Detection of PVL and mecA Genes in *Staphylococcus aureus* isolated from Iraqi Patients

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Abstract

The bacterial infection *Staphylococcus aureus* considered as Gram-positive, and widely distributed bacterium that commonly infect the skin and mucous membranes and those infections would be a cause of death and illness. The present study was aimed to detect the PVL and mecA genes of *S. aureus* isolated from SSTIs in Baghdad, Iraq by RT-PCR. This study included 96 *S. aureus* isolates from SSTIs and identified by Vitek. The results showed 61 (63.5%) isolates were positive for PVL gene, and for the mecA gene showed 48 (50%). This work presented an effective real-time PCR technique for detecting PVL genes alone or in conjunction with mecA. RT-PCR allows for easier reaction monitoring and eliminates the need for post-PCR processing, saving both resources and time, also it is ideal for diagnostic applications because its simplicity, high sensitivity and specificity. On the other hand it has an option to do all the procedure in an automated mode of action.

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

1. Introduction

*Staphylococcus aureus* is a prominent human pathogen that causes infections in both hospitals and the general public around the world, on the other hand, produces infections with various severity, ranging from simple illnesses to life-threatening situations. *Staphylococcus aureus* capable of living freely in the inanimate environment and spreading from person to person, existing as a colonizer or commensal, hiding in intracellular compartments and, most importantly, inducing 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 from of *S. aureus* is skin and soft tissue infections (SSTIs) (2). The acquisition from the *mecA* gene, which encodes the transpeptidase penicillin-binding protein 2a which 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 -lactam antibiotic resistance (5-7).

Although community outbreaks of MRSA in diverse populations, including American Indian and Alaska Natives, sports teams, prison inmates, and child care attendees, usually involved skin disease, MRSA also can cause severe, sometimes fatal invasive disease (2). Studies of the emergence of community-associated MRSA disease over the past decade determined that isolates causing community-associated and health care–associated MRSA infections were distinct. Isolates from the community were susceptible to most non–β-lactam antimicrobial agents, carried staphylococcal cassette chromosome type IV, and frequently encoded the dermonecrotic cytotoxin known as Panton-Valentine leukocidin (2).

Fast accurate discrimination of MRSA from methicillin-susceptible staphylococci directly from patient blood samples provides data for proper medical decisions regarding antimicrobial therapy, which plays an important role in the reduction of deaths resulting from sepsis. Currently, bacterial culture is required as a standard method for diagnosis of the presence of bacterial pathogens in clinical samples. However, this technique has some disadvantages with regard to desired detection speed and sensitivity (5). Generally, blood culture samples are incubated for 5 days until they show positive signals in continuous monitoring blood culture systems (CMBCSs). Moreover, blood cultures may lead to false-negative results when fastidious or slowly growing bacteria are involved or when samples are obtained after antimicrobial therapy has been started (6). The early diagnosis and adequate treatment of bacterial infections have great impacts on the outcomes for 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). Pantone-valentine Leuko-cidin (PVL) is one of seven leukocidins produced by these bacteria. PVL, a custom composed of two proteins named F which has 32KDa and S protein 38 KDa and those proteins controlled by Lukf/PV genes (9).

Real-time PCR is significantly faster than conventional PCR and other detection methods. The combination of excellent sensitivity and specificity, low contamination risk, ease of performance, and speed has made real-time PCR technology appealing to clinical microbiology laboratories (8). In this study, a multiplex real-time PCR assay using probes specific for S. aureus and a methicillin resistance gene was tested for the rapid detection and identification of MRSA, methicillin-susceptible S. aureus (MSSA). Accurate information on the scope and magnitude of MRSA infections in the Iraqi population is needed. Therefore, current study was designed to detect the PVL and mecA genes of S. aureus isolated from SSTIs in Baghdad, Iraq by RT-PCR.

2. Materials and Method

From June 2020 to January 2021, 96 isolates of S. aureus have been isolated from pus of subjects suffering from cutaneous infections, chronic ulcer or wounds, those patients have been collected while they were visiting Al-Yarmook teaching hospital in Baghdad, Iraq for medical consultant. Vitek-60 microbiology machine have been used to confirm the S. aureus, and bacterial isolates were incubated for 18 to 24 hours at 37 °C after the cultivation on blood agar.

2.1. DNA extraction

The DNA was extracted from all S. aureus utilizing commercial kit (Zymo, USA) and then stored at -20 C. Primers to detect the genes were mentioned previously by Roberts, O'Shea (10). The cycling program have been mentioned in the previous paper and as follow: it started by pre-denaturation of 98 C for 6 min, and then 30 cycles from first step 95 C for 15 sec, 55 C for 5 sec and final step at 72 C for 10 sec.
2.2. Real-time PCR assay

The multiplex real-time PCR TaqMan assay was carried out with the Real-MRSA and Real-MRCoNS multiplex real-time PCR assay kits (M&D, Wonju, Republic of Korea) using the CFX-96 real-time PCR system (Bio-Rad, Hercules, CA), which was used for thermocycling and fluorescence detection. The real-time 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; distilled water (DW) was added for a final volume of 20 μl. The specific primers and probes used for the identification of Staphylococcus species and S. aureus and for detection of antibiotic resistance are listed in Table 1. The multiplex real-time PCR assay detected the 16S rRNA, nuc, and mecA genes simultaneously, in a single tube, by incorporating three target-specific TaqMan probes labeled with different fluorophores (Cy5, HEX, and FAM). To determine the assay specificity, primers and probes for detecting the 16S rRNA, nuc, and mecA genes were tested with 88 nonstaphylococcal reference strains, including 37 Gram-positive and 23 Gram-negative bacteria. The multiplex real-time PCR assay for detecting the 16S rRNA, nuc, and mecA genes yielded negative results with all nonstaphylococcal reference strains; thus, no cross-reactivity was detected.

Positive and negative controls were included throughout the procedure. No-template controls with sterile DW instead of template DNA were incorporated in each run under the following conditions: 95°C for 3 min and 35 cycles of 95°C for 20 s and 60°C for 40 s in a single real-time PCR assay. The bacterial load was quantified by determining the cycle threshold (CT), which is the number of PCR cycles required for the fluorescence to exceed a value significantly higher than the background fluorescence level.

Oligonucleotide primers to detect the PVL toxin genes (lukS-PV, lukF-PV) were designed from a conserved region of a previously published sequence (GenBank accession number, X72700 as described by Lina et al., 1999). The primers were designed with LightCycler Probe Design Software Version 1.0 (Idaho Technology Inc., 2001) and amplify a 258 bp region across the lukS/lukF gene junction. Primers are designed as follows: PVLSC-F, GCTCAGGAGATACAAG and PVLSC-R, GGATAGCAAAAGCAATG. Amplification
and detection was carried out on a SmartCyclerTM (Cepheid, Sunnyvale, CA) using a LightCycler1 FastStart DNA Master SYBR Green I kit (Roche Applied Bioscience, Indianapolis, IN). The cycling conditions were as follows: after an initial denaturation step of 98 °C for 5 min, samples completed 30 cycles of amplification (15 s of denaturation at 95 °C, 5 s of annealing at 55 °C and 10 s of extension at 72 °C). Since SYBR Green binds to any double stranded DNA, it was essential to perform a melt curve analysis directly following amplification (5 s at 95 °C, 15 s at 65 °C, then ramp from 65 to 95 °C at 0.1 °C/s).

Primers and probes used in this study are as follows:

<table>
<thead>
<tr>
<th>Target and primer/probe name</th>
<th>Nucleotide sequence (5’ to 3’) 16S rRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S-472F</td>
<td>AGTGATGGAAGGTCTTCGGATCGTAAA</td>
</tr>
<tr>
<td>16S-575R</td>
<td>CGTGGCTTTCTGATTAGGTACCGTC</td>
</tr>
<tr>
<td>16S-513P</td>
<td>GGAAGAACAAYGTTGTAAGTAACTRTGCACRT</td>
</tr>
<tr>
<td>mecA-1501F</td>
<td>GCTCAAATTTCAACAAAAAATTTAGATAATG</td>
</tr>
<tr>
<td>mecA-1598R</td>
<td>TGAAAGGATCTGACTGGTAAATCAGT</td>
</tr>
<tr>
<td>mecA-1542P</td>
<td>AGCTGATTACGGTTACGGAAGGTGA</td>
</tr>
</tbody>
</table>

3. Results and Discussion

The resulted amplification curves of RT-PCR, are shown in figure 1 each curve represent the amplification of single PVL gene. The results showed 61 isolates were positive for PVL gene which mean those samples possess the PVL toxin. And for the MecA gene
amplification are illustrated in figure 2 and showed 48 curves and also that mean those samples are possess this gene.

Figure 1. The result of RT qPCR amplification of *PVL* gene

Figure 2. The result of RT qPCR amplification of *mec A* gene

Molecular assays, including the *mecA* gene, are now regarded standard methods for detecting 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. Due to its high virulence and pathogenicity, *Staph*
*S. aureus* carrying the PVL gene has global worldwide interest. It can cause infections from the skin and soft tissues, also life-threatening diseases such haemorrhagic pneumonia. Young people who have no prior risk factors are also in high risk of infections, and it 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 percent of women's and 58.3 percent of men's (14). PVL gene was shown to be high in children under the age of 14 in an Indian study (15). PVL positively *S. aureus* has been linked to SSTIs; in point of fact, most investigations have found that PVL gene frequency is higher in pus specimens from SSTIs than in blood, urine, or sputum samples (16). According to Akram, Izhar (17) 186 of 384 *S. aureus* isolates tested positive for the PVL gene. The PVL gene was found in 49 percent of people. In *S. aureus*, the PVL gene was found in 44.9 percent of males and 53.5 percent of females. The PVL gene was found in the highest frequency in the paediatric age group. In this investigation, TaqMan real-time PCR was used to investigate a series of samples in order to detect PVL, and *Mec A* genes positive *S. aureus* isolates. The PVL-encoding gene were found (63.5%) , and (50%) of *Mec A* gene of isolates , according to the findings of this study disagree with study conducted by Shariati, Validi (18) found that (10.7%) of isolates contained the PVL- gene, which is consistent with studies conducted in other regions of the world (16, 19).

**Conclusions**

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

**Ethical approval**

All investigations were conducted in accordance with the ethical committee of Al-maarif University College.

**References**


