

MOLECULAR DETECTION of PANTON VALENTINE LEUKOCIDIN (PVL) GENES in METHICILLIN-RESISTANCE STAPHYLOCOCCUS AUREUS (MRSA) ISOLATED FROM BURNS INFECTION

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Abstract:

Background: Methicillin resistance *Staphylococcus aureus* (MRSA) infection related to community has been enhanced through the world. One of the significant cytotoxins elaborated by a few strains of *S. aureus* is the Panton Valentine leukocidin (PVL), coded by two genes, lukS-PV and lukF-PV which is element of toxin that created pores in the membranes of cells, the role of PVL in the disease process severity of *Staphylococcus aureus* till known is debated.

Amis: This study was planned to detect the Panton Valentine leucocidin (PVL) genes and MRSA isolated from Burn wound infection in community and hospital acquired settings.

Material and methods: Cross-sectional study involved 200 patients who presented with burn wounds from third-degree or fourth-degree. The bacteria from all samples were undergone to Cefoxitin disc diffusion testing utilization a 30 µg cefoxitin disc for methicillin resistant determination. Following DNA extraction, conventional polymerase chain reaction (PCR) was used to detect PVL genes.

Results: Twenty-eight samples were positive culture for *Staph. aureus*, of which 19 (67.8%) patients presented with CAI, and 9 (32.1%) belong Hospital acquired infection. A total of 23 (82.1%) showed methicillin resistance. Out of 28 isolates, 7(25%) isolates were harboring PVL gene, all of which were Methicillin resistance community acquired infection. None of *S. aureus* isolates from hospital acquired infection had PVL.

Conclusion: This study indicated high prevalence of PVL among community acquired infection MRSA isolates, and the lack of this gene in Hospital acquired infection.

Keywords: *Panton Valentine Leukocidin, Methicillin resistance, community acquired infection, Hospital acquired infection.*

INTRODUCTION

Staphylococcus aureus is a standout amongst the most widely recognized and significant human pathogens related to an expansive range of disease. It is a noteworthy reason for emergency nosocomial infections, particularly in wound infection and catheter device associated disease. Expanding drug unresponsiveness's among *S. aureus* mainly to methicillin make infection with these bacteria worldwide dangers. (1)

MRSA infection related to the community has been increased through the world and one of the notably clinical manifestation is skin and soft tissue infections, which contributed to ability of this pathogen to overwhelm natural barriers of the host leading to invade both surface and deeper tissues (2).

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 *Staph. aureus* till now is a debate matter (3). Some epidemiological studies reported that the high virulence of community-acquired infection (CAI) MRSA is related to PVL; other evidence suggested the role of PVL in promoting pathogenicity indirectly by inducing expression of other virulence factors (4).

The *Staph. aureus* confines with PVL are quickly spreading and they cause various types of skin manifestations range from breast abscesses, pyomyositis, necrotizing fasciitis and pneumonia (5).

The isolation of antibiotic resistant strains *Staph. aureus* from clinical samples especially those harboring PVL toxins should be kept under check through accurate and prompt monitoring evaluation (5).

This study was proposed to detect the PVL genes and MRSA isolated from Burn wound infection in community and Hospital settings.

MATERIAL AND METHODS

The current investigation involved samples which were collected from 8 burn units in Baghdad, all burn patients are transferred from either the emergency department unit of the Hospital or from other regional Hospitals in the country.

This cross-sectional study was carried out in a total of 200 patients who presented with burn wounds from third-degree or fourth-degree during a period from the first of November 2018 to the end of January 2019. Demographic and clinical characteristics from cases were collected using a structured questionnaire. The current investigation was authorized by the institution review group of college of Medicine/ Al-Nahrain University.

Sample collection and processing :

Two hundred burn wound samples were collected aseptically, from two patients' groups as follow.

Group I: community acquired infection group (CAI) included 100 patients presented with third-degree or fourth-degree burn; who were either admitted inpatients within 24 hours in emergency departments or ICUs of hospitals before referrer to burn unit, with no history of prior hospitalization.

Group II: Hospital acquired infections (HAI) included 100 hospitalized patients presented with third-degree or fourth-degree burn; who were admitted more than 48 in the burn unit.

Isolation and identification of *Staphylococcus aureus*

The isolated *Staphylococcus aureus* bacteria from all samples were identified firstly according to Phenotypic characteristics, including the type of hemolysis on blood agar media in addition to size, shape, color of the colonies; Then a final identification was performed according to the growth on mannitol fermentation in addition to API Staph Strip system (6).

Phenotypic detection of MRSA:

The 28 *Staphylococcus aureus* isolates undergone to Cefoxitin disc diffusion method utilizing a 30 µg cefoxitin disc. Subsequently colonies grew, direct suspension of the colonies was carried out to obtain a turbidity equivalent to a 0.5 McFarland standard and processing in plates containing Mueller Hinton agar medium, the results were interpreted according to CLSI guidelines 2013. An inhibition zone diameter of ≤ 21 mm was reported as methicillin resistant and ≥ 22 mm was reported as methicillin sensitive. The standard isolates for MRSA from central public health laboratory ATCC25923 were used as control.

DNA extraction

The DNA of *Staphylococcus aureus* were extracted according to the manufacture instructions by using Promega genomic DNA purification kit (Madison, USA)

Primers for the detection of genes for PVL

Identification of PVL genes were 'Luk-PV-1 (ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A)' and 'Luk-PV-2(GCA TCA AGT GTA TTG GAT AGC AAA AGC)' which intensify a 433 base pair fragment specific for lukS/F –PV genes, encoding the PVL S/F bicomponent proteins as referred by McClure JA *et al* (7).

RESULTS

Patient baseline characteristics

There was no significant association between sex of participant and *Staph aureus* culture results, close rate of positive cultures was seen in male 20 (71.4%) than female 8 (28.5%), as shown in table (1).

In this study, out of 28 positive cultures for *Staphylococcus aureus*, these bacteria were recovered from 19 (67.8 %) patients presented with community infection, while 9 (23.1%) from these bacteria were isolated from nosocomial acquired infection, the observed difference is statistically significant *P*. value 0.041 table (2).

Detection of MRSA isolates.

A total of 23 (82.1%) out of 28 *Staphylococcus aureus* isolated from both community acquired infection and hospital acquired infection were showed methicillin resistance by Oxacillin screening test, of 10 community acquired infection 8 (80%) Methicillin resistance while 15(83.3 %) out of 18 hospital acquired infection considered Methicillin resistance table (3).

PCR for detection of PVL gene.

Out of 28 *Staphylococcus aureus* isolated 7(25%) isolates were harboring PVL gene figure (1), all 7 isolates were Methicillin resistance community acquired infection, none of hospital acquired *S. aureus* isolates had PVL

DISCUSSION

Coagulase positive *S. aureus* is the major cause of nosocomial and community acquired infection, MRSA is one of the major emerging global health problem pathogens that responsible for increasing morbidity and mortality caused by *S. aureus* (8)

In current study it was found that among 28 patients positive for *Staphylococcus aureus* infection, 20(71.4%) were males and 8(28.5 %) were females; there was no statistically significant difference between gender distribution ($p= 0.222$) such result was in accordance with study of den Heijer *et al* (2013) who reported that males were more likely than females to be *Staphylococcus aureus* carriers (9).

The same result was obtained by Jarvis WR *et al* (2012) who mentioned that male gender was a significant risk for infection with methicillin resistant *Staphylococcus aureus* (10). A study by Lamers *et al* (2011) proved a robust evolutionary relationship between clinical infection and nasal colonization isolates (11).

Melles *et al* (2004) mention evidence that virtually any *S. aureus* genotype carried by a human host can cause an intrusive infection in different site over the body (12).

The isolation rate of MRSA in this study found to be 82.1% among all *Staphylococcus aureus* isolates, this is in agreement with other studies that indicated an overall high rate of frequency MRSA observed in the last years (13- 15).

Worldwide development of MRSA is profound public health issue and obstacle to clinicians. Its significant reasons of community- and health care-associated infections such as superficial skin and soft tissue infections (SSTI) to intrusive infections, sepsis, and death. (16).

Study the occurrence of methicillin resistance *S. aureus* is important to advising public health policy and planning a framework of approaches to deal with further infection caused by this type of bacteria (16).

The current investigation found that in virtually all cases, frequently of *Staph. aureus* infection was more among the community acquired infection patients 19(67.8%) which clearly reflects the community source of this bacteria in current study. Similar results were observed in other studies (17,18).

One of the most important pathogens that causes skin and soft tissue infections is *Staphylococcus* species particularly MRSA, many studies have reported that, places that provide great proximity among individuals are a major element in the acquisition of methicillin resistance *Staph. aureus* infections (19).

Methicillin-resistance in *Staphylococci* stand for non-response to all of the β -lactam antibiotics and their derivatives, and this type of bacterial strain considered problematic in treatment of bacterial infection in both community and hospital acquired infection , of 10 community acquired infection 8(80 %) Methicillin resistance while 15(83.3 %) out of 18 hospital acquired infection considered Methicillin resistance ,the high rate of resistance to Methicillin in current study provides evidence that the misuse and overuse of the β -lactam antibiotics and their derivative in Iraq, as a country where antibiotics are sold over the counter.

Moreover, such results may also give attention to the overuse of β -lactam antibiotics in animal food such as in broiler which in turn transfers to humans by various means such as meat and egg consumption, contact with chicken and egg; such result in harmony with the

Release of Panton Valentine leucocidin lead to destruction of macrophage cells particularly polymorphonuclear and mononuclear cells in addition to that it has necrotic effect and accelerating apoptosis thereby enhance the pathogenicity of *S. aureus* which related to morbidity and mortality (23).

In the current study, the PVL genes were detected in 7(25%) all of them were Methicillin resistance community acquired infection, none of hospital acquired *S. aureus* isolates had PVL. Such finding in harmony with other results reported increase rate of PVL among MRSA isolates (24,25) such results are worrisome because the related toxin is responsible for many skin infections, sepsis and severe necrotizing pneumonia.

Accordingly, all isolated that harboring PVL genes in current study belong to community acquired infection such result is in line with and correlate well with many other studies which reported that PVL genes can be approved as marker of community acquired *Staphylococcus aureus* (26,27)

CONCLUSION

This study found that high prevalence of PVL among community acquired infection MRSA isolates, and the absence of this gene in Hospital acquired infection.

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Table (1): Distribution of *Staphylococcus aureus* isolated from wound according to gender.

Gender	Culture of <i>Staphylococcus aureus</i>		P.value
	Growth negative	Growth positive	
Male	102 (59.3%)	20 (71.4%)	0.222
Female	70 (40.7%)	8 (28.5%)	

Table (2): Distribution of culture positive *Staphylococcus aureus* in nosocomial and Community infection.

Source of infection	Culture of <i>Staphylococcus aureus</i>		P. value
	Growth negative	Growth positive	
Community	81 (47.0%)	19 (67.8%)	0.041

Nosocomial	91 (52.9%)	9 (32.1%)	
Total	172 (100%)	28 (100%)	

Table (3): Susceptibility of *S. aureus* to Methicillin in both CAI and HAI

Methicillin sensitivity	CAI		HAI		Total isolates	
	No.	%	No.	%	No.	%
Methicillin resistance	8	80	15	83.3	23	82.1
Methicillin sensitive	2	20	3	16.6	5	17.8

CAI: Community acquired infection

HAI: Hospital acquired infection

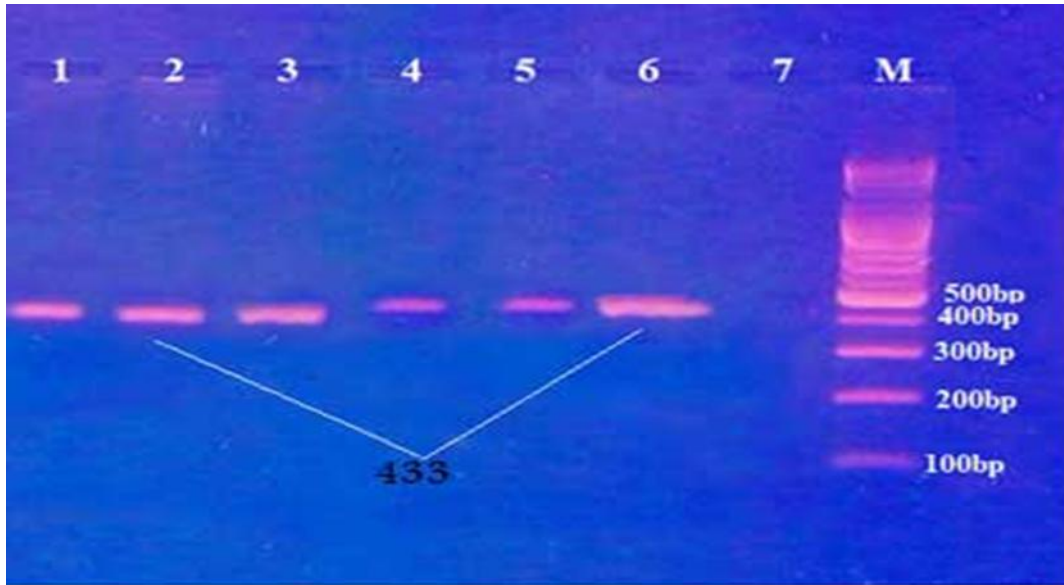


Figure (1): PCR amplification (433bp) for PVL gene in *S. aureus* isolates with 100bp DNA ladder