Detection of Class I Integrons in Staphylococcus aureus Isolated From Clinical Samples

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1. Background

The role of staphylococcus aureus as a pathogen causing serious infections in humans has long been confirmed (1). S. aureus is considered as an opportunistic pathogen, especially in people who are immunosuppressed and in wounds caused by severe burns (2). S. aureus is the second most common cause of nosocomial infections after Escherichia coli, constituting about 20% of all nosocomial infections (3). In recent years, in despite of introducing a variety of antibiotics, this organism has caused severe infections in burns as well as in different parts of the body, due to acquired drug resistance. Widespread inappropriate use of antibiotics in treating bacterial infections has led to the selection and circulation of resistant strains and the growing risk of transferring resistant genes to sensitive bacteria. One of the causes of antibiotic resistance in S. aureus strains is the gain of resistance genes including integrase and qac/sul.

Objectives: The purpose of this study was to investigate the presence of class 1 integron in S. aureus isolated from clinical samples for the first time in Iran.

Materials and Methods: This descriptive study was performed on 200 strains of S. aureus isolated from patients admitted to Baqiyatallah Hospital in Tehran in 2013. These strains were confirmed using biochemical and serological tests and the presence of class 1 integron was determined by polymerase chain reaction (PCR).

Results: Among the 200 samples, 1% of the strains (two isolates) contained the class 1 integron gene. The results of this study showed that the highest frequency of the obtained samples belonged to males and the isolates occurred mostly in individuals aged 51-60 years old. The highest number of strains was found in wound samples. The strains were most frequently isolated from the emergency ward and the intensive care unit (ICU).

Conclusions: Findings of this study showed that integron can have a limited frequency in S. aureus isolated from clinical sample in Tehran.

Keywords: Staphylococcus aureus; Drug Resistance; Polymerase Chain Reaction
for resistance to quaternary ammonium compounds and disinfectants; 2) the sul1 gene, which codes for resistance to sulfonamides. 3) ORF5, which codes for a protein with unknown function (7).

Currently, five classes of integrons have been identified based on the heterogeneity of integrase proteins. These integrons can be located in integrated sequences (IS), plasmids capable of conjugation, transposons or chromosomes, and all can be used as a means of genetic material transfer between species (7). Since the discovery of antibiotics, their inappropriate prescription in bacterial infections has led to selection and spread of resistant strains of bacteria, turning them to a global problem (8-10). The main outcome of this wrong treatment strategy has been the replacement and development of some colonies from resistant strains of bacteria rather than sensitive strains (10). These strains can transfer the resistance factors to other strains and even sensitive species of bacteria and create an exponentially growing population of resistant bacteria. The presence of integron, found in bacterial species, has created a two-fold capacity to obtain the resistance factors. Strains containing integron cannot be determined by routine microbiological methods and their presence is therefore missed. The presence of integron in bacteria, whether lacking or possessing the gene cassette, is just the tip of the iceberg that potentially may create a catastrophic and uncontrollable spread of such resistant bacteria (11).

Screening for integrons in bacterial strains and its relationship with the severity and extent of antibiotic resistance can be helpful in management of infection. In addition, this study provided national and international information about the extent and frequency of integron sequences among strains of S. aureus in Iran. Simultaneous occurrence of multiple drug resistance in bacteria has been associated with integron class 1. No report has been provided on these important genetic elements in S. aureus in Iran.

2. Objectives
This study was performed to detect and identify class 1 integron in S. aureus isolates.

3. Materials and Methods
This descriptive study was performed on 200 S. aureus isolates from patients admitted to Baqiyatallah Hospital in Tehran in 2013. These strains were confirmed as S. aureus by biochemical and serological tests and the presence of class 1 integron was determined by polymerase chain reaction (PCR). Gram-positive isolates with positive DNase, catalase, mannitol and coagulase tests were considered as S. aureus (12).

3.1. DNA Extraction
The bacteria were cultured in Luria-Bertani (LB) medium (CinnaGen, Iran) and incubated at 37°C overnight. The inoculated medium was centrifuged at 5000 rpm for six minutes. The supernatant was removed and the pellet was washed with 200 µL distilled water and the previous step was repeated once more; 100 µL Tris-EDTA (TE) buffer, 10 µL sodium acetate and 10 µL lysozyme 100 were added and the final mixture was incubated for half an hour at room temperature and then for 30 minutes at 43°C. Afterwards, 20 µL of proteinase K was added and the mixture was maintained at 60°C for one hour. Thereafter, 20 µL 20% SDS was added and the final volume was incubated for 10 minutes at 37°C, followed by adding 160 µL chloroform and centrifuging at 10000 rpm for 10 minutes. Afterwards, 70 µL cold isopropanol and 5 µL NaCl 5N were added to the supernatant and it was incubated for two hours at 20°C and also one hour at -70°C and then centrifuged at 13000 rpm for 20 minutes. The supernatant was discarded and ethanol, 70%, 750 µL was added to the pellet and it was centrifuged with the previous conditions. The supernatant was removed and the pellet was incubated for one hour at 37°C to dry out and then 50 µL TE buffer was added to the dried pellet.

3.2. Polymerase Chain Reaction to Detect integrase and qac/sul1 Genes
This technique was used to screen for the integrase and qac/sul1 genes in the extracted DNA from samples of S. aureus (Table 1).

The PCR reaction mixtures included 2 µL 10 x buffer, 2.5 µL dNTP 10 Mm, 2 µL and 1.5 µL MgCl2, 50 mM for the integrase and qac/sul1 genes, respectively, 1 µL of each primer (10 mM). 2 µL Taq DNA polymerase 0.5 U, 1 µL template DNA, and 14 and 14.5 µL deionized distilled water for the integrase and qac/sul1 genes, respectively. The final volume was 25 µL (all of the materials were purchased from CinnaGen Company, Iran).

The PCR reactions had 35 cycles, including 45 seconds at 94°C for denaturation, 50 seconds at 45°C for annealing, and one minute at 72°C for amplification of the target gene (extension). After the PCR reaction, the PCR product was investigated on a 1% agarose gel and the bands were visualized in a transilluminator under the UV light.

Table 1. Nucleotide Sequences of Primers and the Polymerase Chain Reaction Product Size (13, 14)

<table>
<thead>
<tr>
<th>Primer</th>
<th>Target Gene</th>
<th>Primer Sequence</th>
<th>PCR Product bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward-inti</td>
<td>integrate</td>
<td>GTCGGTCAAGGTTTCTG</td>
<td>490</td>
</tr>
<tr>
<td>Revers-inti</td>
<td>integrate</td>
<td>GCCAATTTTGCAGCATG</td>
<td>490</td>
</tr>
<tr>
<td>Forward-qac</td>
<td>conserved</td>
<td>ATTCGCAATTTGTTGCGAAGT</td>
<td>570</td>
</tr>
<tr>
<td>Revers-sul-3</td>
<td>conserved</td>
<td>TCCATCGATAATAACTCTAA</td>
<td>570</td>
</tr>
</tbody>
</table>
4. Results

The results of this study showed that the highest frequency of the obtained samples belonged to males (69.3%). The mode of age frequency was in the 51-60 age groups (20.3%). The samples were most frequently isolated from wounds (59.9%), sputum (25%) and bronchoalveolar lavage (15.1%). The emergency ward and the intensive care unit (ICU) were the most infected sites of the hospital (18.2% and 19.8%, respectively). PCR detection revealed that only 1% (two isolates) of 200 *S. aureus* isolates had the integrase and *qac/sul* genes, which are responsible for the presence of class 1 integron (Figures 1 and 2).

4.1. Polymerase Chain Reaction Detection of the integrase Gene

PCR was performed to detect the integrase gene. The PCR products were run on 1% agarose gel. The integrase gene of *S. aureus* was visualized at the 490-bp region (Figure 3).

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**Figure 1.** Frequency of the *Staphylococcus aureus* Isolates in Different Age Groups

![Bar chart showing frequency distribution by age group.](chart1.png)

*S. aureus* was mostly isolated from the individuals 51-60 years old.

**Figure 2.** Frequency of Samples in Different Wards

![Bar chart showing frequency distribution by ward.](chart2.png)

The *S. aureus* samples were mostly isolated from the emergency ward and ICU (18.2% and 19.8%, respectively).
4.2. Polymerase Chain Reaction Detection of the qac/sul1 Gene

PCR was performed to detect the qac/sul1 gene. The PCR product was visualized on 1% agarose gel at the 570-bp region (Figure 4).

5. Discussion

The spread of antibiotic resistance genes including integrons has become a major problem in treatment of infections caused by pathogenic bacteria (15). Over the past few decades, different genera of S. aureus have increasingly become resistant to commonly used antibiotics (16). The rise of resistance to antibiotics may be due to improper use of antimicrobial agents in animal feeds or inappropriate use of antibiotics in treating infections (17). Recently, the emergence of strains with multi-antibiotic resistance has caused some problems in treatment of infections in humans and animals (18). According to national and international reports, the issue of multidrug resistance of S. aureus has emerged in the recent few years and has become a public health problem in Iran (19). The aim of this study was to identify and investigate the prevalence of class 1 integron in S. aureus strains isolated from clinical samples. Our findings indicated that the integrase gene was observed only in two isolates (1%) among 200 samples of S. aureus.

Antunes and colleagues used the integrase gene as a target for identification of class 1 and 2 integrons and found that the frequency of integrin in Salmonella enterica was 75% (20). Krauland et al. also used both regions as targets for identification of integrons and showed that clonal expansion and horizontal transfer of the integrase gene has led to the worldwide spread of resistance to antimicrobial drugs against Salmonella (15). Rodriguez et al. used variable regions as targets for identification of integrons in Salmonella samples (21). In another study by Xu et al. in 2007 in North China on 53 isolates of methicillin-resistant coagulate-negative S. aureus, 30 isolates had class 1 integron (13). In 2008, Zhenbo Xu and colleagues reported methicillin-resistant S. aureus in China for the first time, in which they showed that six strains of S. aureus had class 1 integron (14).

Zhang et al. in 2004 studied 33 isolates of Salmonella among healthy people in China and found that most of them were resistant to sulfamethoxazole, tetracycline, and streptomycin. All of the isolates were susceptible to ceftriaxone and 11 isolates had class 1 integron (22). In another study, Mirnejad et al. identified class 1 and 2 integrons in Acinetobacter baumannii strains and showed that 42% and 82% of the strains contained class 1 and class 2 integrons, respectively (23). Few studies have been performed on integrons in clinical strains of S. aureus in the world. However, there is no report of that from Iran. Further research is required in this field. On the other hand, geographical maps of antibiotic resistance can be used to guide clinicians in the choice of antibiotics, before culture results become available (24). Prudence and foresight in this area has been limited to the countries that are aware of their past and present reports in this field. Therefore, the results from other parts of the world may not be useful for policy makers of public health in Iran. Undoubtedly, further studies are required to achieve these goals, especially in the field of epidemiology and identification of dominant strains using sensitive genotypic and phenotypic typing techniques in the country.

Our findings indicated that integrons can be found limitedly among S. aureus isolated from clinical samples in Tehran. The surveillance on antibiotic resistance including integron screening as an indicator and sign of acquisition and spread of antibiotic resistance can be an important strategy in combating antibiotic resistance in S. aureus.

Authors’ Contributions
Abbas Ali Imani Fooladi supervised, developed the study...
concept, and design and critical revision of the manuscript; Emad Yahaghi researched and contributed to the development drafting of the manuscript and critical revision of the manuscript; Mohsen Amin was the main editor and contributed to the design of this research; Reza Mirnejad and Reza Nezamzade advised and contributed to the design of this research.

References