aureus* (MRSA) has changed in the past few years due to the encroachment of community associated MRSA (CA-MRSA) strains in to health care settings [10]. The aims of the present study were to determine the prevalence of CA-MRSA in health care institutions, and whether carriage of PVL could be used as a surrogate marker for CA-MRSA.

MATERIALS AND METHODS

A total of 100 clinical isolates of *Staphylococcus aureus* strains were isolated from various clinical samples during the period of Nov 2012 to October 2013 from tertiary care hospitals in TamilNadu. which includes Dharmapuri (Govt Dharmapuri Medical College Hospital), 50 strains; and Theni (Govt Theni Medical College Hospital), 50 strains; the collected isolates were inoculated onto Blood Agar and incubated at 37°C for 24 hours, identified by standard biochemical tests [11]and confirmed by Tube coagulase test and DNase test and they were tested for antibiotic susceptibility by the Kirby Bauer disc diffusion method on Muller Hinton agar (Hi media Laboratories Pvt. Ltd, India) and were interpreted in accordance with the CLSI guidelines[15]. Methicillin resistance was detected by taking Cefoxitin disc (30µg) as a surrogate marker. Amikacin (AK) 30µg, Ceftriaxone (CTR)30 µg, Ciprofloxacin (CIP)5 µg, Clindamycin (CD) 2 µg, Cotrimoxazole (COT)25 µg, Erythromycin (E) 15 µg, Fusidic acid(FC)10 µg, Gentamicin (GEN)10 µg, Linezolid (LZ)15 µg, Penicillin G (P)10 U, Pristinomycin (RP)15 µg, Rifampicin(RIF) 5 µg, Teicoplanin(TEI) 30 µg, Tetracycline(TE)30 µg and Mupirocin(MU)5 µg. Vancomycin resistance was screened by BHI Vancomycin screen Agar(6µg/ml). Patient data were collected to distinguish CA-MRSA strains from HA-MRSA strains by epidemiological criteria, as defined by the Center for Disease Control and Prevention (CDC), on the basis of which, all the isolates were HA-MRSA.

Molecular detection of PVL MRSA was carried out using Triplex PCR (Table:2), [13] to detect *mec* A gene which confers resistance to Methicillin, *fem* A gene to differentiate *Staphylococcus aureus* from CONS and *pvl* gene, which codes for Panton-Valentine leucocidin toxin. SCC*mec* typing by PCR; SCC*mec* types were determined by a multiplex PCR strategy which generates a specific amplification pattern for SCC*mec* types I to V (Table: 3)[14].

RESULTS

Eighty four MRSA strains were isolated from patients at two Tertiary care hospitals. According to the CDC definition [15], all the MRSA were Hospital associated MRSA (HA-MRSA). Most MRSA strains were isolated from patient in the Department of Surgery(34%), followed by patient in the Department of internal medicine(21%) and Department of Orthopaedics (17%). The age of the patient ranged from 2 days to 70 years. Ninety five patients (93%) were inpatients and five (7%) were outpatient at the time of the first MRSA detection. Eighty three (83%) isolates were isolated from skin and soft tissue specimens, nine (9%) from blood, five (5%) from urine, three (3%) from other body sites. The MRSA isolates were analysed by SCC*mec* typing. Four different SCC*mec* types were detected, of which traditional nosocomial SCC*mec* type I (n=22) and III (n=13), were seen representing 42% of all isolates, whereas SCC*mec* type II was completely absent. The majority of MRSA isolates carried SCC*mec* type V (n=33; 39%). Surprisingly only few isolates showed SCC*mec* type IV (n=4; 5%) and 14% were non-typeable (n=12;). The MRSA isolates were screened for the presence of the PVL genes (*luk*S-PV and *luk*F-PV) by PCR. Thirty isolates (36%) carried the genes for PVL; of which (67%) were strongly associated with SCC*mec* type V, (10%) SCC*mec* type I, (10%) SCC*mec* type III, (3%) SCC*mec* type IV and (17 %) were Non-Typeable (Figure.1). A high proportion of PVL positive isolates showed resistance to Clindamycin (43%), Erythromycin (100%), Ciprofloxacin (97%), Gentamicin (83%) and Cotrimoxazole (97%).

MRSA strains carrying SCC*mec* type I were resistant to

Table 1. : Antibiotic resistance among MRSA to various antibiotics with respect to SCC*mec* types:

SCC*mec* type	No of MRSA strains	No. (%) of HAMRSA strains(a)	No (%) of PVL-positive strains	No.(%) of strains resistant to									
				CIP	CLI	SXT	ERY	GEN	LIN	MLS	RIF	TET	VAN
I	22	22(100)	3(10)	19(86)	3(14)	16(73)	19(86)	12(55)	2(9)	14(64)	0	9(41)	0
Ii	-	-	-	-	-	-	-	-	-	-	-	-	-
Iii	13	13(100)	1(3)	12(92)	2(15)	13(100)	13(100)	12(92)	0	11(85)	1(8)	11((85)	0
IV	4	4(100)	1(3)	3(75)	2(50)	2(50)	4(100)	2(50)	0	1(25)	0	2(50)	0
V	33	33(100)	20(67)	30(91)	11(33)	30(91)	33(100)	18(55)	1(3)	18(55)	3(9)	10(30)	0
NON TYPEABLE	12	12(100)	5(17)	12(100)	3(25)	12(100)	8(67)	7(58)	0	6(50)	1(8)	6(50)	0

a On the basis of CDC definition (15)

b CIP,Ciprofloxacin; CLI, Clindamycin; SXT, Cotrimoxazole; ERY, Erythromycin; GEN, Gentamicin; LIN, Linezolid; MLS, Streptogramin B; RIF, Rifampicin; TET, Tetracycline; VAN, Vancomycin;

Details of the primer sequence used and amplicon size of target gene mentioned below:

Table: 2 : Triplex PCR by the method of Nagarajan *et.al.*, 2013

Target genes	Primer sequence	Amplicon size (bp)
femA	F: 5' – AAAAAAGCACATAACAAGCG – 3' R: 5' – GATAAAGAAGAAACCAGCAG – 3'	132
mecA	F: 5'-TGCTATCCACCCTCAAACAGG-3' R: 5'-AACGTTGTAACCACCCCAAGA-3'	286
Pvl	F:5'–ATCATTAGGTAAAATGTCTGGACATGATCCA–3' R: 5'– GCATCAASTGTATTGGATAGCAAAAGC– 3'	441

Table: 3 : SCC*mec* typing by the method of Boye *et al.,* 2007.

Target genes	Primer sequence	Amplicon (bp)
ccrA2-B2	β – 5'-ATTGCCTTGATAATAGCCYTCT-3' α3-5'- TAAAGGCATCAATGCACAAACACT-3'	937
ccrC	ccrCF- 5'-CGTCTATTACAAGATGTTAAGGATAAT-3' ccrCR- ccrCCCTTTATAGACTGGATTATTCAAAATAT	528
IS 1272	1272F1-5'- GCCACTCATAACATATGGAA-3, 1272R1- 5'-CATCCGAGTGAAACCCAAA-3'	415
IS431	5RmecA- 5'-TATACCAAACCCGACAACTAC-3' 5R431- 5'-CGGCTACAGTGATAACATCC-3'	359

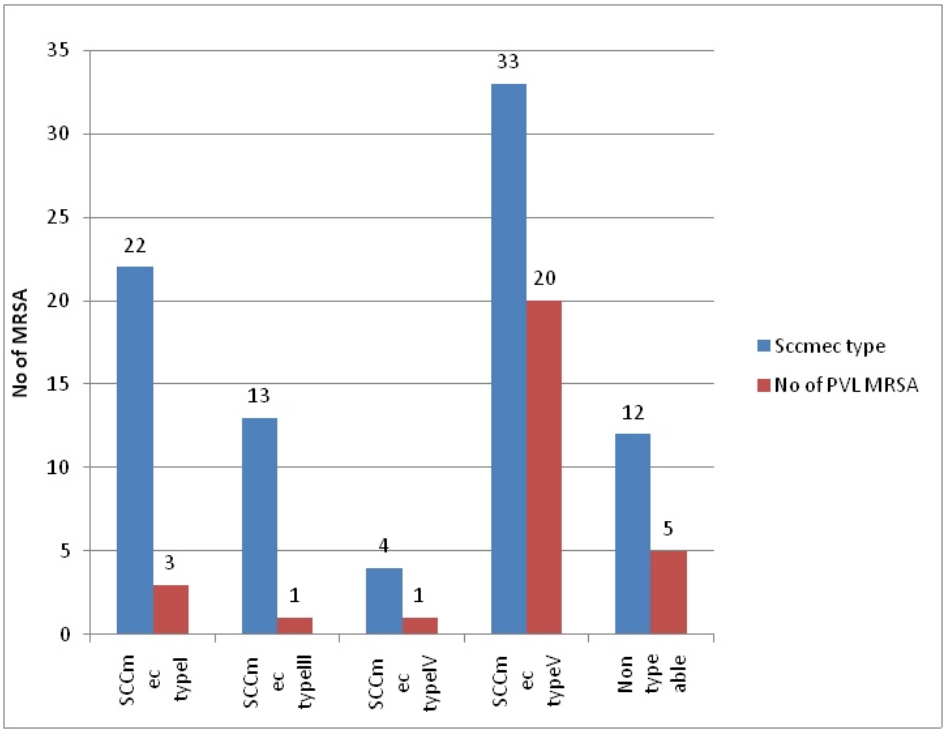

Figure 1 : Distribution of various SCC*mec* types and PVL amongst the MRSA isolates

Ciprofloxacin (86%), Cotrimoxazole (73%), Erythromycin (86%), Gentamicin (55) and Tetracycline(41%). Strains carrying SCC*mec* type III were resistant to Ciprofloxacin (92%), Cotrimoxazole(100%), Erythromycin(100%), Gentamicin (92%) and Tetracycline (85%). MRSA isolates carrying SCC*mec* type IV were resistant to Ciprofloxacin (75%), Clindamycin (50%), Cotrimoxazole(50%), Erythromycin (100%), Gentamicin(50%) and Tetracycline(50%). A high proportion of the *SCCmec* type V isolates showed resistance to Ciprofloxacin (91%), Cotrimoxazole (91%), Erythromycin (100%), Gentamicin(55%) and Tetracycline(30%) (Table.1.). All MRSA isolates were susceptible to Vancomycin.

DISCUSSION

In the present report, we provide retrospective epidemiological and molecular typing data of 84 MRSA isolates collected during a one year period (Nov 2012 to Oct 2013) in the tertiary care hospitals in Tamilnadu. SCC*mec* types I and III have been reported to be the most frequent nosocomial MRSA strains [8,9]. Surprisingly , these types were either absent (Type II) or present in very low proportion (42%; Type I and III) in our hospitals, whereas overall predominance of MRSA strains carrying SCC*mec* types V and IV (44%), which are traditionally attributed to CA-MRSA strains. These data confirm a tendency seen in previous studies from India[19] and international studies that reported the spread of CA-MRSA SCC*mec* type V and IV strains in hospital settings in both Europe and United states[8,16,20]. To our knowledge, such high proportion of SCC*mec* type V as were found in our hospitals have not been reported earlier in hospital settings. The predominance of HA-MRSA strains carrying a CA genotype in our hospitals is significantly higher than reports from previous studies in India[19].

PVL (Panton Valentine Leukocidin) exotoxin production is one of the classical features of CA-MRSA [17,18] . We identified much higher rate of PVL positive MRSA strains (36%) in our study which was strongly associated with SCC*mec* type V(67%), and to a lesser extent SCC*mec* type IV(3%). 10 % of PVL MRSA strains carried SCC*mec* type I, while 3% carried SCC*mec* type III and 17% were non-type able.

The PVL MRSA isolates showed higher resistance towards various antibiotics than the PVL negative MRSA isolates, viz.,Ciprofloxacin (97%), Clindamycin (43%), Erythromycin (100%), Gentamicin (83%) and Cotrimoxazole (97%).

CONCLUSION

This study demonstrates high prevalence of MRSA (84%) than the previous reports in India [21, 22]. The high proportion of HA-MRSA strains carrying SCC*mec* types V, together with the higher prevalence of PVL positive MRSA strains suggest strong infiltration of CA-MRSA in the hospital setup. The high degree of antibiotic resistance among the PVL positive isolates indicates that differences in antibiotic resistance pattern between HA - MRSA and CA-MRSA is getting lower. Hence, the presence of PVL among HA-MRSA requires further research to confirm if PVL is a surrogate marker for CA-MRSA. There is a need to control the probable infiltration of CA-MRSA in the hospital settings.

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Conflicts of Interest **:**

None.

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