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Distribution of *spa* Types, Integrons and Associated Gene Cassettes in *Staphylococcus aureus* Strains Isolated From Intensive Care Units of Hospitals in Tehran, Iran

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Abstract

Background: Nosocomial *Staphylococcus aureus* is known as an important clinical pathogen in health care, hospital, and community settings. One of the serious threats associated with clinical isolates of *Staphylococcus aureus* is multi-drug resistance associated with integrons.
Objectives: The objective of the present study was to investigate antimicrobial susceptibility patterns, frequency of class 1 and 2 integrons, and associated gene cassettes in different *spa* types of *Staphylococcus aureus* isolated from intensive care units (ICUs).
Methods: During a five-month descriptive cross-sectional study, 80 *Staphylococcus aureus* strains isolated from hospitalized patients in ICU wards in five hospitals of Tehran, Iran were investigated. *Staphylococcus aureus* isolates were submitted to susceptibility testing and Polymerase Chain Reaction (PCR) to detect *mecA* gene, class 1 and 2 integrons, and associated gene cassettes. All the isolates were genotyped by staphylococcal protein A (*spa*) typing.
Results: The overall prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) was found to be 86.2%. All the isolates were susceptible to vancomycin, teicoplanin and linezolid and resistant to penicillin and ampicillin. All the 80 *Staphylococcus aureus* isolates were observed to be multi-drug resistant. Class 1 and 2 integrons were commonly found in 56.3% and 18.7% of the isolates, respectively. Six different gene cassettes were detected in class 1 integron (*aadA2*, *aadB*, *blaoxa*, *aacA4*, *cmlA6*, and *catB*) and three were found in class 2 (*dfrA1*, *aadA1*, and *sat2*). Gene cassette arrays *aadA*, *aadB*, *blaoxa*, and *aacA* were common in the two integron classes of *Staphylococcus aureus* isolates. Five different *spa* types of t790, t030, t969, t7580 and t1425 were identified among our isolates where *spa* type t790 was the most predominant *spa* type among integron-bearing *Staphylococcus aureus* strains.
Conclusions: The present study reports on a high rate of multi-drug resistance, the predominance of the frequency of class 1 integron, and the emergence of *spa* type t790 among Iranian *Staphylococcus aureus* strains. The results revealed that the dissemination of multi-drug resistance among *Staphylococcus aureus* isolates may be associated with the presence of integrons. Therefore, continuous surveillance to monitor integrons and the associated gene cassettes among nosocomial pathogens, especially *Staphylococcus aureus*, is essential.

Keywords: Integron, MRSA, *Staphylococcus aureus*

1. Background

Staphylococcus aureus (*S. aureus*) is the major cause of infection in either hospitals or within communities across the world (1) causing a variety of illnesses that can range from mild skin infections and wound infections to endocarditis, pneumonia, bacteremia and life-threatening diseases. *Staphylococcus aureus*, as one of the most prevalent pathogens in hospitals, can easily be transmitted by direct contact (including contaminated hands or droplet transmission) and indirect contact (such as environment or hospital air) between patients and medical staff (2). The most important factor contributing to the successful extensive distribution of this nosocomial pathogen is stated to be its remarkable ability to acquire resistance to new antimicrobial agents (3).

Shortly after the introduction of penicillin as a first therapeutic option for the treatment of infections caused by penicillin-resistant *S. aureus*, Methicillin-Resistant *Staphylococcus aureus* (MRSA) emerged in the 1960s, and

since the 1980s, MRSA strains have become endemic in hospitals worldwide with associated significant patient morbidity and mortality. To the extent that MRSA have emerged as a major public health concern (4). Methicillin resistance primarily results from the presence of *mecA* gene, which encodes a modified penicillin-binding protein (PBP2a) and has low affinity for β-lactams. During the past several decades, in spite of introducing a variety of therapeutic measures including antibiotic therapy, MRSA strains have shown a remarkable ability for rapid development of multi-drug resistance (MDR) (5).

The widespread emergence of MDR *S. aureus*, as a common cause of nosocomial infections, is becoming a serious concern in global public health. The increase of resistance does not only lead to increase of economic burden but it also may cause serious therapeutic problems as well as exacerbates infection control in hospitals. Although the mechanisms of resistance among bacteria are very diverse, horizontal gene transfer has already been proved as the most important mechanism for the dissemination of antimicrobial resistance in microbial populations. Also, the role of integrons as a genetic element in horizontal transfer of antibiotic resistance has been well established (6).

Integrons are a vital element in the spread of MDR, particularly in gram-negative pathogens. They are normally motionless but can be transferred through mobile genetic element, e.g., plasmids and transposons. The basic structure of integrons is composed of 5'and 3'-conserved segments with gene cassettes containing antibiotic resistance genes. Essential components of the 5' region consist of an integrase gene (*intI*) encoding an integrase, a recombination site (attI), and a strong promoter gene. To date, several classes of integrons have been described based on the integrase genes (7). Class 1 integrons are distributed in both gram-positive and especially in gram-negative bacteria isolated from clinical samples. They are often associated with lateral transfer of antibacterial resistance genes. Class 2 integrons are less common compared to class 1 integrons and have frequently been reported in gram-negative bacteria. Reports on other classes of integrons are scarce (8). Although the role of integrons in the development of MDR in gram-negative bacteria is definite, relatively little data exists on the prevalence of integrons in gram-positive bacteria, especially in *S. aureus* isolates.

2. Objectives

The present study was an attempt to understand the possible presence and thus dissemination of different classes of integrons and associated gene cassettes among *S. aureus* isolates recovered from hospitalized patients in intensive care units (ICUs) of hospitals in Tehran, Iran. We also intended to detect different molecular types of integron-bearing *S. aureus* strains by *spa* typing.

3. Methods

3.1. Sampling and Data Collection

During a five-month period, 1st of April to 31st of August 2015, a total of 80 *S. aureus* isolates were recovered from various clinical specimens of hospitalized patients in ICU wards in five hospitals of Tehran, Iran: one in northern Tehran (A, general and governmental hospital), one in central Tehran (B, specialized and sub specialized private hospital), one in southern Tehran (C, general and governmental hospital), one in eastern Tehran (D, general and private hospital), and one in western Tehran (D, specialized and sub specialized governmental hospital). In the present study, all of the hospitalized patients in the ICU, who were found to have *S. aureus* infection were included. Duplicate isolates and patients who had a history of previous use of antibiotics before sampling were excluded from the study. According to the inclusion and exclusion criteria, 290 clinical specimens obtained from hospitalized patients at the ICU were included and 65 samples were excluded from our study. The research was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran [94-1293]. Written informed consent was obtained from the patients to use their samples for research purposes. It is important to note that all the equipment and kits in this research were calibrated and certificated by the manufacturer. All the obtained biological samples were transported to the laboratory within two hours of collection and were processed immediately. Identification of *S. aureus* isolates was performed based on standard microbiological procedures such as colony morphology, gram staining, growth on mannitol salt agar, and production of catalase, coagulase and DNase. All the isolates were also evaluated for the presence of the *femA* and *nucA* genes via the polymerase chain reaction (PCR) (9).

3.2. Antimicrobial Susceptibility Testing

In vitro susceptibility testing was performed using a panel of 16 antibiotic disks for all the isolates by the Kirby-Bauer disk diffusion procedure, according to the guidelines of the clinical and laboratory standards institute (CLSI) (10). The following antimicrobial drugs were used in the present survey: penicillin (PG 10 μg), ampicillin (AP 10 μg), teicoplanin (TEC 30 μg), ceftriaxone (CRO 30 μg), gentamicin (GM 10 μg), kanamycin (K 30 μg), amikacin (AK 30 μg), tobramycin (TN 10 μg), linezolid (LZD 30 μg), erythromycin (E 15 μg), gatifloxacin (GAT 5 μg), clindamycin (CD 2 μg), levofloxacin (LEV 5 μg), ciprofloxacin (CIP 5 μg),

and trimetoprim-sulfamethoxazole (TS 25 μg). The minimum inhibitory concentration (MIC) for vancomycin was determined with E-test strips (AB Biodisk, Sweden), according to the manufacturer's instructions. For all the isolates, intermediate sensitivity was scored as resistance. Multidrug Resistance (MDR) was defined as resistance to three or more unique antibiotic classes in addition to resistance to beta-lactams (11). All antibiotic disks used in the current research were obtained from Mast co., UK. A standard reference strain, *S. aureus* ATCC25923, was used as a quality control strain in every test run. *Staphylococcus aureus* isolates were preserved in Tryptic Soy Broth (TSB; Merck co., Germany) containing 20% glycerol at -70°C until further molecular analysis.

3.3. Methicillin-Resistant Staphylococcus aureus Screening

Methicillin resistance was detected using a cefoxitin disc (30 μg) and an oxacillin disc (1 μg) on Mueller Hinton agar plates supplemented with 4% NaCl in accordance to the clinical and laboratory standards institute (CLSI) guidelines (10). Isolates with phenotypic resistance to oxacillin were also tested for the presence of the *mecA* gene using PCR.

3.4. Extraction of Plasmid and Genomic DNA

Genomic DNA of the strains was extracted using the commercial kit InstaGene Matrix (BioRad, Hercules co., CA, USA) along with the addition of lysostaphin (Sigma-Aldrich co., USA) to a final concentration of 15 μg/mL. The Qiagen Plasmid Midi Kit was used for plasmid DNA extraction, according to the manufacturer's instructions.

3.5. spa Typing

Spa typing was performed as described by Harmsen et al. for all MRSA isolates. For *spa* typing, the polymorphic X region of the protein A gene (*spa*) was amplified by PCR (12). The PCR products were subjected to DNA sequence analysis, and their nucleotide sequences on both strands were determined using an ABI Prism 377 automated sequencer (Applied Biosystems, Perkin-Elmer co., Foster City, CA). Sequence editing was done using Chromas software (version 1.45, Australia). Edited sequences were assigned to particular *spa* types according to the guidelines described by a Ridom *Spa*Server database (http://www.*spa*server.ridom.de).

3.6. Detection of Integrons

The presence of class 1 and 2 integrons was investigated using PCR with degenerate primers, described by Moura et al. (13). Polymerase chain reaction conditions for amplification of 280 bp fragment of the int1 and 232 bp fragments of the int2 by thermocycler (Eppendorf co., Hamburg, Germany) were as follows: initial denaturation for five minutes at 94°C, 35 cycles of denaturation at 94°C for 45 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for one minute. The final extension was carried out at 72°C for five minutes.

3.7. Detection of Gene Cassettes Inserted in the Variable Regions and Sequencing

Amplification of the variable region between class 1 and 2 integrons was performed using primer pairs introduced by Moura et al. (13). The PCR products of variable regions were purified by the QIAquick Gel Extraction kit (Qiagen co., Hilden, Germany). Purified PCR products were subjected to sequencing with an ABI Prism 377 automated sequencer (Applied Biosystems, Perkin-Elmer co., Foster City, CA) in both directions. The sequences were assembled making use of the SeqMan program within the Lasergene suite version 7 (DNAstar Inc., Madison, WI, USA). The BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against GenBank database and the Integron Database INTEGRALL (http://integrall.bio.ua.pt/) were performed repeatedly for sequence comparison and annotation.

3.8. Statistical Analysis

Statistical analysis was carried out using the SPSS, version 18.0 software (SPSS Inc., Chicago, IL). Chi-square was run to determine the P value. A P value of less than 0.05 was considered statically significant.

4. Results

During the study, a total of 80 *S. aureus* isolates were obtained from 290 clinical specimens isolated from hospitalized patients in ICU wards of five hospitals in Tehran. The average age of the participants was 39 (median 41.8, ranging from 9 months to 69 years of age). In the present investigation, 72.5% of the patients were female and 27.5% male; the M:F ratio was 0.37. The age distribution was 10% for patients aged equal or less than 18 years, 77.5% for 19 to 59 years, and 12.5% for equal or above 60 years. Clinical specimens included wound (n = 28, 35%), blood (n = 15; 18.8%), sputum (n = 8; 10%), ear secretions (n = 7; 8.7%), catheter (n = 7; 8.7%), body fluids (bronchoalveolar lavage and cerebrospinal fluid) (n = 6; 7.5%), urine (n = 5; 6.3%) and pus (n = 4; 5%).

4.1. Antimicrobial Resistance Phenotypes

All *S. aureus* isolates were subjected to antimicrobial susceptibility testing. The result of antimicrobial susceptibility test of 80 *S. aureus* clinical isolates revealed the rates

of resistance to the majority of tested antibiotics ranging between 37.5% and 80 %. All the isolates were 100% susceptible to vancomycin, teicoplanin and linezolid, and 100% resistant to penicillin and ampicillin. Based on the results obtained in the present study, the lowest levels of resistance (37.5%) were related to levofloxacin. None of the observed strains were sensitive to the antimicrobial agents. Also, it was found that all the isolates were MDR. In particular, 25 (31.3%) of the isolates were resistant to nine antibiotics, 19 (23.8%) were resistant to ten antibiotics, 14 (17.5%) were resistant to eight antibiotics, and 11 (13.8%) were resistant to eleven antibiotics. Simultaneous resistance to five, seven, four, twelve and six antibiotics was seen in four, three, two and one isolates, respectively. The results of antimicrobial susceptibility testing of isolates are shown in Table 1. Overall, 69 (86.2 %) isolates were resistant to methicillin and were confirmed as MRSA based on the detection of the *mecA* gene.

4.2. Detection of Class 1 and Class 2 Integrons and Characterization of the Variable Region

The class 1 and 2 integrons were commonly found in 45 (56.3%) and 15 (18.7%) isolates, respectively. The existence of class 3 integron was not confirmed in any of the MDR strains. Co-existence of class 1 and 2 integron was detected in eight isolates (10%). The frequencies of different classes of integrons among 80 *S. aureus* isolated from ICU wards are presented in Table 2. The results indicated that the relationship between resistance to ceftriaxone, gentamicin, kanamycin, tobramycin, and ciprofloxacin and the presence of integron was statistically significant. The correlation between these two factors is given in Table 1. Out of 45 isolates carrying class 1 integrons, 38 (84.4%) were located on plasmids and seven (15.6%) on chromosomes while out of fifteen isolates carrying class 2 integrons, ten (66.7%) were located on plasmids and five (33.3%) on chromosomes. Accordingly, the positive rate of both classes 1 and 2 integrons in plasmid DNA was higher than that of genomic DNA. All the integron-bearing *S. aureus* strains were screened for variable regions. Different sizes of the variable region of integron were subjected to sequencing. The results of sequencing of the integron cassette region revealed six different gene cassettes (*aadA2*, *aadB*, *blaoxa*, *aacA4*, *cmlA6* and *catB*) in class 1 integrons and three different gene cassettes (*dfrA1*, *aadA1*, and *sat2*) in class 2 integrons. The numbers of cassette genes in class 2 integrons were much more limited compared with those of class 1 integrons. In three (37.5%) isolates harboring both class 1 and 2 integrons simultaneously, PCR could not amplify the variable region as well as three (20%) isolates harboring class 2 integrons. All the *S. aureus* isolates were *spa* typed and five different *spa* types were discriminated (t790 in 26 strains; t030 in 18 strains; t969 in 14 strains; t7580 in 12 strains; t1425 in 10 strains). Genotypic characteristics of 80 integron-bearing *S. aureus* strains are summarized in Table 3.

4.3. Spa Type t790

The most predominant *spa* type in the present study was *spa* type t790, all of which were MRSA. The majority of *spa* type t790 (69.2%) were obtained from wound infections. All the patients infected with *spa* type t790 were in the age group of 19 to 59 year-olds. Amongst these t790 *spa* types, 18 (69.2%) carried integron class 1. None of the strains carried integron class 2. Co-existence of class 1 and 2 integrons was detected in eight isolates (30.8%). The integron cassette regions in three strains, simultaneously harboring class 1 and 2 integrons, were not amplified by PCR. The MDR pattern among *spa* type t790 isolates included resistance to 11 (26.9%), 10 (23.1%), 9 (42.3%) and 8 (7.7%) antibiotics. The most prevalent gene cassette among *spa* type t790 isolates was *blaoxa* gene cassette leading to resistance to beta-lactam antibiotics.

4.4. Spa Type t030

The second most common *spa* type identified in the current survey was *spa* type t030. The majority of *spa* type t030 (33.3%) were obtained from blood infections. The presence of class 1 and 2 integrons in strains with *spa* type t030 was confirmed in 50% and 44.4% of isolates and only a single isolate did not harbor an integron. Interestingly, the cassette region of class 2 integron in three strains could not be amplified by PCR. The most gene cassette type among *spa* type t030 isolates was aad gene cassette conferring resistance to aminoglycosides. The results showed that seven (38.9%) isolates were resistant to at least nine antibiotics, seven (38.9%) were resistant to at least eight antibiotics, two (11.1%) resistant to at least nine antibiotics, one (5.6%) resistant to eleven antibiotics, and one (5.6%) resistant to five antibiotics.

4.5. Spa Type t969

Fourteen (17.5%) patients were observed to be infected by isolates belonging to this *spa* type. These isolates were recovered from blood (n = 4; 28.6%), catheters (n = 4; 28.6%), wounds (n = 3; 21.4%) and pus samples (n = 3; 21.4%). The majority of the infected patients were elderly; 11.3% over 60. Nine (64.3 %) isolates were positive for class 1 integron and the remainder (35.7%) was integron-negative. Isolates with *spa* type t969 had diverse antibiotic resistance patterns.

Table 1. Antibiotic Resistance Pattern and Association With the Existence of Integron in *Staphylococcus aureus* Strains Isolated From Intensive Care Units[a]

Antibiotics	Antibiotic Susceptibility (n = 80)			Integron Positive (n = 68)			Inetgron Negative (n = 12)			P Value
	R	I	S	R	I	S	R	I	S	
Penicillin	80 (100)	0	0	68 (100)	0	0	12 (100)	0	0	-
Ampicillin	80 (100)	0	0	68 (100)	0	0	12 (100)	0	0	-
Vancomycin	0	0	80 (100)	0	0	68 (100)	0	0	12 (100)	-
Teicoplanin	0	0	80 (100)	0	0	68 (100)	0	0	12 (100)	-
Ceftriaxone	58 (72.5)	2 (2.5)	20 (25)	55 (80.9)	2 (2.9)	11 (16.2)	3 (25)	0	9 (75)	0.001[b]
Gentamicin	60 (75)	2 (2.5)	18 (22.5)	56 (82.4)	0	12 (17.6)	4 (33.3)	2 (16.7)	6 (50)	0.022[b]
Kanamycin	55 (68.8)	5 (6.2)	20 (25)	55 (80.9)	3 (4.4)	10 (14.7)	0	2(16.7)	10 (83.3)	0.001[b]
Amikacin	48 (60)	3 (3.7)	29 (36.3)	45 (66.2)	1 (1.4)	22 (32.4)	3 (25)	2 (16.7)	7 (58.3)	0.108
Tobramycin	40 (50)	0	40 (50)	40 (58.8)	0	28 (41.2)	0	0	12 (100)	0.001[b]
Linezolid	0	0	80 (100)	0	0	68 (100)	0	0	12 (100)	-
Erythromycin	63 (78.8)	6 (7.5)	11 (13.7)	58 (85.3)	3 (4.4)	7 (10.3)	5 (41.7)	3 (25)	4 (33.3)	0.055
Gatifloxacin	45 (56.3)	0	35 (43.7)	40 (58.8)	0	28 (41.2)	5 (41.7)	0	7 (58.3)	0.430
Clindamycin	52 (65)	5 (6.2)	23 (28.8)	45 (66.2)	2 (2.9)	21 (30.9)	7 (58.3)	3 (25)	2 (16.7)	0.492
Levofloxacin	30 (37.5)	2 (2.5)	48 (60)	24 (35.3)	2 (2.9)	42 (61.8)	6 (50)	0	6 (50)	0.528
Ciprofloxacin	64 (80)	1 (1.2)	15 (18.8)	58 (85.3)	0	10 (14.7)	6 (50)	1 (8.3)	5 (41.7)	0.042[b]
Trimetoprim- Sulfamethoxazole	38 (47.5)	7 (8.7)	35 (43.8)	28 (41.2)	7 (10.3)	33 (48.5)	10 (83.3)	0	2 (16.7)	0.082

[a] Values are expressed as No. (%).
[b] Significant values.

Table 2. Frequency of Integrons Found in 80 *Staphylococcus aureus* Isolates From Intensive Care Units

Integron Class	No. (%)
Integron class 1	45 (56.3)
Integron class 2	15 (18.7)
Integron class 1 and 2	8 (10)
Integron class 3	0
Without integron	12 (15)
Total	80 (100)

4.6. Spa Type t7580

The other *spa* type identified in the study was *spa* type t7580. These isolates were isolated from ear (n = 3; 25%), urine (n = 3; 25%), wounds (n = 3; 25%), sputum (n = 2; 16.7%), and body fluids (n = 1; 8.3%). Class 1 and 2 integrons were detected in 50% and 33.3% of isolates, respectively. Two isolates (16.7%) were integron-negative. The dominant resistance profile among these *spa* types included resistance to 10 antibiotics (41.7%).

4.7. Spa Type t1425

This *spa* type was mostly isolated from sputum. The frequencies of class 1 and 2 integrons were identical (30%). Forty percent of the isolates did not harbor any integron. Ninety percent of the patients infected with this *spa* type were under 18 years of age. Isolates with this *spa* type were remarkably less antibiotic resistant.

4.8. Nucleotide Sequence Accession Numbers

The nucleotide sequence obtained in this study are available in the GenBank nucleotide database under accession numbers LC093087, LC093088, LC093089, LC093090, LC093091, LC093092, LC093093, LC093094, LC093095, LC093096 from the gene cassette of class 1 integrons and LC093990, LC093991 and LC093992 from the gene cassette of class 2 integrons

5. Discussion

Integrons, genetic elements containing gene cassettes carrying antibiotic resistance genes, are linked to MDR and subsequently constrict the therapeutic options and aggravate clinical outcomes (14). The role of class 1 and 2 integrons in the dissemination of antibiotic resistance genes in gram-negative bacteria is well documented. Nevertheless, little is known about the prevalence of class 1 and 2 integrons in gram-positive bacteria leading to MDR distribution (8). The present study concentrated on the carriage of class 1 and 2 integrons and associated gene cassettes in *S. aureus* strains recovered from clinical samples of patients in ICUs and also detection of different *spa* types in Tehran, Iran.

It has been reported that HA-MRSA from diverse geographical locations have remarkably different genetic

characteristics (15-17). In the current survey, the prevalence of HA-MRSA isolates was 86.2%, which was higher than that in Taiwan (1), Nigeria (18), Hungary (17, 19), Egypt (16), Serbia (20) and Croatia (15). The increased rate of resistance to methicillin among the isolates obtained in our study could be attributed to the longer period of the study, the sample type, investigated population and different wards of the hospital.

The MRSA strains are usually resistant to macrolides, lincosamides, aminoglycoside, and approximately all currently available beta-lactam antimicrobial agents, like penicillin and cephalosporins (21-23). The susceptibility pattern revealed that vancomycin, teicoplanin, and linezolid had good activity against *S. aureus* infections, while penicillin and ampicillin had the lowest antibacterial effect on *S. aureus* isolates. The results were in agreement with previous findings from Italy (24), Croatia (15), Taiwan (1), Serbia (20), and Turkey (25).

Although previous studies have revealed the emergence of MRSA with reduced susceptibility or gradual increased resistance to vancomycin in Iran (26), the results obtained in the present study can help infer that proper antibiotic prescription protocols, extensive surveillance programs, and standard principles of infection control in health care systems may have led to a decrease in vancomycin resistance in MRSA isolates.

The results demonstrated that resistance to ciprofloxacin (80%), erythromycin (78.8%), gentamicin (75%), ceftriaxone (72.5%), kanamycin (68.8%), and clindamycin (65%) was relatively high while approximately less than half of the strains were resistant to levofloxacin (37.5%) and trimethoprim-sulfamethoxazole (47.5%). This is largely in accordance with the findings reported by Ko et al. in a study of 74 MRSA strains isolated from 12 Asian countries (27). Antimicrobial resistance patterns revealed that all the isolates (100%) were MDR, which is in accordance with the findings of Xu et al. in China (6), yet in comparison to the previous investigations in Serbia (83.9%) (20) and Taiwan (75.8%), (1) a high frequency of MDR *S. aureus* was observed in our study. Studies have shown that the frequency of MDR *S. aureus* isolates seems to vary by region (6, 15, 18, 24, 28). Increase in the frequency of MDR *S. aureus* is a challenging and serious public health concern especially in ICUs.

As mentioned, integrons are widely known for their role in the dissemination of antibiotic resistance, particularly among gram-negative pathogenic bacteria. Class 1 integrons are the most prevalent class among mobile integrons. The classification of different integrons is based on the relative homology of *intI* (7). In this survey, the presence of class 1 and 2 integrons was confirmed in 45 (56.3%) and 15 (18.7%) isolates, respectively. These findings are consistent with previous studies in which the rate of detection of integron class 1 was more than integron class 2 (6, 28, 29). In the study of Xu et al. in China, out of 30 S. auerus isolated from environment and surgical patients, 16 (53%) clinical and environmental isolates were positive for the class 1 integrase gene (6). In a study conducted by Guney et al. that investigated the presence of class 1 integron in MRSA isolated from a 1150-bed training and research hospital located at Black Sea region in Turkey, one hundred clinical MRSA isolates were investigated. The result revealed that none of the isolates harbored class 1 integron (29). The high prevalence of class 1 integron in our survey strongly demonstrated that class 1 integron might serve as a reservoir for antimicrobial resistance in Iranian *S. aureus* strains.

Six different gene cassettes (*aadA*, *aadB*, *blaoxa*, *aacA4*, *cmlA6* and *catB*) in class 1 integron and three different gene cassettes (*dfrA1*, *aadA1* and *sat2*) in class 2 integron were identified. Given the previous researches and Integron Database INTEGRALL (http://integrall.bio.ua.pt/): *aadA* gene cassette conferred resistance to streptomycin and spectinomycin; aadB gene cassette conferred resistance to gentamicin, tobramycin, kanamycin, dibekacin and sisomicin; blaoxa gene cassette conferred resistance to beta-lactam antibiotics such as ampicillin, cephalothin, oxacillin and cloxacillin; *aacA* gene cassette confers resistance to amikacin, dibekacin, isepamicin, netilmicin, sisomicin and tobramycin; *cmlA6* and *catb* gene cassettes conferred resistance to chloramphenicol; and *sat2* gene cassette confers resistance to streptothricin. Overall, nine different gene cassettes were detected. In the present study, cassette genes encoding resistance to aminoglycosides (*aadA*, *aadB*) and beta lactams (*blaoxa*) were the most predominant cassettes in the class 1 integron followed by *aacA* gene cassette. The cmlA6 gene cassette was found to be rare in class 1 integron. In a study conducted by Xu et al. on nosocomial MRSA strains sampled during 2001 to 2006, it was found that seventy-six out of 179 (42.5%) of the tested strains carried class 1 integrons and four unique arrays of gene cassettes (*aadA2*, *aacA4- cmlA1*, *dfrA17-aadA5* and *dfrA12-orfF-aadA2*) were the most frequently detected resistance genes found in clinical isolates (30). It is established that class 1 integrons are associated with a variety of resistance gene cassettes, yet aminoglycoside resistance determinant are detected frequently (6, 30). It should also be noted that this integron had been reported to be associated with Tn3 transposon family (Tn21 or Tn1696) therefore widespread distribution of class 1 integron could be attributed to the spread of integron-containing transposon (7, 8). In class 2 integron, the most predominant cassette gene was *dfrA1* that conferred resistance to trimethoprim. The literature review showed that class 2 integrons are associated with

the Tn7 transposon and dihydrofolate reductase enzyme encoded by the *dfr* gene, which is located on Tn7 (8). The frequency of class 1 and 2 integrons in plasmid DNA was higher than that of genomic DNA, which is largely in accordance with the findings of Ren et al. (31).

In the present study, we made an attempt to determine different types of *spa* in integron-bearing *S. aureus* strains. The main *spa* types from our survey were t790, t030, t969, t7580 and t1425 respectively. In agreement with earlier reports, we found *spa* type t790 with a high percentage of MDR and integron class 1 (69.2%) as the most common *spa* type among our isolates (21). Although t790 was previously reported as a predominant community-acquired MRSA (CA-MRSA) clone in Iran (21), Germany (32) and Ireland (33), in our study t790 was detected as the predominant *spa* type among the HA-MRSA isolates. It can be deduced that CA-MRSA *spa* types have successfully established themselves in Iranian hospitals and health care settings.

The majority of t030 strains from our study were resistant to ciprofloxacin, erythromycin, gentamicin and clindamycin. Class 1 integron was the most frequent integron among this *spa* type. The most common gene cassette was aad that conferred resistance to aminoglycosides. These findings are consistent with the study of Chen et al. in China in 2010. They demonstrated that *spa* t037, which was prevalent before year 2000, was rapidly replaced by the t030 MRSA clone, which emerged in 2000. Since then, t030 (73.3%) has become the most popular MRSA clone. Antibiotic susceptibility testing exhibited that predominant antibiotic resistance profile among t030 isolates included resistance to tetracycline, erythromycin, gentamicin, ciprofloxacin, clindamycin and rifampin. Another interesting finding was that the first patient with t030 MRSA was found at an ICU (34). Considering the high resistance of this *spa* type to many antibiotic agents and easy transmission, special care must be taken to avoid the spread of this particular type. In spite of high frequency of integron class 1 and diverse antibiotic resistance patterns of t969, t7580 and t1425 types, they had limited frequency in *S. aureus* isolates in our study. Previous studies have also confirmed that *spa* type t969 has low frequency compared with other *spa* types (35, 36).

A major strength of this study was that it was performed on *S. aureus* strains isolated from hospitalized patients in ICUs, in order to study the distribution of integron and associated gene cassettes in different *spa* types of nosocomial *S. aureus*; however our study had limitations, which was the modest sample size and the impossibility of using other methods such as pulsed-field gel electrophoresis (PFGE) and Multilocus sequence typing (MLST).

5.1. Conclusion

To summarize, our study revealed that a large proportion of *S. aureus* strains in our hospitals harbored an integron, which may lead to dissemination of multiple antibiotic resistance. This dilemma emphasizes that antibiotic resistance remains a problem and strategies to control *S. aureus* infections must be revised. We also confirm the presence t790, t030; t969, t7580 and t1425 with a high level of multiple antibiotic resistance in Iran. Therefore, it can be suggested that in order to understand the prevalence and epidemiology of integron in different molecular tapes of *S. aureus*, further studies are required.

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Footnotes

Authors' Contribution: Mehdi Goudarzi and Hossein Goudarzi conceived and designed the experiments. Sima sadat Seyedjavadi, Mehdi Azad and Mehdi Goudarzi performed the experiments. Sima sadat Seyedjavadi, Mehdi Azad, Mehdi Goudarzi and Hadi Azimi analyzed the data. Mehdi Goudarzi and Hossein Goudarzi Contributed to reagents/materials/analysis tools. Sima sadat Seyedjavadi, Mehdi Azad, Mehdi Goudarzi, Hossin Goudarzi and Hadi Azimi wrote the paper.

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Table 3. Genotypic Characterization of 80 *Staphylococcus aureus* Isolates From Hospitalized Patients at the Intensive Care Unit

spa type/ Kreiswirth IDs	Site of Isolation	Resistance Profile	Integron Type	Gene Cassette
t790/TJEJNF2MOMOKR	Wound	PG, AP, E, GM, CD, K, AK, GAT, TN, TS	1	*aadA2*
	Wound	PG, AP, CIP, E, GM, CD, K, AK, GAT, TN, TS	1	*aadB-aadA1-cmlA6*
	Wound	PG, AP, CIP, E, GM, CD, K, AK, GAT, TN, TS	1	*aadB-aadA1-cmlA6*
	Wound	PG, AP, CIP, E, GM, CD, K, AK, GAT, TN, TS	1,2	*orfD-aacA4-catB8*
	Wound	PG, AP, CIP, E, K, GM, CD, AK, GAT, TN, TS	1,2	*orfD-aacA4*-catB8
	Wound	PG, AP, E, CRO, K, CD, AK, GAT, LEV	1,2	*orfD-aacA4-catB8*
	Wound	PG, AP, CIP, CRO, CD, AK, GAT, TN, TS	1	*aadA2*
	Wound	PG, AP, CIP, E, GM, CD, AK, TN, TS	1,2	*dfrA1*
	Wound	PG, AP, CIP, E, GM, K, AK, TN, TS	1,2	*dfrA1*
	Wound	PG, AP, CIP, GM, CD, AK, TN, TS	1,2	-
	Wound	PG, AP, CIP, E, GM, CRO, CD, AK, TN	1	*blaoxa2*
	Wound	PG, AP, CIP, E, GM, CRO, CD, AK, GAT, TN, LEV,	1	*aacA4*
	Wound	PG, AP, CIP, GM, CRO, CD, AK, GAT, TN, LEV	1	*blaoxa2*
	Wound	PG, AP, CIP, GM, CRO, CD, AK, TN, LEV	1	*blaoxa2*
	Wound	PG, AP, CIP, E, CRO, AK, GAT, TN	1	*blaoxa2*
	Wound	PG, AP, CIP, E, CRO, CD, AK, GAT, TN	1	*aacA4*
	Wound	PG, AP, CIP, E, CRO, CD, K, AK, GAT, LEV	1	*blaoxa2*
	Wound	PG, AP, CIP, E, CRO, K, AK, GAT, TS	1,2	-
	Ear	PG, AP, CIP, E, GM, CRO, CD, K, GAT, TS	1,2	-
	Body fluids	PG, AP, CIP, E, GM, CD, K, AK, TN	1	*blaoxa2*
	Blood	PG, AP, CIP, E, GM, CRO, CD, K, AK, GAT, TN	1	*blaoxa2*
	Blood	PG, AP, CIP, E, GM, CRO, AK, GAT, TN	1	*blaoxa2*
	Blood	PG, AP, CIP, E, GM, CRO, CD, K, GAT, TN, LEV	1	*blaoxa2*
	Blood	PG, AP, CIP, E, GM, CRO, CD, K, GAT, TN	1	*aacA4*
	Blood	PG, AP, CIP, E, GM, CRO, CD, K, AK, GAT	1	*blaoxa2*
	Body fluids	PG, AP, CIP, E, CRO, CD, GAT, TN, LEV	1	*blaoxa2*
t030/WGKAQQ	Wound	PG, AP, CIP, E, CRO, CD, GAT, TN, TS	1	*aacA4*
	Ear	PG, AP, CIP, E, CRO, CD, GAT, TN, TS, LEV	1	*aadA2*
	Urine	PG, AP, CIP, E, GM, CRO, CD, K, GAT, TN, TS	2	*dfrA1-sat2-aadA1*
	Body fluids	PG, AP, CIP, E, GM, CRO, CD, K, GAT, TS	2	*dfrA1-sat2-aadA1*
	Blood	PG, AP, CIP, E, GM, CD, TN, TS, LEV	1	*aadA2*
	Urine	PG, AP, CIP, E, GM	-	-
	Wound	PG, AP, CIP, E, GM, CD, TN, TS, LEV	2	*dfrA11*
	Blood	PG, AP, E, GM, CD, K, AK, TN, LEV	2	*dfrA11*
	Blood	PG, AP, CIP, E, CD, K, AK, TN, TS	2	*dfrA11*
	Blood	PG, AP, CIP, GM, CD, K, AK, GAT, TN	2	-
	Blood	PG, AP, CIP, E, GM, K, AK, TN	2	-
	Blood	PG, AP, CIP, CRO, CD, K, AK, TN	2	-
	Wound	PG, AP, CIP, GM, CRO, CD, AK, TN	1	*aadA2*

	Ear	PG, AP, CIP, E, GM, CRO, AK, LEV	1	*orfD-aacA4-catB8*
	Ear	PG, AP, CIP, E, GM, CRO, K, AK	1	*aadA2*
	Body fluids	PG, AP, CIP, E, GM, CRO, K, AK, LEV	1	*aadA2*
	Body fluids	PG, AP, CIP, E, GM, CRO, AK, LEV	1	*aadB*
	Pus	PG, AP, CIP, E, GM, CRO, K, TS	1	*aadB*
t969/WGAQQ	Blood	PG, AP, CIP, E, CRO, K, TS, LEV	1	*aadB*
	Blood	PG, AP, E, GM	-	-
	Catheter	PG, AP, CIP, GM, CRO, K, AK, TS	1	*aacA4*
	Catheter	PG, AP, CIP, E, GM, CRO, CD, K, AK, TS	1	*aacA4*
	Blood	PG, AP, E, GM, CRO, CD, AK	-	
	Blood	PG, AP, E, GM, CRO, CD, K, AK, LEV	1	*aadB*
	Catheter	PG, AP, CIP, GM, CRO, K, AK, LEV	1	*aadB*
	Catheter	PG, AP, CIP, K, AK	-	-
	Pus	PG, AP, CIP, E, GM, K, AK, TN, TS, LEV	-	-
	Pus	PG, AP, CIP, E, GM, AK, TN, TS, LEV	1	*aadB*
	Pus	PG, AP, E, CRO, CD, K, AK, GAT, TN	-	
	Wound	PG, AP, CIP, E, GM, CRO, CD, K, AK, TN, TS	1	*blaoxa2*
	Wound	PG, AP, CIP, E, GM, CD, K, GAT, TN, TS	1	*aadB*
	Wound	PG, AP, E, GM, CRO, CD, K, TS, LEV	1	*blaoxa2*
t7580/YHHGW2MBQBLO	Sputum	PG, AP, CIP, E, CRO, CD, K, GAT, TS	1	*orfD-aacA4-catB8*
	Sputum	PG, AP, CIP, E, CRO, CD, K, GAT	-	-
	Ear	PG, AP, E, GM, CRO, K, GAT	-	-
	Wound	PG, AP, CIP, E, GM, CRO, CD, K, GAT, LEV	1	*aadB*
	Ear	PG, AP, CIP, GM, CRO, GAT, TS, LEV	1	*aadB*
	Ear	PG, AP, CIP, GM, CRO, GAT, TS	1	*aadB*
	Urine	PG, AP, CIP, E, GM, CRO, K, GAT, TN, TS	1	*aadB*
	Wound	PG, AP, CIP, E, GM, CRO, K, AK, GAT, TN, TS, LEV	2	*dfrA1-sat2-aadA1*
	Urine	PG, AP, CIP, CRO, CD, K, GAT, TN, TS	2	*dfrA1*
	Urine	PG, AP, CIP, E, GM, CRO, CD, K, AK, GAT	2	*dfrA1-sat2-aadA1*
	Body fluids	PG, AP, E, GM, CRO, CD, K, GAT, TS, LEV	2	*dfrA1*
	Wound	PG, AP, CIP, E, GM, CRO, CD, K, TS, LEV	1	*aadB*
t1425/ I2Z2EGMM	Catheter	PG, AP, CIP, E, GM, CRO, K, AK, GAT, TS	1	*aadB*
	Catheter	PG, AP, CIP, E, GM, CRO, K, AK, GAT, LEV	1	*aadB*
	Catheter	PG, AP, CIP, E, GM, CRO, CD, K, AK, GAT, LEV	1	*aadB-aadA1-cmlA6*
	Wound	PG, AP, CIP, E, GM, CRO, CD, K, GAT, LEV	2	*dfrA1*
	Sputum	PG, AP, CIP, E, GM, CRO, CD, K, GAT, TS, LEV	2	*dfrA11*
	Sputum	PG, AP, GM, CRO, K, AK, GAT, TS, LEV	2	*dfrA11*
	Sputum	PG, AP, CRO, CD, K	-	-
	Sputum	PG, AP, CRO, K, GAT	-	-
	Sputum	PG, AP, CIP, CRO, CD, K	-	-
	Sputum	PG, AP, E, GM	-	-

Abbreviations: AK, amikacin; AP, ampicillin; CD, clindamycin; CIP, ciprofloxacin; CRO, ceftriaxone; E, erythromycin; GAT, gatifloxacin; GM, gentamicin; K, kanamycin; LEV, levofloxacin; LZD, linezolid; PG, penicillin; TEC, teicoplanin; TN, tobramycin; TS, trimetoprim- sulfamethoxazole; VA, vancomycin.