Microbes and Infectious Diseases 2025; 6(2): 659-669

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Molecular study of *Staphylococcus aureus* accessory gene regulator (*agr*) types in Tanta University Hospitals

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ARTICLE INFO

Article history:
Received 11 October 2024
Received in revised form 5 November 2024
Accepted 10 November 2024

Keywords:
Staphylococcus aureus
Agr
Biofilm
antimicrobial resistance
virulence.

ABSTRACT

Background: *Staphylococcus aureus* has developed antibiotic resistance, a global challenge that makes managing infections difficult. The accessory gene regulator (*agr*) is a quorum-sensing system virulence regulator. **Objectives:** The objective of this study was to investigate the relations between *agr* types, antimicrobial resistance, and biofilm formation among *S. aureus* isolates. **Methodology:** Various clinical samples from patients with nosocomial infections in ICUs of Tanta University Hospitals were included. *S. aureus* isolates were identified, antibiotic sensitivity testing was done using Kirby-Bauer disk diffusion method, *mec*A gene screening by PCR, the tissue culture plate technique was utilized to evaluate the biofilm formation, and *agr* typing was detected by multiplex PCR. **Results:** Seventy-six *S. aureus* isolates were obtained; they were highly resistant to ampicillin (90.8%), imipenem (73.7%) and no resistance for vancomycin or linezolid in all isolates. 56 isolates (73.68%) were MRSA while 20 isolates (26.31%) were MSSA. There is a significant variation in biofilm formation between MSSA and MRSA isolates. Both biofilm-forming and non-forming isolates exhibited a rise in antibiotic resistance. Among all isolates, *agr* I was the commonest type (61.8%) followed by *agr* III (18.4%). The existence of *agr* I was linked to biofilm development. Isolates with *agr* I were resistant to Erythromycin, Gentamycin, Ciprofloxacin, Levofloxacin, and Tetracycline among MRSA isolates. **Conclusion**: High rate of antibiotic resistance, and biofilm production among *agr*-positive *S. aureus* isolates were detected in ICUs. Therefore, the development of a new effective anti-virulence agent is promising for the treatment of *S. aureus* infection.

Introduction

Staphylococcus aureus (*S. aureus*) is a well-known and prevalent bacterial opportunistic pathogen, that causes severe skin infections, respiratory tract infections, surgical site infections, endocarditis, osteomyelitis, sepsis, bacteremia as well as implant-associated diseases [1, 2]. It is regarded as one of the main contributors to infections detected in both hospitals and the community [3]. Antibiotic resistance has emerged in *S. aureus* which is a global concern causing challenges in the management of infection in clinical practice worldwide [4].

Many virulence factors that *S. aureus* possesses are responsible for its broad range of infections as surface-associated factors, hemolysins and enzymes, and super antigenic toxins [5]. Furthermore, *S. aureus* may create biofilms in

DOI: 10.21608/MID.2024.327713.2279

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tissues as on the surfaces of biological materials and medical equipment [6]. Biofilm growth greatly enhances S. aureus pathogenicity, as it may hide them from the human immune system's damage and reduce resistance to drugs, which can lead to severe and persistent relapsing infections [7, 8].

Quorum sensing is mechanism, by which bacterial cells coordinate and communicate based on population density [9]. First reported in 1986, the accessory gene regulator (*agr*) encodes a quorum-sensing mechanism that functions as a master regulator of the expression of virulence genes. The *agr* operon includes the genes for *agr* A, *agr* B, *agr* C, and *agr* D. *S. aureus* isolates can be divided into four *agr* different groups (*agr* I, *agr* II, *agr* III, and *agr* IV) based on the sequences of the *agr C* gene (which encodes the receptor of the autoinducing peptide, AIP) and the *agr D* gene (which encodes cyclic AIP) [10, 11]. The *agr* system plays a dominant role of staphylococcal pathogenesis by controlling the virulence factors, biofilm formation, and the expression of antimicrobial resistance genes, so it represents a clear example of the link between virulence and resistance [12,13].

The *agr* approach is a useful molecular tool that could be used to track and identify *Staphylococcal* clones and determine the source of nosocomial infections in hospital settings, as management of these infections is a significant problem [14].

Novel strategies disrupting the systems that control the expression of the *agr* system are a key goal of anti-virulence drugs, which aid in treating *S. aureus* infections [15-17].

Understanding the genetic components helps in monitoring, controlling, and treating *S. aureus* infections. Considering this, the objective of the present study was to study the molecular characters of the *agr* system of *S. aureus* isolates from Intensive Care Units (ICUs) of Tanta University Hospitals and to investigate possible correlation with their antibiotic resistance pattern and biofilm formation ability.

METHODS

This cross-sectional study was carried out on 180 different clinical samples from patients admitted to ICUs of Tanta University Hospitals with symptoms and signs of infections that appeared at least 48h after admission. All participants provided written informed consent. It was conducted in the Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University, from February to July of 2024. The study was approved by Tanta University Faculty of Medicine's Ethics and Research Committee, with approval code (3624PR227/6/23). The principles of the Helsinki Declaration were followed throughout the whole process.

Staphylococcus aureus isolation and identification:

Different clinical samples were collected (wound swabs, pus, urine, sputum, blood). The sample was collected subsequently; the samples were promptly transferred to the microbiology lab under aseptic conditions. Samples were cultivated on nutrient agar, MacConkey agar, and blood agar (**Oxoid, UK**), followed by a 24 to 48-hour aerobic incubation period at 37˚C. Colonies were determined by colonial morphology, Gram staining, and biochemical reactions by clinical laboratory guidelines [18].

Antimicrobial susceptibility testing:

As per Clinical and Laboratory Standard Institute (CLSI) recommendations, the antimicrobial sensitivity of *S. aureus* isolates was assessed by Muller Hinton agar (Oxoid, UK) plates using a modified Kirby Bauer disc diffusion method. Antibiotics used were cefoxitin (30 µg), oxacillin (1 µg), erythromycin (15 µg), ciprofloxacin (5 µg), gentamycin (10 µg), tetracycline (30 µg), linezolid (30 µg), vancomycin (30 µg), ampicillin (10 µg), imipenem (10 µg), levofloxacin (5 µg), rifampicin (5 µg), cotrimoxazole (25 µg), and clindamycin (2 µg) (Hi-Media). The plates were incubated for 24 hours at 37°C, and then the inhibitory zones were measured and assessed by the CLSI-recommended protocols [19].

Methicillin resistance *S. aureus* (MRSA) screening:

Isolates of *S. aureus* were screened phenotypically using the cefoxitin disk diffusion method for methicillin resistance by inoculation of the tested isolate on a Muller Hinton agar (MHA) plate , and a cefoxitin (FOX 30 µg) disk was applied to the plate and incubated at 37˚C for 24 h. The inhibition zones were measured, and those less than 21 mm in diameter were considered MRSA according to CLSI [19].

Biofilm formation Assay:

S. aureus isolates were tested for their ability to form biofilms using the quantitative tissue culture plate (TCP) method as described in a

previous study [20]. Each isolate was tested in triplicates. 100 µl of bacterial suspension were loaded into each well of a 96-well flat bottom microtiter plate and incubated at 37˚C for 24 h. *S. aureus* ATCC 27543 at the same concentrations was used as a positive control, and sterile MH broth was used as a negative control. After incubation, the wells were washed, and fixed with methanol, and the adherent cells were stained using 0.1% (v/v) crystal violet, then rinsed and air-dried. The optical density of the stained biofilm was measured by an ELISA microplate reader at 570 nm. Biofilm production was interpreted and categorized into non-biofilm producers, mild, moderate, or strong biofilm [21].

Genomic DNA extraction:

Following the manufacturer's recommendations for Gram-positive bacteria, the DNA of the *S. aureus* isolates was extracted using the ABT bacterial DNA microextraction kit (Applied Biotechnology, Egypt). Using agarose gel electrophoresis and a nanophotometer (Thermo NanoDrop 2000; Thermo Scientific), the concentration and purity of the genomic DNA were evaluated. -20°C was used to store the extracted DNA.

Detection of *mec*A gene:

Methicillin resistance was further confirmed by amplification of the *mec*A gene by PCR using the following primers: F: 5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3' and R: 5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3'. *S. aureus* American Type Culture Collection (ATCC) 43300 served as its positive control. The thermocycler was programmed for: 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 1 min, and final extension step at 72 °C for 6 min were performed after the initial denaturation at 95°C for 3 min [22].

Accessory gene regulator (*agr*) typing:

S. aureus isolates were tested for the identification of *agr* specificity groups using multiplex PCR-based assays using the primers and amplification conditions as previously described [11] and been shown in **Table 1.** Positive control strains were *S. aureus* National Collection Type Culture (NCTC) 10652 for *agr* Group I; *S. aureus* NCTC 10654 for *agr* Group2 and *S. aureus* ATCC 27664 for *agr* Group 3. As follows: first denaturation at 94°C for 5 minutes; then 30 cycles at 94°C for 1 minute, annealing for 1 minute at 55°C, and extending for 1 minute at 72°C before doing a final extension at 72°C for 5 minutes. The amplicons were electrophoresed in a 1.5% agarose gel, stained with ethidium bromide, and then exposed to ultraviolet light for visualization. Using the GeneRuler 100 bp plus DNA ladder (Thermo Scientific, Waltham, MA, USA), the lengths of the PCR products were calculated, as illustrated in **Figure 1**.

Statistical Analysis:

Version 20.0 of the IBM SPSS software package (Armonk, NY: IBM Corp.) was used to put data into the computer and analyze them. Chi-square tests were employed to compare several groups. The chi-square is corrected using Fisher's exact test. The student t-test was utilized to compare two groups under study for quantitative variables that are regularly distributed. P-values less than 0.05 were regarded as statistically significant.

RESULTS

Seventy-six *Staphylococcus aureus* isolates were obtained out of 180 different clinical samples from the ICUs. Depending on the cefoxitin disc and PCR for detection of the *mec*A gene, 73.68% (56/76) of *S. aureus* isolates were MRSA, while 26.31% (20/76) were MSSA. Males were more frequently infected than females in both MRSA and MSSA, with percentages (69.6% and 60%) respectively. The mean age of *S. aureus* infections was (49.57 ± 11.38). No significant difference was seen between MSSA and MRSA regarding age and sex. The highest percentage of *S. aureus* isolates was recovered from urine samples and the lowest percentage was from blood samples with no significant difference between MSSA and MRSA. However, a statistically significant difference was found between MSSA and MRSA regarding biofilm formation. The highest percentage of positive biofilm producers in both MSSA isolates and MRSA isolates were moderate biofilm producers in percentage (40% and 44.6%) respectively. Strong biofilm producers were (35.7% and 15%) in MRSA and MSSA respectively. Furthermore, the most prevalent *agr* type among all *S. aureus* isolates was type I followed by type III, type II, and type IV in percentage (61.8%, 18.4%, 2.6%, and 1.3%) respectively without significant difference between MSSA and MRSA isolates as described in **Table 2** and **Figure 1.**

Regarding the antibiotic resistance patterns, *S. aureus* isolates had the highest resistance towards ampicillin, imipenem, cefoxitin with resistance rates of 90.8%, 73.7%, and 73.7%,

respectively. No resistance was detected toward vancomycin and linezolid. On comparing the resistance pattern among MSSA and MRSA, all MRSA isolates had higher resistance than MSSA to all tested antibiotics with high statistical significance except for clindamycin and rifampicin as shown in **Table 3**. Correlations between *agr* types and biofilm formation revealed that *agr* I was closely associated with biofilm formation in both MSSA and MRSA isolates with P value= (0.048* and 0.002*) respectively as demonstrated in **Table 4**.

Moreover, correlations between biofilm formations and antibiotic resistance in MRSA demonstrated that biofilm-positive and negative isolates exhibited a variable degree of antibiotic resistance. Biofilm-positive MRSA isolates were resistant to imipenem, ampicillin, ciprofloxacin, levofloxacin, tetracycline, erythromycin, cotrimoxazole, clindamycin, and gentamycin in percentages (100%, 100%, 80%, 80%, 80%, 50%, 45%, 25%, and 20%) respectively. However, all non-biofilm former isolates were sensitive to clindamycin, ciprofloxacin, levofloxacin, and tetracycline. There was a statistically significant difference between the two *S. aureus* groups in terms of clindamycin, ciprofloxacin, levofloxacin, tetracycline, erythromycin, and gentamycin **(Table 5).**

As regards the relation between *S. aureus* isolates with *agr* I and antibiotic resistance, it was detected that MRSA isolates with *agr* I were significantly correlated with erythromycin, gentamycin, ciprofloxacin, levofloxacin, and tetracycline resistance ($p<0.05$), while *agr* I in MSSA isolates was non-significantly associated with antibiotic resistance, as shown in **Table 6.**

Table 1. List of primers, with oligonucleotide sequences, amplicon size, which were used to detect various agr genes of Staphylococcus aureus.

Gene	**Primers**	**Product Size (bp)**
***pan-agr* F**	5-ATG CAC ATG GTG CAC ATG C-3	
***agr* I R**	5-GTC ACA AGT ACT ATA AGC TGC GAT-3	441
***agr* II R**	5-TAT TAC TAA TTG AAA AGT GGC CAT AGC-3	575
***agr* III R**	5-GTA ATG TAA TAG CTT GTA TAA TAA TAC CCA G-3	323
***agr* IV R**	5-CGA TAA TGC CGT AAT ACC CG-3	659

Table 2. Comparison between MSSA and MRSA isolates regarding demographic data sample type, biofilm formation and *agr* types.

	Total (n = 76)	**MSSA (n = 20)**	**MRSA (n = 56)**	**Test of Sig.**	**P-value**
Sex					
Male	51 (67.1)	12 (60.0)	39 (69.6)	χ^2= 0.621	0.431
Female	25 (32.9)	8 (40.0)	17 (30.4)		
Age (years)					
Min – Max.	24.0 – 72.0	24.0 – 68.0	24.0 – 72.0	t= 0.280	0.780
Mean ± SD.	49.57 ± 11.38	48.95 ± 12.18	49.79 ± 11.18		
Sample					
Sputum	19 (25.0%)	3 (15.0%)	16 (28.6%)	FET= 5.769	0.202
Urine	31 (40.8%)	11 (55.0%)	20 (35.7%)		
Pus	9 (11.8%)	3 (15.0%)	6 (10.7%)		
Wound	9 (11.8%)	3 (15.0%)	6 (10.7%)		
Blood	8 (10.5%)	0 (0.0%)	8 (14.3%)		
Biofilm					
Negative	9 (11.8%)	6 (30.0%)	3 (5.4%)	c^2= 8.572*	0.008*
Positive	67 (88.2%)	14 (70.0%)	53 (94.6%)		
Weak	11 (14.5%)	3 (15.0%)	8 (14.3%)	FET= 1.465	0.560
Moderate	33 (43.4%)	8 (40.0%)	25 (44.6%)		
Strong	23 (30.3%)	3 (15.0%)	20 (35.7%)		
***agr* type**					

Non	12 (15.8%)	5 (25.0%)	7 (12.5%)	FET= 7.407	0.085
I	47 (61.8%)	11 (55.0%)	36 (64.3%)		
II	2 (2.6%)	2 (10.0%)	0 (0.0%)		
III	14 (18.4%)	2 (10.0%)	12 (21.4%)		
IV	1 (1.3%)	0 (0.0%)	1 (1.8%)		

Table 3. Antibiotic resistance patterns among Staphylococcus aureus isolates.

Antibiotics	**Abbreviations Disc content (ug)**	**Total *S.aureus* (n = 76)**	***MSSA* (n = 20)**	***MRSA* (n=56)**	***P-value***
Clindamycin	DA 2 ug	8 (10.5%)	0 (0.0%)	8 (14.3%)	FE P= 0.102
Erythromycin	E 15 ug	37 (48.7%)	2 (10.0%)	35 (62.5%)	<0.001*
Gentamycin	CN (10 ug)	23 (30.3%)	0 (0.0%)	23 (41.1%)	0.001*
Oxacillin	OX (5 ug)	56 (73.7%)	0 (0.0%)	56 (100.0%)	<0.001*
Cefoxitin	FOX (30 ug)	56 (73.7%)	0 (0.0%)	56 (100.0%)	<0.001*
Cotrimoxazole	COT (25 ug)	24 (31.6%)	0 (0.0%)	24 (42.9%)	<0.001*
Ciprofloxacin	CIP (5ug)	30 (39.5%)	0 (0.0%)	30 (53.6%)	<0.001*
Levofloxacin	LEV (5 ug)	30 (39.5%)	0 (0.0%)	30 (53.6%)	<0.001*
Vancomycin	VA (30 ug)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
Linezolid	LZD (30 ug)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
Imipenem	IPM (10 ug)	56 (73.7%)	0 (0.0%)	56 (100.0%)	<0.001*
Tetracycline	TET (30 ug)	32 (42.1%)	2 (10.0%)	30 (53.6%)	0.001*
Rifampicin	RD (5 ug)	1 (1.3%)	0 (0.0%)	1 (1.8%)	FE P=1.000
Ampicillin	AMP (10 ug)	69 (90.8%)	13 (65.0%)	56 (100.0%)	P=<0.001*

Table 4. Correlations between agr types and biofilm formation among MSSA and MRSA isolates.

		agr type					FET	P- value
		Non	I	II	III	IV		
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)		
MSSA	Biofilm	(n = 5)	(n = 11)	(n = 2)	(n = 2)	(n = 0)		
	Non	4 (80.0%)	1 (9.1%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	12.891*	0.048*
	Weak	0 (0.0%)	1 (9.1%)	1 (50.0%)	1 (50.0%)	0 (0.0%)		
	Moderate	1 (20.0%)	6 (54.5%)	1 (50.0%)	0 (0.0%)	0 (0.0%)		
	Strong	0 (0.0%)	3 (27.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
MRSA	Biofilm	(n = 7)	(n = 36)	(n = 0)	(n = 12)	(n = 1)		
	Non	1 (14.3%)	2 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	20.830*	0.002*
	Weak	5 (71.4%)	2 (5.6%)	0 (0.0%)	1 (8.3%)	0 (0.0%)		
	Moderate	1 (14.3%)	16 (44.4%)	0 (0.0%)	7 (58.3%)	1 (100%)		
	Strong	0 (0.0%)	16 (44.4%)	0 (0.0%)	4 (33.3%)	0 (0.0%)		

FET: Fisher Exact test

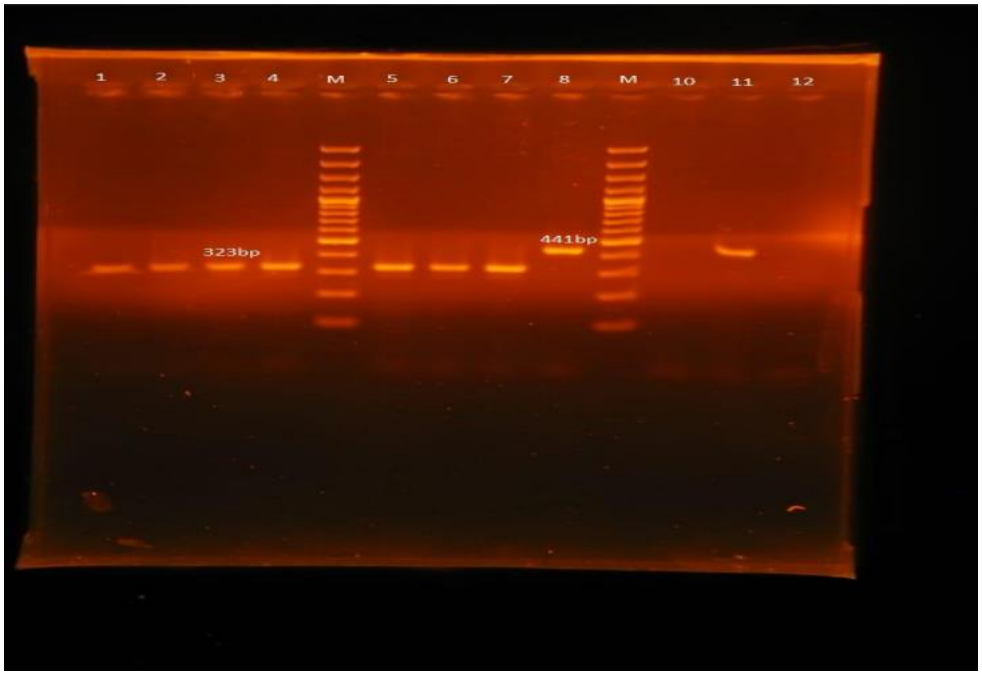
Table 5. Correlations between biofilm formation and antibiotic resistance pattern among MRSA isolates (n =56).

	Biofilm				FET	p
	Non (n = 3)	Weak (n = 8)	Moderate (n = 25)	Strong (n = 20)		
	No. (%)	No. (%)	No. (%)	No. (%)		
Clindamycin	0 (0.0%)	2 (25.0%)	1 (4.0%)	5 (25.0%)	5.168	0.147
Erythromycin	3 (100.0%)	2 (25.0%)	20 (80.0%)	10 (50.0%)	10.466*	0.008*
Gentamycin	2 (66.7%)	2 (25.0%)	15 (60.0%)	4 (20.0%)	8.926*	0.023*
Oxacillin	3 (100.0%)	8 (100.0%)	25 (100.0%)	20 (100.0%)	–	–
Cefoxitin	3 (100.0%)	8 (100.0%)	25 (100.0%)	20 (100.0%)	–	–
Cotrimoxazole	1 (33.3%)	7 (87.5%)	7 (28.0%)	9 (45.0%)	8.775*	0.022*
Ciprofloxacin	0 (0.0%)	6 (75.0%)	8 (32.0%)	16 (80.0%)	14.735*	0.001*
Levofloxacin	0 (0.0%)	6 (75.0%)	8 (32.0%)	16 (80.0%)	14.735*	0.001*
Vancomycin	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	–	–
Linezolid	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	–	–
Imipenem	3 (100.0%)	8 (100.0%)	25 (100.0%)	20 (100.0%)	–	–
Tetracycline	0 (0.0%)	6 (75.0%)	8 (32.0%)	16 (80.0%)	14.735*	0.001*
Rifampicin	0 (0.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	2.862	1.000
Ampicillin	3 (100.0%)	8 (100.0%)	25 (100.0%)	20 (100.0%)	–	–

Table 6. Correlations between agr type I and antibiotic resistance pattern among S. aureus isolates.

	agr type					
	MSSA		χ^2 (^{FE}p)	MRSA		χ^2 (p)
	Non Type I (n = 9)	Type I (n = 11)		Non Type I (n = 20)	Type I (n = 36)	
	No. (%)	No. (%)		No. (%)	No. (%)	
Clindamycin	0 (0.0%)	0 (0.0%)	–	3 (15.0%)	5 (13.9%)	0.013(^{FE}p=1.000)
Erythromycin	0 (0.0%)	2 (18.2%)	1.818 (0.479)	6 (30.0%)	29 (80.6%)	14.021(<0.001*)
Gentamycin	0 (0.0%)	0 (0.0%)	–	1 (5.0%)	22 (61.1%)	16.725(<0.001*)
Oxacillin	0 (0.0%)	0 (0.0%)	–	20 (100%)	36 (100%)	–
Cefoxitin	0 (0.0%)	0 (0.0%)	–	20 (100%)	36 (100%)	–
Cotrimoxazole	0 (0.0%)	0 (0.0%)	–	10 (50.0%)	14 (38.9%)	0.648 (0.421)
Ciprofloxacin	0 (0.0%)	0 (0.0%)	–	17 (85.0%)	13 (36.1%)	12.355*(<0.001*)
Levofloxacin	0 (0.0%)	0 (0.0%)	–	17 (85.0%)	13 (36.1%)	12.355*(<0.001*)
Vancomycin	0 (0.0%)	0 (0.0%)	–	0 (0.0%)	0 (0.0%)	–
Linezolid	0 (0.0%)	0 (0.0%)	–	0 (0.0%)	0 (0.0%)	–
Imipenem	0 (0.0%)	0 (0.0%)	–	20 (100%)	36 (100%)	–
Tetracycline	0 (0.0%)	2 (18.2%)	1.818 (0.479)	17 (85.0%)	13 (36.1%)	12.355*(<0.001*)
Rifampicin	0 (0.0%)	0 (0.0%)	–	1 (5.0%)	0 (0.0%)	1.833(^{FE}p=0.357)
Ampicillin	8 (88.9%)	5 (45.5%)	4.105 (0.070)	20 (100%)	36 (100%)	–

Figure 1: Agarose gel electrophoresis of amplified agr genes stained with ethidium bromide. M: 100bp DNA size marker, Lanes (1 to 7): Positive for agr III (323bp), Lane (8, 11): Positive for agr I (441 bp), Lane (10, 12): Non typable .



Abbreviations: DNA; deoxyribonucleic acid; bp; base pair.

Discussion

Managing *S. aureus* infections is a notable issue in healthcare settings worldwide due to the rise of multidrug resistance [23]. The accessory gene regulatory (*agr*) system is a key regulatory and control mechanism in *S. aureus* infection which controls its pathogenic [12]. Overall, in this study, MRSA represented (73.68%) of the total *S. aureus* isolates, while (26.3%) were MSSA. These findings align with that reported in Assiut, where MRSA was (73%) and higher than that previously reported in different areas in Egypt; Cairo, and Zagazig (67% for each), Alexandria, Mansoura, and Tanta (61%, 59%, and 40%, respectively) [24]. The high MRSA incidence in Egypt might be attributed to several variables as insufficient infection control programs. The most frequent complaints from healthcare professionals included workload, poor resources, few opportunities for infection control training, and a shortage of staff [25]**.** Moreover, antibiotic self-medication and improper antibiotic usage are common in Egypt [26].

Most *S. aureus* isolates (88.2%) were positive biofilm producers with a significant difference in biofilm formation between MRSA and MSSA. Other investigations also found similar results [27, 28]. Conversely, Lai et al. found no significant variance in the biofilm formation between MRSA and MSSA [29]. The variation observed in the abilities of biofilm formation might be due to strain differences, environmental factors, and geographical settings, as well as different laboratory detection methods, and different biofilm-associated genes [23].

In the present study, the greatest proportion of *S. aureus* isolates (40.8%) was recovered from urine samples, with the prevalence of MRSA and MSSA (35.7% and 55%), respectively. In contrast, Iraq and Australia had lower percentages of MRSA in urine samples (7.7% and 4.06%, respectively) [30].

Our results found that the predominant type was *agr type* I (61.8%), *agr* type III (18.4%), *agr* type II (2.6%), and *agr* type IV (1.3%). Also, *agr* group I was the most prevalent type in MRSA isolates and MSSA in percentage (63.4%, 55%, respectively). In consistent with our results *agr* group I was dominated in several of studies [11, 31]. However, *agr* II was the predominant cause of nosocomial infections in a different study **[**32]. Differences in infection management practices, together with ecological, and regional

considerations, might be the cause of these discrepancies in the distribution of *agr* groups [17].

Concerning the antimicrobial resistance, *S. aureus* isolates in the present study noticed a great resistance rate toward ampicillin (90.8%), imipenem (73.7%), and cefoxitin (73.7%). These resistance rates were higher than the study results of Derakhshan et al. [12].

Moreover, another study reported high resistance rates of MRSA isolates to the majority of antibiotics compared to MSSA except for clindamycin and rifampicin [33]. In addition, no vancomycin or linezolid-resistant strain was observed in the current study which was inconsistent with the results of other studies [27, 34]. This might be due to their limited usage because of their unavailability, expensive costs, and serious adverse effects.

On the contrary, vancomycin resistance was noticed in 23.4% of MRSA isolates, as reported by El Sawy et al. [33]. Therefore, culture sensitivity testing should be done to guide the use of vancomycin for the treatment of MRSA isolates. Moreover, Linezolid resistance was observed in the study results of Che et al. [35].

The current work showed that *agr* I was significantly associated with biofilm production in MSSA and MRSA isolates, with P value= 0.048* and 0.002*, respectively. In agreement with our results**,** Chan et al. showed a significant association between *agr* I and biofilm production among MSSA isolates [27]. But *S. aureus* isolates with *agr* group II were associated with strong biofilm producers in different studies [31, 33]. However, Yang et al. found no significant difference between biofilm production and *agr* groups [36]. These variances may be due to strain differences brought about by microbial adaptability and regional factors.

Our study revealed a significant differences between biofilm-producer and non-producer isolates of MRSA toward cotrimoxazole, ciprofloxacin, levofloxacin, tetracycline, erythromycin, and gentamycin. These results are supported by previous studies [37]. Reduced antibiotic diffusion through the biofilm matrix and diminished bacterial strain variations in metabolic activity could explain association between biofilm and antibiotic resistance [38].

As regards the correlation between antibiotic resistance pattern and *agr* genotypes, MRSA isolates with *agr* I were significantly correlated with erythromycin, gentamycin, ciprofloxacin, levofloxacin, and tetracycline resistance ($p<0.05$), while *agr* I in MSSA isolates was non-significantly associated with antibiotic resistance. These results were matched with the results of Saedi et al. who defined a major link between *agr* I and tetracyclin, erythromycin, clindamycin, and ciprofloxacin resistance [34]. Also our results were coincided with results of the study done by Chan et al. who found a significant association between *agr* I and fluoroquinolones resistance ($p < 0.05$) [23]. Whereas Javdan et al. reported a significant correlation between *agr* I and antibiotic resistance to cefoxitin ($p = 0.04$) [14].

The study findings suggest targeting biofilm formation and *agr*-regulated resistance mechanisms is a potential score to develop new antibacterial agents. Therefore, **the limitations of this study** were the small sample size, all samples were from single healthcare setting, and investigations were done for only *agr* gene. So future studies are recommended to ensure analysis of a large number of samples from different centers, also including samples from community- acquired infections to study their virulence with regard to different quorum sensing genes.

Conclusions

There was a significant increase in the percentage of MRSA isolates with the ability for biofilm formation among *S. aureus* isolated from ICU patients with nosocomial infections. Among the isolated *S. aureus*, the primary *agr* gene was *agr* I. Moreover, *agr* I, the predominant gene in MRSA isolates, was closely associated with the formation of biofilm and antibiotic resistance. So, *agr* molecular typing is an effective method for controlling *S. aureus* infections through the development of new promising anti-virulence agents targeting *agr* system.

Declarations:

Not applicable consent for publication.

Material and data accessibility: Data are available upon request.

Funding: No grants from funding organizations were given.

The author(s) declare no potential conflicts of interest to the authors.

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