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Original Article

Detection of Panton-Valentine leukocidin and *MecA* Genes in *Staphylococcus aureus* isolated from Iraqi Patients

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Abstract

A gram-positive bacterium, *Staphylococcus aureus*, which is widely distributed is considered as a bacterial infection that commonly infects the skin and mucous membranes. Such infections can be the cause of death and illness. In the present study by using reverse transcription-polymerase chain reaction (rt-PCR) the Panton-*Valentine leukocidin* (PVL) and *mecA* genes of *S. aureus* which were isolated from skin and soft tissue infections (SSTIs) in Baghdad, Iraq were investigated. This study included 96 *S. aureus* isolated from SSTIs and identified by Vitek. The results showed that 61 (63.5%) and 48 (50%) of the isolates were positive for PVL and *mecA* genes, respectively. This work presented an effective real-time PCR technique for detecting PVL genes alone or in conjunction with *mecA*. The rt-PCR allows for easier reaction monitoring and eliminates the need for post-PCR processing, saving both resources and time. Moreover, it is ideal for diagnostic applications because of its high sensitivity, simplicity, and specificity. Besides, the rt-PCR has an option to do all the procedures in an automated mode of action.

Keywords: *MeC A* gene, *PVL* gene, Real-Time PCR, *Staphylococcus aureus*

1. Introduction

Staphylococcus aureus is a prominent human pathogen that causes infections in both hospitals and the general public around the world. It leads to infections with various degrees of severity, ranging from simple illnesses to life-threatening situations. The *S. aureus* has the ability to live freely in the nonliving environment and transmission between individuals. On the other hand, *S. aureus* can hide in intracellular compartments. All in all, it is well documented that the S. aureus can induce various forms of human disease (1). Infections caused by *S. aureus*, above all by antibiotic-resistant strains, have reached epidemic proportions globally. The most prevalent clinical manifestation form of *S. aureus* is skin and soft tissue infection (SSTI) (2). The acquisition from the *mecA* gene encodes the transpeptidase penicillin-binding protein 2awhich is a molecular characteristic for methicillin-resistant *S. aureus* (MRSA) strains (3). The *mecA* gene is found on the staphylococcal cassette chromosome *mecA*, a movable genetic element that has been described in at least 13 distinct kinds (4). New *mecA* homologues, *mecB, C, and D*, have recently been discovered to confer ß-lactam antibiotic resistance (5-7).

In diverse populations around the globe the outbreaks of MRSA usually involved skin disease. Nevertheless, MRSA can cause acute and in some cases fatal invasive illnesses (2). Over the past decade, several investigations on the emergence of community-associated MRSA disease have been done and identified that isolates causing community-associated

and healthcare-associated MRSA infections were distinct from each other. It is revealed that the staphylococcal which carried type IV cassette chromosome isolated from the community were susceptible to most non–β-lactam antimicrobial agents, frequently encoded the dermonecroticcytotoxin which is known as Panton-Valentine leukocidin is encoded by the staphylococcal type IV cassette chromosome (2).

Rapid accurate differentiation between MRSA and methicillin-susceptible staphylococci which is obtained from patient blood samples provides data for proper medical decisions regarding antimicrobial therapy. This rapid and accurate discrimination has a pivotal role in the prevention of deaths resulting from infection. It is well documented that for the bacterial diagnosis in the clinical samples practitioners have to apply a bacterial culture. By the way, the bacterial culture has some disadvantages such as limitation in the detection speed and sensitivity (5).

Blood samples are generally cultured and incubated for a period of 5 days. This takes place until the positive signals in continuous monitoring blood culture systems are detected. A disadvantage of blood cultures would be the false-negative results. This may happened in different conditions as follows: 1) in case of the presence of fastidious or slowly growing bacteria, and 2) in samples which are obtained from patients who are treated with antimicrobial agents (6). In patients with systemic infection early diagnosis and proper antimicrobial therapy played a pivotal role to cure patients with systemic infections.

Leukocidins are virulence factors with two parts and pyrogenic super-antigenic poisons that can disrupt host cell membranes and influence immunological responses by activating immune cells (8). The *PVL* is one of seven leukocidins produced by these bacteria. The *PVL*, a custom composed of two proteins named F, has 32KDa and S protein 38 KDa; these proteins are controlled by Lukf/PV genes (9).

It is approved that the real-time polymerase chain reaction (rt-PCR) is dramatically faster than conventional PCR and other diagnostic methods. Due to its high sensitivity, high specificity, very low risk of contamination, and simplicity the rt-PCR has considered as an appealing method in clinical microbiology laboratories (8). In the current research, a multiplex rt-PCR assay using specific probes for *S. aureus* and a methicillin resistance gene detection was used for the rapid and accurate discrimination of MRSA, methicillin-susceptible *S. aureus*. Accurate data is needed on the MRSA infections in the Iraqi population. Therefore, the current research was designed to investigate the PVL and mecA genes of *S. aureus* isolated from SSTIs in Baghdad, Iraq by rt-PCR.

2. Material and Methods

From June 2020 to January 2021, 96 isolates of *S. aureus* were isolated from the pus of subjects suffering from cutaneous infections and chronic ulcers or wounds. The subjects were selected from the patients who visited Al-Yarmook teaching hospital in Baghdad, Iraq for medical consultations. Vitek-60 microbiology machine (Biomerieux, USA) was used to confirm the *S. aureus*, and bacterial isolates were incubated for 18-24 h at 37 °C after the cultivation on blood agar.

2.1. DNA Extraction

The DNA was extracted from all *S. aureus* by a commercial kit (Zymo, USA) and stored at -20 C. Primers to detect the genes were mentioned in a previous study byRoberts, O'Shea (10). The cycling program was mentioned in the previous paper which is as follows: it started by pre-denaturation at 98 °C for 6 min, and then 30 cycles from the first step at 95 °C for 15 sec, 55 °C for 5sec, and finally at 72 °C for 10 sec.

2.2. Real-Time Polymerase Chain Reaction Assay

The Real-MRCoNS multiplex rt-PCR and Real-MRSA assay kits (M&D, Wonju, Republic of Korea) were used for the completion of the multiplex rt-PCR TaqMan assay. The rt-PCR amplification was performed in a total volume of 20 µl containing 10 µl of 2× Thunderbird probe quantitative PCR mixture (Toyobo, Osaka, Japan), 5.0 µl of primer and TaqMan

probe mixture, and 5 µl of template DNA.Eventually, distilled water was added for a final volume of 20 µl.

3. Results and Discussion

The resulted amplification curves of RT-PCR, are shown in figure 1, and each curve represents the amplification of a single *PVL* gene. The results showed that 61 isolates were positive for the *PVL* gene which indicated that those samples possess the *PVL* toxin. The *mecA* gene amplification are illustrated in figure 2 and showed 48 curves. This findings have implied that those samples are possess this gene. Molecular assays, including the *mecA* gene, are now regarded as standard methods for detecting the genes and discriminating *S. aureus* from other species using PCR (11). According to Perez, Dias (12), the PCR methodology is now utilized as a gold standard approach for evaluating other traditional procedures.

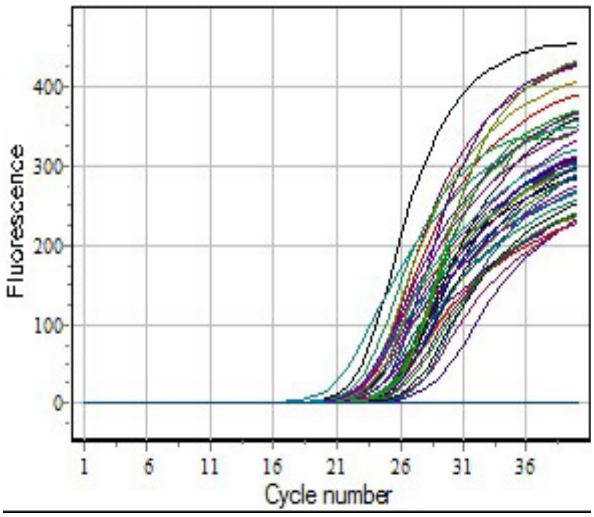

Figure 1. Results of quantitative reverse transcription-polymerase chain reaction amplification of Panton-Valentine leukocidin gene.

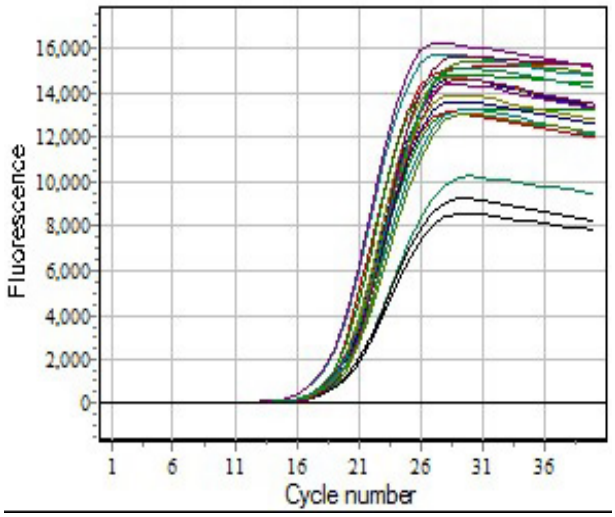

Figure 2. Resultsof quantitative reverse transcription polymerase chain reaction amplification of the *mecA* gene.

Due to its high virulence and pathogenicity, *S. aureus* carrying the *PVL* gene has global worldwide interest. It can cause infections in the skin and soft tissues as well as life-threatening diseases, such as hemorrhagic pneumonia. Young people who have no prior risk factors are also at high risk of infections which contributes to a high rate of morbidity and mortality (13). In 2009, researchers in Iran discovered that the *PVL* gene was present in 41.67% and 58.3% of women and men, respectively (14). Based on the results of a study performed in India, the prevalence rate of the *PVL* gene was shown to be high in children under the age of 14 (15).

The *PVLS. aureus* has a positive relationship with SSTIs; in fact, most investigations have found that the frequency of the *PVL* gene is higher in pus specimens collected from SSTIs, compared to blood, urine, or sputum samples (16). According to the results of a study conducted by Akram, Izhar (17), 186 (49%) out of 384 *S. aureus* isolates tested positive for the *PVL* gene. Moreover, they found that 44.9% and 53.5% of the positive isolates were collected from male and female subjects, respectively. The *PVL* gene was found in the highest frequency in the pediatric age group.

In the present study, TaqMan real-time PCR was used to investigate a series of samples to detect isolates *that* tested positive for *PVL* and *mecA* genes *S. aureus*. Based on the results, the *PVL* and *mecA* genes were found in 63.5% and 50% of the isolates, respectively. The findings of this study are inconsistent with those of a study conducted by Shariati, Validi (18) which indicated that 10.7% of isolates contained the *PVL*-gene. Their finding is consistent with those of the

studies conducted in other regions of the world (16, 19).

4. Conclusion

The RT-PCR allows for easier reaction monitoring and eliminates the need for post-PCR processing, saving both resources and time. Moreover, it is ideal for diagnostic applications as they are simple to perform, have high sensitivity and specificity, and can be automated.

Authors' Contribution

Study concept and design: R. N. H.
Acquisition of data: S. A. K. J.
Analysis and interpretation of data: R. N. H.
Drafting of the manuscript: S. A. K. J.
Critical revision of the manuscript for important intellectual content: S. A. K. J.
Statistical analysis: R. N. H.
Administrative, technical, and material support: R. N. H. and S. A. K. J.

Ethics

All investigations were conducted in accordance with the Ethics Committee of Al-Maarif University College, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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