



Original article

Panton-Valentine Leukocidin gene positive methicillin resistant *Staphylococcus aureus*, the community strains causing infections in intensive care unit - High risk of outbreak and preventing strategies

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Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) is an important pathogen that causes hospital acquired infections recorded in the intensive care unit (ICU). Most of the MRSA isolates carry mecA gene which is a molecular marker for methicillin resistance. There are two types of MRSA, community acquired (C-MRSA) and hospital acquired (H-MRSA), both of these contain mecA gene. The Panton-Valentine Leukocidin (PVL) gene is normally present in C-MRSA infections which are now found to be widespread in hospital setting. Our objective was to assess the presence of PVL gene in mecA gene positive MRSA isolates from ICUs. This was a cross sectional study in ICUs of a tertiary care hospital over a period of 8 months (June 2013 to January 2014). Total of two hundred patients admitted in the ICUs who were suspected to have acquired infection 48 hours after admission were included in the study. By routine bacteriological examination and disc diffusion sensitivity testing with 30µg cefoxitin discs, MRSA strains were isolated. Polymerized chain reaction (PCR) was performed to detect mecA gene and PVL gene using specific primers. Cefoxitin disc diffusion screening showed 112 positive MRSA strains among which the mecA gene was detected in 104 strains and was absent in the remaining eight strains of total MRSA. Among the 104 mecA gene positive MRSA strains, 46 (44%) strains contained PVL gene. Our results indicate a higher prevalence of PVL-positive MRSA strains in the ICUs compared to many earlier studies. These strains were susceptible only to very few antibiotics and the empirical treatment options should be planned accordingly. Awareness of intensive care physicians and proper training of health care workers in the ICU could lower the magnitude of this problem.

Key words: C-MRSA, H-MRSA, ICU, mecA gene, PVL gene

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Methicillin resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen that causes severe morbidity and mortality worldwide. MRSA strains are endemic in most of the hospitals and account for 29%–35% of all clinical isolates¹.

Almost all ICU acquired *Staphylococcus aureus* infection recorded in the Intensive care units (ICUs) are MRSA. The actual reservoirs of MRSA in institutions are infected in-patients, while transient hand carriage of the organism by health care workers facilitates patient-to-patient transmission².

Most clinical isolates of MRSA have the *mecA* gene which encodes production of PBP2a, a modified penicillin binding protein with low affinity for β -lactam antibiotics³. The *mecA* gene plays a major role in conferring resistance to β -lactam antibiotics and is the key molecular marker of methicillin resistance.

The Pantone Valentine Leukocidin (PVL) toxin is a bipartite toxin comprising two proteins, which are Pantone-Valentine Leukocidin F ("LukF-PV") and Pantone-Valentine Leukocidin S ("LukS-PV"). This toxin is encoded by PVL gene commonly found in MRSA strains which cause community acquired infections⁴.

There are two types of MRSA, community acquired (C-MRSA) and hospital acquired (H-MRSA) and both of these contain *mecA* gene. As the *mecA* gene is essential for acquiring resistance of MRSA strains, the PVL gene is an additional factor which is seen only in community acquired strains⁵.

The PVL-positive MRSA strains were thought to be associated with C-MRSA infections earlier, which are now widespread in hospital ICUs as indicated by few studies^{5,6}. The high incidence of MRSA infection associated with PVL positive strains in the ICU suggests the possibility of a hospital outbreak due to the presence of PVL gene.

More over it is essential to distinguish PVL positive MRSA strains as their antibiotic susceptibility pattern differs from that of PVL negative MRSA strains⁷. These PVL positive MRSA strains are susceptible to many non β -lactam antibiotics especially to Clindamycin⁷. Ultimately the treatment regimen changes if there is presence of PVL gene in MRSA strains, detection of which assists intensive care physicians to choose appropriate empirical therapy. The purpose of this study was to assess the presence of PVL gene in *mecA* gene positive MRSA isolates from ICUs.

Materials and methods

Setting and design

It was a cross-sectional study in Intensive Care Units (ICUs) of a tertiary care hospital over a period of 8 months (June 2013 to January 2014).

Study population

Total of two hundred patients admitted in the ICUs who were suspected to have acquired infection after admission.

Exclusion criteria

- Patients with known infection at the time of admission
- Patients in incubation period at the time of admission (disease manifestation **within** 48 hours of admission)
- Patients on antibiotic therapy
- Patients with immune-suppression

After obtaining Institutional Ethical Committee clearance, various clinical samples such as blood, CSF, pus, urine, sputum, aspirated fluids, etc. were collected from patients suspected to have acquired infection after getting admitted in ICU. The samples were subjected to routine bacteriological examination and identified as *Staphylococcus aureus*. The disc diffusion sensitivity testing was done with 30 μ g cefoxitin discs as per CLSI guidelines (CLSI 2010) from which 112 MRSA strains were isolated and stored in nutrient agar vials at -20°C for further detection of *mecA* and PVL genes. Susceptibility to various antibiotics such as erythromycin (15 μ g), ciprofloxacin (5 μ g), gentamicin (30 μ g), clindamycin (2 μ g) and tetracycline (30 μ g) was determined by Kirby-Bauer disk diffusion method.

American Type Culture Collection (ATCC) *S. aureus* 29213 (methicillin-susceptible), ATCC *S. aureus* 43300 (methicillin-resistant) and ATCC *S. aureus* 49775 (PVL gene positive) served as the reference strains for quality control.

Mec A detection by PCR

The *mecA* gene was amplified with two oligonucleotide primers. Forward primer: 5'CTGGTGAAGTTGTAATCTGG-3', backward primer: 3'ATCGATGGTAAAGGTTGGC-5' which gave a PCR product of 533 bp. The PCR was performed with an initial denaturation step done at 94°C for 1 minute which continued for 30 cycles each for 30 seconds. The primary annealing of the template and the primers took place at 48°C-54°C for 30 seconds in each cycle. The extension step

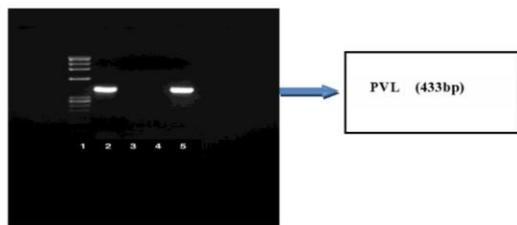
by Taq polymerase was done at 72°C for 1 minute. The bases are coupled to the primer at the 3' side. The final extension was done for 5-10 minutes.

Agarose gels were prepared with TAE buffer and added ethium bromide 1µgm/15ml gel. 5 µl of PCR product from each sample was mixed with 1µl of sample buffer and loaded on 1% agarose and electrophoresis done at 80 volt for 25 to 30 minutes. The band of product was observed by UV transilluminator and documented by gel analyser machine⁸.

PVL gene detection by PCR (Fig 1)

PCR was performed in the same manner using oligonucleotide sequence available at the GenBank data library (accession number, X 72700) usually ranging from 15-30 bases as primers to detect PVL gene. PVL gene was amplified using the following primers. F: 5' ATCATTAGGTAAAATGTCTGGACATGATC-3', B: 3'- GCATCAAGCTGTATTGGATAGCAAAGC-5'.

Positive PCR products were identified by sequencing and comparison with X2700 sequences⁹.



1-Molecular ladder ; 2,5-Positive samples ; 3,4-Negative samples.

Fig 1. PVL gene in PCR

Result

The highest incidence of infection by PVL producing MRSA is noted in the age group ranging between 50 and 60 years. 73% of them were males and 27% were females.

The sample-wise distribution of all MRSA strains are presented in table 1. The largest number of samples isolated were pus samples from wound infections.

Table 1: MRSA strains from various samples

Sample	(Total no = 112)
Pus	41(37%)
Sputum	24 (21%)
Urine	21(19%)
Blood	17 (15%)
Aspirated fluids	9 (8%)

From a total of 200 samples, cefoxitin disc diffusion screening showed 112 positive MRSA strains, for which PCR was performed to detect mec A gene and PVL gene. The mec A gene was detected in 104 strains and was absent in the remaining eight MRSA strains. Among the 104 mecA gene positive MRSA strains, 46 (44%) strains contained PVL gene (Fig 2).

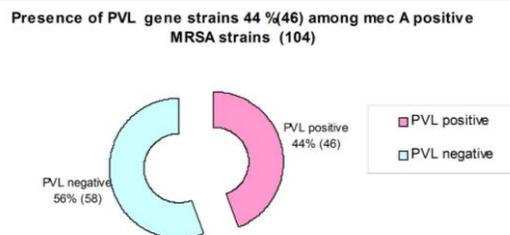


Fig 2. Presence of PVL gene among mec A positive MRSA strains

Most of the PVL producing MRSA strains were resistant to gentamycin (n=27, 59%) and ciprofloxacin (n=35, 76%). A good number of strains were susceptible to erythromycin, tetracycline and clindamycin as shown in table 2.

Table 2: Susceptibility of PVL positive strains (total = 46) to various antibiotics

Antibiotic	Susceptible	Resistant
Gentamicin	19 (41%)	27 (59%)
Erythromycin	41(89%)	5 (11%)
Tetracycline	38 (83%)	8 (17%)
Ciprofloxacin	11 (24%)	35 (76%)
Clindamycin	34 (74%)	12 (26%)

Discussion

In the present study the incidence of infection by PVL producing MRSA is highest in sixth decade which is contradictory to study report by Nandita et al⁷ in which it was in the third decade. This could be justified as the immunity decreases in elderly age group they are more prone for infection even with less virulent organisms. The PVL toxin does not have any role in enhancing virulence and in fact the PVL producing MRSA strains are considered to be less virulent compared to other MRSA strains because of their predominant presence in community acquired infections^{7,10,11}.

Most of the patients were males (73%) in this study which correlates with Nandita et al⁷. Among all clinical samples, pus from wound infections was in highest number from which PVL positive MRSA were isolated. It was similar to Nandita et al in which the commonest clinical manifestation was

surgical site infection followed by abscesses⁷. But earlier, pneumonia and bacteraemia accounted for the majority of MRSA infections in hospital due to hospital acquired MRSA strains¹.

PVL-positive MRSA strains were previously responsible for community acquired infections which are now widespread in hospital setting⁵. In the present study conducted in the ICU, 46 (44%) of PVL positive MRSA strains were isolated among all the MRSA strains. It is relatively higher compared to Nandita et al and less when compared with D'Souza et al^{7,12}. In the study by Nandita et al, 38 % of the PVL positive MRSA strains were from patients who had hospital acquired infections signifying the presence of these strains in the hospital environment⁷. In the study conducted by D'Souza et al in Mumbai, 67% of the MRSA strains causing hospital acquired infections carried the PVL gene¹².

Ramdani-Bouguessa et al studied 21 community-acquired infections of which PVL-positive isolates were 18 (86%) and 40 hospital-acquired infections of which 27 (67.5%) were PVL positive⁵.

There is a possibility that the concerned patients had been nasal carriers at the time of admission¹³. Added to that, the health care workers could have disseminated these strains from patient to patient in the ICU^{14,15}. This hand carriage depends on the carriage rate of the locality and a high carriage rate always has elevated risk of hospital acquired infections.

Another important issue of concern in our study was that most of the PVL positive MRSA strains exhibited resistance to many antibiotics. High level of resistance was shown to gentamycin (n=27, 59%) and ciprofloxacin (n=35, 76%). Ramdani-Bouguessa et al also detected more number of multidrug-resistant PVL-positive MRSA from hospital-acquired infections and the resistance was found to be more to gentamicin and ofloxacin⁵.

Though there are several methods to detect mec A gene, PCR appears to be rapid, sensitive and specific assay compared to other molecular techniques and MIC of Methicillin or Oxacillin¹⁶.

Conclusion

Our results indicate a very high prevalence of PVL-positive MRSA strains in the study area. These strains were resistant to multiple antibiotics, including gentamicin and ciprofloxacin.

Since most of the PVL-positive MRSA strains showed susceptibility to erythromycin, tetracycline and clindamycin in our study, the treatment options are restricted only to these antibiotics.

The Intensive Care Physicians must be well aware of this condition and must have the knowledge about the empirical treatment based on the susceptibility pattern of the strains circulating in the hospital. Strict Surveillance Strategies should be proposed in high risk areas such as the ICU and the existing antibiotic policy of the hospital should be revised. The health care workers must be insisted to follow the proper hand washing technique to prevent hand carriage of the organisms.

Conflict of interest: Nil

Acknowledgements: Nil

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