Investigating the Correlation Between the Panton-Valentine Leukocidin (PVL) Gene and Methicillin-Resistant Staphylococcus aureus (MRSA) in Community-Acquired Soft Tissue Infections

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Abstract

Introduction: MRSA, methicillin-resistant Staphylococcus aureus, poses a considerable threat to human health by causing infections in both community and healthcare environments. Among the numerous virulence factors possessed by S. aureus, the Panton-Valentine Leukocidin gene (PVL) stands out as particularly significant. The current study aimed to detect the prevalence of the PVL gene in community-acquired MRSA isolated from skin and soft tissue infections (SSTIs).

Subjects and Methods: Among 109 S. aureus isolates collected from SSTIs, MRSA isolates were detected by the cefoxitin disc diffusion method and cultured on an ORSAB (oxacillin resistance screening agar base) medium. Polymerase chain reaction (PCR) was used to detect the PVL gene in MRSA isolates.

Results: Forty-one MRSA (37.6%) isolates were detected among S. aureus isolated from community-acquired SSTIs by different phenotypic and genotypic methods. The majority of community-acquired MRSA cases (97.5%) tested positive for the pvl gene.

Conclusion: Our study revealed a significant prevalence of PVL among community-acquired MRSA isolates.

Keywords: MRSA; Staphylococcus aureus; PVL; SSTIs.

1. Introduction

*Staphylococcus aureus* (*S. aureus*) is a significant human pathogen that is linked to community- and hospital-acquired illnesses all over the world [1]. A potentially fatal staphylococcal infection’s prognosis was improved by the discovery of penicillin, but after years of widespread use, resistance emerged due to the production of lactamases [2]. Despite the fact that methicillin is lactamase-resistant, methicillin-resistant *S. aureus* (MRSA) strains have been identified quickly following methicillin use [3]. Since 1960, MRSA, also known as hospital-associated MRSA (HA-MRSA), has been recognized as a virulent disease in hospitals [4]. The epidemiology of MRSA has changed since 1990 as a result of the emergence of community-acquired MRSA (CA-MRSA)
outside of hospitals [5]. The Panton-Valentine Leukocidin gene (PVL) is S. aureus's most serious toxin [6]. A phage-encoded two-component toxin that can be introduced into the S. aureus genome [7]. Particularly in skin and soft tissue infections (SSTIs), PVL-toxin is regarded as the most significant marker of CA-MRSA [8].

The study was aimed at detecting the incidence of MRSA in S. aureus isolated from SSTIs and the PVL gene prevalence in CA-MRSA.

2. Subjects and methods

2.1. Identification of MRSA

The detection of MRSA was carried out across 109 S. aureus isolates obtained from SSTIs by the disk-diffusion method using a cefoxitin (30 g) disc and oxacillin resistance screening agar base (ORSAB) medium. Isolates that had an inhibition zone to Cefoxitin diameters of less than 21mm [9, 10] and/or characteristic growth on ORSAB medium (Oxoid Ltd., Hampshire, UK) were considered MRSA. ORSAB medium employs aniline blue to detect mannitol fermentation, which leads to the formation of vibrant blue colonies indicating the presence of S. aureus. ORSAB was enhanced with lithium chloride, polymyxin B, and oxacillin as per the instructions provided by the manufacturer and subsequently incubated at a temperature of 35–37 °C for 24 hours. MRSA grows on this medium and yields blue colonies [11].

2.2. Antibiotic susceptibility test

Antibiotic sensitivity tests were done for MRSA isolates by the Kurdy-Bauer method. The antibacterial activities of antibiotics were assessed by measuring the inhibition zones in mm [12].

The following discs of antibiotics (Oxoid) were used: Cefoxitin (FOX) 30 μg, Amoxicillin/Clavulanic acid (AMC) 30 μg, Erythromycin (E) 15 μg, Tetracycline (TE) 30 μg, Linezolid (LNZ) 30 μg, Gentamycin (CN) 10 μg, Ciprofloxacin (CIP) 5 μg, Sulphamethoxazole/Trimethoprim (SXT) 25 μg, Imipenem (IPM) 10 μg, Cefotaxime (CTX) 30 μg, Cefepime (FEP) 30 μg [13, 14].

Strains that were identified as MRSA were stored in Eppendorf tubes with broth and glycerol tubes at -80°C for further molecular work.

2.3. Molecular Methods

DNA extraction

By using the boiling procedure reported by Zhang et al., (2012) DNA was extracted from MRSA strains as follows: MRSA plate cultures that were freshly grown overnight were necessary [15]. The day before PCR, a single colony of MRSA was chosen using a sterile culture stick, and a thick stripe of the bacteria was formed on a Mannitol salt agar plate and incubated for 18 hours at 37 °C.

A 1.5 ml micro-centrifuge tube was filled with around 75 μl of sterile, distilled water. A small number of bacteria from the dense overnight streak was collected using a sterile culture stick. To create a cloudy solution, the bacteria were stirred in sterile
water. Every sample underwent this procedure.

To lyse the bacteria and liberate its nucleic acids, the tubes were heated in a dry heat block for 10 min at 95 °C. The samples were then taken out of the heat block and let to cool for five minutes. The samples were then centrifuged at 13,000 rpm for 1 minute to produce a clear supernatant that included nucleic acids and a pellet of cellular debris. For later usage, the DNA (clear supernatant) was kept in storage at -20 °C [16].

**Detection of Pvl gene**

The pvl gene products (LukS and LukF, respectively) were then amplified by PCR using a 433-bp overlap-region fragment of genomic DNA the primer sets that were used to find the pvl genes [17]. The following conditions were used for the amplifications: pre-denaturation for 2 minutes at 94°, then 25 cycles of 94° for 15 seconds, 55° for 15 seconds, and 72° for 15 seconds. and a final extension phase lasting five minutes at 72 °C [18]. The samples were examined on a 2% agarose gel stained with ethidium bromide in 0.5x TBE buffer, and the DNA bands were visualized using a UV transilluminator.

**3. Results**

By phenotypic methods using a disc diffusion approach with a 30 μg Cefoxitin disc and ORSAB medium (Figure 1), 41 MRSA (37.6%) isolates were detected from 109 S. aureus isolated and identified from SSTIs.

![Figure 1: MRSA Identification by using ORSAB.](image_url)
In the current study, resistance rates were the highest with Cefoxitin (100%), Amoxicillin/Clavulanic Acid (100%), Cefotaxime (100%), and also (100%) with Cefepime, while the lowest resistance was with Ciprofloxacin (12.2%), Sulphamethoxazole/Trimethoprim (12.2%), Imipenem (14.6%), and Linezolid (24.4%) (Figure 2; Table 1).

**Figure 2:** Antibiotic susceptibility among MRSA.

**Table 1:** Antibiotic susceptibility among MRSA.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Resistance</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin (FOX)</td>
<td>41 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Erythromycin (E)</td>
<td>9 (22%)</td>
<td>22 (53.7%)</td>
<td>10 (24.4%)</td>
</tr>
<tr>
<td>Tetracycline (TE)</td>
<td>20 (48.8%)</td>
<td>7 (17.1%)</td>
<td>14 (34.1%)</td>
</tr>
<tr>
<td>Gentamycin (CN)</td>
<td>10 (24.4%)</td>
<td>19 (46.3%)</td>
<td>12 (29.3%)</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>26 (63.4%)</td>
<td>3 (7.3%)</td>
<td>12 (29.3%)</td>
</tr>
<tr>
<td>Sulphamethoxazole/Trimethoprim (SXT)</td>
<td>5 (12.2%)</td>
<td>20 (48.8%)</td>
<td>16 (39%)</td>
</tr>
<tr>
<td>Linezolid (LNZ)</td>
<td>10 (24.4%)</td>
<td>0 (0%)</td>
<td>31 (75.6%)</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic acid (AMC)</td>
<td>41 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Imipenem (IPM)</td>
<td>6 (14.6%)</td>
<td>2 (4.9%)</td>
<td>33 (80.5%)</td>
</tr>
<tr>
<td>Cefotaxime (CTX)</td>
<td>41 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cefepime (FEP)</td>
<td>41 (100%)</td>
<td>0 (0%)</td>
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</tbody>
</table>
Almost all CA-MRSA (97.5%) were PVL-positive (Figure 3), with an amplicon size of 433 bp.

**Figure 3:** PVL gene of MRSA. All samples (15 samples) are positive (band at 433).

### 4. Discussion

The incidence of CA-MRSA increased in the early years of this century [19]. MRSA is found in SSTIs due to the exposure of deep tissues to the pathogen [20]. The objective of this research was to analyse the incidence of MRSA among *S. aureus* strains isolated from SSTIs and determine the incidence of the PVL gene within the community-acquired MRSA isolates.

In the present research, resistance rates were the highest with cefoxitin (100%), amoxicillin/clavulanic acid (100%), cefotaxime (100%), and cefepime (100%), while the lowest resistance was with ciprofloxacin (12.2%), sulphamethoxazole/trimethoprim (12.2%), imipenem (14.6%), and linezolid (24.4%). This disagrees with the results of Zuma et al. (2017), who found the resistance rates for Ciprofloxacin were high (60.6%) and 78.7% for Erythromycin, while the lowest was observed with Gentamycin (16.4%), Tetracycline, and Linezolid (4.9%) [21]. The resistance rate was low for Sulphamethoxazole/Trimethoprim in the current study (12.2%); in the study by Zuma et al. (2017), the resistance rate was 11.5% [21].

The PVL, which is a powerful serious cytotoxin and essential virulence factor of *S. aureus*, was first reported in 1932. This toxin can disrupt leukocyte membranes, cause marked tissue necrosis, and lead to enhanced virulence [22]. PVL-producing MRSA can cause mild SSTIs, but necrotizing pneumonia and severe cases of sepsis were also
documented [23]. CA-MRSA containing the PVL gene has spread worldwide, with various incidences all over the world [24].

PVL is present in most CA-MRSA and is rarely isolated from HA-MRSA [25]. In the current research, we found the PVL gene in 97.5% of the studied CA-MRSA. This agrees with Bhatta et al. (2016) who considered the PVL gene an important marker of community-acquired strains, as they found 75 PVL-positive out of 83 CA-MRSA isolates with a positive predictive value of 95% [25]. Our findings were much lower than those of Moghadam et al. (2017), where PVL-positive MRSA strains were 52.94% [26]. 20% of S. aureus isolates from SSTIs were PVL-positive, according to data from the United Kingdom [27]. This is a worrying indicator, as the prevalence rate of PVL-positive S. aureus has increased from less than 2% [28].

Conclusion

PVL-positive MRSA is a rapidly emerging phenomenon, as we found the PVL gene in 98% of the studied CA-MRSA isolated from SSTIs.

Ethical approval and consent to participate: The study protocol was approved by the Research Ethics Committee, Faculty of Medicine at Fayoum University, Egypt, number 16 (Code: D68). Adequate precautions have been taken to safeguard data confidentiality during data collection, storage, analysis, and dispensation.

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Conflicts of Interest: All authors declare no conflict of interest.

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