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Investigating the Panton-Valentine Leukocidin Gene in Methicillin-Resistant Staphylococcus aureus from Diabetic Foot Infections

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* is a significant pathogen associated with multidrug resistance among diabetic foot infections. The *Panton-Valentine Leukocidin (pvl)* gene is an MRSA marker frequently present in community-acquired infections; it causes deep skin and soft tissue infections and osteomyelitis. **Aim of the study:** This study aimed to detect the prevalence of the *PVL* gene in MRSA isolates among diabetic foot infections (DFIs). **Patients and methods:** A cross-sectional study conducted from August 2023 to January 2024 investigated MRSA in 150 patients with DFIs at Al-Manathira General Hospital in Al-Najaf, Iraq. *Staphylococcus aureus* isolates were diagnosed using the Vitek® 2 technique. Methicillin-resistant *S. aureus* isolates were detected by a phenotypic method depending on cefoxitin disk diffusion and confirmed by a genotypic method by PCR targeting the *mecA*, and then the *pvl* gene was investigated. The genotypic methods employed particularly primers targeting the *mecA* gene and *pvl* gene using the monoplex PCR technique. **Results:** The findings demonstrated that 95 (93.1%) of the 102 *S. aureus* isolates were resistant to cefoxitin, and according to the findings of the molecular study, only 96 (94.11%) isolates showed the *mecA* gene. Regarding the *pvl* gene, 97 (95.09%) of isolates produced positive findings. **Conclusions:** Diabetic foot infections exhibited a significantly high presence of the *pvl* gene within MRSA strains. These were confirmed using PCR, the gold standard method for detecting the *mecA* gene associated with methicillin resistance.

Keywords: Diabetic foot infections, MRSA, PVL gene, mecA gene, S. aureus.

Article Information

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INTRUDUCTION

Hearing problems are common neurological deficit, and diabetes mellitus is a common cause for these problems. The organ of Corti which is essential for hearing is at the risk of damage by high blood sugar (1). Hearing impairment affects the quality of life of diabetic patients, many researchers found hearing impairment in diabetic patients (2). Diabetes mellitus can cause sensorineural hearing loss (SNHL) because of neuropathy and microvascular complications of diabetes mellitus. The presence of comorbidities in diabetic patients such as hypertension and atherosclerotic vascular diseases, also can affect hearing (3). Some histopathological studies

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revealed a neurological and vascular damage in the inner ear of diabetic patients (4). It is well known now that increased oxidative stress has an important role in the development of long term complications of diabetes mellitus (5). Diabetes-relatedhearing impairment is of gradual onset but progressive and bilateral sensorineural deficit, which affect higher more than lower frequencies(2). The present study aims to study the relation of sensorineural hearing with diabetic mellitus in comparison to non-diabetic control.

PATIENTS AND METHODS

A cross-sectional study was conducted from August 2023 to January 2024 on 150 patients with diabetic foot infections (112 males and 38 females) at Al-Manathira General Hospital in Al-Najaf governorate. S. aureus isolates were diagnosed using the Vitek® 2 technique (Biomerieux, France) after bacterial cultures on mannitol salt agar and blood agar were incubated at 37°C for 18–24 hours (10).

Methicillin-resistant S. aureus was detected according to the CLSI 2020, and cefoxitin inhibition zones of 21 mm or less were considered indicative of MRSA(11).

The extraction of DNA was carried out using the Wizard® Genomic DNA Purification Kit (Promega, USA) and then assessed for purity and concentration using a nanodrop spectrophotometer.

The monoplex PCR technique was carried out using a master mix (Promega, USA), and specific primers described by (12, 13) for the macA gene and pvl gene were designed by Macrogen, Korea, as shown in Table 1, under the reaction conditions demonstrated in Table 2.

The Study Design

It is a prospective case-control study.

RESULTS

Out of 150 wound swabs from DFIs, 102 S. aureus isolates constituted 95 (93.1%) phenotypically methicillin-resistant S. aureus, depending on cefoxitin disc diffusion, as demonstrated in Figure 1.

A polymerase chain reaction (PCR) assay was employed to detect the mecA gene, which is a marker for methicillin resistance. Out of 102 S. aureus isolates, 96 (94.11%) detected the presence of the mecA gene, indicating a concerningly high frequency of MRSA, as shown in Figure 2.

Our findings in Figure 3 show a very high prevalence (95.09%) of the pvl gene among the MRSA isolates.

96 (94.11%) of the mecA gene of MRSA isolates harbored a pvl gene associated with the mecA gene, while one MRSA isolate harbored the pvl gene, and the mecA gene was absent, as demonstrated in Figure 4.

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Figure 1. Shows Identifying methicillin-resistant S. aureus using cefoxitin disc diffusion technique.

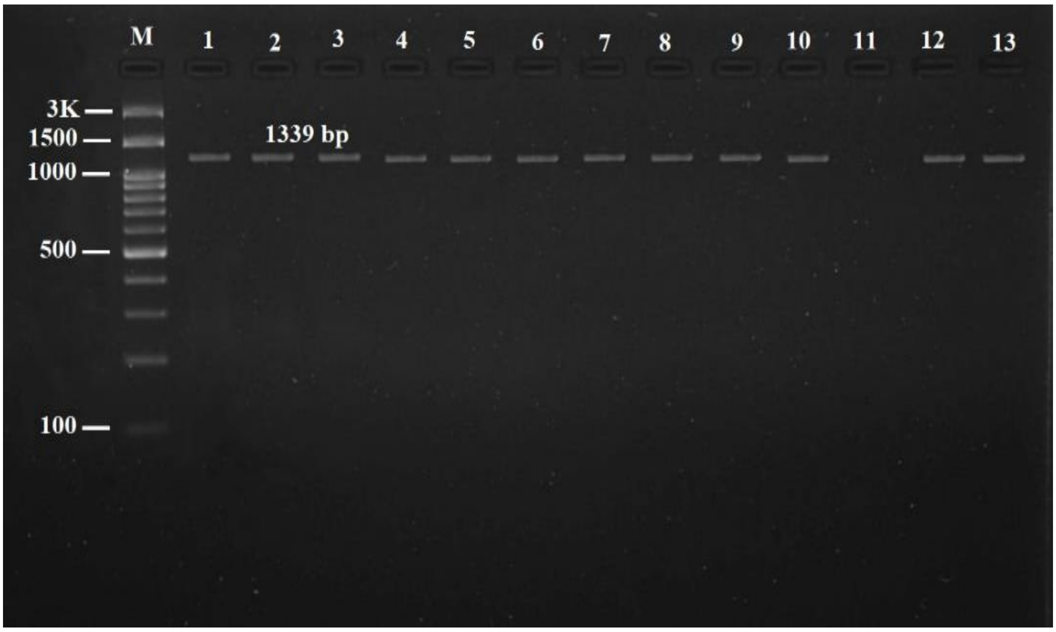

Figure 2. Visualized using agarose gel electrophoresis, PCR products from the mecA gene in MRSA isolates display a consistent band size of 1339 base pairs, with positive results present in lanes 1–10 and 12–13. Interestingly, the isolate in lane 11 lacks this band, indicating a negative mecA gene result. Line M: DNA markers (100–3000 bp) were considered controls.

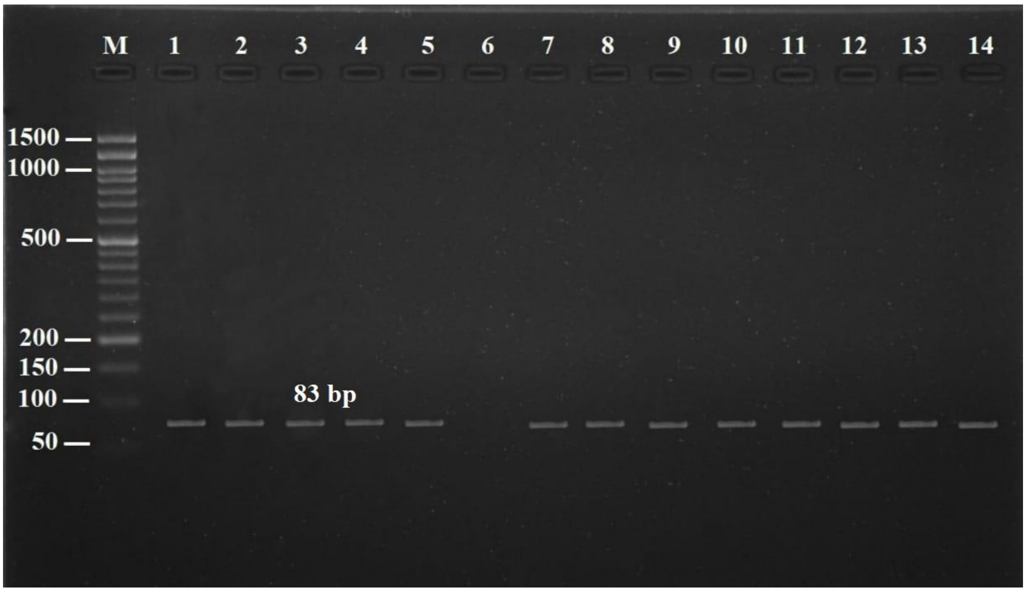

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Figure 3. Visualized using agarose gel electrophoresis, PCR products from the PVL gene in MRSA isolates display a consistent band size of 83 base pairs, with positive results present in lanes 1–5 and 7–14. The isolate in lane 6 lacks this band, indicating a negative PVL gene result. Line M: DNA markers (50–1500 bp) were considered controls.

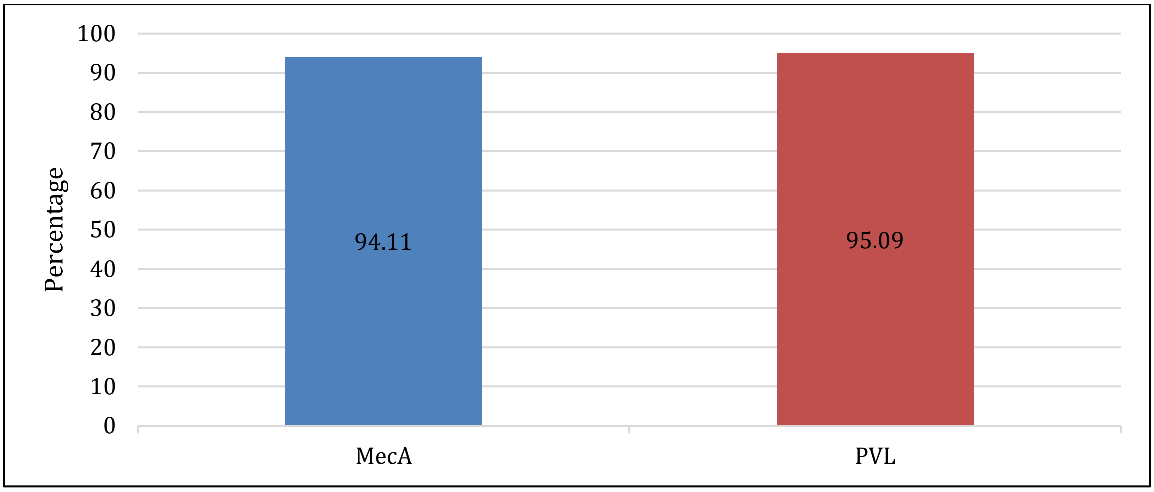

Figure 4. Percentage of mecA gene and PVL gene among MRSA isolates.

DISCUSSION

Staphylococcus aureus is a common infection in hospitals and communities. S. aureus infections, particularly MRSA, are a growing public health concern. The global epidemiology of MRSA infections is evolving rapidly (5). This study evaluated 150 wound swabs from DFIs, of which 102 S. aureus isolates were identified from diabetic foot infections. This study is consistent with the findings of (14). The findings indicated that of the 102 isolates of S. aureus, 95 (93.1%) exhibited resistance to the cefoxitin antibiotic. As shown in Figure 1. The results of this study are consistent with previous studies conducted by (2, 15). The findings presented in this study contradict the findings published by (16, 17).

In the current study, PCR analysis confirmed the presence of the mecA gene in 96 of 102 isolates, highlighting a concerningly high prevalence of MRSA (94.11%) as shown in Figure 2. Our findings detected a mecA gene prevalence of 94.11% in S. aureus isolates, consistent with the high rates reported by (94% and 94.33%, respectively), (17,18). Ahrabi et al. reported a lower prevalence (54.54%), suggesting potential regional or methodological differences(19).

The PVL gene was detected in a remarkable 95.09% of the studied S. aureus isolates, further indicating its prevalence within the community, as shown in Figure 3. Panton-Valentine leukocidin (PVL), a significant virulence determinant of Staphylococcus aureus, is encoded by the LukS-PV and LukF-PV genes (20). PVL production occurs in diverse S. aureus strains, including both MSSA and MRSA (21). Our results are consistent with those of (22), who showed a high PVL prevalence in MRSA (100%). However, they differ from the results (26.6%), (49%), and (0%), respectively,(2,23,24).

Our research, as shown in Figure 4, revealed a very high co-occurrence of the pvl and mecA genes in all the S. aureus isolates we examined. This finding indicates that the pvl gene might play a role in increasing both the virulence and antibiotic resistance of S. aureus bacteria.

Panton-valentine leukocidin (PVL), a potent toxin, serves as a significant indicator for community-acquired MRSA, a strain often implicated in skin and soft tissue infections, including abscesses, furuncles, and cellulitis(2, 25). MRSA isolates may be transmitted from community to hospital or hospital to community due to their high pathogenicity(26).

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Evidence gathered from various countries suggests a concerning rise in the prevalence of the Panton-Valentine leukocidin (PVL) gene within methicillin-resistant Staphylococcus aureus (MRSA) isolates(2). Our study identified a high prevalence of PVL in MRSA strains (75%), consistent with our study conducted by (27). D'Souza et al. found that PVL-positive isolates were prevalent among methicillin-resistant Staphylococcus aureus (MRSA) at a rate of 64%(28). The prevalence of PVL is lower in various regions of the world, being much lower in areas like France, and the UK (5%, and 4.9%, respectively) (24). These findings highlight the significant geographical variation in PVL incidence across different communities.

CONCLUSIONS

The current study showed a high prevalence of the pvl gene among MRSA isolates with diabetic foot infections. It serves as a community-associated MRSA marker. The PCR study is effective in detecting pvl and mecA genes.

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