DOI: JCDR/2012/3981:1986

Original Article

Microbiology Section

The Prevalence and the Risk Factors Which are Associated with *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* Which Harboured the Panton-Valentine-Leukocidin Gene in Sikkim

KUNSANG ONGMOO BHUTIA, T. SHANTIKUMAR SINGH

ABSTRACT

Background: The clinical sequelae of PVL-positive *S. aureus* infection tend to be more severe than those of the PVL-negative ones. The strong association of the PVL toxin in the MRSA isolates suggests the methcillin-resistance which contributes to the success of these PVL-positive isolates.

Aims: To estimate the burden and the risk factors which were associated with *S. aureus* and MRSA which harboured the *pvl* gene in Sikkim.

Settings and Design: A point prevalence study was conducted during a period of one year from August 2010 to 2011 in the teaching hospitals.

Materials and Methods: A total of 119 clinical strains of *S. aureus* were subjected to monoplex *(fem-A)* and multiplex PCR (*mec-A* and *pvl*) respectively.

Statistical Analysis: The data were analyzed by using Pearson's Chi-square test. The *p*-value of <0.05 was taken as statistically significant.

Results: Out of 119 isolates of *S. aureus*, 117 (98.31%), 49 (41.17%) and 54 (45.37%) isolates were positive for the *fem-A* (internal control), *mec-A* and the *pvl* genes respectively. Out of the 117 *fem-A* positive isolates, 47 (40.17%) and 52 (44.44%) were found to be *mec-A* (MRSA) and pvl positive. Among the 54 pvl positive isolates, 47 (87.03%), 5 (9.25%) and 2 (3.7%) were reported from the MRSA (*fem-A* and *mec-A* positive), MSSA (*fem-A* positive and *mec-A* negative), and the MRCoNS (*fem-A* negative and *mec-A* positive) isolates respectively. The PVL toxin was significantly higher in the MRSA (87.03%), as compared to the MSSA (9.25%). Among the risk factors which were studied, patients with a previous history of antibiotic intake and hospitalization and who attended the surgery and the burn/ICU units were more positive for the MRSA and the PVL-*S. aureus* infections as compared to the negative patients.

Conclusion: The high prevalence of the PVL toxin among the *S. aureus* population, which was mainly reported from the MRSA isolates, irrespective of their types i.e. CA or HA-MRSA, revealed that PVL was not a reliable marker for the CA-MRSA strains. Rather, it may be hypothesized that the MRSA strains may be important reservoirs of the PVL toxin and that it may be slowly acquired by the MSSA strains.

Key Words: MRSA, PVL, Risk-factor, *S.aureus,* Sikkim

INTRODUCTION

Staphylococcus aureus expresses a variety of virulence factors, including Panton-Valentine-leukocidin (PVL). It is a cytotoxin that causes leucocyte destruction and tissue necrosis, which is produced by fewer than 5% of the *S. aureus* strains [1]. PVL positive *S. aureus* causes diverse clinical syndromes, ranging from superficial skin and soft tissue infections to various deep-seated infections such as pneumonia, infective endocarditis, osteomyelitis, and enterocolitis [1].

Reports have suggested the strong association of the PVL toxin in the community-associated (CA) MSSA, and the CA-MRSA infections [2]. However, in recent years, the strains of MRSA which carried the *pvl* gene are increasingly being reported in hospital-associated (HA) infections [3], and to a lesser extent in CA- infections [4], thus revealing that the *pvl* gene is no longer a reliable marker for the CA-MRSA infections. The established risk factors for the MRSA infection include recent hospitalization or surgery, residence in a long-term-care facility, dialysis, and indwelling percutaneous medical devices and catheters. Recently, cases of MRSA have been documented in healthy, community-dwelling persons who did not have the established risk factors for the MRSA acquisition. Because they are apparently acquired in the community, they are often referred to as community-acquired MRSA [5]. The present study was conducted to determine the burden of the PVL toxin in the general *S. aureus* population and in the MRSA strains in Sikkim, and to determine the risk-factors which are associated with it. Secondly, it was done to investigate whether the *pvl* gene carriage in CA-MRSA still hold true for its differentiation from the HA-MRSA isolates.

MATERIALS AND METHODS

Design and Settings of Study

Point prevalence study which was conducted on 119 *S. aureus* strains which were isolated from various clinical specimens during a period of one year from August 2009 to 2010 in teaching hospitals.

Case Definition and Source of the Data

Hospital-associated *S. aureus* isolates, as ones which were cultured from clinical specimens, were obtained at > 72 hours after the hospital admission of the patients or from patients with a history of hospitalization within six months of *S. aureus* isolation date.

Community-associated *S. aureus* isolates which were cultured during the first 72 hours of a patient's hospital admission, from outpatients or from patients with no h/o hospitalization within six months of the *S. aureus* isolation date were used. The data of the patients were obtained from the laboratory investigation registers and from the medical record files. The data of the study subjects included the basic demographic profiles, the date of hospital admission and discharge, clinical findings, culture site, status of the patients (IP/OPD), clinical history, and the department which was visited.

Identification of the *S. aureus* Isolates

All the isolates were identified as *S. aureus* by using conventional techniques, including the slide and the tube coagulase test, the DNase test (Hi-Media), the Phosphatase test (Hi-Media) and the Modified OF test (Hugh-Leifson test). The *S. aureus* strains were inoculated into semi-solid nutrient agar in screw capped vials and they were stored at -20ºC for further molecular analysis.

DNA Isolation

The test inoculum was prepared by inoculating two to three isolated colonies of *S. aureus* into 3 to 4 ml of BHI broth (Hi- Media), and it was incubated overnight at an ambient temperature of 35-37 ºC. Then, DNA was extracted by using the HiPurA™ Bacterial and Yeast Genomic DNA Miniprep Purification Spin kit (Hi-Media).The DNA concentration was determined as micrograms per milliliter according to the A_{260} values by using a Nanodrop ND-1000 Spectrophotometer.

Monoplex PCR for the Detection of the *fem-A* gene

To validate, all the isolates which were tested was *S. aureus*. The primers, GFEMAR-1(5'-AAA AAAGCACATAACAAGCG-3') and GFEMAR-2 (5'-GATAAAGAAGAAACCAGCAG-3'),with a 132 bp amplicon size for the amplification of the *fem-A* gene (internal control) were selected from a published sequence of Mehrotra *et al* [6], and they were checked for their specificity against available *S. aureus* genomes by using the BLAST utility which was available through the National Center for Biotechnology Information website (www.ncbi.nim.nih.gov) under GenBank Accession no (X17688.1). They were commercially obtained from Sigma-Aldrich, Bangalore. PCR was performed by using the Taq PCR Master Mix Kit (Qiagen). A 25-µl final reaction volume mixture was prepared, which consisted of 12.5 µl of the master mix, 1µl of each of the forward and reverse primers (0.4µM), 7.5 µl of RNase free water and 3µl of the DNA template. PCR was performed by using a thermocycler (Biometra) with the thermocycling conditions of initial denaturation (95ºC,5 min), followed by 35 cycles of the three step cycling conditions of denaturation (94ºC, 30 sec), annealing (57ºC, 1 min) and extension (72ºC, 30 sec), followed by the final extension (72ºC,7 min) and soaking at 4ºC. Then, 5µl of the amplified products were mixed with 2µl of ethidium bromide (Fermentas) and loaded on a 2% agarose gel (Amresco) along with GeneRuler ™ 100 bp Plus DNA Ladder, (Fermentas), followed by electrophoresis at 100 volts for 50-60 min. The gel was visualized under a UV transilluminator (Bio-Doc analyzer, Biometra).

Multiplex PCR for the Detection of the *mec-A* and the *pvl* Genes

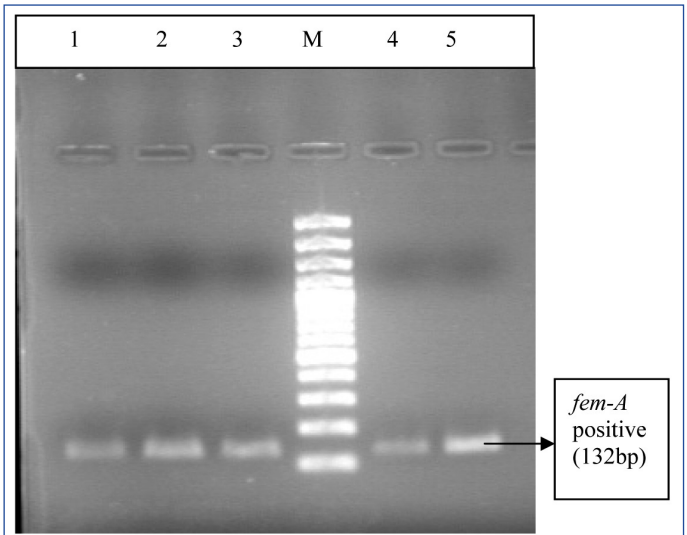
The primers for the amplification of the *mec-A* and the *pvl* genes were MECAP4 (5'-TCCAGATTA CAACTTCACCAGG-3'), and MECAP7(5'-CCACTTCATATCTTGTAACG-3') under (GenBank Accession No-Y00688) with a 162 bp amplicon size for the *mec-A* gene as was described by Oliveria *et al* [7] and *luk-PV*-1 (5'-ATCATTAGGTAAAATGTC TGGACATGATCCA-3') and *luk-PV*-2 (5'-GCATCAAGTGTATTGGATAGCAAAAGC-3') under (GenBank accession no-X72700) with a 433 bp amplicon size as was described by McClure *et al* [8] respectively. The primers were blasted and commercially obtained (Sigma-Aldrich, Bangalore). PCR was performed by using a Qiagen Multiplex PCR kit with slight modifications. A 25-µl final reaction volume mixture was prepared, which consisted of 12.5 µl of the mastermix, 2.5 µl of the primer mix (0.2µM of each primer), 3µl of DNA template and 7µl of RNase free water. The DNA samples were subjected to thermocycling conditions, with the initial inactivation step (95ºC,15min), the three step cycling condition of denaturation (94ºC, 30 sec), annealing (60ºC, 90 sec) and extension (72ºC, 90sec) for 35 cycles, with the final extension (72ºC,10 min) and soaking at 4ºC. Then, 5µl of the amplified products were mixed with 2µl of ethidium bromide (Fermentas) and loaded on a 2% agarose gel (Amresco) along with GeneRuler™ 100bp Plus DNA Ladder (Fermentas), followed by electrophoresis at 100 volts for 50-60min. The gel was visualized under a UV transilluminator (Bio-Doc analyzer, Biometra).

Statistical Analysis

The categorical variables were analyzed by using Pearson's Chi-square test. A *p*-value of <0.05 was taken as statistically significant.

RESULTS

A total of 119 conventionally identified isolates of *S. aureus*, 117 (98.31%), 49 (41.17%) and 54 (45.37%) were positive for the *fem-A* (internal control), *mec-A* and the *pvl* genes, as was detected by monoplex [Table/Fig-1] and multiplex PCR [Table/Fig-2] respectively. Out of the 117 *fem-A* positive *S. aureus* isolates, 47 (40.17%) and 52 (44.44 %) were found to be *mec-A* (MRSA) and pvl positive. Among the 54 pvl- *S. aureus* isolates, 47 (87.03%), 5 (9.25%) and 2 (3.7%) were reported to be *mec-A*

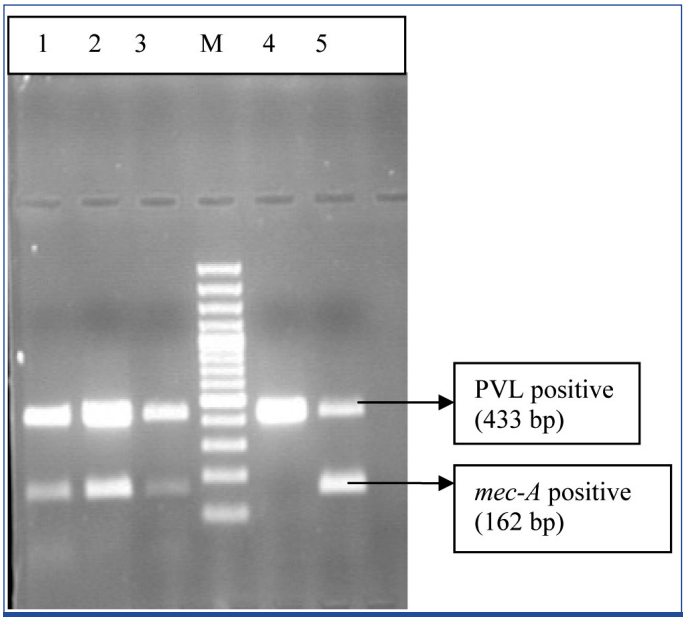


[Table/Fig-1]: Monoplex PCR for the detection of *fem-A* (162bp), Lane 1 to 5 =Positive for *fem-A* gene, M= (Marker 100 bp)

positive (MRSA), *mec-A* negative (MSSA) and *fem-A* negative, *mec-A* positive (MRCoNS) *S. aureus* isolates respectively. Out of the 117 *S. aureus* isolates respectively, 34 (29.05%) and 13 (11.11%) were categorized as CA- and HA-MRSA, and 50 (42.73%) and 20 (17.09%) were categorized as CA- and HA-MSSA respectively. All the CA- and HA-MRSA isolates were positive for the *pvl* gene, whereas in comparison, the *pvl* gene were detected in only four (8%) and one (5%) isolates among the CA- and the HA-MSSA isolates [Table/Fig-3].

Comparison of the Patients' Characteristics with the MRSA and the MSSA Infections

No significant difference was seen in the MRSA patients' demographic (gender, age-group and ethnicity) and clinical (status of the patients, settings for acquiring infections and clinical characteristics) profiles in comparison to those of the MSSA patients. The mean age ± SD of the patients with the MRSA and the MSSA infections were found as 25.23 ±16.86 and 23.59 ± 17 respectively. However, a significant number of patients with the MRSA infections had a history of antibiotic intake (36.17%) and hospitalization (34.04%) against 7 (10%) and 5(7.14%) in the MSSA patients. A majority of the infections were reported from the ICU and the burn units. In comparison, the MSSA infections were reportedly higher in the patients who were attending the orthopaedic and other units of the hospitals, whereas the incidence of both the MRSA and the MSSA infections were found to be highest in patients who attended the surgery department. A significant difference was also seen in the distribution of the PVL toxin in the MRSA (100%) and the MSSA (7.14%) isolates [Table/Fig-4].



[Table/Fig-2]: Multiplex PCR (*mec-A* and PVL gene). Lane 1,2,3,5 = Positive *mec-A* (162bp) and PVL (433bp), M = Marker (100bp DNA ladder), Lane 4 = Negative *mec-A* (162bp) positive PVL (433 bp)

	PVL gene	
Types of S.aureus (n=117)	Positive (n=52)	Negative (n=65)
CA-MRSA (n=34)	34	0
HA-MRSA (n=13)	13	0
CA-MSSA (n= 50)	4	46
HA-MSSA (n=20)	1	19

[Table/Fig-3]: Distribution of *pvl* gene in CA and HA-MRSA and MSSA isolates.

Characteristics	MRSA (n=47)	MSSA (n=70)	p-value
Male Female	30 (63.82%) 17 (36.17%)	48 (68.57%) 22 (31.42%)	p > 0.5
Age (Mean ±SD)	25.23 ±16.86	23.59 ± 17	
PVL toxin	47 (100%)	5 (7.14 %)	p <0.001*
Age group			
0-10	12 (25.53%)	18 (25.71%)	p> 0.5
11-20	7 (14.89%)	15 (21.42%)	
21-30	13 (27.65%)	18 (25.71%)	
>31	15 (31.91%)	19 (27.14%)	
Ethnicity			
Nepali	29 (61.70%)	51 (72.85%)	p > 0.1
Bhutia & Lepchas	9 (19.14%)	13 (18.57%)	
Others	9 (19.14%)	6 (8.57%)	
Status of patients			
Out-patients (OP) In-patients (IP)	20 (42.55%) 27 (57.44%)	28 (40%) 42 (60%)	p >0.5
Clinical characteristics			
Abscess	15 (31.91%)	26 (37.14)	p >0.5
CSOM/Pneumonia	7 (14.89%)	6 (8.57%)	p > 0.1
Cellulitis	8 (17.02%)	15 (21.42%)	p> 0.5
Burn case	7 (14.89%)	5 (7.14%)	p > 0.1
Blood stream infections (BSI)	5 (10.63%)	5 (7.14%)	p > 0.5
Bone and joint infections	6 (12.76%)	12 (17.14%)	p > 0.5
H/O of trauma and accident	8 (17.02%)	5 (7.14%)	p > 0.05
Diabetic mellitus	6 (12.76%)	6 (8.57%)	p > 0.1
Previous h/o antibiotic intake	17 (36.17%)	7 (10%)	p < 0.001*
Previous h/o hospitalization	16 (34.04%)	5 (7.14%)	p < 0.001*
Settings of infections acquired			
Community Hospital acquired	34 (72.34%) 13 (27.65%)	50 (71.42%) 20 (28.57%)	p > 0.5
Department			
Surgery	24 (51.06%)	32 (45.71%)	p < 0.05*
Orthopaedics	6 (12.76%)	12 (17.14%)	
Burn unit/ICUs	12 (25.53%)	7 (10 %)	
Others	5 (10.63%)	19 (27.14%)	

[Table/Fig-4]: Comparison of patient's characteristics with MRSA and MSSA infections.

Note: *Significant p-value.

Comparison of the Patients' Characteristics with the PVL-MRSA and the PVL-MSSA Infections

Both the PVL positive MRSA and MSSA isolates were found to be higher in males (63.82% and 80%) as against the females (36.17% and 20%).The mean ages in both the groups were also found to lie within the similar range, i.e. 25- 30 years. (25.23±16.86 and 30± 17.39). However, the PVL-MRSA were found to be higher in the hospitalized patients (57.44%), and the PVL-MSSA were more in the out-patients (80%). The PVL-MRSA was found to be prevalent in different ethnicities and age groups, as against the PVL-MSSA [Table/Fig-5]. The clinical profiles of the PVL-MRSA patients were varied in comparison to those of PVL-MSSA, which was reported from patients with abscess (60%) and bone and joint infections (40%) only. Both the PVL-MRSA and the MSSA isolates were

Characteristics	PVL-MRSA (n=47)	PVL-MSSA (n=5)	p-value
Male Female	30 (63.82%) 17(36.17%)	4 (80%) 1(20%)	p > 0.5
Age (Mean ±SD)	25.23±16.86	30± 17.39	
Age group			
0-10	12 (25.53%)	1 (20%)	p > 0.10
11-20	7 (14.89%)	0	
21-30	13 (27.65%)	2 (40%)	
>31	15 (31.91%)	2 (40%)	
Ethnicity			
Nepali	29 (61.70%)	5 (100%)	p > 0.10
Bhutia & Lepchas	9 (19.14%)	0	
Others	9 (19.14%)	0	
Status of patients			
Out-patients In-patients	20 (42.55%) 27 (57.44%)	4 (80%) 1(20%)	p > 0.5
Clinical characteristics			
Abscess	15 (31.91%)	3 (60%)	p > 0.10
CSOM/Pneumonia	7 (14.89%)	0	p > 0.10
Cellulitis	8 (17.02%)	0	p > 0.10
Burn case	7 (14.89%)	0	p > 0.10
Blood stream infections	5 (10.63%)	0	p > 0.10
Bone and joint infections	6 (12.76%)	2 (40%)	p > 0.10
H/O of trauma and accident	8 (17.02%)	0	p > 0.10
Diabetic mellitus	6 (12.76%)	0	p > 0.10
Previous h/o antibiotic intake	17 (36.17%)	0	p > 0.10
Previous h/o hospitalization	16 (34.04%)	0	p > 0.10
Settings of infections acquired			
Community Hospital-acquired	34 (72.34%) 13 (27.65%)	4 (80%) 1(20%)	p > 0.10
Department			
Surgery	24 (51.06%)	3 (60%)	p > 0.10
Orthopaedics	6(12.76%)	2 (40%)	
Burn unit/ICUs	12 (25.53%)	0	
Others	5 (10.63%)	0	

[Table/Fig-5]: Comparison of patient's characteristics of PVL positive MRSA and MSSA isolates.

found to be higher in community-acquired infections (72.34% and 80%) in comparison to their incidence in hospital-acquired infections (27.65% and 20%). The highest number of PVL-MRSA were isolated from the patients who attended the surgery department (51.06%), followed by the burn /ICU units (25.53%), the orthopaedic unit (12.76%), and other departments (10.63%). The PVL-MSSA isolates were present only in the patients who visited the surgery (60%) and the orthopaedic (40%) departments [Table/Fig-5].

Comparison of the Patients' Characteristics with the PVL Positive and Negative S. aureus Infections

The prevalence of PVL positive and negative *S. aureus* isolates, in terms of gender, ethnicity, mean age ± SD, settings of the infections acquired, status of patients (OP/IP) and age-group wise was found to be comparable. [Table/Fig-6]. However, the PVL positive *S. aureus* was significantly higher in patients with a previous h/o antibiotic intake (32.69%) and hospitalization (30.76%) and in patients who were admitted in the burn/ICU units in comparison to the PVL negative group [Table/Fig-6].

DISCUSSION

The data on the prevalence of MRSA among the Indian isolates of *S. aureus* is extensive in the literature [9,10]. However, the screening for the PVL toxin among them was initiated recently [11]. Due to the increased infiltration of the CA-MRSA strains into the hospital setting in the recent years, and their association, mainly with the skin and soft-tissue infections, has gained importance in the detection of the PVL toxin in the MRSA population [12]. In this study, the prevalence of the PVL toxin among the general *S. aureus* population was found to be 44.44%

Characteristics	PVL positive S.aureus (n=52)	PVL negative S.aureus (n=65)	p-value
Male Female	34 (65.38%) 18 (34.61%)	44 (67.69%) 21 (32.30%)	p > 0.5
Age (Mean ±SD)	25.69 ± 19.09	24.75 ± 17.89	
Age group			
0-10	13 (25%)	17 (26.15%)	p > 0.5
11-20	7 (13.46%)	15(23.07%)	
21-30	15 (28.84%)	16 (24.61%)	
>31	17 (32.69%)	17 (26.15%)	
Ethnicity			
Nepali	34 (65.38%)	46 (70.76%)	p > 0.10
Bhutia & Lepchas	9 (17.30%)	13(20%)	
Others	9 (17.30%)	6 (9.23%)	
Status of patients			
Out-patients In-patients	24 (46.15%) 28 (53.84%)	24 (36.92%) 41 (63.07%)	p > 0.5
Clinical characteristics			
Abscess	18 (34.61%)	23 (35.38%)	p > 0.50
CSOM/Pneumonia	7 (13.46%)	6 (9.23%)	p > 0.10
Cellulitis	8 (15.38%)	15 (23.07%)	p > 0.1
Burn case	7 (13.46%)	5(7.69%)	p > 0.1
Blood stream infections	5 (9.61%)	5(7.69%)	p > 0.5
Bone and joint infections	8 (15.38%)	10 (15.38%)	p > 0.5
H/O of trauma and accident	8 (15.38%)	5(7.69%)	p > 0.10
Diabetic mellitus	6 (11.53%)	6(9.23%)	p > 0.5
Previous h/o antibiotic intake	17 (32.69%)	7 (10.76%)	p < 0.01 *
Previous h/o hospitalization	16 (30.76%)	5 (7.69%)	p < 0.01 *
Settings of infections acquired			
Community Hospital-acquired	38 (73.07%) 14 (26.92%)	46 (70.76%) : 19 (29.23%)	p > 0.5
Department			
Surgery	27 (51.92%)	29 (44.61%)	p < 0.05 *
Orthopaedics	8 (15.38%)	10 (15.38%)	
Burn unit/ICUs	12 (23.07%)	7 (10.76%)	
Others	5 (9.61%)	19 (29.23%)	

[Table/Fig-6]: Comparison of patient's characteristics of PVL positive and negative *S.aureus* infections.

Note: *Significant p-value.

(52/117), which was very high as compared to that which was reported by McClure *et al* [8] in <5%of *S. aureus* population. But it was comparable to the rate (37.20%) that was reported by Lina *et al* (64 out of 172) [1].

The three major genotypic markers that distinguish the CA from the HA-MRSA isolates: their genetic lineage (ST), the architecture of their mobile genetic element (*SCCmec* type) and th presence of the PVL toxin [13]. Epidemiologic and clinical data have also suggested that the high virulence potential of CA-MRSA is associated with the bearing of the PVL toxin [2]. Therefore, the PVL locus represents a stable genetic marker of the CA-MRSA strains [13].

This study has evaluated the presence of the PVL toxin as a marker to validate the genotypic definition of the CA-MRSA isolates. The PVL toxin was detected in all the MRSA isolates, irrespective of their types. On the contrary, several-studies have reported the presence of the PVL toxin in all the CA-MRSA isolates, whereas its presence was not reported from HA-MRSA , thus concluding that the PVL toxin was a reliable marker of the CA-MRSA infections [2,11,13]. However, the findings of this study were in agreement with few reports which said that the presence of the PVL toxin could not be used as a sole marker for CA-MRSA [3,4]. Our findings suggested that though the prevalence of the PVL toxin varied in different geographical regions, in our region, the MRSA strains may be important reservoirs of the PVL toxin, which was now being slowly acquired by the MSSA strains.

Among the risk factors which were studied, a previous history of hospitalization and antibiotic intake were found to be significantly associated with the MRSA infections. Similarly, many studies reported prior antibiotic use [9,10,14], a prior h/o of hospitalization [10,14] as an important risk factors for the acquisition of the MRSA infections. In our study, the MRSA infections were also significantly higher in the patients who were admitted to the surgery and the burn/ICU units. Similarly, Narezkina *et al* found that MRSA were significantly higher in the surgery and the burn/ ICU units [15]. A recent study from New Delhi too reported that MRSA accounted for ≥95% of all the *S. aureus* infections in burn patients [16]. On the contrary, a study from Assam [17], reported that the rate of the MRSA infections was highest among the patients in the orthopaedic unit (34%), followed by the surgery department (18%). We also noticed a high difference in the distribution of the PVL toxin in the MRSA (100%) and the MSSA (7.14%) populations, whereas Campbell and colleagues [18], in 2008, reported that the presence of the PVL toxin was very high in both the groups, the MRSA (89%) and the MSSA (75%) isolates respectively. However, this distribution was found to be statistically insignificant. An increase in the incidence of the MRSA infection among the patients in the surgery and the ICU/burn units, may be due to the high association of the MRSA strains in the skin and soft tissue infections. The presence of the PVL toxin may serve as an added virulence factor, complicating the MRSA infections in patients who were admitted to these critical units (ICUs/burn) of the hospitals.

In this study, we too evaluated the risk factors which were associated with the PVL-MRSA and the MSSA infections. We found that PVL-MRSA was higher in males than in females (63.82% and 36.17%) and mainly in younger adults (25.23±16.86 years). Similarly, some studies have reported a higher incidence of PVL-MRSA in male patients than in females [4,19]. It was more prevalent in young adults, with the mean or median age ranging from 20-25 years [4,19,20]. On the contrary, Denis *et al* [20], in 2005, reported a higher prevalence of PVL-MRSA in females than in males (56% and 44%). Our findings suggested that young male adults were more vulnerable to the PVL-MRSA infections, than older people and children.

As in our study, Roosney *et al* [4], in 2007, found that a majority of the PVL-MRSA were associated with skin and soft-tissue infections, particularly from abscesses (52%). However, they reported a slightly higher incidence in Blood stream infections (16% vs. 10.63%) and a lesser incidence in pneumonia (4% vs 14.89%) than in our study. Boakes *et al,* in [19], found that very less PVL-MRSA was associated with bacteraemia (1.75%) and pneumonia (2.63%), as compared to that in our study. Similarly, Denis *et al* [20], in 2005, in Belgium, found that a majority of the PVL-MRSA were present in subcutaneous abscesses (50%), than in cellulitis (6.25%),and bacteraemia (6.25%). In agreement to this, a recent study by Muttaiyah *et al* [21], in 2010, too mentioned that a majority of the PVL-positive MSSA infections were reported from SSTI (48%), and bone and joint diseases (31%) and that only a very less percentage of catheter-related infections (1%), primary bacteraemia and endocarditis (0%) were reported. This data indicated that both the PVL-MRSA and MSSA were mainly associated with skin and soft-tissue infections, mainly abscesses. However, severe infections like BSI and pneumonia are more likely to be associated with PVL-MRSA than PVL-MSSA, suggesting that the methicillin resistance in the PVL-*S. aureus* had contributed to the success of these strains in being associated with life-threatening infections (BSI and pneumonia) as compared to the relatively mild skin and soft-tissue infections.

Based on the settings of acquiring infections, we found that a majority of the PVL-MRSA infections were community-acquired (72.34%) than hospital acquired infections (27.65%). This was comparable to a finding from the study of Roosney *et al* (2007) in which the community acquired infections were 76% and the hospital acquired infections were 24% [4]. On the contrary, Denis *et al,* in 2005, in Belgium, found that a very high percentage of PVL-MRSA were community-acquired (97.56%) and that merely 2.44% were hospital-acquired [20]. The changing epidemiology of the PVL-MRSA strains over the years, had begun to be reported from the HA-infections as well, may be due to their pre-existing colonization with the PVL positive CA-MRSA isolates that had found a portal of entry during the invasive procedures which were performed in the hospitals. The majority of the PVL-MRSA patients had a h/o of trauma and accident, hospitalization, antibiotic intake and self-reported diabetic mellitus. However, none of these risk factors were reported from the compared group. A study reported that 31.25% patients with PVL-MRSA had a h/o previous antibiotic therapy, which was comparable to our finding (34.04%), whereas 12.5% had a h/o of hospitalization, which is comparatively less as compared to our findings (36.17%) [20]. These were the established risk factors for the acquisition of the MRSA infections were and found to be associated with the PVL-MRSA infections as well. These showed no significant alterations in the established risk factors, even with the acquisition of the PVL toxin in these groups (MRSA and MSSA), but the presence of the PVL toxin may serve as an added virulence factor, complicating the MRSA infections. However, the incomparable numbers of PVL-MRSA and MSSA may limit these findings, which need to be further validated.

In the present study, the prevalence of PVL-*S. aureus* was found to be 44.44%. In comparison, a higher prevalence of over 90%. was

reported by Campbell *et al* [18] and a lower prevalence of < 5% was reported by McClure *et al* [8].

No significance difference was seen in the patient's demographic and clinical profiles, except that as compared to the above groups, the patients with a previous h/o antibiotic intake, hospitalization and those who were admitted to the surgical and burn/ICU units were more prone to the PVL-*S. aureus* infections. The mean age of the patients was comparable in both the groups, mainly those of younger adults. On the contrary, in a recent study by Muttaiyah *et al* (2010), ethnicity, younger age, and community-onset infections, were all found to be independently associated with the presence of the PVL-positive MSSA infections [21].

In conclusion, the high prevalence of the PVL toxin among the *S. aureus* population and a higher prevalence which was reported from the MRSA isolates irrespective of their types ie. community-associated (CA) or hospital-associated(HA) MRSA, suggested that the presence of the PVL toxin was no longer a reliable marker for the CA-MRSA infections. Rather, it may be hypothesized that the MRSA strains may be the important reservoirs of the PVL toxin which was slowly being acquired by the MSSA strains. This study also indicated that PVL appeared to be a possible virulence factor that caused the success of these MRSA strains in being associated with infections which ranged from mild skin and soft-tissue infections to life-threatening infections like BSI and burn infections. Therefore, specific therapeutic approaches which could target the PVL toxin may be helpful in treating the severe PVL-MRSA infections in these vulnerable groups.

ACKNOWLEDGEMENT

We would like to thank the Dean, Sikkim Manipal Institute of Medical Sciences. We would also like to acknowledge Mr. Arum Prakash (CEO), Dr. Samar Husain Naqvi and Ms Shilpie Biswas of Genetix-Biotech, New Delhi.

REFERENCES

[1] Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, *et al*. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 1999;29(5):1128-32.

[2] Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, *et al*. Community-acquired methicillin resistant *Staphylococcus aureus* which carry the Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis* 2003;9(8):978-84.

[3] Maree C, Daum R. Boyle-Vavra S, *et al*. A rapid, temporal increase in the community-acquired methicillin resistant *Staphylococcus aureus* strains which caused nosocomial infections. In: *Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington,DC, 2006.

[4] Roosney AS, Shore AC, Morgan PM, Fitzgibbon MM, O'Connell B, Coleman DC. The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) which harboured the Panton Valentine Leukocidin genes (*pvl*) revealed that *pvl* was a poor marker for the community-acquired MRSA strains in Ireland. *J Clin Microbiol* 2007;45(8):2554-63.

[5] Naimi TS, LeDell KH, Boxrud DJ, Groom AV, Steward CD, Johnson SK, *et al*. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota,1996–1998. *Clin Infect Dis* 2001;33(7):990–6.

[6] Mehrotra M, Wang G, Johnson WM. Multiplex PCR for the detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J Clin Microbiol* 2000;38(3):1032–5.

[7] Oliveira DC, de Lencastre H. A multiplex PCR strategy for the rapid identification of the structural types and the variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002;46(7):2155–61.

[8] McClure JA, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, *et al*. A novel multiplex PCR assay for the detection of the Staphylococcal virulence marker, the Panton-Valentine Leukocidin genes and the simultaneous discrimination of the methicillin-susceptible from the resistant Staphylococci. *J Clin Microbiol* 2006;44(3):1141–4.

[9] Srinivasan S, Sheela D, Shashikala, Mathew R, Bazroy J, Kanungo R. The risk factors and associated problems in the management of infections which are caused by methicillin resistant *Staphylococcus aureus*. *Indian J Med Microbiol* 2006;24(3):182-5.

[10] Vidhani S, Mathur MD, Mehndiratta PL, Rizvi M. Methicillin-Resistant *Staphylococcus aureus* (MRSA): the associated risk factors. *Indian J Pathol Microbiol* 2003;46(4):676-9.

[11] D'Souza N, Rodriques C, Mehta A. Molecular characterization of methicillin-resistant *Staphylococcus aureus* with the emergence of epidemic clones of sequence type (ST)22 and ST 772 in Mumbai, India. *J Clin Microbiol* 2010;48(5):1806-11.

[12] Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K, Miller LG. Community-associated methicillin-resistant *Staphylococcus aureus* isolates which cause healthcare-associated infections. *Emerg Infect Dis* 2007;13(2):236-42.

[13] Boyle-Vavra S, Daum RS. Community-acquired methicillin resistant *Staphylococcus aureus*: the role of Panton-Valentine Leukocidin. *Lab. Invest* 2007;87(1):3-9.

[14] Hidron AI, Kourbatova EV, Halvosa JS, Terrell BJ, McDougal LK, Tenover FC, *et al*. Risk factors for colonization with methicillin resistant *Staphylococcus aureus* (MRSA) in patients who were admitted to an urban hospital: Emergence of community-associated MRSA nasal carriage. *Clin Infect Dis* 2005;41(2):159–66.

[15] Narezkina A, Edelstein I, Dekhnich A, Stratchounski L, Pimkin M, Palagin I. Prevalence of methicillin-resistant *Staphylococcus aureus* in different regions of Russia: results of a multicenter study. Institute of Antimicrobial Chemotherapy, Smolensk.12 th *Europeon Congress of Clinical Microbiology and Infectious Diseases*. 24-27 April, Italy, Milan. Poster # P-481.

[16] Duggal S, Kaur N, Hans C. An investigation of MRSA from the burns ward: the importance of hand hygiene. *JCDR* 2011;5(3):476-9.

[17] Sarma JB, Ahmed GU. Characterisation of the methicillin resistant *S. aureus* strains and the risk factors for their acquisition in a teaching hospital in north east India. *Indian J Med Microbiol* 2010;28(2):127-9.

[18] Campbell SJ, Deshmukh HS, Nelson CL, Bae IG, Stryjewski ME, Federspiel JJ, *et al*. Genotypic characteristics of the *Staphylococcus aureus* isolates from a multinational trial of complicated skin and skin structure infections. *J Clin Microbiol* 2008;46(2):678–84.

[19] Boakes E, Kearns AM, Ganner M, Perry C, Hill RL, Ellington MJ. Distinct bacteriophages which encoded the Panton-Valentine leukocidin (PVL) protein among international methicillin-resistant *Staphylococcus aureus* clones which harbored PVL. *J Clin Microbiol* 2011;49(2): 684–92.

[20] Denis O, Deplano A, De Beenhouwer H, Hallin M, Huysmans G, Garrino MG, *et al*. Polyclonal emergence and importation of community-acquired methicillin-resistant *Staphylococcus aureus* strains which harboured the Panton-Valentine leucocidin genes in Belgium. *J Antimicrob Chemother* 2005;56(6):1103-6.

[21] Muttaiyah S, Coombs G, Pandey S, Reed P, Ritchie S, Lennon D, *et al*. Incidence, risk factors, and outcomes of Panton-Valentine leukocidin-positive, methicillin-susceptible *Staphylococcus aureus* infections in Auckland, New Zealand. *J Clin Microbiol* 2010;48(10):3470–74.

AUTHOR(S):

1. Ms. Kunsang Ongmoo Bhutia
2. Dr. T. Shantikumar Singh

PARTICULARS OF CONTRIBUTORS:

1. Lecturer, Department of Microbiology, Sikkim Manipal Institute of Medical Sciences.
2. Professor & HOD, Department of Microbiology, Sikkim Manipal Institute of Medical Sciences.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Ms. Kunsang Ongmoo Bhutia,
Department of Microbiology,
Sikkim Manipal Institute of Medical Sciences,
5th Mile Tadong, East Sikkim-737102
Phone: 9932010640
E-mail: ankusang@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS:

None.

Date of Submission: **Sep 24, 2011**
Date of Peer Review: **Dec 31, 2011**
Date of Acceptance: **Jan 17, 2012**
Date of Publishing: **May 01, 2012**