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Detection of Panton-Valatin Lecucidin and Meca Genes in *Staphylococcus Aureus* Isolated from Al-Najaf Patients

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Abstract
Methicillin.-resistant. Staphylococcus. Aureus poses a significant. Challenge in managing the infections it can cause. The Panton-Valentine leukocidin (PVL) is a significant factor contributing to the virulence of Staphylococcus aureus (SA) and has been associated with potentially life-threatening infections. The aim of this study was to investigate the frequency of methicillin resistant Staphylococcus aureus (MRSA) within Staphylococcus aureus (SA) isolates, together with the detection of the Panton-Valentine Leukocidin gene (PVL). The utilization of the disk diffusion method was employed as a means to detect the presence of methicillin resistance. Both cefoxitin and oxacillin disks were employed for this purpose. The molecular investigation employed particular primers designed to target the mecA gene and pvl gene using the polymerase chain reaction (PCR) method. The results indicated that among the 30 isolates of Staphylococcus aureus (SA) that were diagnosed, 25 were determined to be strains of methicillin-resistant Staphylococcus aureus (MRSA). In addition, the MRSA isolates demonstrated resistance to both cefoxitin and oxacillin. According to the findings of the molecular investigation, it was observed that 83.3% of the isolates obtained from (SA) exhibited the presence of the mec a gene. Regarding the pvl gene, it was observed that 26.6% of the tested isolates yielded positive results, resulting in the identification of a total of 8 positive isolates.

Keywords: PVL Gene, Meca Gene, S Aureus

1. Introduction

Staphylococcus aureus is a bacterial species that possesses the ability to induce pathogenicity in humans, while also exhibiting a commensal relationship with its host. It is widely recognized that Staphylococcus aureus has the ability to colonize around 30 percent of the human population. Concurrently, it functions as a noteworthy the determinant plays a crucial role in the development of bacteremia and endocarditis (IE), as well as other infections such as osteoarticular, cutaneous and subcutaneous, pleuropulmonary, and device-associated infections [1]. It is widely considered that the horizontal transfer of genes responsible for antibiotic resistance, as well as the synthesis of various virulence factors, play a crucial role in the establishment and persistence of drug-resistant strains of S. aureus inside hospital environments [2]. Staphylococcus aureus exhibits a diverse array of virulence factors that are crucial to its capacity for host tissue colonization and invasion, evasion of the host's immune system, and acquisition of nutrients [3]. The occurrence of Methicillin-resistant Staphylococcus aureus (MRSA) outbreaks has emerged as a significant issue in healthcare facilities on a global scale. The current treatment interventions for managing MRSA infections are restricted to glycopeptides, linezolid, tigecycline, and cefazoline [4]. The emergence of methicillin resistance is facilitated by the presence of the mecA gene and is acquired through horizontal gene transfer, specifically involving a mobile genetic element known as staphylococcal cassette chromosome mec (SCCmec). The occurrence of methicillin resistance in Staphylococcus aureus bacteria can be conceptualised as a result of the crosslinking of peptidoglycans within the bacterial cell wall. PBP2a demonstrates a reduced affinity for β-lactam antibiotics, resulting in resistance to the entire class of these antimicrobial drugs [6].

Leukocidins are considered virulence factors consisting of two components. They possess pyrogenic properties and act as super-antigenic poisons, capable of disrupting host cell membranes and modulating immunological responses through the activation of immune cells [7]. The PVL is one of the seven leukocidins that are produced by these bacteria. The PVL, a custom consisting of two proteins known as F and S, has a molecular weight of 32KDa and 38KDa, respectively. These proteins are regulated by the Lukf/PV genes [8].

The initial attribution of Pantone Valentine leucocidin (PVL) expression as a distinctive characteristic of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) was subsequently challenged by a growing number of publications documenting the presence of PVL-negative CA-MRSA strains [9]. Panton-Valentine leukocidin (PVL) is synthesized by the expression of the lukF-PV and lukS-PV genes, which are situated within the genomes of several lysogenic bacteriophages [10].

2. Methods

2.1 Samples

Between September 2022 and January 2023, a total of 300 samples were obtained from various sources including pus swabs, urine samples, seminal fluid, high vaginal swabs, and burn swabs. The samples were obtained from patients who sought medical consultations at Al-Furet al-Awsat hospital and AL-Najaf Teaching Hospital in AL-Najaf City, Iraq. The Vitek-2 microbiology equipment, manufactured by Biomatrix in the United States. The bacterial isolates were cultured on blood agar and thereafter incubated at a temperature of 37 °C for a duration of 18-24 hours.

2.2 Diagnosis of Methicillin Resistent S. Aureus (MRSA)

The impartial of this study was to conduct a disk diffusion test on methicillin-resistant Staphylococcus aureus (MRSA) isolates. Both oxacillin and cefoxitin, in addition to methicillin, were utilized in this study to identify MRSA isolates. The utilization of methicillin was not implemented in this research to determine MRSA isolates, as this particular antibiotic is susceptible to experimental variables. Additionally, it has been reported that certain studies investigating MRSA detection may have yielded inaccurate results due to the inability to identify resistance when employing methicillin [11]. Nevertheless, the utilization of oxacillin in tests is more prone to identify resistance compared to the use of methicillin. This is due to oxacillin's higher resilience to degradation during storage and its increased ability to detect staphylococcal isolates that exhibit hetero resistance. (CLSI, 2021) [12]. (Table 1)

Table 1: Antibiotics Disk

Antibiotics	Symbol	Concentration
Cefoxitin	FOX	30 µg
Oxacillin	OX	30 µg

The determination of oxacillin inhibition zones for resistance isolates in the Disk diffusion test was conducted at a maximum diameter of 10mm, as specified by the Clinical and Laboratory Standards Institute (CLSI, 2021) [12]. Similarly, the determination of cefoxitin inhibition zones for resistant strains in the Disk diffusion test was performed at a maximum diameter of 21mm. (CLSI, 2021) [12].

2.3 DNA Extraction

The genomic DNA of bacterial isolates was extracted using the Genomic DNA extraction Mini Bacteria Kit (Favorgen). The DNA that was obtained was assessed using a Nanodrop instrument (THERMO, USA) to determine its concentration in nanograms per microliter (ng/µL). Additionally, the purity of the DNA was evaluated by measuring the absorbance at wavelengths of 260 nm and 280 nm.

2.4 PCR Assay

The polymerase chain reaction (PCR) was conducted using a master mix provided by Promega. The Polymerase Chain Reaction (PCR) experiment was conducted to determine the presence of the mecA and PVL genes in Staphylococcus aureus (Table 2).

Table 2: The polymerase chain reaction (PCR) primers

Gene	Temperature(°C) /Time					Cycles number
	Initial Denaturation	Condition of one cycle			Final Extension	
		Denaturation	Annealing	Extension		
Pvl	94/2min	94/1min	51/45sec	72/45sec	72/4min	33cycle
MecA	94/4 min	94/45 sec	60/30sec	72/45sec	72/4 min	35cycle

Table 3: PCR Program used in the study

Primer		Sequence	Amplicon	
MECA	F	GTG.AAG.ATA.TAC.CAA.GTG.ATT.	147pb	(Karmakar *et al*, 2018) [22]
	R	ATG.CGC.TAT.AGA.TTG.AAA.GGAT.		
PVL	F	ATCATTAGGTAAAATGTCTGGACATGATCCA	433pb	(Karmakar *et al*, 2018) [22]
	R	GCATCAAGTGTATTGGATAGCAAAAGC		

3. Result and Discussion

3.1 Isolation of Bacteria and Diagnosis

The current investigation involved the inclusion of 30 isolates of Staphylococcus aureus that were obtained from various sources such as urine, high vaginal swab, pus, wound, and seminal fluid. All the isolates exhibited the capacity to proliferate on mannitol salt agar, which is widely recognized as a selective and differential medium for the Staphylococcus genus [13]. The colonies exhibited a spherical shape, a polished surface, an elevated structure, a mucous consistency, and a lustrous appearance as shown in Fig 1.

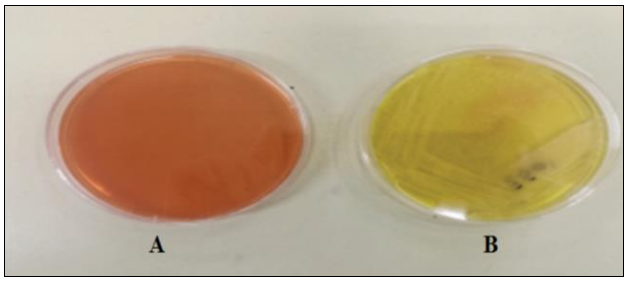

Fig 1: Mannitol salt agar medium (A); colonies of Staphylococcus aureus ferment mannitol and form large golden colonies surrounded by wide yellow zones (B)

A comprehensive microscopic analysis was conducted on all 30 isolates subsequent to the application of Gram staining. The resulting observations revealed the presence of Gram-positive cocci, which were organized in a cluster resembling an irregular arrangement of grape-like structures. To facilitate further identification, the catalase test was conducted and yielded positive findings for all 30 isolates. A coagulation assay was conducted in order to determine the species of the bacterial isolates, and it was observed that all isolates exhibited coagulation capability. The isolates were subjected to colony characterization following subculturing on 5% human blood agar and incubation at 37˚C for 24 hours. The colonies exhibited a substantial, round, and golden appearance, encompassed by a distinct zone of hemolysis. The ß-hemolysis type is frequently observed on blood agar.

3.2 Detection of Methicillin Resistant S. aureus (MRSA)

In accordance with the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI, 2021) [12], the Disk Diffusion Test was conducted using both oxacillin and cefoxitin for the purpose of testing MRSA isolates. The determination of oxacillin inhibition zones for resistance isolates in the Disk diffusion test was conducted at a maximum diameter of 10mm, as specified by the Clinical and Laboratory Standards Institute (CLSI, 2021) [12]. Similarly, the determination of cefoxitin inhibition zones for resistant strains in the Disk diffusion test was conducted at a maximum diameter of 21mm, as recommended by CLSI (2021) [12]. The findings indicated that of the 30 isolates of S. aureus, 25 (83.3%) exhibited resistance to both medicines (See Fig 2).

Fig 2: Detection of Methicillin resistance for S. aureus isolates

The findings presented in this study align closely with the research conducted by Al-Maliki (2014), which reported a resistance rate of approximately 80.3%. Furthermore, the study found that 16.4% of isolates exhibited moderate resistance, while 3.3% shown sensitivity. Al-Geobory (2015) demonstrated that the resistance rate was approximately 90.90%. In a study conducted by Al-Dahbi (2016), it was demonstrated that the rate of resistance reached approximately 94.3%. The findings presented in this study are contradictory to the findings reported by Peck *et al*. (2017), who observed that 51.4% of the isolates exhibited resistance to Methicillin, while 48.6% shown sensitivity.

3.3 Detection of Methicillin Resistant by PCR

A Polymerase Chain Reaction (PCR) was conducted on a total of 30 Staphylococcus aureus isolates in order to ascertain the existence of mecA genes, as illustrated in Fig 2. The findings of the study revealed that 25 out of 30 isolates were found to possess the mecA gene, hence showing a high prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) among the isolates.

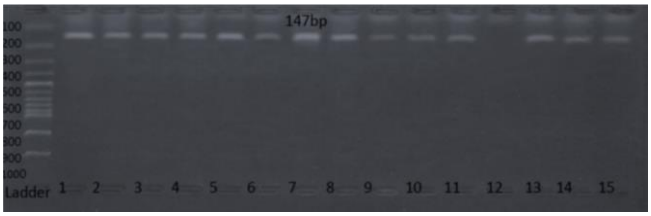

Fig 3: PCR product to MecA in 1% agarose gel electrophoresis, Voltage 85V, Time 60 min and 5 µl of PCR product put in all well. DNA ladder 100bp, and MecA positive band in 147bp

The findings indicated that 83.3% (25 out of 30) of Staphylococcus aureus isolates harbored the mecA gene, suggesting that the majority of the isolates were methicillin-resistant Staphylococcus aureus (MRSA). Research conducted in India examined a total of 17 isolates, from which 7 instances were found to be positive for the mecA gene [18]. In a study conducted by Sajith *et al*. [19], it was reported that among 35 isolates of MRSA, 33 (94%) were found to possess the mecA gene, as indicated by the collected PCR data. In a separate study conducted by Zeinalpour *et al*. [20], it was shown that the presence of the mecA gene was detected in 54.54% of the analyzed samples. In congruence with prior investigations on phenotypic methicillin resistance outcomes, it was shown that all the isolates under examination included the mecA gene, hence confirming their classification as methicillin-resistant Staphylococcus aureus (MRSA) isolates [21].

3.4 Detection of Panton-Valentine Leucocidin (pvl Gene)

The findings of the study indicate that the Staphylococcus aureus sex isolates examined in this research possess the PVL gene. Specifically, the presence of this gene was seen in 20% of the isolates, as illustrated in Fig 4.

Panton-Valentine leukocidin (PVL) represents a significant virulence determinant of Staphylococcus aureus (S.A), with its expression being governed by the LukS-PV and LukF-PV genes [22]. Panton-Valentine leukocidin (PVL) has been observed to be generated by many strains of Staphylococcus aureus (SA), including Methicillin-Sensitive SA (MSSA) and Methicillin-Resistant SA (MRSA) [23]. In a study conducted by Akram and Izhar [24], it was found that out of 384 S. aureus isolates tested, 186 (49%) exhibited a positive presence of the PVL gene. Ritz *et al*. [25] have provided evidence indicating that the prevalence of PVL S. aureus is elevated in infections attributed to methicillin-resistant Staphylococcus aureus (MRSA), ranging from 74% to

100%.

The results of this investigation demonstrate a lack of agreement with the data from a previous study conducted by [26], which reported a prevalence of 10.7% of isolates containing the PVL gene. This finding is in agreement with a previous study conducted by [27]. The findings indicate that the presence of the PVL and mecA genes was observed in 63.5% and 50% of the isolates, respectively.

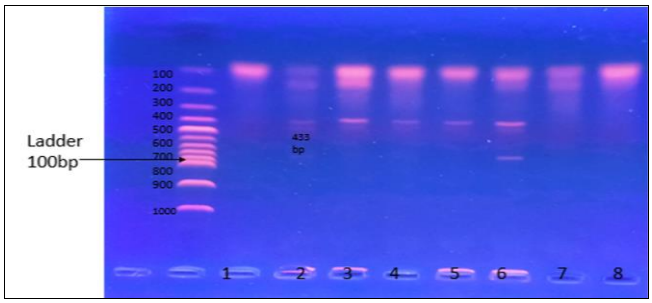

Fig 4: The PCR product of the pvl gene was analyzed using 1% agarose gel electrophoresis. The electrophoresis was conducted at a voltage of 100 V for a duration of 90 minutes. Each well was loaded with 5 µL of the PCR product. The DNA sample used in this experiment is a DNA ladder with a length of 100 base pairs (bp). The PCR products observed in lanes 1, 2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 14, 15, and 17 correspond to a positive case band with a size of 433 base pairs

The findings of our investigation indicate that all isolates harboring the pvl gene exhibit methicillin resistance, so suggesting that the presence of pvl gene confers heightened virulence and resistance in bacteria. Based on the data shown in Fig 5.

The Panton-Valentine leukocidin (PVL) is frequently employed as an indicator for community-acquired methicillin-resistant Staphylococcus aureus (MRSA), which is accountable for infections affecting the soft tissues and deep layers of the skin [28, 29]. Nevertheless, the prevalence of PVL among MRSA isolates exhibits variability on a global scale.

Evidence from multiple countries indicates a rising occurrence of PVL among MRSA isolates [30, 31]. In a study conducted by Subarna Roy *et al*. in India, the researchers observed a prevalence of 62.85% for Panton-Valentine leukocidin (PVL) among methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-sensitive Staphylococcus aureus (MSSA) strains. Specifically, the prevalence of PVL was found to be 85.1% among MRSA strains and 48.8% among MSSA strains. These findings suggest a greater prevalence of PVL among MRSA strains compared to the results obtained in our own study [32]. A study conducted by D'Souza *et al*. in Mumbai, India, revealed a prevalence rate of 64% for PVL positive isolates among methicillin-resistant Staphylococcus aureus (MRSA) [33]. The prevalence of PVL in different regions of the world has been documented to be lower, with reported rates of 5% in France, 4.9% in the UK, 8.1% in Saudi Arabia, and 14.3% in Bangladesh [24-26]. These findings indicate significant variation in the incidence of PVL across different geographical locations and populations.

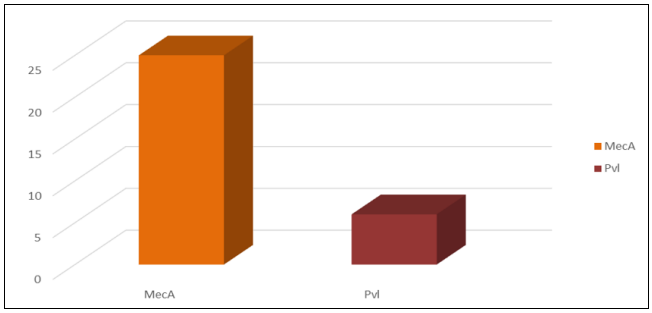

Fig 5: Association between mecA and PVL

4. Conclusion

From this study, we conclude that both disk diffusion method as well molecular identification by PCR are excellent methods to detect (MRSA) and that all pvl positive isolates were (MRSA) as well, reflecting an association between the two traits.

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