

# DETECTION OF THE PVL GENE AMONG STAPHYLOCOCCUS AUREUS ISOLATES FROM PATIENTS WITH SKIN AND SOFT TISSUE INFECTIONS AND ITS ASSOCIATION WITH DISEASE SEVERITY

Alaa A. Khaleel

Department of Medical Biotechnology,  
College of Science, Tikrit University, Tikrit 34001, Iraq  
Corresponding author's: alaa92@tu.edu.iq

## Abstract:

**Background:** Staphylococcus aureus is a major cause of skin and soft tissue infections (SSTIs). It remains unknown if Pantón–Valentine leukocidin (PVL) and its virulence factors contribute to more severe diseases. **Aim:** To establish the prevalence of the pvl gene of Staphylococcus aureus among SSTI isolates and assess the association with disease severity.

**Materials and Methods:** A cross-sectional study was conducted in Baghdad between December 2024 and January 2026 involving 71 patients with suspected SSTIs. Standard microbiological methods were used to identify the bacteria, and the VITEK 2 system confirmed the results. We evaluated 41 S. aureus isolates in total. Real-time PCR was used to find the pvl gene, and statistical analysis was used to see how it was related to the severity of the disease.

**Results:** The pvl gene was detected in 24 (58.5%) isolates. A significant association was found between pvl positivity and increased disease severity ( $p = 0.032$ ), with moderate and severe infections more common among PVL-positive cases. However, correlation analysis showed only a weak relationship. White blood cell counts were higher in moderate and severe cases but were not statistically significant.

**Conclusion:** PVL-positive Staphylococcus aureus strains are prevalent among SSTI patients in Baghdad and may contribute to more severe infections, although disease severity remains multifactorial.

**Keywords:** Staphylococcus aureus, Pantón–Valentine leukocidin, SSTIs, Molecular detection, Clinical severity.

## Introduction

Skin and soft tissue infections (SSTIs) represent a big global public health challenge for the healthcare systems of the world. Some infections are mild, such as superficial ones, while others are severe and require hospitalisation or surgery. One of the most common germs responsible



for such infections is *Staphylococcus aureus*. That's because it mutates and is very dangerous. *Staphylococcus aureus* with methicillin-resistant strains particularly, constitutes an issue of increasing complexity. The increasing number of such infections poses many challenges in curative processes of skin and soft tissue infections (SSTIs), As a result, research will need to be enhanced on the virulence factors that aggravate disease [1,2]. *Staphylococcus aureus* is regarded as a disease-causing agent due to the presence of numerous virulence factors like toxins, enzymes as well as its potential to evade immune defense mechanisms. Pantone–Valentine leukocidin (PVL) is a bifunctional toxin that perforates cellular membranes and induces the death of leukocytes. It is conducive to bacterial survival and tissue damage [3]. PVL-positive strains have been associated with more severe clinical signs and symptoms such as abscesses, necrotizing infections and recurrent SSTIs. There is still some doubt about precisely how much this alters the severity of disease. Molecular diagnostics have come a long way now and in particular polymerase chain reaction (PCR) operating in real time enables detection that is both quick and accurate of virulence genes such as *pvl* directly from clinical isolates [4]. Real-time PCR can identify extremely virulent strains relatively easily compared to other methods. This might affect the clinical decision making and control strategy for infection [5]. Recent studies have revealed that *pvl* gene is present in different levels in *Staphylococcus aureus* isolates from multiple countries. This suggests that we require local epidemiological data to elucidate transmission and the impact of the PVL-positive strains in clinical care [6]. However, though research is intensive on it [7], there is still poor information regarding how common and the importance of *pvl* gene is in treating *Staphylococcus aureus* isolates from SSTIs in Middle Eastern countries (mainly Iraq). Moreover, it is still unclear how the presence of *pvl* gene correlates with clinical severity, in particular with clinical/laboratory parameters like systemic inflammatory response and white blood cell counts [8,9,10]. This study aimed to determine the prevalence of *pvl* gene in *Staphylococcus aureus* isolates of individuals with skin and soft tissue infections and its relationship with disease severity and certain clinical parameters.

## 2. Materials and Methods

### 2.1 Study Design and Setting

This cross-sectional study was conducted in Baghdad between December 2024 and January 2026. Clinical specimens were obtained from patients with skin and soft tissue infections (SSTIs) at the dermatology and surgical outpatient clinics, as well as from individuals hospitalized at Baghdad Teaching Hospital. The study included participants of both genders, aged 12 to 67 years.

### 2.2 Study Population and Sample Collection

A total of 71 patients who were clinically suspected of having SSTIs were included in the study. Whenever possible, specimens like wound swabs and pus aspirates were taken from infected areas without any contamination before antibiotics were given. All samples were quickly sent to the microbiology lab in sterile conditions for processing.



### 2.3 Isolation and Identification of *Staphylococcus aureus*

Blood agar and mannitol were used to grow samples. Salt agar was used and the samples were kept at 37 °C for 24 to 48 hours. We did a preliminary identification based on the shape of the colonies, Gram staining, catalase, and coagulase tests. The automated VITEK 2 system (bioMérieux, France) was used to confirm the final identification. Out of 71 samples, 41 isolates were identified as *Staphylococcus aureus* and chosen for molecular analysis.

### 2.4 White Blood Cell Count (WBC) Measurement

An automated hematology analyzer (HORIBA, France) was used to find the white blood cell (WBC) counts as part of the complete blood count (CBC). Peripheral venous blood samples were obtained in EDTA tubes and analyzed following the manufacturer's guidelines. The acquired WBC values served as a measure of a systemic inflammatory and were incorporated into the subsequent statistical analysis.

### 2.5 DNA Extraction

The Geneaid DNA Extraction Kit (Geneaid Biotech Ltd., Taiwan) was used to get genomic DNA from confirmed isolates, following the manufacturer's instructions. In short, the bacterial cells were broken open, and DNA was cleaned up by spinning it through a column. A spectrophotometer was used to measure the DNA's concentration and purity. The DNA that was taken out was kept at -20 °C until it was needed.

### 2.6 Detection of the *pvl* Gene by Real-Time PCR

#### 2.6.1 Primers and Probe

A real-time polymerase chain reaction (PCR) test that targeted the nucleotide sequence (GenBank accession number X72700.1) was utilized in order to identify the Pantone–Valentine leukocidin (*pvl*) gene. The primers and probe were adopted from a previously validated study [7], as follows: forward primer (*pvl*-F) 5'-AAATGCTGGACAAAACCTTCTTGG-3', reverse primer (*pvl*-R) 5'-TTTGCAGCGTTTTGTTTTTCG-3', and probe (*pvl*-Probe) 5'-VIC-AAATGCCAGTGTTATCC-MGBNFQ-3'. The expected amplicon size was 108 bp.

#### 2.6.2 Real-Time PCR Amplification Conditions

For this study, we used quantitative real-time polymerase chain reaction (qPCR) amplification in a total reaction volume of 20 µL with an input of 10 µL 2× qPCR master mix, 0.5 µL forward primer, 0.5 µL reverse primer, 0.25 µL probe, 2 µL template DNA, and nuclease-free water to achieve the final volume. All amplification steps were conducted on a real-time qPCR equipment (SaCycler-96, Sacace Biotechnologies, Italy). The conditions included thermal cycling: the initial strip separation at 95°C for 5 minutes, followed by 40 cycles of strip separation at 95°C for 15 seconds, and then annealing and elongation at 60°C for 60 seconds. Fluorescence readings during the annealing and elongation phases are recorded, with a cycle threshold value (Ct) < 35 as positive values for this experiment.



### 2.7 Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to demonstrate the potency of PCR amplification, observing many PCR products. We used 1× TAE solution to make a 1.5% agarose gel which we stained with ethidium bromide. A 100 bp DNA ladder was located in the wells next to 5 µL PCR product with loading dye. We performed electrophoresis for about 45 to 60 min at a voltage of 80–100 V. UV transillumination was used to test for DNA bands, and the DNA ladder was used to evaluate the expected band size (108 bp).

### 2.8 Assessment of Clinical Severity

Mild, moderate, and severe skin and soft tissue infections (SSTIs) were classified using standardized clinical criteria according to established guidelines. Mild infections were characterized as localized involvement of the skin without systemic symptoms; they involved mild redness, swelling, warmth, or pain, and did not require hospitalization or invasive treatment. Moderate infections were characterized by systemic manifestations including fever ( $>38\text{ }^{\circ}\text{C}$ ), elevated white blood cell count ( $>12,000\text{ cells/mm}^3$ ), or tachycardia. Such infections necessitated systemic antibiotic therapy and careful clinical management; there were no signs of organ failure. Severe infections were characterized by widespread or rapidly advancing disease linked to systemic inflammatory response syndrome (SIRS), indicators of deep tissue involvement such as abscess formation necessitating drainage or necrotizing infection, hemodynamic instability, or the requirement for surgical intervention and/or admission to the intensive care unit. The attending physicians made the classification based on their clinical evaluation and the lab results at the time the patient came in.

### 2.9 Statistical Analysis

Statistical analysis was carried out with the software SPSS version 26.0 (IBM, USA). The categorical variables were presented as frequencies and percentages, and a chi-square test or Fisher's exact test was used, depending on the circumstances, to assess the association between the presence of the pvl gene and the severity of clinical symptoms. A p-value smaller than 0.05 was considered to be statistically significant.

### 2.10 Ethical Considerations

Our study received ethical approval from the Ethics Committee (Tikrit College of Medicine) at Tikrit University. We obtained written informed consent from all participants in our study before collecting the samples, in accordance with the Declaration of Helsinki.

## 3. Results

### 3.1 Demographic and Clinical Characteristics

A total of 41 confirmed *Staphylococcus aureus* isolates were included in this study. The age ranged from 12 to 67 years, with a mean age of  $34.6 \pm 12.8$  years. Males constituted 23 (56.1%), while females were 18 (43.9%). Most patients were from urban areas 25 (61.0%), whereas 16



(39.0%) were from rural settings. Regarding educational level, 15 (36.6%) had primary education, 17 (41.5%) secondary education, and 9 (22.0%) university level. Comorbidities were present in 16 (39.0%) patients, as show in Table 1.

**Table 1.** Characteristics of the Patients' Demographics and Clinicals (n = 41)

Variable	Category	No. (%)
Age group	≤20	10 (24.4)
	21–40	17 (41.5)
	>40	14 (34.1)
Sex	Male	23 (56.1)
	Female	18 (43.9)
Residence	Urban	25 (61.0)
	Rural	16 (39.0)
Education	Primary	15 (36.6)
	Secondary	17 (41.5)
	University	9 (22.0)
Comorbidity	Yes	16 (39.0)
	No	25 (61.0)

### 3.2 Detection of the pvl Gene

The Pantone–Valentine leukocidin (pvl) gene was successfully detected using real-time PCR in the examined *Staphylococcus aureus* isolates. Out of the total 41 isolates, **24 (58.5%)** were positive for the pvl gene, whereas **17 (41.5%)** were negative. The amplification curves of positive samples showed clear exponential fluorescence signals with cycle threshold (Ct) values ≤35, confirming the presence of the target gene. In contrast, negative samples showed no detectable amplification within the defined cycle range, as show in Table 2.

**Table 2.** Distribution of pvl Gene Among *Staphylococcus aureus* Isolates (n = 41)

Variable	Category	No. (%)
pvl Gene	Positive	24 (58.5)
	Negative	17 (41.5)
Ct Value Range (Positive cases)	≤30	15 (62.5)
	31–35	9 (37.5)

### 3.3 Identification of *Staphylococcus aureus*

Out of the total 71 clinical samples collected from patients with skin and soft tissue infections, **41 (57.7%)** isolates were confirmed as *Staphylococcus aureus* using standard microbiological methods and the automated VITEK 2 system. *Staphylococcus aureus* isolates exhibited characteristic growth on Mannitol Salt Agar, evidenced by mannitol fermentation, which



caused a distinct color change of the medium from red to yellow due to acid production. Figure 1 shows some typical characteristics of cultures.

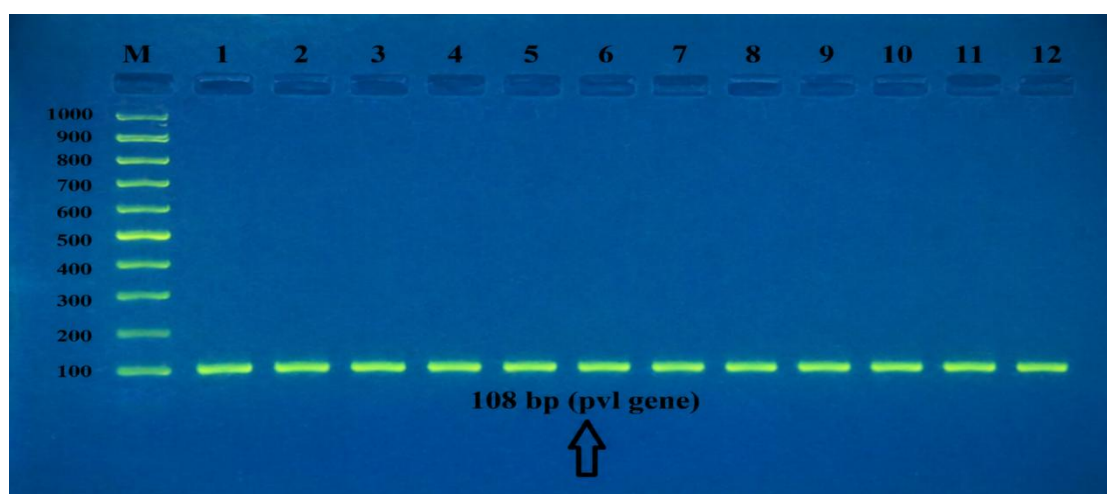


**Figure 1.** Proliferation of *Staphylococcus aureus* on Mannitol Salt Agar indicating mannitol Fermentation resulting in a color transition of the medium from red to yellow.

### 3.4 Agarose Gel Electrophoresis Findings

Agarose gel electrophoresis confirmed successful amplification of the target gene. Clear and distinct DNA bands corresponding to the expected amplicon size (108 bp) were observed in pvl-positive samples, whereas no bands were detected in negative samples or the negative control, confirming the specificity of the amplification, as show in Figure 2.

**Figure 2.** Shows typical results from agarose gel electrophoresis, with clear DNA bands at about 108 bp in pvl-positive samples and no bands in negative samples or the negative control. This shows that the amplification was specific.



### 3.5 Association Between pvl Gene and Clinical Severity



The severity of the infections was as follows: 11 (26.8%) were mild, 16 (39.0%) were moderate, and 14 (34.1%) were severe. Moderate and severe infections were more common in pvl-positive isolates than in pvl-negative isolates. Statistical analysis revealed a significant correlation between the presence of the pvl gene and heightened disease severity ( $p = 0.032$ ), as illustrated in Table 3.

**Table 3.** Association Between *pvl* Gene and Clinical Severity

Severity	pvl Positive	pvl Negative	Total	p-value
Mild	4	7	11	
Moderate	10	6	16	
Severe	10	4	14	
<b>Total</b>	<b>24</b>	<b>17</b>	<b>41</b>	<b>0.032</b>
Note: p-value was calculated using the Chi-square test.				

### 3.6 Correlation Analysis Between PVL Gene and Clinical Variables

Spearman correlation analysis was performed to evaluate the relationship between pvl gene positivity and selected clinical parameters. The analysis demonstrated a weak positive correlation between the pvl gene and clinical severity ( $r = 0.17$ ), as well as hospitalization ( $r = 0.11$ ), surgical intervention ( $r = 0.13$ ), and fever ( $r = 0.23$ ). However, these correlations were not statistically significant ( $p > 0.05$ ). In contrast, clinical severity showed strong positive correlations with hospitalization ( $r = 0.78$ ,  $p < 0.01$ ), surgical intervention ( $r = 0.65$ ,  $p < 0.01$ ), and fever ( $r = 0.74$ ,  $p < 0.01$ ), indicating that increased severity was closely associated with worse clinical outcomes. A weak and non-significant correlation was observed between pvl gene positivity and white blood cell count ( $r = -0.12$ ). White blood cell (WBC) counts were higher in moderate and severe cases compared to mild infections ( $13,200 \pm 3,100$  vs  $8,600 \pm 2,200$  cells/mm<sup>3</sup>), although the difference was not statistically significant ( $p = 0.078$ ). As shown in Table 4.

**Table 4.** Spearman Correlation Matrix Between PVL Gene and Clinical Variables

Variable	PVL gene	Severity	Hospitalization	Surgery	Fever	WBC count
PVL gene	1	0.17	0.11	0.13	0.23	-0.12
Severity	0.17	1	0.78**	0.65**	0.74**	0.05
Hospitalization	0.11	0.78**	1	0.53**	0.61**	-0.09
Surgery	0.13	0.65**	0.53**	1	0.58**	-0.02
Fever	0.23	0.74**	0.61**	0.58**	1	-0.03
WBC count	-0.12	0.05	-0.09	-0.02	-0.03	1
Note: Values represent spearman correlation coefficient ( $\rho$ ). ** indicates statistically significant correlation at $p < 0.01$ .						



---

#### **4. Discussion**

The present study confirmed *Staphylococcus aureus* in 41 out of 71 clinical samples collected from patients with skin and soft tissue infections (SSTIs), representing 57.7% of suspected cases, which reinforces its well-established role as a leading pathogen in wound and skin infections. Almuhayawi et al. reported a recent statement in the literature that was corroborated by the data [12], thus highlighting the clinical relevance of *S. aureus* for wound infections. Furthermore, the integration of Mannitol Salt Agar and automated VITEK 2 system supported the identification performance in accordance with previous regional investigations [13], which reinforced the importance of phenotypic-automated methods for accurately detecting *S. aureus* isolates.

The *pvl* gene was identified in 58.5% of *S. aureus* isolates in this study showing a high prevalence of PVL-positive strains in SSTI patients. Such data is in line with findings obtained by Leistner et al. [14], which showed a strong correlation between PVL-expressing *S. aureus* strains and cutaneous infections and recurrent disease. However, the prevalence was much greater compared to local Iraqi studies, such as Hussein et al. [15] reporting on a *pvl* gene in only 9.6% of isolates obtained from diabetic foot infections. This disparity might be due to the acute nature and pus-filled lesions characteristic of SSTIs, rather than chronic diabetic foot infections with the involvement of many types of bacteria that alter the vascular and metabolic state of the host. El Aila et al. [16] also reported a decreased PVL burden, about 29.8%, at Gaza hospitals, adding evidence to the idea that geographical variations, demographic characteristics, type of infection, and local epidemiological variation play a major role in PVL prevalence.

On the contrary, the prevalence of PVL identified in the present study has been found to coincide with that in some European settings reporting that PVL-positive *S. aureus* strains were closely associated with SSTIs contracted in community settings. For example, studies in Germany have demonstrated that PVL-positive isolates were frequently associated with recurrent abscesses and furunculosis. This is consistent with the observation that PVL is more frequent in isolates derived from purulent skin lesions than isolates taken from other infection sites [17]. An association between the presence of the *pvl* gene and illness severity ( $p = 0.032$ ) was found in this study. This implies that PVL-positive isolates are more likely to be associated with moderate-to-severe disease. This observation is biologically plausible since PVL has been demonstrated to influence leukocytes, thereby influencing host immune reaction, leading to necrotic tissue diseases, presenting clinically as abscess, widespread inflammatory process, or requiring surgical actions. Garbo et al. [18] previously emphasized the severity of PVL-associated infections, particularly invasive ones. Chi-square analysis revealed a strong correlation, while Spearman correlation analysis illustrated only a weak positive correlation of *pvl* positivity with illness severity. This contradiction is scientifically plausible, as illness severity is a multi-dimensional endpoint of virulence factors beyond a single one. The host's immune status, bacterial burden, depth of infection, timing of medical interventions, patients' health issues, and antibiotic resistance can all influence clinical outcomes. Danjean et al. [19] highlighted that the epidemiology and consequences of PVL-positive *S. aureus* are intimately connected to clonal diversity and the transmission process, as opposed to just the *pvl* gene. The incidence of severe and moderate



infections described with PVL Positive isolates in the present study was consistent with other studies, which have reported that PVL-producing strains were associated with deep tissue involvement, abscess formation and necrotizing infections. Similarly, Aoki et al. [20] also described that PVL-positive community-acquired MRSA strains have an important role in skin infections, especially when pus-filled lesions developed. On the other hand, the poor relationship between positivity of pvl gene and WBC count suggests that PVL alone does not regulate systemic inflammatory responses. WBC count was higher in moderate and severe than in mild cases, but also not significantly. That may be because the responses of leukocytes on various hosts and other clinical conditions — the length of time the infection was present, whether an antibiotic had been employed before or how different people's immune systems are affects how well leukocytes respond [21].

These links (strong associations between clinical severity and hospitalization, surgical intervention, fever) substantiate the clinical classification used in this study. These results have demonstrated that as a disease progresses, there is a rise in the clinical burden as well as an increase in the need for more complex care. Similar findings have been recorded in earlier studies, which indicate more prevalent and more aggressive disease treatment as a result of PVL-positive SSTIs [22]. Regional epidemiological analyses should be conducted as there is considerable variability of PVL prevalence geographically. Al-Saleh et al. [23] also revealed that PVL-positive MRSA strains were not distributed evenly across Gulf countries. Local monitoring information is crucial for treatment planning and infection control. To this end the real-time PCR method employed in this study has an enormous methodological advantage, enabling rapid, accurate and simple-to-interpret identification of pvl. Molecular methods are particularly beneficial because phenotypic characterisation is not enough to ascertain which strains produce PVL. Müller et al. [24] emphasize the necessity of swift assessment of PVL in clinical practice as a guideline for treatment options and optimal infection control practices. However, PVL should be considered a contributing virulence factor rather than merely a measure of illness severity. Klein et al. [25] reported PVL-positive *S. aureus* strains in mild and severe clinical presentations. Infection results can be attributed to a combination of germs, individual and environmental influence. Accordingly, up to now this study has collected local data on the high prevalence of PVL-positive *S. aureus* isolates in Baghdad patients with SSTI, as well as on pvl gene and clinical severity being positively correlated in the study population. This is consistent with Thakar et al. [26], defining similar percentages of PVL in *S. aureus* isolates and proving the implication for PVL in pus and wound infections. Collectively, the results support the potential integration of molecular detection of the pvl gene as part of clinical surveillance programs and indicate its applicability for further clinical studies in treatment of SSTIs.

### **Conclusion**

After investigating the prevalence of the pvl gene in strains of *Staphylococcus aureus*, it can be concluded that pvl is a significant feature, particularly among patients with skin and soft tissue infections who were admitted to the Baghdad clinic settings. According to the findings, PVL-



positive strains exhibited significantly increased disease severity and significant association with the expression of the pvl gene, i.e., PVL-positive strains could be correlated with more severe disease. These variables in the pathogenesis of the disease cannot be directly attributed to a single virulence determinant, due to a weak correlation. Such findings, coupled with the ability to accelerate diagnosis and management of high-risk infections through the integrated molecular detection of virulence genes (predominantly pvl) into the existing diagnostic and surveillance protocols, call for further study. This research provides important regional data that may help elucidate PVL-positive *Staphylococcus aureus* epidemiology and clinical manifestations in Iraq. Longitudinal studies should be carried out with other virulence and resistance markers and at larger sample sizes to better inform about the contribution of PVL in disease progression. Further rigorous studies, focusing on characterization of MRSA, are in order to clarify the clinical importance of PVL-positive strains.

## Acknowledgment

We extend our thanks and gratitude to the administration of Baghdad Teaching Hospital for giving us the opportunity to collect samples and facilitating our research mission. We also thank all the laboratory staff and the patients participating in this study.

## Funding

This research did not obtain any specific financing from public, commercial, or non-profit entities.

## Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## References

1. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical microbiology reviews*, 28(3), 603-661.
2. Lakhundi, S., & Zhang, K. (2018). Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. *Clinical microbiology reviews*, 31(4), 10-1128.
3. Shallcross, L. J., Fragaszy, E., Johnson, A. M., & Hayward, A. C. (2013). The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. *The Lancet infectious diseases*, 13(1), 43-54.
4. Jin, Y., Zhou, W., Ge, Q., Shen, P., & Xiao, Y. (2024). Epidemiology and clinical features of Skin and Soft Tissue Infections Caused by PVL-Positive and PVL-Negative Methicillin-Resistant *Staphylococcus aureus* Isolates in inpatients in China: a single-center retrospective 7-year study. *Emerging Microbes & Infections*, 13(1), 2316809.



5. Galia, L., Ligozzi, M., Bertoncetti, A., & Mazzariol, A. (2019). Real-time PCR assay for detection of *Staphylococcus aureus*, Panton-Valentine Leucocidin and Methicillin Resistance directly from clinical samples. *AIMS microbiology*, 5(2), 138.
6. Adeyemi, F. M., Oyedara, O. O., Yusuf-Omoloye, N. A., Ajigbewu, O. H., Ndaji, O. L., Adegbite-Badmus, M. K., ... & Oluokun, T. E. (2024). Guardians of resistance and virulence: detection of *mec*, *femA*, *Van*, *pvl*, *hlg* and *spa* genes in methicillin and vancomycin-resistant *Staphylococcus aureus* from clinical and food samples in Southwestern Nigeria. *BMC microbiology*, 24(1), 498.
7. Al Emon, A., Hossain, H., Chowdhury, M. S. R., Rahman, M. A., Tanni, F. Y., Asha, M. N., ... & Rahman, M. M. (2024). Prevalence, antimicrobial susceptibility profiles and resistant gene identification of bovine subclinical mastitis pathogens in Bangladesh. *Heliyon*, 10(14).
8. McGee, L., Chochua, S., Li, Z., Mathis, S., Rivers, J., Metcalf, B., ... & Beall, B. W. (2021). Multistate, population-based distributions of candidate vaccine targets, clonal complexes, and resistance features of invasive group B streptococci within the United States, 2015–2017. *Clinical Infectious Diseases*, 72(6), 1004-1013.
9. Gomez-Gamboa, L., Perozo-Mena, A., Bermudez-Gonzalez, J., Villavicencio, C., Villasmil, J., Ginestre, M. M., & Velasquez, J. (2021). Detection of carbapenemase-producing bacteria in a public healthcare center from Venezuela. *The Journal of Infection in Developing Countries*, 15(01), 163-167.
10. ALdai, E. D. A. (2023). Molecular Detection of Panton-Valentine Leukocidin Gene of *Staphylococcus aureus* Clinical Isolates From Different Hospitals in Khartoum state (Doctoral dissertation, ALNEELAIN UNIVERSITY).
11. Galia, L., Ligozzi, M., Bertoncetti, A., & Mazzariol, A. (2019). Real-time PCR assay for detection of *Staphylococcus aureus*, Panton-Valentine Leucocidin and Methicillin Resistance directly from clinical samples. *AIMS microbiology*, 5(2), 138.
12. Almuhayawi, M. S., Alruhaili, M. H., Gattan, H. S., Alharbi, M. T., Nagshabandi, M., Al Jaouni, S., ... & Elnosary, M. E. (2023). *Staphylococcus aureus* induced wound infections which antimicrobial resistance, methicillin-and vancomycin-resistant: assessment of emergence and cross sectional study. *Infection and Drug Resistance*, 5335-5346.
13. Al-Ghazal, S. A., & Al-Hassnawi, H. H. (2024). Molecular detection of pore-forming leuko toxin in methicillin resistant *Staphylococcus aureus* isolated from skin infection. *Medical Journal of Babylon*, 21(1), 186-190.
14. Leistner, R., Hanitsch, L. G., Krüger, R., Lindner, A. K., Stegemann, M. S., & Nurjadi, D. (2022). Skin infections due to Panton-Valentine leukocidin-producing *S. aureus*. *Deutsches Ärzteblatt International*, 119(45), 775.
15. Hussein, S. Z., & Saleh, G. M. (2024). Molecular Detection of Virulence Factors Genes for *Staphylococcus aureus* in Diabetic Foot Ulcers in Iraq. *Ibn AL-Haitham Journal For Pure and Applied Sciences*, 37(3), 98-105.



16. El Aila, N. A., Al Laham, N. A., & Naas, T. (2023). Prevalence of *mecA* and Panton-Valentine Leukocidin Genes in *Staphylococcus aureus* Clinical Isolates from Gaza Strip Hospitals. *Microorganisms*, 11(5), 1155.
17. Rentinck, M. N., Krüger, R., Hoppe, P. A., Humme, D., Niebank, M., Pokrywka, A., ... & Leistner, R. (2021). Skin infections due to Panton-Valentine leukocidin (PVL)-producing *S. aureus*—Cost effectiveness of outpatient treatment. *PLoS One*, 16(6), e0253633.
18. Garbo, V., Venuti, L., Boncori, G., Albano, C., Condemi, A., Natoli, G., ... & Colomba, C. (2024). Severe Panton–Valentine-Leukocidin-Positive *Staphylococcus Aureus* Infections in Pediatric Age: A Case Report and a Literature Review. *Antibiotics*, 13(12), 1192.
19. Danjean, M., Courbin, V., Ho, A., Bousquet, A., Rodriguez, C., Jacquier, H., & Woerther, P. L. (2025). Molecular epidemiology of panton valentine leukocidin-producing *Staphylococcus aureus* infections, Djibouti, 2018–2023. *PLOS Neglected Tropical Diseases*, 19(9), e0013544.
20. Aoki, A., Hatamiya, Y., Fukamatsu, H., Sugiyama, S., Yamamoto, T., & Aoyama, Y. (2025). Panton-Valentine Leukocidin-Producing Community-Acquired Methicillin-Resistant *Staphylococcus aureus* in Skin and Soft Tissue Infections: Clinical and Epidemiological Insights From Japan. *The Journal of Dermatology*, 52(11), 1682-1690.
21. Grebe, T., Rudolf, V., Gouleu, C. S., Löffler, B., Adegnika, A. A., Shittu, A. O., ... & Schaumburg, F. (2022). Neutralization of the *Staphylococcus aureus* Panton-Valentine leukocidin by African and Caucasian sera. *BMC microbiology*, 22(1), 219.
22. Müller, E., Monecke, S., Armengol Porta, M., Narvaez Encalada, M. V., Reissig, A., Rüttiger, L., ... & Ehricht, R. (2025). Rapid Detection of Panton–Valentine Leukocidin Production in Clinical Isolates of *Staphylococcus aureus* from Saxony and Brandenburg and Their Molecular Characterisation. *Pathogens*, 14(3), 238.
23. Al-Saleh, A., Shahid, M., Farid, E., & Bindayna, K. (2022). Trends in methicillin-resistant *Staphylococcus aureus* in the Gulf Cooperation Council countries: antibiotic resistance, virulence factors and emerging strains. *Eastern Mediterranean Health Journal*, 28(6), 434-443.
24. Boucherabine, S., Nassar, R., Mohamed, L., Habous, M., Nabi, A., Husain, R. A., ... & Senok, A. (2025). Methicillin-resistant *Staphylococcus aureus*: the shifting landscape in the United Arab Emirates. *Antibiotics*, 14(1), 24.
25. Klein, S., Hannesen, J., Zanger, P., Heeg, K., Boutin, S., & Nurjadi, D. (2020). Entry of Panton–valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* into the hospital: prevalence and population structure in Heidelberg, Germany 2015–2018. *Scientific Reports*, 10(1), 13243.
26. Thakar, V. H., Kumar, M., Meera, M., Deepa, D., Aishwarya, B., Bharati, D., ... & Shailaja, S. (2025). Prevalence and Outcome of Infections Caused by *Staphylococcus aureus* Strains Harboring the Panton-Valentine Leukocidin Gene. *Cureus*, 17(4).

