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A Detailed Study of Antimicrobial Sensitivity Pattern of Penton Valentine Leucocidin Gene Positive and Negative *Staphylococcus aureus* from Pus Samples

Asma Akram, [2]Mateen Izhar, [2]Anwaar Basheer, [1]Mariya Ali, [3]Asma Yaqoob, [4]Asim Saifullah
[1]*Department of Pathology, Continental Medical College, Lahore*
[2]*Department of Microbiology, Shaikh Zayed Medical Complex, Lahore*
[3]*Department of Pathology, Fatima Memorial Medical and Dental College, Lahore*
[4]*Department of Pediatric Orthopedic Surgery, Children Hospital, Lahore*

ABSTRACT

Introduction: *Staphylococcus aureus* harboring Panton Valentine Leucocidin gene are emerging and spreading worldwide. PVL gene was first identified by Noel Panton and Francis Valentine in 1932 who IC Pakistan only limited data is available regarding the effect of PVL gene on sensitivity pattern of *Staphylococcus aureus.* Therefore, this study was conducted to understand the antimicrobial sensitivity pattern of both PVL positive and negative *Staphylococcus aureus* isolates. **Aims & Objectives:** This study was conducted to understand the antimicrobial sensitivity pattern of both PVL positive and PVL negative *Staphylococcus aureus* isolated from pus samples received from various indoor and outdoor departments of a tertiary care hospital of Lahore. **Place and duration of study:** Microbiology and Molecular Biology Laboratory Shaikh Zayed Hospital Lahore. Duration of study is one year after the approval of research topic. **Material & Methods:** A total of 384 *Staphylococcus aureus* isolates from skin and soft tissue infections were identified and selected. Their antimicrobial sensitivity testing was done by Kirby disc diffusion method using Muller Hinton agar. **Results:** Frequencies of PVL gene in MRSA and MSSA were 51% and 44% respectively. Frequency of PVL gene was also found to be high in Ciprofloxacin sensitive, Gentamicin sensitive, Erythromycin resistant and fusidic acid resistant isolates. **Conclusion:** Almost half of *Staphylococcus aureus* isolates were found PVL positive. They were mostly multidrug resistant. The PVL positive *Staphylococcus aureus* isolates showed high resistance against antibiotics than PVL negative isolates.

Key words: PVL (Panton Valentine Leucocidin), MRSA (Methicillin Resistant *Staphylococcus aureus)*

INTRODUCTION

In genus *Staphylococci* there are more than 26 species included till date. *Staphylococcus aureus* is one of the most critical and harmful pathogen among these *Staphylococci.*[1] It is the most widely recognized microbe that causes skin and soft tissue diseases in both children and adults.[3] *Staphylococcus aureus* also cause life-threatening infections, for example, hemorrhagic pneumonia and endocarditis.[4] *Staphylococcus aureus* colonize different body sites however nasal pit is the main site of colonization. Pathogenic factors produced by *Staphylococcus aureus* render this organism exceedingly pathogenic.[5] They include surface proteins, toxins and enzymes.[6] In the past, *Staphylococcus aureus* caused high mortality due to its pathogenicity. This mortality was reduced to sufficiently low levels due to discovery of Penicillin in 1940s. But relieve from Penicillin was brief as Penicillin resistant *Staphylococcus aureus* (PRSA) emerged rapidly. Methicillin, a beta lactamase-insensitive beta-lactam prepared in late 1950s was a good alternative to PRSA but Methicillin resistant *Staphylococcus aureus* that were resistant to commonly available beta lactamase from hospital settings. It caused an increased use of antibiotic of last resort vancomycin as a treatment option for MRSA. Initially, the rates of MRSA increased slowly and they were mostly isolated from hospital settings but later dramatic increase in frequency of MRSA occurred[2] and it was followed by isolation of MRSA from community settings from the people that were previously healthy and had no or very little chance of carrying MRSA.[7] Community acquired *Staphylococcus aureus* infection was previously caused by Methicillin sensitive

Staphylococcus aureus.[8] It was also observed that in many areas of world CA-MRSA had become more common than CA-MSSA.[9] Moreover, CA-MRSA is more infectious than HA-MRSA. The clinical picture of HA-MRSA infection is less severe than CA-MRSA but it is difficult to treat as compared to CA-MRSA.[2] Methicillin resistance in *Staphylococcus aureus* is mediated by acquiring *mecA* gene that encodes altered penicillin binding protein that has low affinity for beta lactams. Both MRSA and MSSA can carry PVL gene (Panton Valentine Leucocidin gene) which makes them more aggressive and pathogenic. It was discovered by Van deVelde in 1894 who found its capability of lysing leucocytes and is named after Sir Philip Noel Panton and Francis Valentine who related it with skin and soft tissue diseases in 1932.[5] PVL gene is acquired by *Staphylococcus aureus* by viruses called Prophages which carry various genes between bacteria.[10] These toxins destroy the outer membranes of white blood cells by the combine action of 2 secretory proteins named S and F by making pores in the membranes of cell and causing leakage of cellular contents through that pores resulting in cell death.[11] Purified PVL is only toxic to white blood cells and macrophages in humans and rabbits, they are not toxic for erythrocytes.[12] In Nepal a study was carried out in 2014 with sample size of 73 including MRSA and MSSA and they were isolated from various samples; among them PVL gene was 26.1% in MRSA and 51.9% in MSSA.[13] While in India a study was conducted in which overall prevalence of PVL was 62.85% and among MRSA and MSSA it was 85.1% and 48.8% respectively.[14] Limited work has been done on PVL gene and effect of harboring gene on organism's sensitivity pattern in Pakistan. So, this study was planned to observe the drug resistance pattern of PVL positive and PVL negative *Staphylococcus aureus* collected both from indoor and outdoor.

Abbreviations:

CA-MRSA: Community Acquired Methicillin Resistant *Staphylococcus aureus*; CLSI: Clinical and Laboratory Standards Institute; HA-MRSA: Hospital Acquired Methicillin Resistant *Staphylococcus aureus*; MDR: Multidrug Resistant; PCR: Polymerase Chain Reaction; PVL: Panton Valentine Leukocidin;

MATERIAL AND METHODS

Approval and consent to participate

Ethical approval to conduct the study was obtained from the Institutional Review Board (IRB), Federal Postgraduate Medical Institute, Shaikh Zayed Hospital, and Lahore.

Collection and Sample Processing

Pus samples from various indoor and outdoor departments of Shaikh Zayed Hospital Lahore were collected. Data related to samples was noted on standardized Proforma.

Samples were processed and *Staphylococcus aureus* was identified according to Laboratory SOPs. Antibiotic susceptibility testing was performed according to CSLI guidelines edition 2016. Following drugs were used; Cefoxitin, Erythromycin, Ciprofloxacin, Gentamycin, Fusidic acid, Penicillin, Vancomycin and Linezolid. *Staphylococcus aureus* showed resistance to at least three antimicrobial drugs were categorized as multidrug resistant.

RESULTS

Table-1 shows 72% resistant in *Staphylococcus aureus* while PVL gene frequency was high in Ciprofloxacin sensitive cases but relationship between drug sensitivity and PVL gene is not significant (p=0.246). While Table-2. Shows 63% resistant in *Staphylococcus aureus* against Erythromycin while PVL gene frequency was high in resistant cases. Table-3 shows high sensitivity against Gentamycin while PVL gene frequency was high in sensitive cases. Table-4 shows high sensitivity against Fusidic acid and PVL gene frequency is also high in sensitive cases. Table-5 and Fig-1 describe that mostly isolates were multidrug resistant and PVL gene frequency was also high in MDR cases while Table-6 and Fig-2 explain that most of *Staphylococcus aureus* were MRSA and half of those MRSA were PVL positive.

Ciprofloxacin	Total n=384	PVL+ve	PVL-ve	p value
Sensitive	92 (24%)	50 (54.3%)	42 (45.65%)	0.246
Intermediate	15 (3.9%)	5 (33.3%)	10 (66.6%)	
Resistant	277(72.1%)	131(47.2%)	146(52.7%)	

Table-1: Penton Valentine Leukocidin gene and Ciprofloxacin

Erythromycin	Total *Staph aureus* n=384	PVL+ve	PVL-ve	p value
Sensitive	130 (33.9%)	61 (47%)	69 (53%)	0.625
Intermediate	11 (2.9%)	4 (36.36%)	7 (63.6%)	
Resistant	243 (63%)	121 (49%)	122 (51%)	

Table-2: Erythromycin and PVL gene relation

Gentamycin	Total n=384	PVL+ve	PVL-ve	p value
Sensitive	235 (61.2%)	120 (51%)	115 (49%)	0.428
Intermediate	2 (0.5%)	1 (50%)	1 (50%)	
Resistant	147 (38.3%)	65 (44.3%)	82 (56%)	

Table-3: Penton Valentine Leukocidin gene and Gentamycin

Fusidic Acid	Total*Staph aureus* n=384	PVL+ve	PVL-ve	p value
Sensitive	240 (62.5%)	120 (50%)	120 (50%)	0.429
Resistant	144 (37.5%)	66 (46%)	78 (54%)	

Table-4: Panton Valentine Leucocidin gene and Fusidic acid

Drug resistance	Total *Staph aureus* n=384	PVL+ve	PVL-ve	p value
MDR	290 (75.5%)	143 (49.3%)	147 (50.7%)	0.555
Non-MDR	94 (24.5%)	43 (46%)	51 (54%)	

Table-5: Relationship of MDR and PVL gene

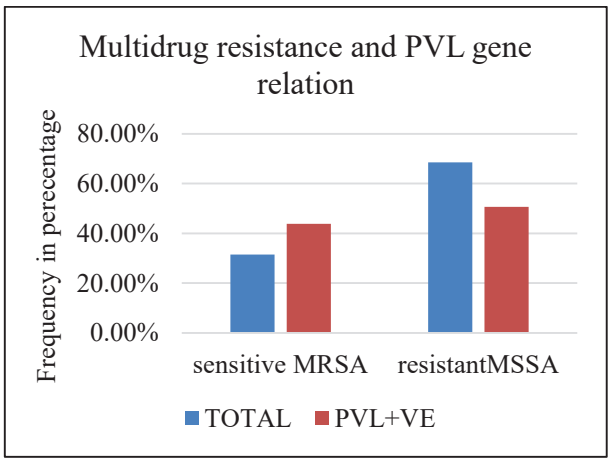

Fig-1: Relation between MDR and PVL gene

Methicillin	Total	PVL+ve	PVL-ve	p value
Sensitive MSSA	121 (31.5%)	53 (43.8%)	68 (56.2%)	0.228
Resistant MRSA	263 (68.5%)	133 (50.6%)	130 (49.4%)	

Table-6: Relationship between Methicillin sensitivity and PVL gene

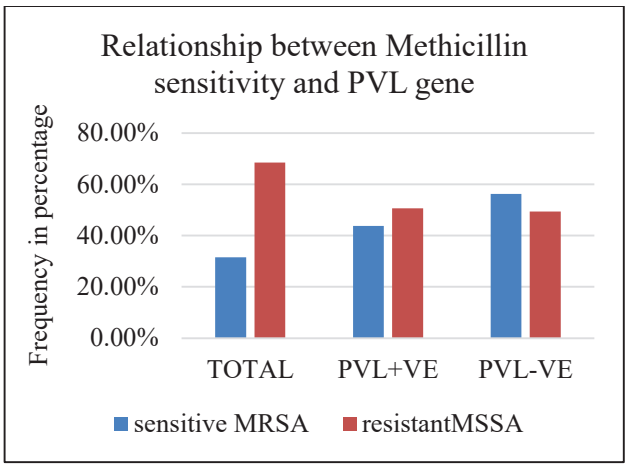

Fig-2: Relation between Methicillin sensitivity and PVL gene

DISCUSSION

Antimicrobial sensitivity of *Staphylococcus aureus* isolates was tested for following purposes: (I) to know MRSA and MSSA (II) to find out the correlation of Ciprofloxacin sensitivity with PVL gene, as it has been considered that PVL gene ratio is high among Ciprofloxacin sensitive *Staphylococcus aureus*[16] (III) To know frequency of MDR isolates (IV) any unusual pattern in AST. All isolates were sensitive to Vancomycin and Linezolid while 97.4% of samples were resistant to Penicillin. In current research, among 384 samples, 68.5% were MRSA and 31.5% were MSSA. Among MRSA 50.6% have PVL gene while in MSSA 43.8% have PVL gene. Suberna Roy et al. from India have reported 85.1% PVL gene in MRSA and 48.8% in MSSA which indicated a higher prevalence of PVL gene than our findings.[14] Another study conducted in Pakistan in 2016 reported 44% MRSA and 55% MSSA while PVL gene frequency in MRSA was 31% and in MSSA it was 18%[15] indicated a lower prevalence of PVL gene than our findings. So, current research work indicates that the frequency of both MRSA and PVL is increasing in our setup with the passage of time. This may be due to unnecessary and excessive use of antibiotics in our setup that is making isolates more antibiotic resistant and causing poor infection control and selection of resistant isolates in the community.

It has been assessed that PVL gene frequency is usually high in Ciprofloxacin sensitive *Staphylococcus aureus*.[16] In 2008, guidelines were published in the UK regarding the treatment of PVL positive *Staphylococcus aureus* infections that emphasized that only Ciprofloxacin sensitive cases should be sent to the laboratory for evaluation of PVL gene.[17] We found high percentage of PVL gene

in Ciprofloxacin sensitive *Staphylococcus aureus* but the difference of percentage was not so high and it was also not statistically proved. In current investigation, Ciprofloxacin resistance in *Staphylococcus aureus* is 72% while it had been reported as low as less than 3% from England and Wales.[6] These results reflect that prevalence of Ciprofloxacin resistance and PVL gene varies greatly between geographical locations and populations. Our investigation shows that more than half of *Staphylococcus aureus* were resistant to Erythromycin and PVL gene frequency was high in Erythromycin resistant *Staphylococcus aureus*. These results are in accordance with the results in Nepal.[5] Current results show that 38% of *Staphylococcus aureus* were resistant to Gentamycin and frequency of PVL gene was high in Gentamycin sensitive cases. Gentamycin resistance in *Staphylococcus aureus* was reported 20% in research from England. Moreover, 37% of *Staphylococcus aureus* were resistant to Fusidic acid while PVL gene frequency was high in Fusidic acid sensitive. Fusidic acid resistance in *Staphylococcus aureus* was reported 33% in research from England and Wales in 2005.[6] Due to sensitivity testing of isolates, it was easy to find out MDR and non-MDR frequency in these isolates.PVL gene frequency was high in MDR. In current study, 75% *Staphylococcus aureus* were MDR and 25% were non-MDR while a study from South India reports 91% MDR isolates.[18]

CONCLUSION

Almost half of *Staphylococcus aureus* isolates were found PVL positive. They were mostly multidrug resistant. The PVL positive *Staphylococcus aureus* isolates showed high resistance against antibiotics than PVL negative isolates.

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The Authors:

Dr. Asma Akram
Senior Demonstrator,
Department of Pathology,
Continental Medical College, Lahore.

Prof. Mateen Izhar
Head, Department of Microbiology and Virology,
Shaikh Zayed Medical Complex, Lahore.

Dr. Anwaar Basheer
Associate Professor,
Department of Microbiology and Virology,
Shaikh Zayed Medical Complex, Lahore.

Dr. Mariya Ali
Senior Demonstrator,
Department of Pathology,
Continental Medical College, Lahore.

Dr. Asma Yaqoob
Senior Demonstrator,
Department of Pathology,
Fatima Memorial Medical & Dental College,
Lahore.

Dr. Asim Saifullah
Senior Registrar,
Department of Pediatric Orthopedic Surgery,
Children Hospital, Lahore.

Corresponding Author

Dr. Asma Akram
Senior Demonstrator,
Department of Pathology,
Continental Medical College, Lahore.
E-mail: asmaasim26@gmail.com