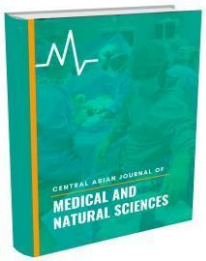

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Molecular Biological Studies of the Agar A Gene in Staphylococcus Aureus (MRSA) and the Discovery of the Association Between Its Gene Expression and Biofilm Formation in Iraqi Patients

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Abstract: Background :From an epidemiological standpoint, MRSA is generally classified into three types based on its origin and prevalence. Each of these types has evolved in different contexts and has its own unique genomic profile . The three types of MRSA are healthcare-associated MRSA (HA-MRSA) . community-associated MRSA (CA-MRSA) . and livestock-associated MRSA (LA-MRSA). Methodology : Samples such as urine, semen, and other bodily fluids were collected from patients. These samples were cultured on mannitol agar and blood agar. After bacterial growth, the bacteria were identified using the VITEK system and tested for methicillin resistance. Bacterial DNA was extracted using a dedicated extraction kit, and gene expression was measured using polymerase chain reaction (PCR).Results : The results of the relationship between bacteria isolated from different body samples and their resistance or sensitivity to the antibiotic methicillin were recorded as follows: 77.8% of the isolated bacteria were methicillin-resistant and distributed across three groups according to the source of isolation, while 22.2% were sensitive, Gene expression analysis revealed that methicillin-resistant bacteria showed increased gene expression in biofilm-producing bacterial samples. Aims od study : The purpose of this study is to clarify the relationship between methicillin-resistant bacteria (MRSA) and Agr A gene expression, and whether this relationship is direct or inverse, that is, whether increased biofilm formation leads to increased gene expression. Conclusion: Staphylococcus aureus (MRSA) strains using modern diagnostic methods such as polymerase chain reaction (PCR). This study demonstrated a correlation between biofilm formation in two strains—methicillin-resistant and methicillin-susceptible—in different patient samples. Additionally, a positive correlation was observed between the gene, Agr A, and biofilm formation in this strain.

Keywords: Staphylococcus Aureus, MRSA, PCR, Agr A, Biofilm, Blood agar.

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1. Introduction

Over the past several decades, the spread and prevalence of MRSA have been constantly evolving, with new MRSA clones emerging in various countries and regions. Therefore, it is necessary to monitor the characteristics, host specificity and transmission routes of new strains and to monitor ongoing surveillance of MRSA in a one-health environment [1],[2] . From an epidemiological standpoint, MRSA is generally classified into three types based on its origin and prevalence. Each of these types has evolved in different contexts and has its own unique genomic profile [3]. The three types of MRSA are healthcare-associated MRSA (HA-MRSA) [4] community-associated MRSA (CA-

MRSA) [5] and livestock-associated MRSA (LA-MRSA) [6]. The emergence of antimicrobial resistance (AMR) poses a significant threat to global public health and economic stability. With the rise of multidrug resistance in MRSA, the resistance mechanisms have become increasingly complex. This section explores the complex resistance mechanisms of MRSA, from genetic determinants to phenotypic adaptations. Detailed research into MRSA resistance mechanisms will not only effectively elucidate the evolution of MRSA but also contribute to the development of new anti-infective drugs and the formulation of control strategies.

The central molecular basis of intrinsic resistance in MRSA is the mecA gene encoded by penicillin-binding protein 2a (PBP2a). This protein provides a robust defense barrier against β-lactam antibiotics by reorganizing the cell wall synthesis pathway [7]. However, this classical mechanism does not fully explain the heterogeneity of resistant MRSA phenotypes and environmental adaptation, i.e., the variation in resistance profiles due to differences in Staphylococcus chromosomal cassette mec (SCCmec) type , Recent studies have revealed that the fem gene family influences the functional efficiency of PBP2a by regulating peptidoglycan precursor metabolism [8]. Meanwhile, the agr quorum sensing system indirectly shapes the resistance phenotype (e.g., biofilm formation and antibiotic osmotic resistance) by dynamically regulating the expression of pathogenic factors [9].

One such factor in the Staphylococcus genus is accessory gene regulators (Agr), known to modulate quorum sensing, which ultimately controls the expression of various pathogenicity genes in these organisms [10]. The P2 and P3 promoters regulate two adjacent transcripts, RNAII and RNAIII, which constitute the agr locus, respectively. The second RNA subunit consists of the agrB, agrD, agrC, and agrA genes [11]. The propeptide, autoinducible peptide (AIP), is encoded by agrD, and the AIP processor is encoded by the agrB gene. A two-component regulatory system consisting of AgrC and AgrA is encoded by the agrC and agrA genes [12]. The AIP propeptide is converted to an octapeptide by AgrB and released into the extracellular space when Agr is activated. The membrane-bound histidine kinase AgrC undergoes autophosphorylation and activation when AIP approaches its threshold, phosphorylating its corresponding response regulator, AgrA [13]. Phosphorylated AgrA increases AIP production by enhancing RNAIII expression through the activation of its RNAII transcript and promoter P3. Pathogenicity is caused by AgrA and RNAIII, two major intracellular effectors that control the expression of toxic factors [14]. The role of functional Agr in disease induction in animal infection models has been extensively studied [13]. It is dysfunction has been associated with reduced susceptibility to thrombin-induced platelet bactericidal protein, vancomycin, and prolonged bacteremia. In terms of biofilm formation ability, Agr-dysfunctional strains typically have greater capacity [15]. The Agr system (agrACDB/RNAIII, accessory gene regulator, quorum sensing system) is the most important of the many systems that regulate gene expression in Staphylococcus aureus (S. aureus). Agr activation is associated with the production of toxins and other factors that cause acute infections.

2. Materials and Methods

Specimen Collection

This study included a total of 100 specimens collected from various body parts and lesions (urinary tract infections, stool, and other bodily fluids) from male and female inpatients and outpatients who received training at the Medical City Clinical Laboratory between January 2026 and May 2026.

Isolation of bacteria :

Following the hospital's routine testing procedures, all specimens were immediately streaked onto nutrient agar (NA) and then streaked onto sectioned MacConkey agar (MA) and blood agar (BA), and incubated at 37°C for 24 hours. The resulting colonies were

Gram-stained and re-streaked onto sectioned mannitol salt agar (MSA). This medium was used for the identification of pathogenic Staphylococcus aureus, and further biochemical tests, such as catalase and coagulase, were performed for more accurate identification of the isolated bacteria.

Molecular Identification of MRSA - Extraction Text

DNA was extracted according to (Vogelstein and C. Jillespie, 1979).Method adopted by the manufacturer of the DNA extraction kit described in Section SAZZS.

PCR Procedure - Excerpted Text

Procedure (Brown, 2002)

a) The Staphylococcus aureus (S. aureus) DNA template was prepared using the method described in Section Methodology
b) Primers (168 tRNA, Agr A, Fnba) were diluted with nuclease-free water according to the manufacturer's instructions.
c) The contents of the master mix were thawed at room temperature before use.
d) 1 µl of template DNA was transferred to a master mix tube, and 1 µl of master mix and 1 µl of each primer were added to each tube.
e) The volume was adjusted to 20 µl with deionized nuclease-free water (10 µl for multiplex PCR, 12 µl for conventional PCR). The mixture was then centrifuged in a mini centrifuge to ensure thorough mixing of the reaction components.
f) f)The tubes were placed in the PCR instrument, and a PCR program with appropriate cycling conditions pre-installed was started. All PCR amplification reactions were performed using a Cleaver Scientific Thermal Cycler 132/89.

PCR mixture without DNA template (non-template negative control).

The sequences of oligonucleotide primers used in conventional PCR and multiplex PCR to detect the presence of, 16S rRNA, AgrA gene were cited from synthesized using Bioneer® (Korea) (Table 1).

Table 1. Primers sequences synthesized using Bioneer® (Korea).

Gene	Primer	Nucleotide sequences (5′ → 3′)
16S rRNA	F	AACTCTGTTATTAGGGAAGAAC
16S rRNA	R	CCACCTTCCTCCGGTTTGTCACC
AgrA	F	TGCCCTCGCAACTGATAATCC
Agr A	R	CCAACTGGGTCATGCTTACGAAT

Statistical Analysis:

The data was statistically analyzed using the statistical analysis software SAS (2012 edition), and significant differences between means were compared using Duncan's test (Duncan, 1955) [16].

3. Results

The experiment recorded one hundred samples distributed in groups according to the distribution in an uneven manner and taken from different locations of the body, where they formed (body fluid, urine samples, and semen samples, while one sample of foot inflammation was recorded) Table (2).

Table 2.

Types of samples	Number	Percentage
Urine	**44**	**44%**
Body fluid	**35**	**35%**
SFA	**20**	**20%**
Leg	**1**	**1%**

Microscopic Examination

Smears of the isolates revealed Gram-positive (purple) spherical cells or cocci arranged in irregular, grape-like clusters. Under a light microscope, single cocci, di cocci, tetra-cocci , and streptococci were also observed.

Related between sample types and Resistance or Sensitive to Methicillin Antibiotics :

The results of the relationship between bacteria isolated from different body samples and their resistance or sensitivity to the antibiotic methicillin were recorded as follows: 77.8% of the isolated bacteria were methicillin-resistant and distributed across three groups according to the source of isolation, while 22.2% were sensitive, as shown in Table 3.

Table 3. Distribution of Antibiotic Resistance or Sensitive to (Methicillin) According to Sample Type.

Sample Type	Resistant (R)	Sensitive (S)	Total
Urine	31	13	44
Body fluid	28	7	32
SFA	18	2	20
Total	**77 (77.8%)**	**23 (22.2%)**	**100**

Table 4. Related between Sample Type and agrA Expression (Mean ± SD).

Sample Type	agrA (Mean ± SD)
Urine	2.05 ± 3.8
Body fluid	4.85 ± 5.6
SFA	4.10 ± 3.9
Leg Pus	6.56

4. Discussion

The quorum sensing system of Staphylococcus aureus is primarily mediated by agr operators and modulates the regulation of number-dependent genes associated with pathogenicity, adhesion, motility, and biofilm formation. In methicillin-resistant Staphylococcus aureus (MRSA), biofilm formation plays a crucial role in persistent infection by promoting bacterial survival on host tissues and medical devices, inhibiting phagocytosis, and limiting antibiotic penetration [17]. In a study detecting biofilms using crystal violet dye, strain VITKV32 produced a robust biofilm, while strains VITKV25 and VITKV39 showed moderate biofilm-forming ability compared to reference MRSA and MSSA strains. These findings are consistent with our research, including a report by [18] which showed that biofilm production in clinical MRSA isolates varies significantly with degree of adhesion, with weaker adhesion strains exhibiting stronger gene expression, and vice versa. Stronger biofilm phenotypes are often associated with the co-expression of resistance determinants and pathogenic factors [18]. These findings highlight the clinical

importance of evaluating biofilm-forming ability in conjunction with identifying resistance characteristics, particularly in multidrug-resistant strains, as biofilm formation increases the risk of chronic infection in addition to antibiotic resistance.

The regulatory network controlling pathogenicity factor expression in Staphylococcus aureus is highly complex, including a two-component regulatory system, a quorum sensing pathway, the general regulator SarA and its analogues, and additional co-regulators [19]. These systems interact intricately to ensure precise control of pathogenicity factor expression. In Staphylococcus aureus, the quorum sensing system is primarily encoded by the agr gene cluster, which regulates the mRNA levels of RNA II and RNA III in response to AIP signaling. These transcripts, in turn, regulate the expression of multiple pathogenicity factors [20]. This study demonstrates potent inhibitory activity against methicillin-resistant Staphylococcus aureus (MRSA). qRT-PCR analysis revealed a concentration-dependent significant decrease in agrA and RNA III expression, indicating dysfunction of the Agr quorum sensing system at the mRNA level. In a study where polymerase chain reaction (PCR) amplification confirmed the presence of mecA and AgrA genes in all three isolates used, MRSA and MSSA reference strains functioned as positive and negative controls, respectively, confirming that the presence of the Agr A gene supports a functional quorum sensing system that controls pathogenicity. Furthermore, the structural susceptibility of agarA to inhibition by small molecules makes it a promising target for pathogenicity [21]. This is consistent with our study, which found that all MRSA strains exhibited Agr A gene expression, with lower gene expression associated with higher strain adhesion. These findings are consistent with recent clinical surveillance data showing that 85% of the same isolates of hospitalized Staphylococcus aureus had the Agr A gene [22]. Thus, these molecular-level confirmations lay the foundation for evaluating transcriptional modifications of pathogenicity and resistance genes to synthetic bacterial treatment.

Previous studies have shown that mutations and changes in lipid composition at the bacterial cell level induce cell lysis, leading to activation of stress response regulatory pathways in Staphylococcus aureus. This contributes to decreased Agr-A gene expression. However, this contradicts our findings [23], which showed that increased bacterial adhesion leads to decreased gene expression. This is because changes in membrane permeability alter signaling via sensor kinases, indirectly inhibiting Agr A activity. Studies conducted in biofilm infection models of bone implants in experimental animals have shown that cytotoxicity and phagocytosis decrease with increasing biofilm density, which is partly dependent on the agar system. However, as reported on [24] strains with defective agar systems form dense and large biofilms. This leads to a similar result to our finding that gene expression was reduced in strains that produced large biofilms. This can be explained in two ways: firstly, Staphylococcus aureus aggregates heterogeneously within the biofilm during agar activity, inducing mutations within it and leading to heterogeneous biofilm formation [25] and secondly, the agar system has different effects on biofilm formation and dispersion [26].

5. Conclusion

The data collected in this study emphasize the need to monitor the spread of methicillin-resistant Staphylococcus aureus (MRSA) strains using modern diagnostic methods such as polymerase chain reaction (PCR). This study demonstrated a correlation between biofilm formation in two strains—methicillin-resistant and methicillin-susceptible—in different patient samples. Additionally, a positive correlation was observed between the gene, Agr A, and biofilm formation in this strain.

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