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DETECTION OF PANTON–VALENTINE LEUKOCIDIN GENE IN METHICILLIN RESISTANCE STAPHYLOCOCCUS AUREUS: CLINICAL AND EPIDEMIOLOGICAL ASPECTS

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ABSTRACT

Introduction: The role of PVL in the disease process and severity of Staphylococcus aureus till now is a debate matter. The data provide substantial evidence that the high virulence potential of CA-MRSA isolates is associated with the expression of PVL. PVL genes carried by HA-MRSA strains have also been recently described in cutaneous and invasive infections. Epidemiology of MRSA strains is constantly changing and is known to vary between hospitals. This study aimed to analyze the prevalence of PVL in MRSA isolates from patients using multiplex PCR. **Material and Methods:** Staphylococcus aureus were isolated from various clinical samples received in Bacteriology Laboratory using standard protocol. Antibiotic susceptibility testing was performed by Kirby – Bauer disc diffusion method. Detection of PVL genes by multiplex PCR. **Results:** The highest prevalence of MRSA PVL positive isolates was from pus sample (74.5%) followed by blood (16.3%). The results of antimicrobial susceptibility testing revealed almost similar pattern of antibiotics resistance among PVL negative MRSA isolates as compared to PVL positive MRSA isolates. **Conclusion:** The detection of PVL-producing MRSA will help us in framing and planning the infection control measures against the MRSA infections.

Keywords: Panton–valentine leukocidin gene; PCR; Antibiotics

INTRODUCTION

Second Staphylococcus aureus is an important human pathogen that causes a diverse range of diseases, from mild superficial skin infection to life-threatening bacteremia and infective endocarditis, as well as toxin-mediated conditions such as toxic shock syndrome [1].Increasing drug resistance among S. aureus and the spread of methicillin resistant Staphylococcus aureus (MRSA) are global threat [2]. MRSA is commonly associated with skin and soft tissue infections (SSTIs), as well as pneumonia, bacteremia, and sepsis [3]. β-lactam antibiotic resistance in MRSA is attributed to the acquisition of mecA gene encoding the transpeptidase penicillin-binding protein 2a and is a molecular hallmark for MRSA strains. The mecA gene is located on a mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec), with at least 13 different types of SCCmec reported to date.(3) One of the significant cytotoxins delivered by certain strains of S. aureus is the Panton Valentine leukocidin (PVL), encoded by two genes, lukS- PV and lukF-PV which is an individual of toxin that made pores in the membranes of cells. The role of PVL in the disease process and severity of S.aureus till now is a debate matter [4]. PVL is a bicomponent pore-forming cytotoxin assembled by LukS-PV and LukF-PV, has been demonstrated to have a significant role in the pathogenesis of MRSA by selectively targeting polymorphonuclear cells, macrophages and monocytes. Epidemiological and clinical data provide substantial evidence that the high virulence potential of CA-MRSA isolates is associated with the expression of PVL. While HA-MRSA strains were initially observed not to be associated with PVL production, PVL genes carried by HA-MRSA strains have also been recently described in cutaneous and invasive infections. Evidently, the epidemiology of MRSA strains is constantly changing and their prevalence as well as molecular characteristics is known to vary between hospitals in different countries, cities within a country, or among wards of a hospital. Therefore, surveillance of the changing epidemiology of MRSA in local healthcare facilities with unique patient population is crucial for obtaining data that may aid empirical therapy and patient management [3] This study aimed to analyze the prevalence of PVL in MRSA isolates from the patients using molecular methods.

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MATERIAL AND METHODS

The study was conducted at a tertiary care hospital from January 2019 to December 2019.The study population comprised of samples obtained from patients of all age and sex who were admitted in the hospital and showed signs & symptoms of infection. The Staphylococcus aureus were isolated from various clinical samples like blood, urine, pus, sputum, pleural fluid, throat swab, high vaginal swab, cerebrospinal fluid, endotracheal secretions, body fluids received in Bacteriology

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Laboratory using standard protocol. Identification of the staphylococcus isolates were done according to the standard protocols. Colony morphology, hemolysis was observed. After which , gram staining was performed and gram positive cocci was identified and subjected for further biochemical testing which included catalase, coagulase test [5].

Antibiotic susceptibility testing was performed by Kirby – Bauer disc diffusion method. MRSA identification was done by Cefoxitin disk diffusion method. Inhibition zone size of≥22mm using Cefoxitin (30µg) disk was taken as sensitive (MSSA) and zone size of ≤21mmwas taken to be Methicillin Resistant Staphylococcus aureus (MRSA) as per Clinical and Laboratory Standards Institute (CLSI) recommendations [6]. Hospital and community associated S. aureus isolates were categorized on the basis of following category: Hospital acquired MRSA (HA-MRSA): Hospital acquired MRSA infection was defined as occurring in a patient whose MRSA isolate was cultured more than 48 hours after admission to the hospital or who had a history of hospitalization, surgery, dialysis or residence in a long term health care facility within 6 months prior to the culture date or who had a indwelling intravenous line, catheter or any other percutaneous medical device present at the time the culture was taken.

Community acquired MRSA (CA-MRSA): Patients were grouped under CA-MRSA if none of the above conditions were met.

Detection of PVL genes by multiplex PCR:

DNA extraction The DNA of Staphylococcus aureus were extracted according to the manufacture instructionsby using Genome Diagnostic DNA extraction kit.Primers for the detection of genes for PVL were 'Luk-PV-1 (ATC ATT AGG TAA AAT GTC TGG ACATGA TCC A)' and 'Luk-PV-2(GCA TCA AGT GTA TTG GAT AGC AAA AGC)' which amplifies a 433 base pair fragment specific for lukS/F –PV genes, encoding the PVL S/F bi-component proteins as referred by McClure JA et al (7). The Real Time PCR comprises of 3 steps which was programmed as follows:1) Denaturation step 95°C for 15 seconds. 2) Annealing step 55°C for 20 Seconds 3) Extension step 72°C for 15 Seconds. After programming, number of cycles was set to 45 cycles. Data analysis was performed with the RotorGene™ software according to the manufacturer's instructions (RotorGene™ 3000 Operator's Manual).

RESULTS

One fifty staphylococcus aureus strains isolated from various samples received in bacteriology laboratory. Out of 150 isolates, 55 (36.66%) were Methicillin resistant staphylococcus aureus (MRSA) and the remaining 95 (63.3%) isolates were Methicillin sensitive Staphylococcus aureus (MSSA) as determined through cefoxitin disc diffusion test. A total of 55 MRSA isolates were included in our study for further analysis. All the 55 MRSA isolates were subjected to PVL gene detection using multiplex PCR. 40 MRSA isolates were found to be PVL positive (72%). Out of which, 13 (32.5%) were classified into HA MRSA and 27 (67.5%) were grouped into CA MRSA. The above classification was based on the criteria mentioned above. (Fig1) Various clinical samples including pus, blood, urine, pleural fluid etc received from different departments were included in our study. The highest percentage of PVL positive MRSA were isolated from pus sample (74.5%) followed by blood (16.3%). The rest of samples showed lower percentage of PVL gene. Among 40 PVL positive MRSA isolates, 61.85% were from males than females with 38.18%.The most affected age group was younger adults (<30 years) with 56.3% followed by older group (>30 years) with 47.7%.

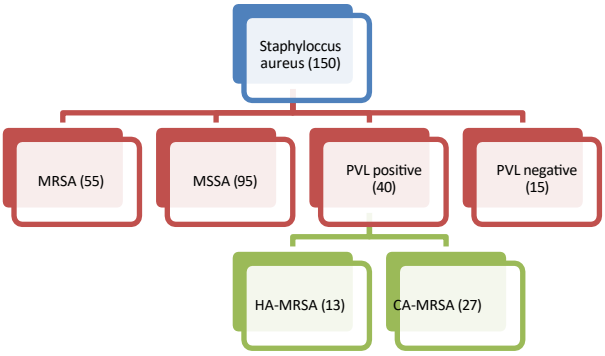

Fig 1: Antibiotic resistance pattern of PVL MRSA isolates

The results of antibiotic susceptibility testing are shown in table 1. Comparison of PVL positive MRSA and PVL negative MRSA antibiotic susceptibility pattern were done. On analysis, similar pattern of antibiotic resistance were noted. Overall, PVL positive MRSA isolates were more susceptible to clindamycin (23.6%) followed by gentamicin (49%). PVL MRSA isolates showed 100% susceptibility against vancomycin and linezolid.

Table1. Antibiotic resistance pattern of PVL MRSA isolates:

Antibiotic	PVl positive MRSA	PVl negative MRSA
Penicillin	40 (100)	15 (100)
Erythromycin	30 (75)	13 (86.6)
Ciprofloxacin	35 (87.5)	10 (66.6)
Levofloxacin	33 (82.5)	12 (80)
Gentamycin	19 (47.5)	9 (53.3)
Clindamycin	10 (25)	3 (20)
Vancomycin	0	0
Linezolid	0	0

DISCUSSION

Pathogenic Staphylococcus aureus is responsible a plethora of nosocomial and community-acquired infections including wound burn infections, skin infections and urinary tract infections (UTI) [7]. Panton–Valentine

commonly used as a marker for community acquired MRSA, responsible for soft-tissue and deep dermal infections. However, the global scenario of PVL among MRSA isolates varies [2]. To study the PVL gene occurrence associated with MRSA holds an important role in community and hospital settings. The detection will help us in framing and planning the infection control measures against the MRSA infections.

PVL prevalence:
The prevalence of PVL gene in MRSA isolates were 72%. Another study done by subarnaroy et al have reported similar prevalence rate of 85% and D'souzaetal reported to have 64% of prevalence rate [9,10]. On the other hand, studies done by Amin etal, Shrestha B et al and Thanna R et al shows low prevalence of PVL gene in MRSA isolates [3,4,11]. PVL positive organism distribution is different in various parts of the world. It appears that PVL carriage depends largely on the geographical location and the organism that are endemic in a particular locality [11]. In turkey, PVL positive MRSA isolates in hospital are reported to range from 1.7 to 20% [3].
In our study PVL gene was detected in 72% of MRSA isolates. The study done by Kunsang et al, PVL gene was detected among all the MRSA isolates (100%), irrespective of their types, which was way higher than reported by our study [12].
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 our study, the prevalence of PVL gene in CA- MRSA was67.5% and in HA-MRSA was 32.5%. Similar finding has been reported by Kunsang et al with 72% prevalence in CA MRSA and 27.6% in HA – MRSA. On the contrary, the study done by other author ,has reported the presence of PVL gene in all the CA- MRSA isolates, whereas its presence was not reported from HA-MRSA [4]. So initially it was concluded that PVL marker was the reliable marker of CA-MRSA infections [12]. But as per our study and few other studies, presence of PVL gene cannot used as a sole marker for CA-MRSA [13, 14].

Sample distribution:
In our study, the highest prevalence of MRSA PVL positive isolates was from pus sample (74.5%) followed by blood (16.3%). The similar findings were reported by Bhatta et al with 75.5% isolation from pus samples and 14.3 % isolation was seen from blood sample [2]. Al-Soud W (2019) have also reported similar prevalence rate with 86% isolation from abscesses [12].

Demographic distribution:
We found that PVL MRSA was higher in males (61.85%) than females (38.18%). Similar results were reported by Bhatta et al with 54% incidence in male and 46% incidence in females. Similarly, Kunsang et al too reported to have PVL MRSA positivity high in males (63.8%) than female (36.15%). On the contrary, Iliya et al. reported PVL MRSA to be higher in females (35.7%) than male (15.4). In our study, most affected age group was young adults (<30 years) with 56.3% followed by old age group is (>30 years) with 47.27%.Similarly, study done by Shrestha B et al. and Unsang et al, most affected age group were below 30 year of age with 37% and 68.5% prevalence respectively. On the contrary, study done by Bhatta DR et al., prevalence of PVL MRSA was higher in children. Our study along with the above studies suggests that young males are more vulnerable to the PVL MRSA, than older people and children.

Antibiotic susceptibility pattern:
The results of antimicrobial susceptibility testing revealed almost similar pattern of antibiotics resistance among PVL negative MRSA isolates as compared to PVL positive MRSA isolates. Overall, PVL MRSA isolates were more susceptible to clindamycin (23.6%) followed by gentamicin (49%). PVL MRSA isolates showed 100% susceptibility against vancomycin and linezolid. According to our study, we can still conclude that vancomycin and linezolid, the commonly used antibiotics against MRSA infections is still reliable and can be used for their treatment. Similar susceptibility pattern is reported by Bhatta DR. et al.
According to the study, low prevalence of PVL positive HA – MRSA as compared to CA-MRSA were noted. By which we can conclude that continuous screening of clinical isolates for the presence of MRSA is required in order to prevent any outbreak of these organisms and infections related to them.
Spread of PVL-producing MRSA from the community into healthcare setting poses a great public health risk and may result in outbreaks affecting vulnerable populations such as neonatal and intensive care units. Screening of virulence characteristics of patient-derived isolates is therefore essential for timely identification of patients carrying multi-drug resistant and virulent bacterial strains that would enable their isolation in hospitals. Continued surveillance and characterization of MRSA isolates in hospitals in the country is imperative for the prevention of spread of virulent nosocomial infections and the implementation of enhanced infection control strategies [3].

Limitations of the study
Our study was done on small number of samples from a single center. So, in order to determine the overall prevalence in this area, large number of samples from different centers needs to be studied. Second major drawback was that genotyping was not performed, which would help in further analysis of the MRSA infections.

CONCLUSION
PVL is a pore-forming toxin largely responsible for skin and soft tissue illnesses. Spread of PVL-producing MRSA from the community into healthcare setting poses a great public health risk and may result in outbreaks affecting vulnerable populations such as neonatal and intensive care units. The detection will help us in framing and planning the infection control measures against the MRSA infections.

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