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Detection of Class I *Integrons* in *Staphyloacoccus aurous* Isolated From Clinical Samples

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Background: *Staphylococcus aurous* is a major pathogen, causing variety of diseases and death in Iran and in the world. Despite the use of a spectrum of new 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. aurous* strains is the gain of resistance genes including *integrase* and *qac/sul*1.

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

Materials and Methods: This descriptive study was performed on 200 strains of *S. aurous* 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. aurous* isolated from clinical sample in Tehran.

Keywords:Staphylococcus aurous; Drug Resistance; Polymerase Chain Reaction

1. Background

The role of *Staphylococcus aurous* as a pathogen causing serious infections in humans has long been confirmed (1). *S. aurous* is considered as an opportunistic pathogen, especially in people who are immunosuppressed and in wounds caused by severe burns (2). *S. aurous* 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 created severe infections in burns as well as in different parts of the body due to acquired drug resistance (4). Misuse of antibiotics in treating bacterial infections has led to the selection of resistant strains, and unfortunately, the risk of transfer of the resistance gene to sensitive bacteria is growing (5). Up until recently, it has been thought that transfer of antibiotic resistance in bacteria takes place mainly through conjugation and transduction by plasmids, phages and transposons carrying the resistance genes. Hall and Collis identified another mechanism of antibiotic resistance gene transfer in 1995, which was delivery by some elements called *integrons* (6). *Integrons* are genetic sets capable of integrating mobile genetic elements called gene cassettes and displacing them. Since *integrons* contain a promoter, they can express genes existing in a gene cassette. Therefore, *integrons* act both as gene expression vectors and as a natural cloning system (7). There are two conserved nucleotide sequences in the 5' and 3' regions of *integrons*. Essential components of the 5' region of all *integrons* are: 1) *integrase* gene (*int I*), coded for recombining the enzyme dedicated to the site; 2) *attI* sequence, a specific recombinant site located adjacent to the *int I* gene; 3) the promoter sequence which is necessary for the expression of genes in the gene cassette integrated in the *integron*. There are three components as follows in the 3' regions of class 1 *integron*: 1) the *qac* gene, which is responsible

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. aurous* 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. aurous* in Iran

2. Objectives

This study was performed to detect and identify class 1 *integron* in *S. aurous* isolates.

3. Materials and Methods

This descriptive study was performed on 200 *S. aurous* isolates 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). Gram-positive isolates with positive DNase, catalase, mannitol and coagulase tests were considered as *S. aurous* (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/sul*1 genes in the extracted DNA from samples of *S. aurous* (Table 1).

The PCR reaction mixtures included 2 µL 10 x buffer, 2.5 µL dNTP 10 Mm, 2 µL and 1.5 µL $MgCl_2$ 50 mM for the *integrase* and *qac/sul*1 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/sul*1 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)

Primer	Target Gene	Primer Sequence	PCR Product, bp
Forward-*int*1	integrate	GTTCGGTCAAGGTTCTG	490
Revers-*int*1	integrate	GCCAACTTTCAGCACATG	490
Forward-*qac*3	conserved segment	ATCGCAATAGTTGGCGAAGT	570
Revers-*sul*1-3	conserved segment	TCCATCGATAATATACCTAA	570

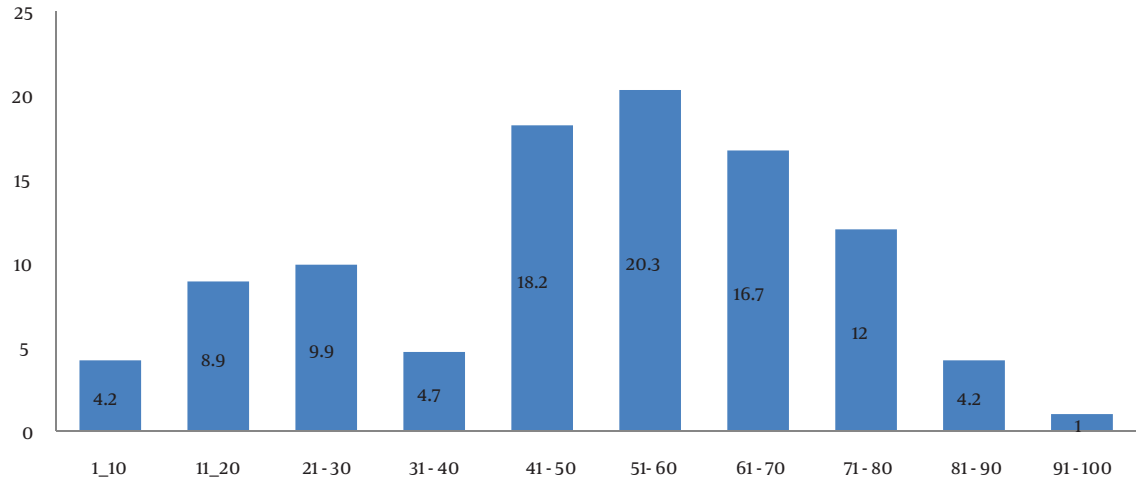
4. Results

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. aurous* isolates had the *integrase* and *qac/sul*1 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

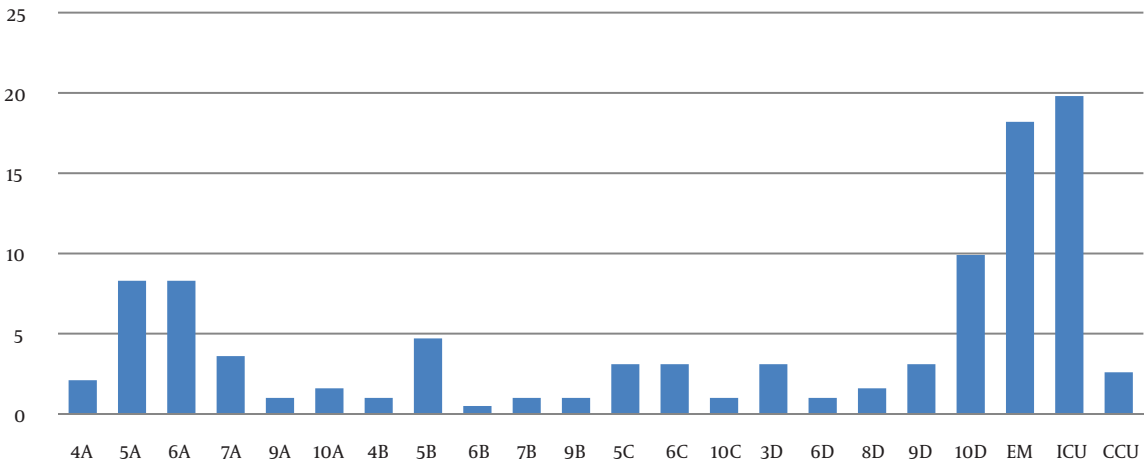
PCR was performed to detect the *integrase* gene. The PCR products were run on 1% agarose gel. The *integrase* gene of *S. aurous* s was visualized at the 490-bp region (Figure 3).

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



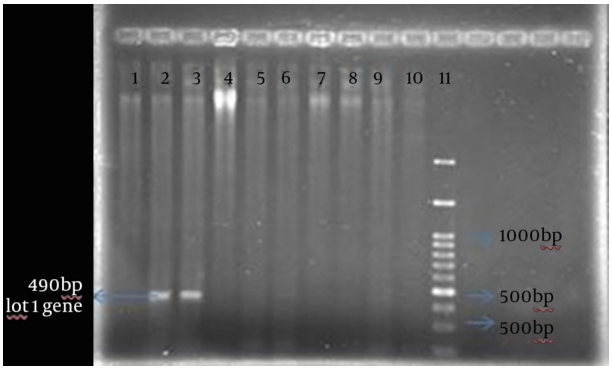
S. aurous was mostly isolated from the individuals 51-60 years old.

Figure 2. Frequency of Samples in Different Wards



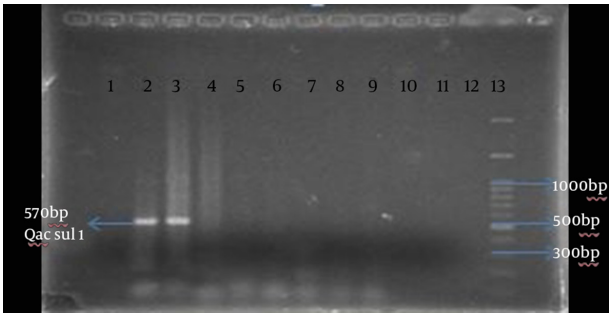
The *S. aurous* samples were mostly isolated from the emergency ward and ICU (18.2% and 19.8%, respectively).

Figure 3. The Polymerase Chain Reaction Products Separated by Electrophoresis on 1% Agarose Gel



Lanes 1, 4, 5, 6, 7, 8 and 9: clinical samples showed the absence of the *integrase* gene; lanes 2 and 3: *int*1 gene; lane 10: negative control; lane 11: low-bp ladder.

Figure 4. The Polymerase Chain Reaction Products Separated by Electrophoresis on 1% Agarose Gel



Lanes 1, 4, 5, 6, 7, 8, 9: clinical samples lacking the *integrase* gene; lanes 2 and 3: *qac*/*sul*1 gene; lane 10: negative control; lane 11: low-bp ladder.

4.2. Polymerase Chain Reaction Detection of the qac/sul1 Gene

PCR was performed to detect the *qac*/*sul*1 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. aurous* 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. aurous* 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. aurous* strains isolated from clinical samples. Our findings indicated that the *integrase* gene was observed only in two isolates (1%) among 200 samples of *S. aurous*.

Antunes and colleagues used the *integrase* gene as a target for identification of class 1 and 2 *integrons* and found that the frequency of *integron* 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 coagulase-negative *S. aurous*, 30 isolates had class 1 *integron* (13). In 2008, Zhenbo Xu and colleagues reported methicillin-resistant *S. aurous* in China for the first time, in which they showed that six strains of *S. aurous* 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. aurous* 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. aurous* 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. aurous*.

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.

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