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Prevalence, Virulence Regulation, and Resistance Mechanisms of Clinical Staphylococcus aureus Isolates: The Role of the agr System and Mobile Genetic Elements in Al-Diwaniyah, Iraq

Hanaa Neamah Abdullah[1], Nada Ahmed Fairooz[2], Bushra Hamad Obaid[2], Sura A Al-Ganahi[3, *], Robeena Farzand[4]

[1]*Ministry of Education, Directorate of Open Education, Diwaniyah Study Center, Diwaniyah 58001, Iraq*
[2] *Polytechnic College, Al-Qadisiyah, Al-Furat Al-Awsat Technical University, Diwaniyah 58001, Iraq*
[3] *Department of Environmental Research and Pollution Prevention, College of Science, University of Al-Qadisiyah, Diwaniyah 58001, Iraq.*
[4] *Warwick Medical School, University of Warwick, Coventry CV4 7AL, United Kingdom*

Corresponding Author Email:
sura.ali@qu.edu.iq

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ABSTRACT

Background: Infections by Staphylococcus aureus are continued as a real issue especially in hospital-acquired disease. It can produce virulent factors that can be controlled by the accessory gene regulator (agr), and it can be affected by mobile genetic elements (MGEs). It should be known their mechanism in local isolates as it is crucial for monitoring infections and treatment approaches.
Objectives: To evaluate S. aureus prevalence in clinical samples and to discover the consequence of the agr system and MGEs in regulating virulence.
Methods: Microbiological procedures were used to gather clinical samples. Also, molecular assays were accomplished to determine agr types, key virulence-associated genes, and MGEs. The data of this study was evaluated to estimate the association among agr function, virulence gene distribution, and resistance traits.
Results: Most clinical samples contain active agr system, with major of agr group I. Virulence factors like enterotoxins, hemolysins, and adhesion-associated genes were extensively spread. MGEs such as pathogenic islands and plasmids were detected and cause antimicrobial resistance and virulence expression. Interestingly, in the isolated samples, active agr systems present a significant association with toxin production more than those with dysfunctional variants.
Conclusion: S. aureus samples show high dominance and genetic variant. Virulence measurements are strongly affected by the agr system and MGEs. Molecular monitoring is important step to display these influences and support active therapeutic and infection-control measures.

Keywords: Staphylococcus aureus, virulence, agr system, mobile genetic elements, prevalence

1. Introduction

Staphylococcus aureus is a bacterium that may be considered the source of clinically important infections. Also, it can be responsible for a wide range of community- and hospital-acquired infections [1]. It may affect superficial skin infection as well as invasive and life-threatening diseases, such as pneumonia, bacteremia, osteomyelitis, and endocarditis [2].

S. aureus pathogenicity depends on the virulence factors to make colonies, avoid host defenses, and

even damage host tissues [3]. The ability of S. aureus to form virulent factors depends on the control of complex regulatory systems. The accessory gene regulator (agr) is key quorum-sensing pathway, organizing the production of surface proteins and secreted toxins in response to bacterial cell density [4, 5]. The agr site is categorized into four main groups (I–IV). These differences have been linked to changes in clinical appearance and disease-causing ability. Isolates with defective agr activity show a constant rise in virulency. On the other hand, these changes may alter some host conditions, highlighting how this regulatory flexibility provides a survival advantage [6,7].

Moreover, the genetic variability of S. aureus strongly affects mobile genetic elements (MGEs). These genetic variabilities include plasmids, prophages, transposons, and pathogenic islands. These elements are essential for horizontal gene transfer, and the host immune system could enhance them by encoding them as factors of antibiotic and toxin resistance. The acquisition and dissemination of these elements facilitate the growth of multidrug-resistant strains. In specific, methicillin-resistant Staphylococcus aureus (MRSA) stays a significant global public health risk [8-10].

It is very important to know the virulence of S. aureus is linked to the agr system, and thus its enhancement by mobile genetic elements (MGEs) in local isolations is very fundamental in clinical management and specifically in public health. Molecular characterization for these factors promotes understanding the mechanisms of pathogenesis and supports the development of successful therapeutic and protection strategies.

2. Materials and Methods

2.1. Sample Collection

In this study 150 clinical specimens were obtained from patients who attending Al-Diwaniyah General Hospital, Al-Diwaniyah, Iraq, during the period from February to September 2025. These specimens involved wound swabs, blood, urine, and respiratory secretions were taken from patients with expected *S. aureus* infections. All samples were sterilized and transported to the microbiology laboratory to examine. Standard microbiological procedures were followed to ensure sample quality and to decrease the risk of contamination via handling and transportation.

2.2. Isolation and Identification of *S. aureus*

All clinical specimens were cultured on mannitol salt agar and blood agar and incubated at 37 °C for 24–48 hours. The colonies of *S. aureus* on mannitol salt agar were smooth, convex, and golden yellow, with the medium changing from red to yellow because of mannitol fermentation Figure 1A. On the other hand, the colonies on blood agar appeared round, shiny, and creamy to golden in color, usually 2-4 mm in diameter, and were surrounded by clear area of beta-hemolysis Figure 1B. The identification was carried out using Gram staining and coagulase tests. Molecular confirmations were performed by polymerase chain reaction (PCR) amplification of the *nuc* gene, which is a species-specific marker for *S. aureus*.

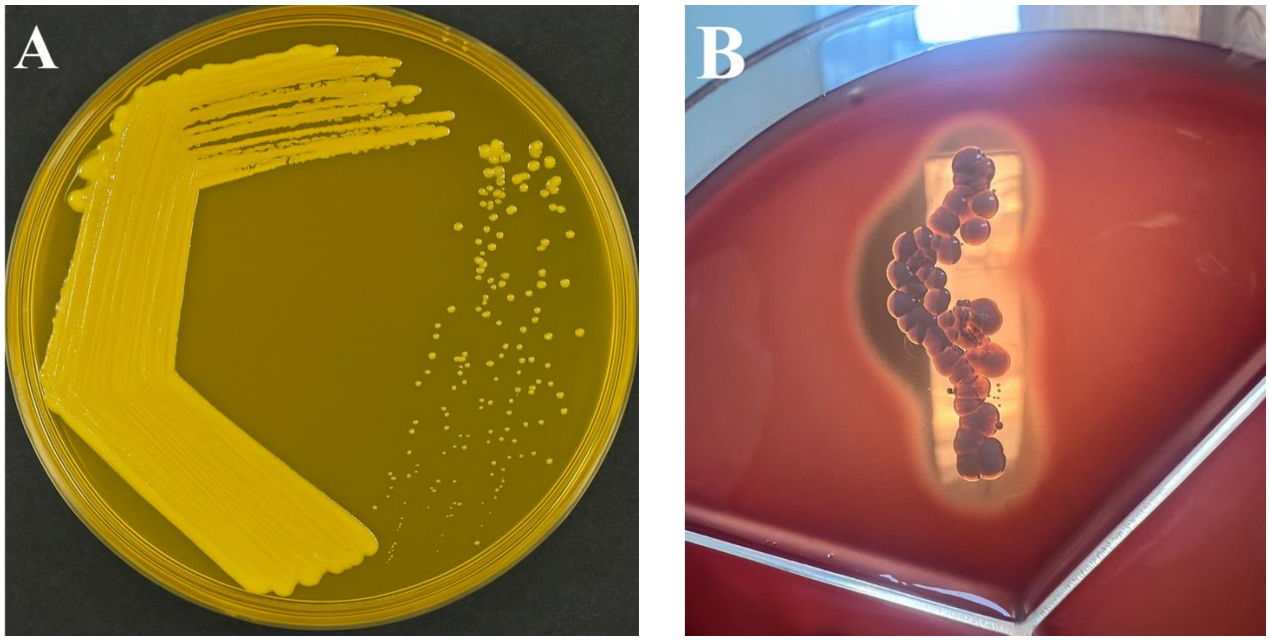

Figure 1. Growth of *S aureus*) On Mannitol Salt Agar (MSA) Showing Mannitol Fermentation with Yellow Colonies. On blood agar, colonies were surrounded by clear zones of β-hemolysis

3. Antimicrobial Susceptibility Testing

The susceptibility antimicrobial of *S. aureus* isolates was detected by the disk diffusion method on Mueller–Hinton agar by Clinical and Laboratory Standards Institute [11]. Some of routinely prescribed antibiotics representing different therapeutic classes was tested, including β-lactams, aminoglycosides, macrolides, tetracyclines, fluoroquinolones, and glycopeptides. After incubation at 37 °C for 18–24 hours, inhibition zone diameters were measured and interpreted according to CLSI breakpoints. Methicillin resistance was specifically confirmed by PCR detection of the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a) and is a definitive marker for MRSA.

4. DNA Extraction

From the overnight cultures of *S. aureus,* DNA were extracted using DNA extraction kit (Qiagen, Germany) by following the manufacturer's procedure. Then, bacterial cells were collected and processed to remove proteins and other contaminants. After that, DNA was purified and eluted in nuclease-free water. Spectrophotometer was used to measure the extracted DNA concentration at 260 nm. Agarose gel electrophoresis was also used to measure the quality of DNA before downstream molecular examinations.

5. Detection of *agr* Types

PCR and primers specific were used to assess the system of (*agr*) in *S. aureus* isolates for *agr* groups I–IV. Each reaction mixture (25 μL) contained DNA, specific primers, dNTPs, Taq DNA polymerase, and buffer. The amplification processes were carried out in the thermal cycler under ideal conditions, consisting of a primary denaturation process, and 30-35 cycles of denaturation, annealing and stretching. PCR products were separated on 2% agarose gel stained with ethidium bromide and visualized under

UV light. Amplicon size was compared with100 bp DNA ladder, I–IV represents *agr* groups producing fragments of approximately 500 bp (*agr* I), 400 bp (*agr* II), 310 bp (*agr* III), and 210 bp (*agr* IV), respectively.and *agr* types were used as a positive control. While nuclease-free water served as negative control Figure 2.

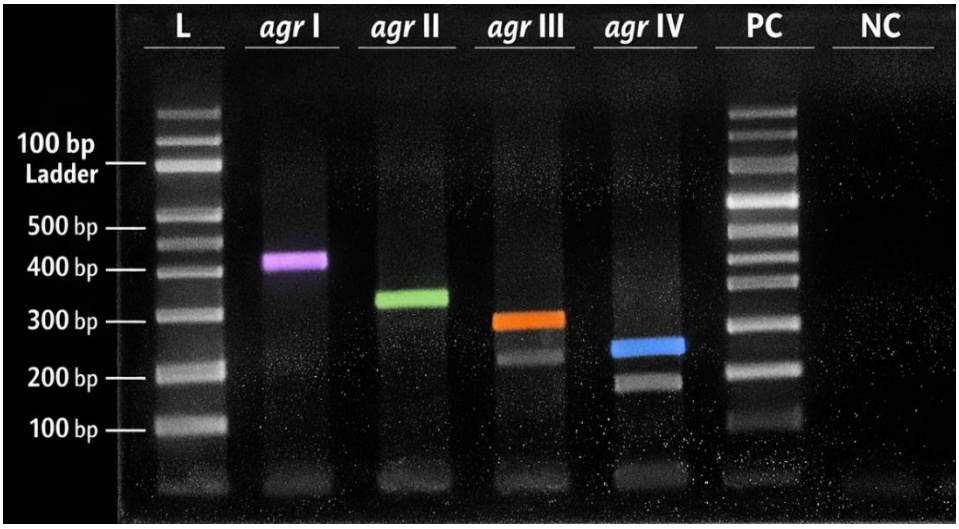

Figure 2. Agarose gel electrophoresis of PCR products showing detection of *agr* types (I–IV) in *S. aureus* isolates. Lane L represents DNA ladder of 100 bp. Lanes I–IV represents *agr* groups. Lane PC represents the positive control strain with known *agr* type, while lane NC represents the negative control.

6. Detection of Virulence and Mobile Genetic Elements

It has been detected that the existence of virulent genes and mobile genetic elements in the *S. aureus* isolates by using PCR. Wanted genes include hemolysins (*hla, hlb*), enterotoxins (*sea, seb, sec*), and adhesion factors (*clfA, fnbA*). Also, markers have been explored associated with mobile genetic elements, such as plasmid-borne resistance genes and staphylococcal pathogenicity islands (SaPIs).

PCR was performed in 25 µL reaction mixture containing DNA, primers, dNTPs, Taq polymerase, and buffer solution. This program was included 30–35 cycles of denaturation, gene-specific annealing, and extension at 72 °C, and finally with extension step. PCR products were separated on 2% agarose gels and examined under UV light. The size of fragments was determined using 100 bp DNA ladder. Reference strains were used as positive controls, and nuclease-free water as negative control.

7. Data Analysis

The distribution of *agr* types and MGEs in *S. aureus* isolates was evaluated using statistical analysis. The relationship among *agr* activity, virulence genes, and antimicrobial resistance were evaluated by chi-square analysis. P value < 0.05 was considered significant and statistical analyses were performed using GraphPad Prism version 10.

8. Results Prevalence of *S. aureus*

A total of 150 clinical specimens was collected from patients in Al-Diwaniyah General Hospital. Out of these, 68 samples (45.3%) were confirmed as *S. aureus* based on culture, biochemical tests, and PCR

amplification of the *nuc* gene. The isolates were obtained from wound swabs (30 isolates, 44.1%), blood samples (15 isolates, 22.1%), urine samples (12 isolates, 17.6%), and respiratory specimens (11 isolates, 16.2%). Wound infections represented the highest proportion of isolates Table 1.

Table 1. Distribution of *Staphylococcus aureus* isolates by sample type

Sample Type	Number of Isolates (n)	Percentage (%)
Wound Swabs	30	44.1
Blood Samples	15	22.1
Urine Samples	12	17.6
Respiratory Specimens	11	16.2
Total	68	100

9. Antimicrobial Susceptibility

Antibiotic resistance was common among the isolates. Penicillin resistance was observed in 64 isolates (94.1%), ampicillin in 60 isolates (88.2%), and erythromycin in 48 isolates (70.6%). Moderate resistance was noted for tetracycline (38 isolates, 55.9%) and gentamicin (28 isolates, 41.2%).

Resistance to vancomycin and linezolid was rare, with only 4 (5.9%) and 2 (2.9%) isolates showing resistance, respectively. The resistance of Methicillin was permitted by the presence of the *mecA* gene in 20 isolates (29.4%). This indicates a notable prevalence of MRSA Table 2 and Figure 3.

Table 2. Antibiotic resistance profile of Staphylococcus aureus isolates (n = 68)

Antibiotic	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Penicillin	64 (94.1)	2 (2.9)	2 (2.9)
Ampicillin	60 (88.2)	4 (5.9)	4 (5.9)
Erythromycin	48 (70.6)	6 (8.8)	14 (20.6)
Tetracycline	38 (55.9)	10 (14.7)	20 (29.4)
Gentamicin	28 (41.2)	8 (11.8)	32 (47.1)
Vancomycin	4 (5.9)	2 (2.9)	62 (91.2)
Linezolid	2 (2.9)	0 (0.0)	66 (97.1)

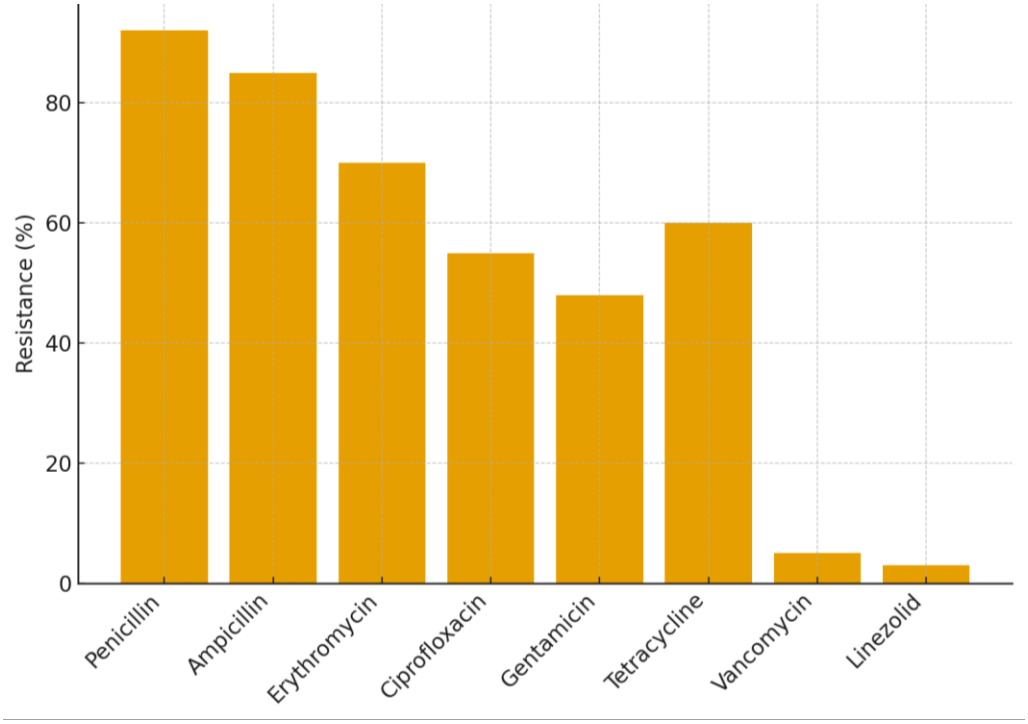

Figure 3. Bar chart shows the percentage of Staphylococcus aureus isolates resistant to commonly tested antibiotics. High resistance was observed to penicillin, ampicillin, and erythromycin, while resistance to vancomycin and linezolid remained low.

10. Distribution of *agr* Types

Multiplex PCR analysis demonstrated that most isolates carried a functional *agr* system. *agr* group I was the most prevalent, found in 28 isolates (41.2%), followed by group II in 18 isolates (26.5%), group III in 12 isolates (17.6%), and group IV in 6 isolates (8.8%). Four isolates (5.9%) did not show detectable *agr* activity Table 3.

Table 3. Distribution of agr groups among S. aureus isolates (n = 68)

agr Group	Number of Isolates (n)	Percentage (%)
Group I	28	41.2
Group II	18	26.5
Group III	12	17.6
Group IV	6	8.8
None detected	4	5.9
Total	68	100

11. Virulence Genes

Screening for virulence determinants revealed that *hla* was present in 50 isolates (73.5%) and *hlb* in 42 isolates (61.8%). Enterotoxin genes were distributed as follows: *sea* in 30 isolates (44.1%), *seb* in 18 isolates (26.5%), and *sec* in 12 isolates (17.6%). Genes associated with adhesion were also common, specifically the *clfA* gene found in 46 isolates (67.6%) and the *fnbA* gene in 40 isolates (58.8%). Isolates that carry virulent

genes were frequently associated with functional *agr* systems. This is suggesting a significant relationship between *agr* activity and pathogen potential Figure 4.

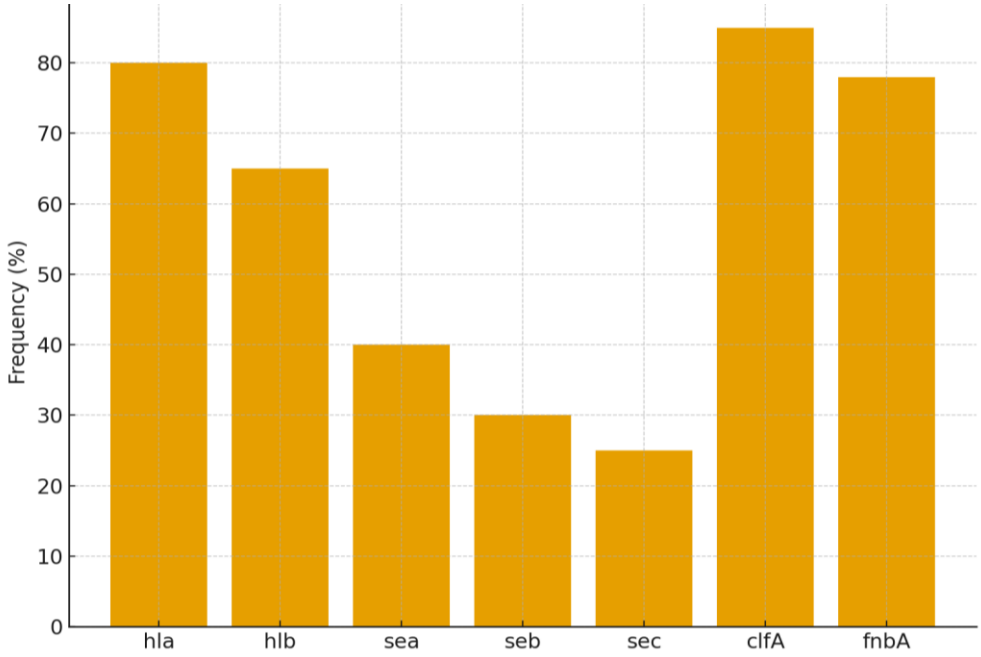

Figure 4. Bar charts show the virulence genes (*hla, hlb, sea, seb, sec, clfA,* and *fnbA*) in *S. aureus* isolates.

12. Mobile Genetic Elements

The genes of plasmid-born resistance were identified in 38 isolates (55.9%). While staphylococcal pathogenicity islands (SaPIs) were found in 24 isolates (35.3%). Isolates that carry MGEs have shown resistance to multiple antibiotic classes. This highlights the function of mobile elements in contributing to antimicrobial resistance and virulence Table 4.

Table 4. Occurrence of plasmid-borne resistance genes and (SaPIs) in *S. aureus* isolates (n = 68)

Mobile Genetic Element	Number of Isolates (n)	Percentage (%)	Association with MDR*
Plasmid-borne resistance genes	38	55.9	Strong
SaPIs	24	35.3	Moderate
None detected	12	17.6	Low
Total	68	100	—
***MDR = multidrug resistance (resistance to ≥3 antibiotic classes).**			

13. Relationship Between *agr*, Virulence, and Resistance

Statistical analysis exhibited significant relationship between the presence of *agr* system and the presence of genes for multiple virulence ($p < 0.05$). Interestingly, isolations with active *agr* system were expected to save combinations such as hemolysin, enterotoxin, and adhesion genes compared to *agr*

activity very weak. Furthermore, isolates that carry MGEs were notably tend to multidrug resistant, presenting resistance to three antibiotic classes Figure 5.

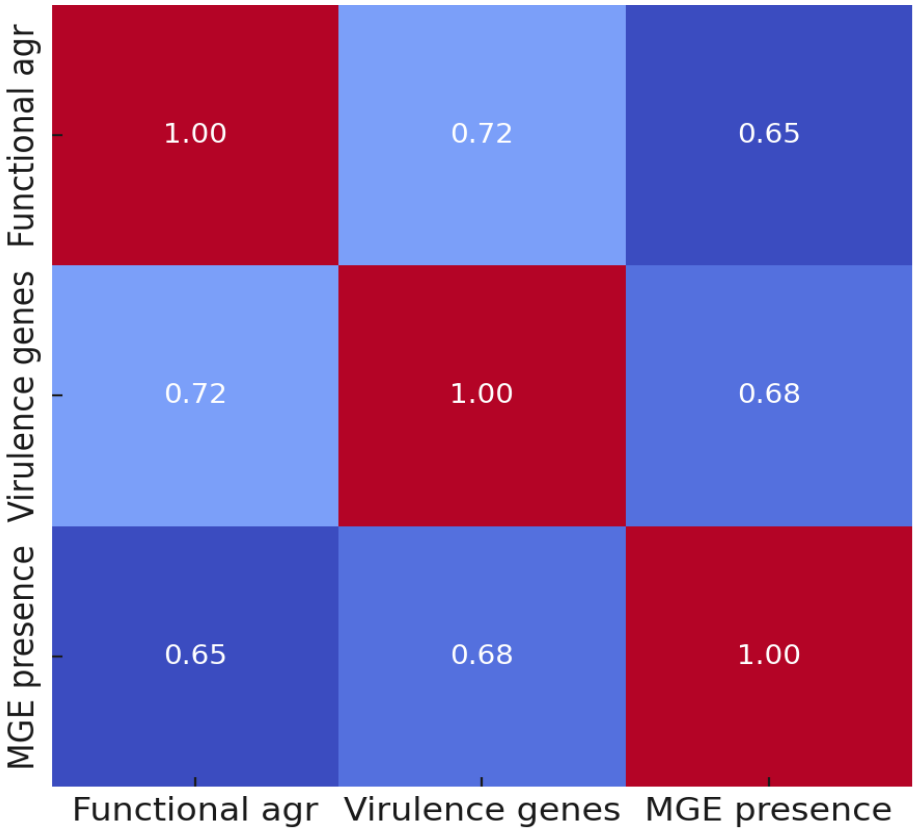

Figure 5. Heatmap illustrates the association between functional *agr* activity, the presence of multiple virulent genes, and MGEs in *S. aureus* isolates from Al-Diwaniyah.

14. Discussion

S. aureus is still the cause of infections in both community and hospital settings. The reason for that is to diverse virulence factors and resistance mechanisms [1]. In this study, 45.3% of *S. aureus* samples from Al-Diwaniyah General Hospital were taken from skin and soft tissue infections. Interestingly, resistance was very high for penicillin (94.1%), ampicillin (88.2%), and erythromycin (70.6%), suggesting widespread β-lactamase activity [12]. MRSA reported for 29.4% of isolates and show the burden of multidrug resistance, whereas vancomycin and linezolid stayed efficient in most cases [13].

Many isolates carried active *agr* loci specifically *agr* group I (41.2%). Even though a few had mutations that cause defect *agr* systems that can lead to infections and biofilm formation. Surprisingly, functional *agr* system was associated with multiple virulence genes and controlling hemolysins, enterotoxins, and adhesion factors [14,15]. Hemolysin (*hla, hlb*) and adhesion genes (*clfA, fnbA*) were widespread in the isolates. while enterotoxin genes (*sea, seb, sec*) appeared in several number of isolates. This indicates potential for tissue damage and toxin-mediated disease [16,17].

MGEs such as plasmids and SaPIs were also observed. These strains that carry these elements tended to be multidrug-resistant and more virulent. This confirms the importance of horizontal gene transfer

[18,19]. The existence of both functional *agr* systems and mobile elements in *S. aureus* explain the ability to survive, avoid defenses, as well as remain in the situation that with under antimicrobial pressure.

Generally, these findings are considered significant as they emphasize the need for continuous molecular surveillance in *S. aureus* populations. The reasons for that are to detect *agr* types, virulence factors, and mobile elements. This can help to guide infection-control actions and the use of antibiotic wisely. Although the small size of samples as well as the single hospital target, this work presents very important and unique awareness of genetic regulation for virulence and resistance. This can change the future research and therapy of MRSA [20,21].

15. Limitations and Future Directions

This study gives very useful understanding for *S. aureus* isolates but still has some limitations. The limited size of samples and all samples have taken from one hospital. For these confines, this study strict generalizability. Thus, future research should include larger number of samples, and many centers to take the sample from. In order to investigate virulence and resistance by understanding *agr* activity, biofilm formation, and interactions with mobile genetic elements.

16. Conclusion

The isolates *S. aureus* in Iraq revealed widespread, considerable genetic diversity, and significant multidrug resistance. The activity of *agr* systems is directly associated with the expression of multiple virulent genes. While MGEs are considered a key factor for both virulence and resistance. These results highlight the importance of integrating molecular surveillance into routine clinical practice to inform treatment decisions and enhance infection control measures.

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Ethical Approval Statement : This study was used discarded clinical specimens collected during routine diagnostic procedures from Al-Diwaniyah General Hospital. No extra samples were taken directly from patients to ensure confidentiality.

Informed Consent Statement: Patients consent was not needed as only discarded clinical specimens were used for this study.

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