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MOLECULAR ANALYSIS OF PANTON VALENTINE LEUKOCIDIN (PVL) GENE IN METICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATED FROM HEALTH WORKERS AT TISHREEN UNIVERSITY HOSPITAL, SYRIA

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ABSTRACT

MRSA poses a major threat to hospital patients as it can be transmitted from health care workers who are asymptomatic carriers of the bacteria. MRSA causes serious infections with a high mortality rate and increases the rate of nosocomial infections, requiring longer hospitalizations and greater use of antibiotics in high-risk hospital units. There is another factor no less important than MRSA, which is PVL. PVL has been associated epidemiologically with virulent and transmissible strains of Staphylococcus aureus. Studies have reported the role of PVL in various deadly diseases, including skin and soft tissue infections, boils, skin abscesses, necrotizing pneumonia, and osteomyelitis. To date, nothing is known about the extent of the prevalence of MRSA among health care workers in Syria in general and in our hospital in particular, and it is not known if there are cases of Positive PVL MRSA among health care workers. Therefore, this study was conducted to determine the prevalence of Positive PVL MRSA among health care workers in Intensive care unit t in Tishreen University Hospital, Syria. **Methods:** 60 nasal swabs taken from healthcare workers in the intensive care unit were examined, regardless of age and gender. The presence of Staphylococcus aureus was detected by traditional methods, then an antibiotic susceptibility test was performed using the Kirby-Bauer method to isolate MRSA. PCR was performed to molecularly detect the PVL gene among the MRSA samples. **Results:** Among 60 nasal swabs from asymptomatic healthcare workers working in the ICU, there were 24 (40%) samples identified as Staphylococcus aureus, of which 20 samples (83.3%) were MRSA. The number of MRSA samples that tested positive for PVL 8 was 40%. **Conclusion:** Screening for the presence of MRSA should be conducted among health care workers because they may be the cause of transmission of infection with highly virulent strains of PVL positive MRSA.

KEYWORDS: MRSA, PVL gene, Health care workers, PCR, intensive care unit.

INTRODUCTION

Staphylococcus aureus is present as part of the normal flora of the skin and mucous membranes such as the nasal cavity. It does not cause harm to internal tissues unless the nasal septum is damaged.[1]

Staphylococcus aureus can cause serious and fatal infections due to the many enzymes and toxins it secretes when it encounters favorable conditions. Therefore, Staphylococcus aureus is the most important pathogen currently causing hospital and community infections.[2]

The emergence of meticillin-resistant Staphylococcus(MRSA) strains that are resistant to many antibiotics and have the ability to rapidly develop resistance to the antibiotics, currently used for treatment, constitutes a major challenge in this era.[2]

MRSA cause serious infections with a high mortality rate and increase the rate of nosocomial infections that require difficult and longer hospitalization and the use of larger quantities of antibiotics in high-risk hospital units. The pathogenicity of Staphylococcus aureus is related to a number of virulence factors, including various bacterial surface components, extracellular proteins and toxins such as Panton Valentine Leukocidin (PVL).[3]

PVL is a virulence factor in S. aureus infection and is a cytolytic that has the ability to destroy leukocytes and invade tissues. PVL is a non-hemolytic cytotoxin that causes toxic and hemolytic changes in polymorphonuclear cells, monocytes and macrophages.[4]

It is encoded by the PVL gene, which includes two exoprotein subunits encoded by Luks-pv and Lukf-pv. These two genes work together as a subunit to form pores by assembling in the cell membranes of host

immune cells, especially leukocytes, monocytes, and macrophages.[5]

Meticillin-resistant S. aureus strains carrying PVL are more virulent and transmissible than PVL-negative strains. These strains occur mainly in young, healthy people with poor health care.(5) PVL-positive MRSA infections are common in Europe and the United States. The first appearance of PVL-positive MRSA infection was observed in the late 1990s, and these strains have spread throughout the world in recent years.[6]

Purpose of the study

Conducting a molecular analysis of the PVL gene and studying its prevalence among MRSA isolates isolated from Health care workers in the intensive care unit at Tishreen University Hospital, Lattakia, Syria.

MATERIALS AND METHODS

- **Materials**

2-1 Place of study

Microbiology Laboratory - Tishreen University Hospital, Lattakia, Syria.

Atomic Energy Commission laboratories, Damascus, Syria.

2-2 Sample collection

60 nasal swabs were collected from healthy, asymptomatic workers in the intensive care unit at Tishreen University Hospital. Samples were collected regardless of age and gender. These samples were tested under sterile conditions in the laboratory department**.**

2-3 Characterization of bacterial strains

Bacterial tests were performed to detect the presence of Staphylococcus aureus. The samples were cultured on blood agar for 24-48 hours aerobically to check for B hemolysis. The identity of the bacteria was determined by observing the shapes of the colonies and performing Gram staining. Biochemical tests were performed to confirm that the staphylococci were S. aureus by culturing them on mannitol salt agar (MSA) medium and performing a coagulase and catalase test. MRSA samples were identified by incubating the strains on Muller-Hinton medium for 24 hours at 37°C using oxacillin (1 µg) and cefoxitin (30 µg) according to CLSI 2021 standards using the Kirby-Bauer method. MRSA samples were identified and the sensitization results were interpreted by measuring the peridisc diameter.

2-4 DNA extraction and detection of PVL gene by PCR

DNA of all samples was extracted using the method used by Hanano et al. 2013, which relied on the use of Liquid nitrogen.[7]

2-5 Molecular analysis

PVL gene was amplified on a thermal cycler.

Forward and reverse primers were designed at the Atomic Energy Commission in Damascus after searching through the NCBI search engine to ensure their specificity and to achieve a PVL product (433pb). (**Table 1**)

Table 1: PCR primers.

Name	Sequence of Primer (5'-3')	PCR product size (bp)
PVL forward	GCAATGAGGTGGCCTTTCCAATACA	433pb
PVL reverse	TGGGGGTAATTCATTGTCTGGCACA	

Table 2: The Programmed Thermal Cycler.

Cycles	Steps	Temperature	Time
First step: 1 cycle	Intitial denaturation	94°C	4 min
Second step: 30 cycle	Denaturation Annealing Extention	94°C 55°C 72°C	45s 45s 30s
Third step: 1cycle	Final extention	72°C	2 min

Each 20 µl PCR mixture consists of 4 µl extracted DNA with 10 µl Thermo Fisher PCR Mix with 0.5 µl reverse primer, 0.5 µl forward primer, and 5 µl DNA-free water.

2-6Electrophoresis of PCR product

After amplifying the gene, electrophoresis was performed on a 1.2% agarose gel containing 0.5 ml ethidium bromide. For comparison, ready to use, Fermentas Gene Ruler 1kb DNA ladder, was used. It was electrophoresed at 100 V for 1 hour and multiple amplified DNA was analyzed by UV radiation with a wavelength of 310 nm. Fragments of DNA 433 bp corresponded amplification of a fragment to the *pvl* genes. **fig. 1.**

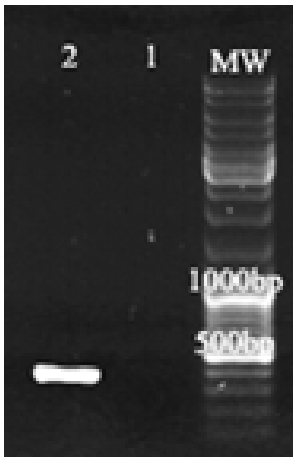

Fig. 1: Pvl gene: path MW: standard molecular marker, path 1: negative test, path 2: pvl gene positive sample.

Data management

The statistical analysis was done using IBM SPSS statistics(Version25) statistical Analysis Software. The values were represented in Number (%) and Mean ± SD.

RESULTS

Among 60 nasal swabs from asymptomatic healthcare workers working in the ICU, there were 24 (40%) samples identified as Staphylococcus aureus, of which 20 samples (83.3%) were MRSA. The number of MRSA samples that tested positive for PVL 8 was 40%.

DISCUSSION

The emergence of antibiotic resistance among bacteria is one of the biggest challenges we face, especially in developing countries such as Syria. In addition, the presence of different types of pathogenic factors in these strains increases their pathogenicity.

The emergence of antibiotic-resistant strains of Staphylococcus aureus is considered one of the biggest challenges facing the world as a whole, especially in developing countries such as Syria, due to war and the poor economic situation, in addition to the indiscriminate use of antibiotics. The presence of different types of pathogenic factors in these strains increases the pathogenicity and virulence of the bacteria.

Among the many virulence factors present in MRSA, PVL is a cytotoxin that targets leukocytes and is responsible for tissue necrosis, skin and soft tissue infections, and necrotic lung disease.[8]

Rates of PVL-positive MRSA colonization among healthcare staff vary greatly. In our study, there were 8 (40%) cases of PVL-positive MRSA nasal colonization, which poses a threat to immunocompromised patients residing in the intensive care unit, as PVL-positive increases morbidity and MRSA aggressiveness.

Some countries have reported high rates of MRSA transmission and cases containing the PVL gene among health care workers. In a study conducted in England, the prevalence of MRSA was 3.7%; 4 of them were PVL positive.[9] In another study in Germany, the prevalence of MRSA was 0.4-4.5%.[10] While the prevalence of MRSA nasal colonization among health care workers in Madagascar was 1.5%.[11] This reflects the large variation in the prevalence of PVL positive MRSA between geographical regions.

Our current study is the first of its kind in Syria, as we have no previous epidemiological studies on the spread of MRSA and the presence of the PVL gene in MRSA among health care workers. The presence of this high percentage of PVL positive MRSA among health care workers raises major health concerns, as health care workers constitute a large reservoir for MRSA bacteria and a potential source of transmission of the disease to patients.

CONCLUSION

When screening for MRSA, it should be taken into account that health care workers may be a potential source of transmission. In addition, PVL-positive healthcare workers have been found to increase the virulence of the disease, so public health measures and preventive measures should be taken to eliminate PVL-positive MRSA.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

REFERENCES

1. Sri Amelia, R. Lia Kusumawati, Mirzan Hasibuan, Lavarina Winda, Ridwan Balatif, Alvin Ivander. Prevalence of Panton-Valentine leucocidin (*pvl*) and exfoliative toxin A (*eta*) gene within methicillin resistant andsusceptible *Staphylococcus aureus* in an urban tertiaryhospital: A molecular epidemiology pilot study [version 2; peer review: 2 approved with reservations]. F1000Research, 2024; 12: 1002. Last updated: 11 JAN 2024.
2. Dilek Dülger, Seda Ekici, Özgür Albuz[3], Ahu Pakdemirli. Investigation of Nasal *Staphylococcus*

aureus carriage in hospital employees and rapid detection of *PVL* and *mecA* genes by RT-PCR. Etlik Vet Mikrobiyol Derg, 2020; 31(1): 47-51.
3. Shallcross L, Fragaszy E, Johnson A, Hayward A. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. Lancet Infect Dis., 2013; 13(1): 43–54.
4. Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine leukocidin–producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis., 1999; 29(5): 1128–1132.
5. Holmes A, Ganner M, McGuane S, Pitt T, Cookson B, Kearns A. Staphylococcus aureus isolates carrying Panton-Valentine leucocidin genes in England and Wales:frequency, characterization, and association with clinical disease. J Clin Microbiol., 2005; 43(5): 2384–2390.
6. Rani Jaiswal, Aditi Garg, Piyush Tripathi, Vimala Venkatesh. Epidemiology of Panton Valentine Leukocidin in clinical *Staphylococcus aureus* isolates - A prospective study at a tertiary care centre in North India. Clinical Epidemiology and Global Health, 2022; 15: 101006.
7. Abdulasamie Hanani, Malek Al-Arfi, Mouhnad Shaban et al .Removal of petroleum- crude oil from aqueous solution by Saccharomyces cerevisiae SHSY strain necessitates at least an inducible CYP450ALK homolog gene. Journal of basic microbiology, 2013; 54(5): 358-368.
8. H. Tabassuma, M. Gulla, A. Rasheeda, A. Banoa, H. Ejazb and N. Javeda. Molecular analysis of Panton-Valentine Leucocidin (pvl) gene among MRSA and MSSA isolates. Brazilian Journal of Biology, 2023; 83: e250351**.**
9. Panagiotis Papastergiou1, and Eleni Tsiouli1. Healthcare-associated transmission ofPanton-Valentine leucocidin positive methicillin-resistant Staphylococcus aureus: the value of screening asymptomatichealthcare workers. Papastergiou and Tsiouli BMC Infectious Diseases, 2018; 18: 484.
10. Ruscher C. Empfehlungen zur Prävention und Kontrolle von Methicillinresistenten Staphylococcus aureus-Stämmen (MRSA) in medizinischen und pflegerischen Einrichtungen—Empfehlung der.
11. Benedikt Hogan1*†, Raphael Rakotozandrindrainy2†, Hassan Al-Emran1, Denise Dekker1, Andreas Hahn1, Anna Jaeger1, Sven Poppert3, Hagen Frickmann4,5, Ralf Matthias Hagen4, Volker Micheel4, Sabine Crusius5, Jean Noel Heriniaina2, Jean Philibert Rakotondrainiarivelo2, Tsiriniaina Razafindrabe2, Jürgen May1 and Norbert Georg Schwarz1. Prevalence of nasal colonisation by methicillin-sensitive and methicillinresistant Staphylococcus aureus among healthcare workers and students in Madagascar. Hogan et al. BMC Infectious Diseases, 2016; 16: 420.