Molecular characterization of methicillin-resistant Staphylococcus aureus recovered from outpatient clinics in Riyadh, Saudi Arabia

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ABSTRACT

Objectives: To examine the recovered strains phenotypically, by conventional methods and genotypically by polymerase chain reaction (PCR), for direct detection of Staphylococcus aureus (S. aureus) 16S ribosomal Ribonucleic Acid (rRNA) gene (which serves as an internal control) and meca gene. Secondly, introduce multiplex PCR targeting at the same time S. aureus 16S rRNA, Panton-Valentine Leucocidin (PVL), and staphylococcal cassette chromosome mec (SCCmec) type IV.

Methods: Thirty-seven strains of S. aureus collected in 2007 from outpatient clinics in King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia, were tested in the College of Pharmacy phenotypically by conventional methods and genotypically by PCR for direct detection of S. aureus 16S rRNA and meca genes. All the 37 strains, were tested also by multiplex PCR targeting at the same time S. aureus 16S rRNA, PVL, and (SCCmec) type IV.

Results: Polymerase chain reaction detected all the 37 bacteriologically positive S. aureus (100%) and the meca gene in all strains phenotypically resistant to methicillin (100%), at the same time it detected the meca gene in 2 strains phenotypically sensitive to methicillin. Only 3 strains (8.1%) recovered from skin and soft tissue infections were positive for PVL and SCCmec type IV.

Conclusion: The PCR assay can be used for rapid detection of S. aureus and meca gene. At the same time the multiplex PCR assay explained in this study is a rapid, sensitive, and reliable test for direct detection of community-acquired methicillin-resistant S. aureus.


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Methicillin-resistant Staphylococcus aureus (MRSA) has been associated with nosocomial infections (hospital-acquired MRSA [HA-MRSA] strains). Despite its spread in hospitals and nursing homes, MRSA has not disseminated into the wider community until recently. Such infections not acquired at a health care setting or institution, are considered community associated MRSA (CA-MRSA), these organisms have recently emerged as an important cause of community-associated staphylococcal infections. Panton-Valentine Leucocidin (PVL), is a highly powerful cytotoxin that affects human and rabbit mononuclear cells. When injected intradermally into rabbits, it induces severe inflammatory lesions, leading to capillary dilation, chemotaxis, polymorphonuclear karyorrhexis, and skin necrosis.

Studies have shown that its toxic effect results from the synergistic action of 2 separate exoproteins, namely, LukS-PV and LukF-PV. These proteins are encoded by 2 contiguous and cotranscribed genes (LukS-PV and LukF-PV), which are carried on temperate bacteriophages. Lysogenic conversion of PVL - negative strains of Staphylococcus aureus (S. aureus) leading to the production of the toxin has been demonstrated. Several authors suggest characterization of the PVL gene in S. aureus isolated from humans, due to its involvement in severe disease conditions among children and humans even with no exposure to health care establishments. The presence of PVL in S. aureus appears to be associated with increased disease severity, ranging from cutaneous infection requiring surgical drainage to severe chronic osteomyelitis, and deadly necrotizing pneumonia. In the United States of America, outbreaks of severe skin infections have occurred in homosexual men, prison inmates, and schoolchildren, similarly, PVL-related skin infections have been reported in the gay community in The Netherlands and in schoolchildren in Switzerland. More recently, cases of community-acquired pneumonia due to PVL-positive S. aureus, have been reported in France, Sweden, The Netherlands, and the United Kingdom. The PVL genes have been identified as a stable marker of community-acquired MRSA strains worldwide, in addition to small mobile staphylococcal cassette chromosome mec (SCCmec) type IV or V genetic element, which harbors the methicillin resistance (mecA) gene, and which is more easily transferred to other strains of S. aureus than the large SCCmec types (type I to III) that are prevalent in HA-MRSA strains. In the future, screening for the PVL virulence factor in S. aureus may become a routine laboratory procedure; therefore, there are many conventional PCR methods for the detection of S. aureus 16S rRNA gene, PVL gene, mecA gene, and SCCmec type IV gene, which require the use of separate assays.

The purpose of this study was, firstly, characterization of the recovered strains phenotypically by conventional methods and genotypically by PCR for direct detection of the S. aureus 16S rRNA gene (which serves as an internal control) and the mecA gene, secondly, to introduce a multiplex PCR target at the same time S. aureus species specific 16S rRNA, (SCCmec) type IV and PVL genes.

Methods. The bacterial isolates used in this study, were 37 strains of S. aureus that were collected in 2007 from outpatient clinics in King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia, including 20 strains recovered from skin and soft tissue infection, 12 strains recovered from abscess, and 3 strains recovered from cellulites, while the other 2 strains were recovered from infected ulcers. All isolates were identified according to colonial and microscopical morphology, catalase and coagulase production, and novobiocin sensitivity. Antimicrobial susceptibility testing to a range of antimicrobial agents including methicillin, oxacillin, chloramphenicol, tetracycline, fusidic acid, gentamicin, erythromycin, ciprofloxacin, and vancomycin was carried out adopting the Kirby-Bauer disk diffusion method using Muller-Hinton broth and agar and antibiotics disks (Oxoid Limited, Hampshire, England), according to the recommendations of the Clinical Laboratory Standards Institute (CLSI) formally the National Committee for Clinical Laboratory Standards, (NCCLS), 2002. Deoxyribonucleic acid (DNA) was extracted from the bacteriologically positive strains. One milliliter of the bacterial suspension was transferred to a microcentrifuge tube with a capacity of 1.5 ml and the genomic DNA was extracted. An aliquot of 5 ml of the supernatant was used as template DNA in the PCR.

For amplification of 16S rRNA gene specific for S. aureus and mecA gene, 2 sets of a primer pairs were used, (TIB, Molbiol, Berlin, Germany) the first pair was (SauF 234: CGA TTC CCT TAG TAG CGG CG and SauR 1501: CCA ATC GCA CGC TTC GCC primers) S. aureus species specific primers, which can amplify 1267 base pair fragments and its annealing temperature is 70°C. The second pair was (MR1: GTG GAA TTG GCC AAT ACA GG and MR2 : TAG GTT CTG CAG TAC CGG AT primers), which can amplify 1399 base pair fragments specific for mecA gene and its annealing temperature is 58°C. Table 1 shows the specificity, nucleotide sequence, size of amplified fragments, and annealing temperature of the primers. All reactions were carried out separately in a final volume of 50µl in micro-amplification tubes (PCR tubes). The reaction mixtures consisted of 5µl of the extracted DNA template from the bacterial isolates, 5µl 10 x PCR buffer (75 mM Tris
Results. The antimicrobial susceptibility patterns of 35 out of 37 tested strains, were resistant to methicillin and oxacillin, which was confirmed by the presence of the mecA gene using the PCR, while the other 2 strains, which appeared methicillin/oxacillin sensitive phenotypically, were positive with PCR and harbored the mecA gene. Seventeen strains out of 37 tested strains (45.6%) were resistant to chloramphenicol, 14 strains (37.9%) were resistant to tetracycline, 11 strains (29.8%) were resistant to fusidic acid, 10 strains (27%) were resistant to gentamicin, and 8 strains (21.7%) were resistant to erythromycin. Only 2 strains (5.4%) were resistant to ciprofloxacin, while all isolates were susceptible to vancomycin. The amplification of 1267 base pair fragments specific for 16S rRNA of S. aureus using SauF234 and SauR1501 primers, revealed positive amplification of 1267 base fragments with all 37 isolates (100%) previously, identified phenotypically as S. aureus with bacteriological examination specific for 16S rRNA of S. aureus as shown in Figures 1a & 1b. For amplification of 1339 base pair fragments specific for the mecA gene using MR1 and MR2 primers, all the 35 strains (100%), which appeared methicillin/oxacillin resistant phenotypically with antimicrobial susceptibility test were positive for amplification of 1339 base pair fragments specific for the mecA gene.
At the same time, the other 2 strains, which appeared methicillin/oxacillin sensitive phenotypically were positive with PCR and harbored the mecA gene as shown in Figures 2a & 2b. Furthermore, Multiplex PCR for detection of S. aureus species-specific 16S rRNA, (SCCmec) type Iva and PVL genes were performed. All the 37 strains (100%) previously identified phenotypically as S. aureus with bacteriological examination were positive for amplification of 756 base fragments specific for 16S rRNA of S. aureus using Staph756F and Staph750R primers, while only 3 strains showed positive amplification of 433 and 450 base pair fragments specific for LukS/F-PV and SCCmec subtype Iva genes using Luk-PV-1 and Luk-PV-2 primers and SCCmec 4a1 and SCCmec 4a2 primers as shown in Figure 3. The 3 isolates harboring the PVL and SCCmec subtype Iva genes were isolated from skin and soft tissue infections.

**Discussion.** The conventional methods used for identification of S. aureus and MRSA isolates are time consuming and the reliability of these methods have been reported to be between 80-95%, while the alternative molecular techniques, mostly based on PCR for amplification of 16S rRNA specific for S. aureus and mecA gene specific for methicillin resistant

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**Figure 1** - Agarose gel electrophoresis showing positive amplification of 1267 base fragments specific for rRNA of S. aureus using SauF234 and SauR1501 primers lanes a) 1, 2, 3, 4, 6, 7, and 8. Lane 5 showing Hae III digest marker. b) 1, 2, 3 4, and 5. Lane 6 and 7 showing negative control while lane 8 showing 100 bp ladder. rRNA - ribosomal ribonucleic acid, S. aureus - Staphylococcus aureus, SauF - Staphylococcus aureus forward, SauR - Staphylococcus aureus reverse

**Figure 2** - Agarose gel electrophoresis showing positive amplification of 1267 base fragments specific for 16S rRNA of S. aureus using SauF234 and SauR1501 primers lanes 2, 3, and 4 and positive amplification of 1339 base pair fragments specific for mecA gene using a) MR1 and MR2 primers lane 5, 6, and 7. Lane 8 showing Hae III digest marker. b) MR1 and MR2 primers lane 5, 6, 7, and 8. Lane 4 showing 100 bp ladder. rRNA - ribosomal ribonucleic acid, S. aureus - Staphylococcus aureus, SauF - Staphylococcus aureus forward, SauR - Staphylococcus aureus reverse, MR - methicillin resistant
have been reported for the rapid and specific detection and characterization of MRSA. Therefore, our study limits for 2 main objectives; Firstly, the use of PCR for rapid and specific detection of 16S rRNA specific for \textit{S. aureus} and the \textit{mecA} gene specific for methicillin/oxacillin resistant. Secondly, the use of a multiplex PCR for detection of 433 and 450 base pair fragments specific for \textit{mecA} gene while lanes 6, 7, and 8 showing amplification of 1267 base fragments specific for 16S rRNA of \textit{S. aureus} using SauF234 and SauR1501 primers. Lane 4 showing HaeIII digestion marker. rRNA - ribosomal ribonucleic acid, \textit{S. aureus} - \textit{Staphylococcus aureus}, SCC\textit{mec} - \textit{Staphylococcal cassette chromosome methicillin}, SauF - \textit{Staphylococcus aureus} forward, SauR - \textit{Staphylococcus aureus} reverse.

Nevertheless, results observed in Figure 3 revealed positive amplification of 756 base fragments specific for 16S rRNA of \textit{S. aureus} with all 37 strains (100%), but only 3 strains (8.1%) out of 37 tested strains showed positive amplification of 433 and 450 base pair fragments specific for \textit{lukS/F-PV} and SCC\textit{mec} subtype IVa genes, such strains could be identified as CA-MRSA as they are harboring the marker genes (PVL and SCC\textit{mec} type IV) of CA-MRSA\textit{v},\textit{w} as shown in Figure 3. The percent of CA-MRSA obtained in this study indicated that there is an increase in the number of patients with CA-MRSA in KSA, which confirm the conclusion of Bukhari et al.\text{i} They recorded that the number of patients with CA-MRSA disease increased from a single patient in 1998 to 15 patients in the year 2000, and they suggested that MRSA is an emerging community pathogen. It is also noticed that the 3 isolates that harbor the PVL gene were isolated from cases of skin and soft tissue infections, which indicated that most cases of CA-MRSA were recovered from skin and soft tissue infections, especially necrotizing skin infection.\text{j} However, the previously mentioned multiplex PCR represents a new tool for simple, rapid, and early reliable testing for detection of CA-MRSA strains.

Therefore, the results of this study indicate that the PCR assay can be used for rapid detection of \textit{S. aureus} and the \textit{mecA} gene and at the same time, the multiplex PCR assay explained in this study is rapid, sensitive, and a reliable test for direct detection of community-acquired MRSA.

References


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