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RESEARCH ARTICLE

Investigation of Panton-Valentine leukocidin gene among Methicillin resistance for *Staphylococcus haemolyticus* isolates from Cesarean section infections in Al-Basrah governate, Iraq

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ABSTRACT

Background: The methicillin-resistant (MR) *staphylococci* are mainly characterized via the presence of the *mecA* gene that encodes the production of a low-affinity Penicillin-binding protein (PBP) known as(PBP2a). the *pvl* as a virulence factor of the synergy-menotropic venom virulence. The *pvl* is a part of family of homeotropic toxin that is composed of two various components and works together on the cellular membrane synergistically.

Methods: One hundred and fifty swab samples were collected from women who had cesarean sections at Al-Basrah Teaching Hospital between October 2022 to January 2023. The Vitek®2 system test revealed for identifying growth. Genomic DNA was extracted from *S.haemolyticus* isolates according to Geneaid kit protocol, the extraction DNA was amplified by using PCR for *mecA* gene detection using a specific primer approximately (533bp) and amplified the *pvl* gene detection using detection using specific primer approximately (433bp).

Results: From October 2022 to January 2023, one hundred and fifty swab samples were collected. The samples showed 57 (38%) positive bacterial growth, distributed 51(89.5%) Gram-positive bacterial isolates, while 6(10.5%) gram-negative bacterial isolates, by Using the Vitek® 2 system, various bacterial species were identified. Out of 34 Gram -positive bacteria isolates, the most predominate *Staphylococcus hemolyticus* 28(82.4%). while the Gram negative bacteria isolates included *Klebsiella spp* 4(66.7%) isolates, , *Escherichia coli* 2 (33.3%) isolates. out of (n = 34) isolates *Staphylococcus spp.* were distributed to 28 (82%) *S.haemolyticus* and 6 (18%) *S.aureus* isolates were gave positive results for detection of the *mec A* gene. While out of (n = 34) isolates were divided into 25 (74%) *Staphylococcus spp* isolates gave a positive result for the detection of the *pvl* gene, while the 9 (26%) isolates were shown negative results for the detection of the *pvl* gene

Conclusions: Most isolates of *S.haemolyticus* and *S.aureus* were producing Methicillin resistance *mecA* gene and Panton-Valentine leukocidin *(pvl)* gene

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INTRODUCTION

The methicillin-resistant (MR) *staphylococci* are mainly characterized via the presence of the *mecA* gene that encodes the production of a low-affinity Penicillin-binding protein (PBP) known as(PBP2a).[1] The *mecA* gene in charge of methicillin resistance, was the first determined in the *S. aureus*, hence many of the *staphylococcal* species were gated to also anchorage.[2] Unfortunately, many methicillin isolates are associated with multi-rug resistance (MDR)to at minimum three antibiotic classes.[3]

In the year 1932, Panton and Valentine described the *pvl* as a virulence factor of the synergy-menotropic venom virulence.[4] *The Panton-valentine leukocidin (pvl)* is a part of the family of hometropic toxins that are composed of two various components and work together on the cellular membrane synergistically. *Luk S-PV* and *Luk F-PV.* are excreted by *S. aureus* previously generating a pore-forming heptamer on polymorph nuclear (PMN) membranes and interacting *pvl* protein with complement receptors on the membranous surfaces of these leukocyte cells.[5,6]

The *pvl* can be caused by invasion inflammation like necrotizing pneumonia. The *pvl* is transferred by methicillin-resistant *S.aureus* (MRSA) and community-associated methicillin-susceptible (CA-MSSA).[7] The *pvl* it is an important pathogenic infection factor, that toxin causes neutrophils to produce proinflammatory cytokines.[5,6] The purpose of this study was to detect pvl gene in methicillin resistance *Staphylococcus hemolyticus* isolates from cesarean section infections from Al-Basrah governorate, Iraq.

MATERIALS AND METHODS

Collection of specimens

One hundred and fifty swab samples were collected from women who underwent cesarean section for both emergency cesarean delivery, and elective cesarean delivery during the period from October 2022 to January- 2023 in Al-Basrah Teaching Hospital were selected depending on their medical history.

Isolation and identification

The samples were collected from patients who underwent cesarean section. The swab samples were cultivated, and the samples that gave positive results for bacterial growth were identified by Vitek® 2 system.

Table 3: Percentage of bacterial species isolated from cesarean section infections.

Total number of isolates	*Gram +ve*	*Gram-ve*	*percentage Total*
57	51(89.5%)*	6(10.5%)	(100%)

The mecA gene Detection.

According to Pournajaf, A. et al.[8], primers utilized for the amplification of *mecA* gene were mentioned in Table 1.

The pvl gene detection

A paired primer was employed for *pvl* amplification was chosen according to[9], (Table 2).

RESULTS

From October 2022 to January 2023, 150 swab samples collected from cesarean section patients at Al-Basrah Teaching Hospital showed 57(38%) positive bacterial growth, 93(62%) negative for bacterial growth, and 51(89.5%) Gram-positive bacterial isolates, while 6(10.5%) Gram-negative bacterial isolates. As shown in table 3.

Identification of bacterial growth by using Vitek® 2 system has emerged various bacterial species, the most predominant were *Staphylococcus hemolyticus*, out of 57 bacterial isolates the 39 (68.42%) isolates, *Staphylococcus aureus* 6(10.53%), *Klebsiella spp* 4(7.00%) isolates, *Staphylococcus saprophyticus* 3 (5.30%) isolates, *Escherichia coli* 2 (3.50%) isolates, *Staphylococcus sciuri* 1(1.75%.) isolate, *Staphylococcus hominis* 1(1.75%) isolate, and *Enterococcus faecium* 1 (1.75%) isolate, as shown in Figure 1.

The results in the present study were emerged that all (n = 34) *Staphylococcus spp.* were distributed to 28 (82%)

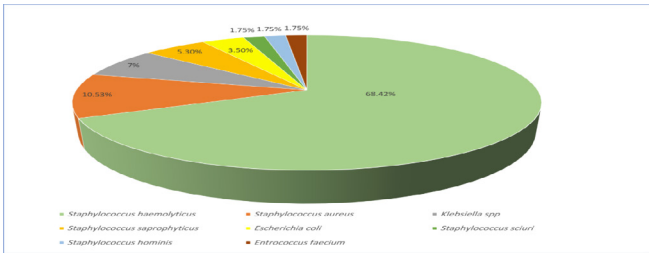

Figure 1: Frequency of bacterial species isolated from cesarean section infections.

Table 1: Specific primers of the *mecA* gene used in PCR.

primers	*Sequence*	*length*	*Size bp*	*Optimizing Ta**
mecA-F	5'-AAAATCGATGGTAAAGGTTGGC-3'	22	533	53°C
mecA-R	5'-AGTTCTGGAGTACCGGATTTGC-3'	22		53°C

*Ta: Annealing temperature.

Table 2: Primers used to amplify of *PVL* gene.

primers	*Sequence*	*length*	*Size bp*	*Optimizing Ta**
lukS-PV	5'-ATCATTAGGTAAAATGTCTGGACATGATCC A-3'	31	433	55 ºC
lukF-PV	5'-GCATCAACTGTATTGGATAGCAAAAGC-3'	27		55 ºC

*Ta: Annealing temperature.

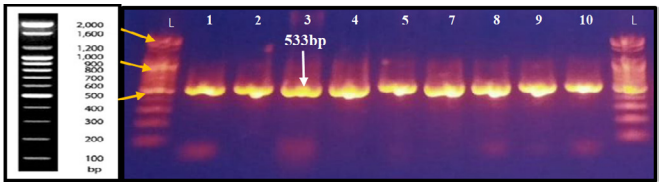

Figure 2: Agarose electrophoresis patterns of *mac A* gene. PCR amplified products, (533bp). band size of bacterial isolates lane (no-1-10) Ladder (100-2000bp), using 1.5% agarose gel, 72V, 45 minutes.

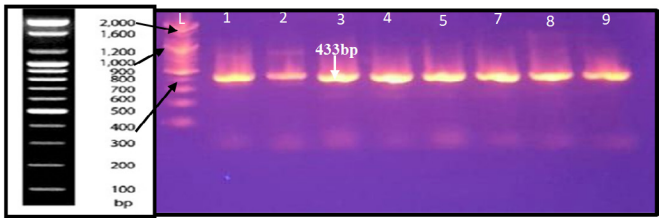

Figure 3: Agarose electrophoresis patterns of PVL gene. PCR amplified products, (433bp). band size of bacterial isolates lane (no-1-9) Ladder (100-2000bp), using 1.5% agarose gel, 72V, 45 minutes.

S.haemolyticus and 6 (18%) *S.aureus* isolates were gave positive results for detection of the *mec A* gene by using PCR technique Figure 2.

While results showed that out of (n = 34) isolates were divided in to 25 (74%) *Staphylococcus spp.* isolates were gave a positive result for the detection of the *pvl* gene, while the 9 (26%) isolates were shown negative results for the detection of the *pvl* gene Figure 3.

DISCUSSION

The study found that Gram-positive bacteria isolates (89.5%) had a higher prevalence than Gram-negative bacteria (10.5%), contradicting previous studies that found a higher prevalence of gram-negative bacteria (63.6%) and gram-positive bacteria (36.4%),[10] these results are not compatible with my study. Whereas, another study's results showed that (68.4%) isolates Gram-negative and (31.6%) gram-positive.[11] This variation could be attributed to environmental factors, geographical location, education level, and patients who took antibiotics before samples.[12-14] Factors such as hormonal changes, depression, menopause, hospital-acquired infections, immunocompromised patients, and long hospital stays can also contribute to the increased risk of infections.[15-17] Antibiotic resistance genes are also a significant factor.[18-20]

The Vitek® 2 system is an automated instrument designed for rapid and accurate identification of most staphylococci in clinical specimens.[15-17] It has been proven effective in detecting Gram-positive cocci and Gram-negative rods, with 99% accuracy and reproducibility confirmed by multiple independent studies.[21] The Colorimetric Vitek® 2 GP card is suitable for clinical samples and has been praised for its performance in detecting gram-positive and Gram-negative rods.[22]

The results of the detection *mecA* gene in the present study showed that all 34(100%) *Staphylococcus spp.* isolates were given positive results for the detection of the *mec A* gene by using the PCR technique. A result of the use of PCR techniques to detect MRSA isolates and decreased conflict for each clinical microbiology laboratory, and necessity the achievement of crucial, active examination procedures. However, the molecular program can promote the identification of community-acquired, and nosocomial resistance *S. aureus* extremely. The other study by[23] reported that in the laboratory culture, antibiotics sensitivity testing (AST) using Oxacillin, methicillin, and Cefoxitin scanner disk diffusion (DD) are the most extensively utilized protocols in vitro for rapidly diagnostic *Staphylococcal* resistance to methicillin. Methicillin-resistant *Staphylococcus aureus* (MRSA) genes encode penicillin-binding protein 2a (PBP2a), the enzyme important for connecting the cell wall of bacteria with peptidoglycan. The PBP2a has less affinity for beta-lactamase, as a result of which leads to resistance to the whole class of antibiograms.[24]

Furthermore, PBP2a, which is producing *mec A* gene alleles. MRSA infections more expanded at a dangerous rate in modern years. MRSA infections have become a greater health concern around the world. MRSA infections are probably to increase.[25] According to our data, and current study, we found that all isolates of MRSA have *bla Z* and *pvl* genes, and that is evidence their active role as a virulence factor for these pathogenic organisms. These bacteria are considered critical pathogens in hospitals, and that are effective treatment becomes difficult.[9]

In the current study the results showed that out of (n = 34) isolates were distributed to *S.haemolyticus* 28 (82%) and *S.aureus* 6 (18%), were divided into 25 (74%) *Staphylococcus spp* isolates were given positive results for detection of the *pvl* gene, while the 9 (26%) isolates were shown negative result for the detection of the *pvl* gene. the *pvl gene* is a one of virulence factor, also a two-component toxin that stimulates the development of a hole in leukocyte of the cell membrane extra receptors. They are two genes (*LukS-PV* and *LukF-PV*), that encode two proteins independently co-transcribed and excreted to generate a complete heptavalent leukcidin.[26,27]

However, the *pvl* gene is a virulence factor that developed by some strains and cause the necrosis of leukocyte lysis a tissues, associated with S. aurous specifically stimulate infections of the skin and soft tissues. Although, can cause infectious inflammation like necrotizing *pneumonia*. Furthermore, the possible risk of spreading in hospitals and considered a major public health problem [27,7], but the *pvl* gene disseminated is low common in MRSA isolates than in MRSA, that is locus was represented as a marker of a stable genetic of community-acquired MRSA (CA-MRSA) strains, so that the toxin may be strongly associated to epidemiological marker for CA-MRSA strains.[28]

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