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Genetic Diversity of Class 1 and Class 2 Integron Gene Cassettes in *Staphylococcus aureus* Isolates from Hospital Wastewater in Tehran, Iran

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Abstract

Background and Objectives: Hospital wastewater is a major reservoir for multidrug-resistant (MDR) bacteria, including integron-bearing *Staphylococcus aureus*. Integrons facilitate the acquisition and dissemination of antimicrobial resistance genes, making their epidemiological characterization essential for infection control.

Methods: In this cross-sectional study, conducted over a 15-month period at two teaching hospitals affiliated with Shahid Beheshti University of Medical Sciences, a total of 120 methicillin-resistant *S. aureus* (MRSA) isolates were collected from hospital wastewater, of which 75 (62.5%) were integron-positive *S. aureus* isolates. The isolates underwent antimicrobial susceptibility testing, integron class determination, and molecular analysis for resistance gene cassettes. Associations between integron classes and antimicrobial resistance profiles were statistically evaluated.

Results: Of the isolates examined, 66.6% carried class 1 integrons, 26.7% carried class 2 integrons, and 6.7% harbored both classes. Carriage of class 1 integrons was significantly associated with resistance to amikacin, penicillin, gentamicin, tetracycline, and erythromycin. Class 2 integrons were significantly correlated with resistance to amikacin, tetracycline, and erythromycin. A total of 16 distinct gene cassette arrays were identified. In class 1 integrons, the most prevalent arrays were *aadB–aacA4–aadA2* (20%), *aadB–catB3* (14%), and *aadB–cmlA6* (12%). In class 2 integrons, *sat2* (25%), *dhfrA1–sat2* (20%), and *dhfrA1* (20%) were most frequent. Multidrug resistance was observed in 94.2% of isolates, with resistance to erythromycin, tetracycline, gentamicin, and amikacin being most common.

Conclusions: This study highlights the predominance of class 1 integrons and their diverse gene cassette arrays in *S. aureus* isolates from hospital wastewater. The significant association between integron carriage and resistance to multiple antibiotics underscores the urgent need for enhanced surveillance and targeted infection control strategies to limit the spread of integron-bearing *S. aureus* within healthcare environments.

Keywords: *Staphylococcus aureus*, Multidrug-Resistant, Hospitals, Wastewaters, Integron, Gene Cassettes

1. Background

Hospital wastewater is a complex and often underappreciated reservoir of pathogenic and drug-resistant microorganisms (1). Among these, *Staphylococcus aureus* is of particular concern due to its ability to persist in diverse environmental conditions and its well-documented role in both community- and hospital-acquired infections (2). The detection of *S. aureus* in hospital effluents not only reflects the clinical burden within the institution but also highlights the potential for environmental dissemination of this pathogen. More critically, hospital wastewater may harbor multidrug-resistant (MDR) strains of *S. aureus*,

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creating a conduit for the transfer of resistance traits to other environmental or clinical bacteria (3). The release of such wastewater into municipal systems without adequate treatment can facilitate the spread of resistance genes in the broader environment, posing serious challenges to public health. Infections caused by MDR *S. aureus* strains are associated with limited treatment options, prolonged hospital stays, increased morbidity, and elevated healthcare costs (3-5). This highlights the importance of environmental monitoring and control of such pathogens in healthcare-associated waste streams (1, 2).

One of the major genetic mechanisms facilitating the emergence and spread of antibiotic resistance in bacterial populations is the presence of mobile genetic elements, particularly integrons. Integrons are DNA elements capable of capturing and expressing gene cassettes, many of which encode resistance determinants against a wide range of antibiotics. These elements consist of an integrase gene (*intI*), a recombination site (*attI*), and a promoter that drives the expression of the integrated cassettes (3, 6). Class 1 integrons are the most prevalent and have been identified in both Gram-negative and, increasingly, Gram-positive bacteria such as *S. aureus*. Through their modular architecture, integrons allow for the accumulation of multiple gene cassettes within a single element, thereby promoting multidrug resistance. The gene cassettes associated with integrons often include resistance to aminoglycosides, sulfonamides, β-lactams, macrolides, and other antibiotic classes. The environmental presence of such integrons, particularly in hospital wastewater, poses a significant threat, as it represents a dynamic reservoir for horizontal gene transfer and the evolution of MDR phenotypes across microbial communities (7).

2. Objectives

The present study was undertaken to investigate the prevalence of integrons in *S. aureus* strains isolated from hospital wastewater samples and to characterize the gene cassettes associated with these integrons. Specifically, this research aimed to determine the frequency of integron carriage and to sequence the resistance gene cassettes integrated within these elements.

3. Methods

3.1. Sample Collection

Hospital wastewater samples were systematically collected on a weekly basis over a 15-month period, spanning from June 2022 to August 2023. A total of 570 samples were obtained from two teaching hospitals affiliated with Shahid Beheshti University of Medical Sciences. For each sampling day, three separate samples were collected using sterile 500 mL polyethylene bottles preloaded with sodium thiosulfate to neutralize residual disinfectants. All samples were immediately transported to the laboratory under cooled conditions and stored at 4°C until processing. Approximately 100 mL of each sample was filtered through a 0.45 µm pore-size membrane filter, after which the retained material was inoculated onto blood agar plates (HiMedia, Mumbai, India) for bacterial culture (8). Only isolates that demonstrated visible bacterial growth on blood agar following membrane filtration were included in the study. Colonies with distinct morphological characteristics were subcultured for purification, and non-viable colonies, mixed cultures, or plates with insufficient growth were excluded.

3.2. Isolation and Identification of Staphylococcus aureus

Presumptive *S. aureus* colonies, characterized by golden-yellow or creamy-white pigmentation, were subjected to preliminary biochemical screening. Catalase activity was confirmed using hydrogen peroxide, and coagulase production was evaluated via the tube method with rabbit plasma (HiMedia, Mumbai, India). Confirmed colonies were further subcultured onto mannitol salt agar and DNase agar (HiMedia, Mumbai, India) for phenotypic verification. For molecular confirmation, genomic DNA was extracted and the presence of the *nucA* gene (270 bp) was assessed using PCR (8).

3.3. Antimicrobial Susceptibility Testing

Antibiotic susceptibility profiles were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2022). The tested antibiotics included amikacin, clindamycin, erythromycin, nitrofurantoin, fusidic acid, mupirocin, rifampin, ciprofloxacin, penicillin, gentamicin, tobramycin, tigecycline, tetracycline, trimethoprim/sulfamethoxazole, and

quinupristin/dalfopristin, which are commonly used for *S. aureus* treatment. The minimum inhibitory concentrations (MICs) of vancomycin and mupirocin (to detect both low-level and high-level resistance) were assessed using the broth microdilution technique in 96-well microtiter plates, as previously described in standard protocols. The D-zone test was also employed to detect inducible clindamycin resistance. Methicillin resistance was screened using a 30 μg cefoxitin disc, and results were interpreted according to CLSI breakpoints. Quality control for all antimicrobial susceptibility tests was maintained by using standard reference *S. aureus* strains: ATCC 25923, ATCC 29213, and ATCC 43300.

3.4. Extraction of Genomic and Plasmid DNA from Staphylococcus aureus

Genomic DNA extraction from *S. aureus* isolates was carried out according to the protocol described by Mostafa et al. (9). PCR amplification products were separated by electrophoresis on a 1% agarose gel and visualized using ethidium bromide staining under ultraviolet light. A 100 bp Plus DNA Ladder (GeneRuler™, Fermentas, Vilnius, Lithuania) was employed as a molecular weight marker to estimate fragment sizes. In parallel, plasmid DNA was isolated from *S. aureus* strains using the Qiagen Plasmid Midi Kit, following the manufacturer's recommended protocol (9).

3.5. Detection of Class 1 and Class 2 Integrons

All *S. aureus* isolates were screened for the presence of class 1 and class 2 integrons using specific primers targeting the *intI1* and *intI2* genes, as previously described by Moura et al. (10).

3.6. Detection and Sequencing of Integron Gene Cassettes

To investigate the gene cassettes embedded within the variable regions of class 1 and class 2 integrons, PCR amplification was performed using specific primer sets described by Moura et al. (10). PCR products exhibiting distinct band sizes upon agarose gel electrophoresis were selected for further analysis. These amplicons were purified using the High Pure PCR Product Purification Kit (Roche, USA) according to the manufacturer's instructions, and then subjected to direct sequencing.

3.7. Statistical Analysis

Comparisons of antibiotic resistance between integron-positive and integron-negative isolates were performed using the Chi-square (χ^2) test. A P-value < 0.05 was considered statistically significant.

4. Results

4.1. Isolation and Antibiotic Susceptibility

A total of 120 *S. aureus* isolates were collected from hospital wastewater samples in Tehran over a 15-month period. All *S. aureus* isolates were confirmed as methicillin-resistant *S. aureus* (MRSA) according to the detection of the *mecA* gene. Among these, 75 isolates (62.5%) were integron positive, and the remaining 45 (37.5%) were integron negative. Overall, all isolates were found to be MRSA, while MDR was confirmed in 94.2% of isolates (n = 113). All strains were resistant to penicillin. The highest level of antibiotic resistance was observed against erythromycin (84.2%), followed by tetracycline (82.5%), gentamicin (74.2%), and amikacin (73.3%). It is worth mentioning that 29 tested isolates were resistant to mupirocin (24.2%). The present analysis revealed that 23 isolates (79.3%) had high-level mupirocin resistance (HLMUPR) and 6 isolates (20.7%) had low-level mupirocin resistance (LLMUPR) patterns. Of the 120 *S. aureus* isolates, resistance to fusidic acid was detected in 9 isolates (7.5%), all of which belonged to MRSA integron-positive strains. According to our results, 56 isolates (46.7%) exhibited constitutive clindamycin resistance [constitutive macrolide-lincosamide-streptogramin B ($cMLS_B$)] phenotypes and 45 isolates (37.5%) displayed inducible clindamycin resistance [inducible macrolide-lincosamide-streptogramin B ($iMLS_{B)}$] phenotypes (Table 1). The results of the disk diffusion method indicated that twenty-two resistance profiles were observed. The top three resistance profiles were as follows: AMK, CLI, ERY, GEN, PEN, TET, TOB (20.8%; 25/120); AMK, ERY, GEN, PEN, TET (11.7%; 14/120); and AMK, ERY, GEN, NIT, PEN, RIF, SYN, TET and AMK, CLI, ERY, GEN, NIT, PEN, RIF, TET (each 10%; 12/120) of isolates.

4.2. Identification of Class 1 and Class 2 Integrons

The integron detection results showed that among the 120 strains of methicillin-resistant *S. aureus*, 75 (62.5%) isolates carried integrons. Integron type I was highly prevalent, representing 66.6% (50/75), followed by type II (26.7%; 20/75). Simultaneous carriage of integron class 1 and 2 was present in 5 isolates of methicillin-resistant *S. aureus* (6.7%). The findings demonstrated that resistance to several different antibiotics is linked

Table 1. Antimicrobial Susceptibility Pattern of Integron Positive and Integron Negative *Staphylococcus aureus* Strains Isolated from Hospital Wastewater [a]

Antibiotics	All Strains of MRSA (n = 120)		Integron-Positive MRSA Isolates (n = 75)		Integron-Positive MRSA Isolates (n = 75)	
	R	S	R	S	R	S
PEN	120 (100)	0 (0)	75 (100)	0 (0)	45 (100)	0 (0)
GEN	89 (74.2)	31 (25.8)	55 (73.3)	20 (26.7)	34 (75.6)	11 (24.4)
AMK	88 (73.3)	32 (26.7)	59 (78.7)	16 (21.3)	29 (64.4)	16 (35.6)
TOB	41 (34.2)	79 (65.8)	38 (50.7)	37 (49.3)	3 (6.7)	42 (93.3)
ERY	101 (84.2)	19 (15.8)	75 (100)	0 (0)	26 (57.8)	19 (42.2)
CLI	56 (46.7)	64 (53.3)	40 (53.3)	35 (46.7)	16 (35.6)	29 (64.4)
TET	99 (82.5)	21 (17.5)	59 (78.7)	16 (21.3)	40 (88.9)	5 (11.1)
TIG	2 (1.7)	118 (98.3)	2 (2.7)	73 (97.3)	0 (0)	45 (100)
RIF	49 (40.8)	71 (59.2)	30 (40)	45 (60)	19 (42.2)	26 (57.8)
NIT	43 (35.8)	77 (64.2)	24 (32)	51 (68)	19 (42.2)	26 (57.8)
SYN	14 (11.7)	106 (88.3)	8 (10.7)	67 (89.3)	6 (13.3)	39 (86.7)
SXT	24 (20)	96 (80)	16 (21.3)	59 (78.7)	8 (17.8)	37 (82.2)
FUS	9 (7.5)	111 (92.5)	9 (12)	66 (88)	0 (0)	45 (100)
MUP	29 (24.2)	91 (75.8)	18 (24)	57 (76)	11 (24.4)	34 (75.6)

Abbreviations: AMK, amikacin; CLI, clindamycin; ERY, erythromycin; FUS; fusidic acid; GEN, gentamicin; MUP, mupirocin; NIT, nitrofurantoin; PEN, penicillin; RIF, Rifampin; SXT, trimethoprim sulfamethoxazole; SYN, quinupristin-dalfopristin; TET, tetracycline; TIG, tigecycline; TOB, tobramycin; MRSA, methicillin-resistant *S. aureus*.

[a] Values are presented as No. (%).

with the presence of integrons. The relationship between the presence of integron class 1 and resistance to amikacin, penicillin, gentamicin, tetracycline, and erythromycin was significant. Resistance to antibiotics including amikacin, tetracycline, and erythromycin in *S. aureus* isolates was associated with the presence of integron class 2. Information about the relationship between integron class 1 and 2 is summarized in Table 2. Of 50 integron class 1-positive isolates, 45 (90%) harbored integron class 1 on the chromosome, and 5 (10%) isolates on plasmid. Among 20 integron class 2-positive isolates, 16 (80%) carried class 2 integrons on the chromosome and 5 (20%) isolates on plasmid. All isolates carrying both class 1 and 2 harbored integrons on the chromosome. The distribution of different classes of integrons and resistance profiles in *S. aureus* strains are presented in Figure 1.

4.3. Characterization of Gene Cassettes

Sequence analyses of PCR products of the integron cassette region revealed 12, 4, and 2 different gene cassettes in class 1, class 2, and class 1+2 integrons, respectively. Overall, 16 distinct kinds of gene cassette arrays were identified. Four (20%) of the class 2 integron-containing isolates did not have any PCR products. In class 1 integrons, the gene cassette arrays *aadB-aacA4-aadA2* (20%), *aadB-catB3* (14%), and *aadB-cmlA6* (12%) were found to be the top three gene cassette arrays, while in class 2 integrons, the gene cassette arrays *sat2* (25%), *dhfrA1-sat2* (20%), and *dhfrA1* (20%) were found to be the most prevalent (Figure 2). Different sizes of gene cassettes of integron class 1 and 2 among 70 methicillin-resistant *S. aureus* isolates are presented in Table 3. In class 1+2 integrons, the gene cassette arrays *blaoxa2* (60%) and *aadA2* (40%) were identified.

4.4. Nucleotide Sequence Accession Numbers

The nucleotide sequence data reported in this article are available in the GenBank nucleotide database under GenBank accession numbers PV870208, PV883328, PV883327, PV935384, PV935386, PV935387, PV940075, PV948192, PV948193, PX021438, PX021568 obtained from the gene cassette of class 1 integrons, and PV948188, PV948189, PV948190, PV948191, obtained from the gene cassette of class 2 integrons. Overall, 16 strains were sequenced.

5. Discussion

The analysis revealed that antibiotics such as tobramycin, fusidic acid, tigecycline, and quinupristin-dalfopristin exhibited the highest levels of activity against the tested *S. aureus* isolates. These results align with previous studies, including those by Rafati Zomorodi et al., who reported strong efficacy for

Table 2. Correlation of Antibiotic Resistance Between Class 1 and Class 2 Integron-Positive and Integron-Negative Methicillin-Resistant *Staphylococcus aureus* Strains

Antibiotics	Class I Integron		Class II Integron		Class I and II Integron	
	R [a]	P-Value	R [a]	P-Value	R [a]	P-Value
PEN	50	0.006 [b]	20	0.055	5	0.11
GEN	45	0.002 [b]	5	0.14	5	0.42
AMK	39	0.003 [b]	18	0.01 [b]	2	0.5
TOB	22	0.06	16	0.08	0	0.47
ERY	50	0.004 [b]	20	0.006 [b]	5	0.08
CLI	25	0.12	15	0.22		0.12
TET	39	0.001 [b]	17	0.045 [b]	3	0.19
TIG	2	0.12	0	0.2	0	0.45
RIF	18	0.11	10	0.3	2	0.41
NIT	19	0.056	5	0.22	0	0.6
SYN	7	0.48	1	0.43	0	0.5
SXT	15	0.74	1	0.11	0	0.34
FUS	9	0.11	0	0.41	0	0.36
MUP	18	0.2	0	0.2	0	0.4

Abbreviations: AMK, amikacin; CLI, clindamycin; ERY, erythromycin; FUS; fusidic acid; GEN, gentamicin; MUP, mupirocin; NIT, nitrofurantoin; PEN, penicillin; RIF, Rifampin; SXT, trimethoprim sulfamethoxazole; SYN, quinupristin-dalfopristin; TET, tetracycline; TIG, tigecycline; TOB, tobramycin.

[a] Values are presented as No.

[b] Statistically significant.

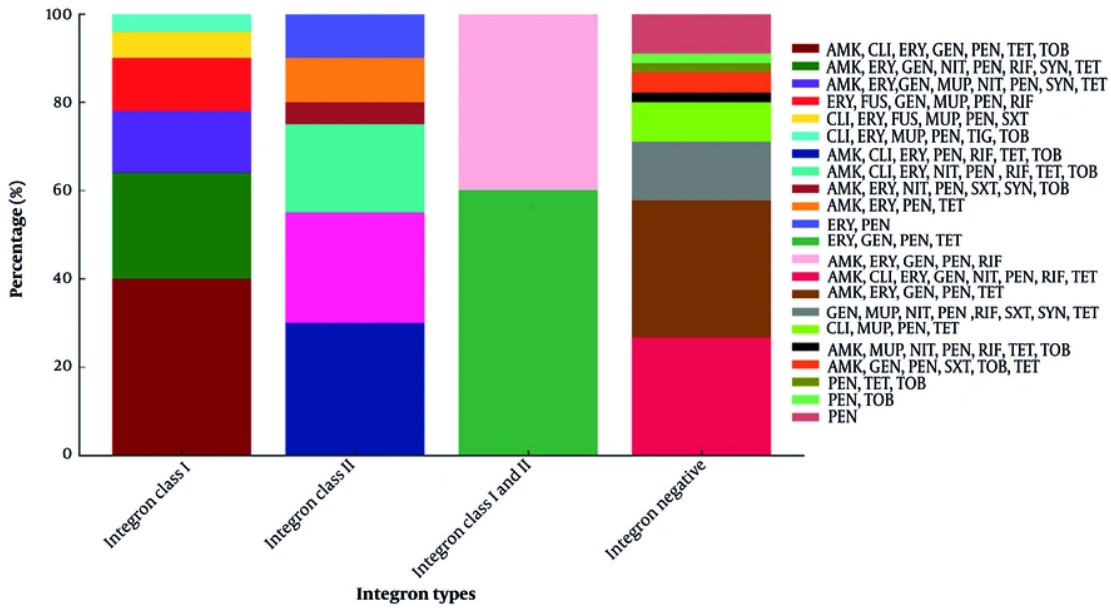

Figure 1. Distribution of resistance profiles by integron types

linezolid and vancomycin (11), and Saedi et al., who observed high susceptibility rates (over 90%) for linezolid, vancomycin, and trimethoprim-sulfamethoxazole (12). Mupirocin continues to serve as a

Table 3. Different Sizes of Gene Cassettes of Integron Class 1 and 2 Among 75 Integron Positive Methicillin-Resistant *Staphylococcus aureus* Isolated from Hospital Wastewater

Integron and Gene Cassette Array	Integron Putative Location	Amplicon Size (bp)	No. of Isolates [a]
Class 1 (50; 66.7)			
aadB	Chromosome	531	3 (6)
aadA2	Chromosome	792	4 (8)
blaoxa2	Chromosome	828	7 (14)
cmlA6	Chromosome	1339	2 (4)
aacA4	Chromosome	801	2 (4)
catB8	Chromosome	901	4 (8)
orfD-blaoxa2	Chromosome	1304	2 (4)
orfD-aacA4	Chromosome	996	5 (10)
aadB-cmlA6	Plasmid	1934	6 (12)
aadB-catB3	Chromosome	1420	5 (10)
aadB- aacA4-aadA2	Chromosome	2691	7 (14)
Putative glucose dehydrogenase- *blaoxa2*-hypothetical protein	Chromosome	1900	3 (6)
Class 2 (20; 26.7)			
dhfrA1	Chromosome	459	4 (20)
sat2	Chromosome	661	5 (25)
dhfrA1-sat2	Plasmid	1214	4 (20)
dhfrA11	Chromosome	474	3 (15)
Without PCR product	Chromosome	-	4 (20)
Class 1 and 2 (5; 6.6)			
aadA2	Chromosome	792	2 (40)
blaoxa2	Chromosome	828	3 (6)

[a] Values are presented as No. (%).

key topical antimicrobial for curbing *S. aureus* transmission and mitigating infection risks across both healthcare and community environments, as supported by earlier studies. In the current investigation, 24.2% of the isolates exhibited resistance to mupirocin, with the majority (79.3%) categorized as high-level mupirocin-resistant (HLMUPR) and the remaining 20.7% classified as low-level mupirocin-resistant (LLMUPR). The detected prevalence of HLMUPR-methicillin-resistant *S. aureus* strains in this study notably surpasses previously reported rates in countries such as China (7%), France (0.8%), and Canada (4.3%) (13-15). This considerable discrepancy underscores the geographic variability in mupirocin resistance patterns among methicillin-resistant *S. aureus* isolates and emphasizes the critical role of regional surveillance in informing effective infection prevention and antimicrobial stewardship efforts.

The distribution of $iMLS_B$ resistance phenotype among *S. aureus* isolates demonstrates considerable variability across different geographical regions and healthcare systems. In the present study, the prevalence of $iMLS_B$ was identified at 37.5%, which is higher than rates reported in Turkey (7.8%) (16), Egypt (7.7%) (17), Iran (8.6%) (18), and Nepal (11.48%) (19), but similar to the prevalence observed in India (37.5%) (20). Prior investigations in Iran have highlighted notable regional differences in $iMLS_B$ rates, ranging from 6% to 32.3%, reflecting the impact of localized antibiotic prescribing practices and diagnostic approaches. In addition, the $cMLS_B$ phenotype was observed in 46.7% of isolates. This finding aligns with previous studies, such as those by Delialioglu et al. (24.3%) (16) and Eksi et al. (20.4%) (21). Nevertheless, other investigations have documented widely differing rates, ranging from 13.1% to as high as 82.9%, depending on the location and study methodology. Such variation likely stems from differences in community and hospital-based macrolide consumption, study population characteristics, and the dissemination of specific clonal lineages. According to our data, the $cMLS_B$ phenotypes were relatively frequent among methicillin-resistant *S. aureus* strains (46.7%), a

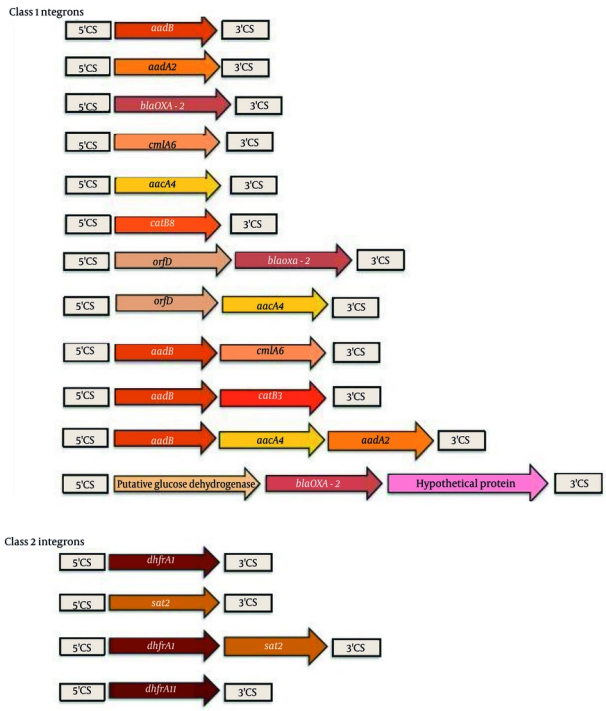

Figure 2. Graphical depiction of the different cassette arrays present in class 1 and class 2 integrons

finding consistent with earlier studies from Nepal, Iran, Egypt, and Turkey (16-19).

Regarding fusidic acid resistance, recent reports from various Asian countries indicate low resistance levels, typically below 10%. Our study identified a fusidic acid resistance rate of 7.5%. These results are consistent with findings from a large multicenter Iranian study, which reported a 3% prevalence of fusidic acid-resistant methicillin-resistant *S. aureus* among 726 *S. aureus* isolates. An upward trend in fusidic acid resistance among methicillin-resistant *S. aureus* isolates has been documented. For instance, data from Kuwait revealed a dramatic rise in resistance rates, escalating from 22% in 1994 to 92% by 2004 (22). While earlier reports from Iran suggested lower resistance levels ranging between 2.5% and 8% (23). Globally, resistance rates differ substantially, ranging from 62.4% in Greece to as low as 0.3% in the United States (24).

It is well established that integrons play a pivotal role in the dissemination of multidrug resistance among pathogenic bacteria (6). In the current study, class 1 integrons were the most frequently detected type, identified in 34.1% of the isolates. Class 2 integrons were present in 14.3% of samples, and a smaller proportion (5.6%) harbored both class 1 and class 2 integrons simultaneously. These findings, consistent with previous literature, emphasize the predominance of class 1 integrons over class 2 in *S. aureus* populations. Our data closely align with the report by Mohammadi et al. in Iran, where class 1 integrons were found in 24.8% of *S. aureus* isolates (25). Similar trends have been reported from clinical samples in Iran. In a multi-center study conducted by Zomorodi et al. on 183 *S. aureus* isolates, integron class 1 was detected in 7.6% of the tested isolates, while none were positive for class 2 (11). In a study performed in Iran on 139 *S. aureus* isolates, Mostafa et al. reported a prevalence of 72.6% and 35.2% of integron class 1 and 2, respectively (9). A study conducted in India in 2015 by Marathe et al. reported that 71% of the identified methicillin-resistant *S. aureus* strains harbored class 1 integrons, while none of the isolates tested positive for class 2 or class 3 integrons, indicating the dominant presence of class 1 integrons in

the studied population (26). In contrast, research conducted by Guney in Turkey reported an absence of class 1 integrons among the examined isolates, emphasizing the substantial regional and bacterial diversity in integron distribution (27). Growing evidence points to class 1 integrons as major contributors to the dissemination of antibiotic resistance, especially in methicillin-resistant *S. aureus* strains. Variations in the occurrence of these genetic elements may stem from factors such as geographic location, clonal differences among isolates, and disparities in antibiotic prescription practices and overuse across regions.

In the current investigation, the most frequently detected gene cassettes within class 1 integrons were *aadB–aacA4–aadA2*, bla_OXA-2, and *aadB–cmlA6*, followed by the *aadB*–catB3 cassette. A study conducted by Mostafa et al. in 2015 similarly identified *aadB*, *aadA2*, and *dhfrA1–sat2*–aadA1 as the predominant cassette arrays. Their research also reported several novel gene cassettes in *S. aureus* isolates harboring class 1 integrons, including *aadB*, oxa2, *aacA4*, orfD–*aacA4–catB8*, *aadB*–catB3, orfD–*aacA4*, and *aadB*–aadA1–*cmlA6*. In contrast, among class 2 integrons, the most common arrays were *dhfrA1–sat2*–aadA1, *dhfrA11*, and *dhfrA1–sat2* (9). Between 2001 and 2006, Xu et al. analyzed a collection of nosocomial methicillin-resistant *S. aureus* isolates and found that 76 out of 179 strains (42.5%) carried class 1 integrons (28). The most common resistance determinants were organized into four distinct gene cassette arrays: *aadA2*, *aacA4–cmlA1*, *dfrA17–aadA5*, and *dfrA12–orfF–aadA2*. Class 1 integrons are recognized for their ability to capture and disseminate diverse antimicrobial resistance genes, with aminoglycoside resistance cassettes being particularly frequent (9). Notably, these integrons have been associated with *Tn3* family transposons, such as *Tn21* and *Tn1696*, suggesting that the extensive distribution of class 1 integrons is likely driven by the mobility of integron-containing transposons (9, 29). Moreover, the distribution of *Tn7* among clinical isolates has been shown to correlate with elevated rates of trimethoprim resistance, a phenotype conferred by the dihydrofolate reductase enzyme encoded by the *dhfr* gene located within *Tn7* (9).

In our analysis, plasmid-borne integrases were detected in 13.3% (10/75) of the isolates, which is lower than those previous reports by Mostafa et al. (36%) and Goudarzi et al. (80%) in *S. aureus* (9, 29). It should be noted, however, that this estimate may be subject to bias, as plasmid and chromosomal DNA can exhibit similar electrophoretic mobility patterns. In a previous investigation, Goudarzi et al. characterized the genetic composition of integrons in *S. aureus* isolates and reported six distinct gene cassettes *aadA*, *aadB*, *bla_OXA*, *aacA4*, *cmlA6*, and *catB* within class 1 integrons. In addition, three gene cassettes *dfrA1*, *aadA1*, and *sat2* were detected in class 2 integrons (29). These findings highlight the genetic diversity of resistance determinants harbored by integrons and underscore their potential role in facilitating the dissemination of multidrug resistance among clinical strains.

Despite the valuable insights provided, this study has several limitations. First, the study was conducted in only two tertiary care hospitals affiliated with Shahid Beheshti University of Medical Sciences, which may limit the generalizability of the findings to other hospitals or regions. Second, only integron-positive *S. aureus* isolates were examined, so the overall prevalence of antimicrobial resistance in the total *S. aureus* population in hospital wastewater may not be fully represented. Third, molecular analysis focused on gene cassette arrays within integrons, but other resistance mechanisms, such as plasmids or transposons outside of integrons, were not evaluated. Finally, environmental factors and temporal variations beyond the 15-month sampling period may influence integron distribution, which were not comprehensively assessed.

5.1. Conclusions

This study shows that a considerable proportion of *S. aureus* isolates in the surveyed hospital wastewater harbor integrons, contributing to the potential spread of multidrug resistance. These findings reinforce the urgency of revising current infection control strategies and highlight that, in some cases, resistance may arise through mechanisms independent of integron carriage.

Footnotes

AI Use Disclosure: The authors declare that no generative AI tools were used in the creation of this article.

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