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ORIGINAL ARTICLE

Genetic and Biofilm Characteristics of *Staphylococcus aureus* Isolates: A Study of the *spa* and *mec*A Genes

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ABSTRACT

Background: *Staphylococcus aureus is a Gram-positive, coccoid bacterium that plays a significant role in both community-acquired and hospital-acquired infections (CAIs and HAIs). It is non-motile, non-spore-forming, and facultatively anaerobic, which allows it to thrive in a variety of environments, including the skin, where it forms part of the normal Microbiota.* ***Objective:*** *This study aims to evaluate the ability of clinical Staphylococcus aureus isolates to produce biofilm, in addition to detecting the presence of the methicillin resistance gene mecA, and typing the spa gene to identify common genotypes.* ***Methodology:*** *Swabs were collected from 250 clinical samples, including specimens from burns, wounds, blood, urine, sputum, the nose, and the middle ear. A biochemical catalase test was performed by transferring a bacterial colony onto a glass slide and adding one drop of 3% hydrogen peroxide (H_2O_2). The tube coagulase test was done to detect the presence of the coagulase enzyme. Human plasma diluted (1:5) with steam-sterilized distilled water was mixed with an equal volume of bacterial culture in the nutrient broth. The spa-1113f/1514R primer set was used in PCR to detect the presence of the spa gene. Also, we used MecA-F/MecA-R to detect the presence of the mecA gene. The sterile microtiter plate method, containing 96 holes, was used to investigate the production of biofilm.* ***Results:*** *Twenty-nine isolates showed strong biofilm production (82.75%), while the intermediate and weak categories were 13.79% and 3.44%, respectively. Regarding the spa gene, three types were shown {t037, n=8 (67.68); t14870, n=2 (16.66); t044, n=2 (16.66)}. The results showed that 100% of the isolates tested positive for the gene, with a significant difference ($P<0.05$). PCR electrophoresis revealed the detection of the mecA gene, which encodes methicillin resistance. The results showed that 100% of the isolates tested positive for the gene, with a significant difference ($P<0.05$).* ***Conclusion:*** *The study showed a high prevalence of the mecA gene, responsible for methicillin resistance. Additionally, the study revealed a higher prevalence of the spa t037 strain compared to other strains.*

INTRODUCTION

Staphylococcus aureus is a Gram-positive, coccoid bacterium that plays a significant role in both community-acquired and hospital-acquired infections (CAIs and HAIs). It is non-motile, non-spore-forming, and facultatively anaerobic, which allows it to thrive in a variety of environments, including the skin, where it forms part of the normal Microbiota[1,2]. While it is commonly present as part of the normal microbiota of the skin, it is a leading cause of hospital-acquired infections (HAIs) and community-acquired infections (CAIs). *S. aureus* typically coexists with other bacterial species in the human body but can act as an opportunistic pathogen, causing infections when it invades other tissues[3]. One of the most concerning strains of *S. aureus* is methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant to the antibiotic methicillin and other β-lactam antibiotics. The emergence of MRSA is a global public health issue, particularly in healthcare settings, where it is a leading cause of bloodstream infections, pneumonia, surgical wound infections, and sepsis[4,5]. The spread of MRSA in healthcare facilities, such as hospitals and nursing homes, poses a significant challenge in infection control, necessitating rapid and accurate detection methods to prevent further transmission[5,6]. The species is classified into two main types: methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA). The emergence of MRSA poses a significant public health challenge due to its resistance to multiple antibiotics. *spa* is essential for rapid typing of MRSA in hospitals because it provides high diagnostic accuracy. This gene contains a region of repeated nitrogenous bases, and *spa* gene patterns are inferred from the loss and gain of these bases and the different order of these repeats[8]. One such method is the *spa* typing technique, which is

essential for accurately identifying MRSA strains in clinical settings. The *spa* gene encodes protein A, a virulence factor that is found predominantly in MRSA strains. *spa* typing allows for the rapid identification of different *S. aureus* clones based on variations in the repetitive sequences within the *spa* gene. This genetic variation leads to differences in the *spa* typing pattern, which can be used to track the spread of MRSA within hospitals and the community[9]. This gene is a virulence factor with a molecular weight of 42 kDa. It is covalently bound to the peptidoglycan layer of *Staph aureus*. Protein A cannot adhere to the bacterial cell wall, is secreted by secretory proteins and is mainly found among methicillin-resistant *Staph aureus* (MRSA) strains[10]. Protein A has several immunosuppressive properties and is one of the important mechanisms for *Staphylococcus aureus* to escape immunity. Protein A binds to the Fcγ portion of IgG, which causes the bacteria to become incorrectly coated with IgG bound, which leads to their non-recognition by neutrophils, thus preventing phagocytosis[11]. This study aims to evaluate the ability of clinical *Staphylococcus aureus* isolates to produce biofilm, in addition to detecting the presence of the methicillin resistance gene *mec*A, and typing the *spa* gene to identify common genotypes.

METHODOLOGY

Culture and identification of *Staphylococcus aureus*

Swabs were collected from 250 clinical samples obtained from various sources, including burns, wounds, and blood, urine, sputum, nose, and middle ear secretion specimens. These were cultured on a selective medium (mannitol salt agar), and identified by the routine bacteriological methods.

A biochemical catalase test was then performed by transferring a bacterial colony onto a glass slide and adding one drop of 3% hydrogen peroxide (H_2O_2). The appearance of bubbles indicated a positive result, confirming the presence of the catalase enzyme[12].

The tube coagulase test, was conducted to detect the coagulase enzyme. Human plasma diluted (1:5) with steam-sterilized distilled water was mixed with an equal volume of bacterial culture in the nutrient broth. Coagulase-positive strains induced plasma clotting within 2–4 hours of incubation at 35°C. In some cases, clot formation was observed after overnight incubation[13].

Investigation of biofilm production of *S. aureus* by the microtiter plate method

The sterile microtiter plate method, containing 96 holes, was used to investigate the production of biofilm in *S. aureus* isolates[14].

DNA Extraction and PCR Amplification

Genomic DNA of bacterial isolates was extracted using the (PrestoTM Mini gDNA) Bacterial Kit for DNA extraction of Gram-positive bacteria according to the manufacturer's instructions (Bioneer/Korea). (table 1). The concentration and purity of DNA extracted from the isolated samples were determined based on the method of Tsouklidis et al[18].

Table1: Optimal conditions for polymerase chain reaction (PCR) to detect genes under study

Amplified gene	Initial denaturation	No. of cycle	Denaturation	Annealing	Elongation	Final extension
Spa	min94°C/1	30	94°C/ 1 min	59°C/1 min	72°C/1 min	72°C/2min
mecA	min94°C/ 1	30	94°C/ 30 sec	57°C/30 sec	72°C/30 sec	72°C/2min

Detection of the *mec*A gene encoding methicillin resistance

Primers were selected to focus on specific parts of the *S. aureus* chromosome to detect the *mec*A virulence gene using PCR technology.

(`5-ACTGCTATCCACCCTCAAAC-3`) *mecA-F*
(`5- CTGGTGAAGTTGTAATCTGG-3`) *mecA-R*

spa Typing

Macrogene, South Korea, examined the nucleotide sequences of the amplified X region of the *spa* gene. Amplified products were extracted from the agarose gel after electrophoretic analysis. The same primers used in the PCR technique were applied for sequencing. The primers used for sequencing the X region of the *spa* gene were as follows:

(5'-TAAAGACGATCCTTCGGTGAGC-3') *spa-1113f*
(5'-CAGCAGTAGTGCCGTTTGCTT-3') *spa-1514r*

A computer program assigned the obtained nucleotide sequences to specific *spa* types. The resulting DNA sequences were initially analyzed using SnapGene software. The FASTA sequence of the X region, located between the conserved SL and SR primers, was then submitted to the *spa*-type website (following the guidelines from the Ridom *spa* Server database, http://www.spaserver.ridom.de) for further typing of the edited sequences into specific *spa* types[15]. This region is evaluated as a series of nucleotide repeats consisting of 24 base pairs, referred to as the repeat identification (r) region. More than 830 specific repeats have been identified. Depending on the types of r and the number

of repeats forming the X region, the result will either be an identified *spa* gene type or a newly discovered type.

Statistical Analysis:

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of different factors in study parameters. Least significant difference-LSD and the T-test were used to significantly compare between means. The chi-square test was used to significantly compare percentages (0.05 and 0.01 probability), in this study.

RESULTS

Number and percentages of bacterial isolates

Figure 1 shows the number and percentages of bacterial isolates. The results indicated bacterial growth in 229 isolates (91.6%), while no bacterial growth was observed in 21 isolates (8.4%).

As for the bacterial growth, the results showed that 80 isolates (32%) were positive for *Staphylococcus aureus*, while 149 isolates (59.6%) were positive for other types of bacteria.

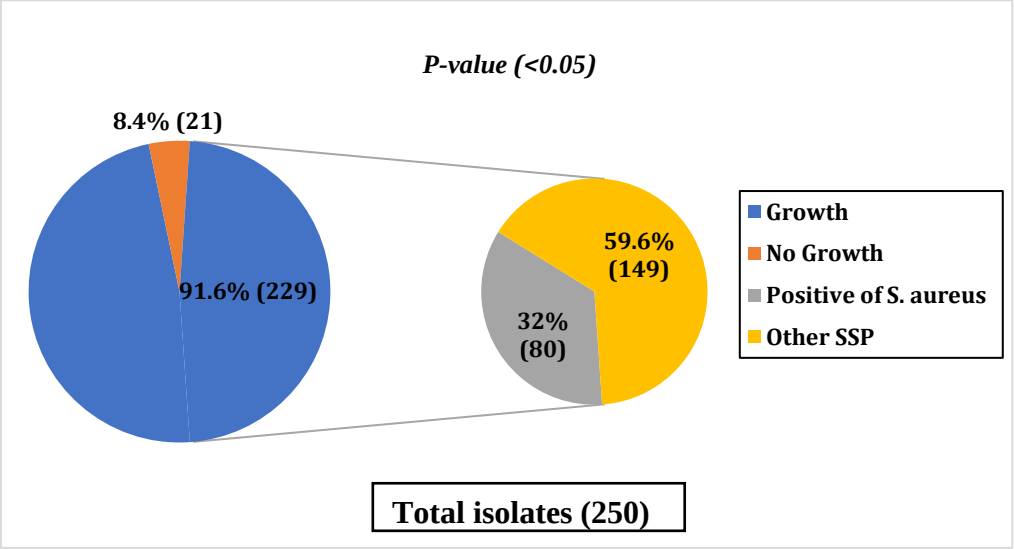

Fig. 1: Numbers and percentages of bacterial isolates isolated from clinical samples

Relationship between biofilm formation and *S. aureus* persistence

Data from Table 2 showed a close relationship between biofilm production in *S. aureus* isolates and their ability to resist antibiotics. When examining the isolates that produced persistent bacteria (29 isolates), 82.75% of them were in the strong biofilm production category, a percentage that far exceeded the moderate and weak categories (13.79% and 3.44%), respectively. As for the isolates that did not produce persistent bacteria (51 isolates), they were distributed as follows: 45.09% in the strong biofilm production category, 31.37% in the moderate category, and 23.52% in the weak category.

Table 2: The relationship between biofilm formation and *S. aureus* persistence

Persistent	Isolates	Isolate number			
		Biofilm formation isolates	Strong biofilm formation	Moderate biofilm formation	Weak biofilm formation
Produce	29	29 (100%)	24 (82.75%)	4 (13.79%)	1 (3.44%)
Non produce	51	51 (100%)	23 (45.09%)	16 (31.37%))	12 (23.52%)
Total Producer%	80	80 (100%)	47 (58.75%)	20 (25%)	13 (16.25%)
Chi-Square (*P-value*)	---	6.050 * (0.0136)	0.021 NS (0.884)	7.200 ** (0.0073)	9.307 ** (0.0023)
.($P \leq 0.01$) **					

Detection of *mecA* gene

The *mecA* gene is a virulence gene and the its detection is standard method for identifying methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. PCR electrophoresis revealed the detection of the *mecA* gene, which encodes methicillin resistance. The results showed that 100% of the isolates tested positive for the gene, with a significant difference (P<0.05), as shown in Figure 3.

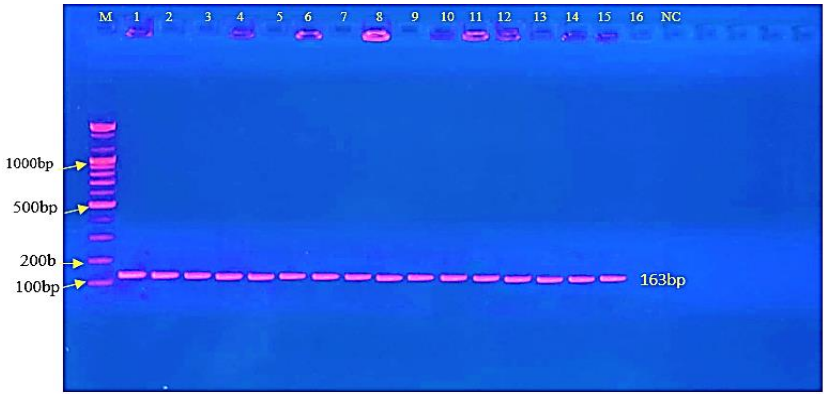

Fig. 3: Electrophoresis of the PCR product of the *mecA* gene (163 base pairs) of *S. aureus* isolates on agarose gel at a concentration of 1.5% and a potential difference of 7 V/cm² for 60 minutes. Lane M represents the ladder marker (100-1500 base pairs); lanes 1-16 are for isolates positive for the *mecA* gene; and lane 17 (N.C.) is a negative control.

Detection of *Spa* gene and typing

Genetically, the results of the polymerase chain reaction (PCR) for the *spa* gene showed that 13 out of 16 isolates (81.25%) of *S. aureus* bacteria were positive, indicating the critical role of the *spa* gene as a virulence marker in *Staphylococcus aureus* infection. The statistical analysis results showed a highly significant difference at $P \leq 0.01$, where the *P-value* was 0.0103, as shown in Figure 2. The resulting DNA sequences were initially analyzed using SnapGene software. The FASTA-type sequence of the X region located between the SL and SR sequences was then submitted to the *spa* type website (guidelines from the Ridom *spa* Server database (http://www.spaserver.ridom.de) were used to assign the edited sequences to specific *spa* types). The duplicates were typed and recognized by SnapGene software. The following *spa* types were identified: (t037, n=8; t14870, n=2; t044, n=2). The genotype of *S. aureus* is shown in Table 3.

Table3. Number and proportions of *spa* types among the studied *Staphylococcus aureus* isolates

***spa* typing**	**No (%)**
t037	**8 (67.68)**
t14870	**2 (16.66)**
t044	**2 (16.66)**
Total	**12 (100)**

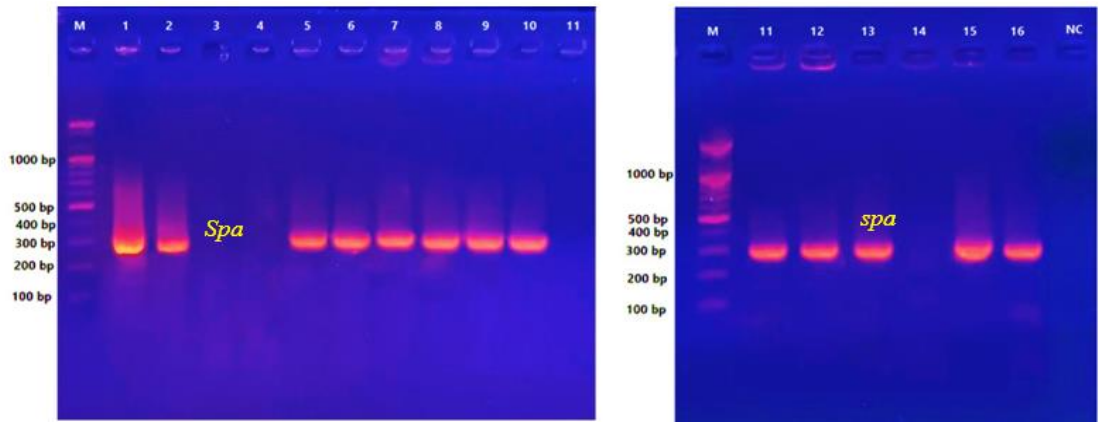

Fig. 2: Agarose gel electrophoresis of *Staphylococcus aureus* (1.5% agarose,7v/cm2 for 60 min) for *spa* gene (Variable). (A): Lane M, (Molecular weight marker); lane 1,2, 5,6,7,8,9,10 (Positive for *spa* gene); lane 3, 4 (Negative control); lane11, N.C. (Negative control). (B): Lane M, (Molecular weight marker); lane 11,12,13,15,16 (Positive for *spa* gene); lane17, N.C. (Negative control)

DISCUSSION

Staphylococcus aureus is a major cause of both community and hospital acquired bacterial infections. Its virulence stems from its high adaptability and ability to survive in diverse environments[18]. A key factor in its virulence is its ability to produce biofilms, a complex aggregate of bacterial cells surrounded by a self-produced matrix of polymeric substances that adheres to both biological and non-biological surfaces[19]. Biofilms play a central role in enhancing bacterial resistance to antibiotics and host immune defense mechanisms, complicating treatment options and increasing the likelihood of recurrence[20]. Several recent studies support this hypothesis. Nguyen etal[21] confirmed that highly biofilm-producing isolates possess complex genetic regulation that contributes to increased antibiotic tolerance. Sharifi etal[22] also indicated that modifying the expression of *ica* genes in *Staphylococcus aureus* increases biofilm thickness and enhances survival in the presence of antibiotics. Javanmard etal[23] emphasized the importance of developing specific antibiotics[24] to target biofilms, demonstrating that combining the use of anti-biofilm compounds with conventional treatments can increase clinical response rates.

The widespread dissemination of the t037 gene is a significant public health concern, as it is closely linked to *Staphylococcus aureus* antibiotic resistance, particularly in methicillin-resistant *S. aureus* (MRSA) infections. Research indicates that this genetic variant is frequently detected in hospital and healthcare settings, where it is transmitted among patients and healthcare personnel due to its high resistance to standard antimicrobial therapies. Studies conducted in hospitals across East Asia have identified the *t037* genotype as one of the most prevalent MRSA isolates collected from patients in intensive care units. These findings highlight the critical need for robust strategies to control its transmission in high-risk healthcare environments[25]. Although the *t14870* and *t044* genotypes were observed at lower frequencies, their presence may be attributed to specific environmental conditions or the emergence of novel genetic mutations. These genotypes might reflect a unique pattern of localized transmission in certain environments or result from geographical variations. The rarity of these genetic types, such as *t14870* and *t044*, could be due to their occurrence in regions with lower population density or limited antibiotic use, factors that may reduce their spread among patients. This aligns with the findings from a previous study[26]. The dissemination of antibiotic-resistance genes, such as t037, presents significant challenges for infection control in healthcare settings. Increased antibiotic usage drives bacterial adaptation, leading to the emergence of resistant strains. This overuse facilitates the development of resistance mechanisms, including the production of genes like *t037*, which are strongly associated with heightened resistance to antimicrobial agents. Such developments pose substantial obstacles for healthcare professionals in managing infections effectively. These findings agree with previously reported observations[27]. On the other hand, the current study did not agree with what was mentioned in Aminshahidi etal Study[28], as this study showed that the *spa t008* pattern was among the most common patterns in Iranian society, indicating a widespread of the pattern in different regions compared to other patterns such as *t037*. Also, the current results did not show any agreement with what was mentioned by Taha etal[29], as this study showed a high prevalence of *spa t021* and *spa t008* patterns in Egyptian hospitals, and concluded that *spa t037* is not among the prevailing patterns in this environment. While the study was very close to the results of a study conducted in Baghdad on *Staphylococcus aureus* isolates[15], the study reported that most infections were contaminated with *S. aureus* t037-sea-positive bacteria, perhaps due to the widespread prevalence of this strain in the hospital environment. Another study conducted in 2014 indicated a wide variety of *spa* styles around the world, it showed that *spa* t037 may not be the dominant style in most regions compared to other styles such as *t008* or *t021*[30].

All isolates gave a positive result for the gene 100%, and the result of the study agreed with the study conducted by researchers Yousaf etal[31] who found that the percentage of *S. aureus* bacteria possessing the gene for methicillin-resistant *S. aureus* (MRSA) reached 61 isolates at a rate of 96.8%. Researchers Sonbol etal[32] in Egypt indicated that the rate of MRSA isolates that gave a positive result for the *mec*A gene was 92.5%, which is close to the result of the current study. Researchers Goudarzi etal[33] also showed that in their study in Iran, that percentage of *S. aureus* bacteria possessing the *mec*A gene reached 70%, which is a lower percentage than our result current study in the. As for local studies, researchers Jaafar etal[34] showed that the percentage of *S. aureus* bacteria possessing the *mecA* gene was 96% in Babylon. The results differed from the results reached by researchers Fajer etal[35] that the percentage of MRSA isolates possessing the *mecA* gene was 63%, and in a local study in Diyala it is less than what the current study has reached.

CONCLUSION

The results of this study confirmed that *Staphylococcus aureus* has a high capacity to produce biofilms, which contributes significantly to its resistance to antibiotics and makes it difficult to eradicate in clinical settings. The study also showed a high prevalence of the *mecA* gene, responsible for methicillin resistance, in most isolates, reflecting the seriousness of these strains as a cause of MRSA infections.

Additionally, the study revealed a higher prevalence of the *spa* t037 strain compared to other strains, indicating its close association with resistant isolates, especially in high-risk healthcare settings.

Ethical approval

The study was conducted in observing with the ethical principles derived from the Declaration of Helsinki. Patients received oral and analytical consent before to sample collection. The study protocol, patient information, and consent form were reviewed and approved by a local ethics committee from the Diyala Health Department in accordance with Administrative Order No. 59149 in October 22, 2023.

REFERENCES

1. Dotel R. Molecular Epidemiology of *Staphylococcus aureus* and Newer Treatment Strategies 2024.
2. Fadhil ZA, Al-Taai HRR, editors. Multiple–loci number analyses of variable-number tandem repeat of molecular detection of *Staphylococcus aureus* isolates. AIP Conference Proceedings; 2023: AIP Publishing.
3. Pal M, Kerorsa GB, Marami LM, Kandi V. Epidemiology, pathogenicity, animal infections, antibiotic resistance, public health significance, and economic impact of staphylococcus aureus: a comprehensive review. American Journal of Public Health Research. 2020;8(1):14-21.
4. Kumar S, Anwer R, Yadav M, Sehrawat N, Singh M, Kumar V. Molecular typing and global epidemiology of Staphylococcus aureus. Current Pharmacology Reports. 2021;7(5):179-86.
5. Al-Jumaily SFS, Al-Taai HRR, Al-Dulaimi AA. Study of Phenotyping and Genotyping Characterization of Methicillin Resistant *Staphylococcus aureus* Isolates. Indian Journal of Forensic Medicine & Toxicology. 2019;13(4):875-80.
6. Foster TJ. Surface proteins of Staphylococcus aureus. Microbiology spectrum. 2019;7(4):10.1128/microbiolspec. gpp3-0046-2018.
7. Jawad Al-Zubaidi SJ. ANTIBACTERIAL ACTIVITY OF ZINC OXIDE NANOPARTICLES AGAINST SOME EFFLUX PUMP GENES IN METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS* ISOLATED FROM WOUNDS AND BURNS INFECTION. Biochemical & Cellular Archives. 2021;21(1).
8. Johnson GA. Antibiotic Resistance Profiles and Population Structure of Healthcare-associated and Community-associated *Staphylococcus aureus* isolated in Fort Portal, Western Uganda: University of Salford (United Kingdom); 2021.
9. Foster TJ, Geoghegan JA, Ganesh VK, Höök M. Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nature reviews microbiology. 2014;12(1):49-62.
10. Naorem RS, Urban P, Goswami G, Fekete C. Characterization of methicillin-resistant *Staphylococcus aureus* through genomics approach. 3 Biotech. 2020;10(9):401.
11. Temming A. Opsonin interactions with immunoglobulin-Fc receptors and their effector functions: A. Robin Temming; 2021.
12. Yuan F, Yin S, Xu Y, Xiang L, Wang H, Li Z, et al. The richness and diversity of catalases in bacteria. Frontiers in microbiology. 2021;12:645477.
13. Authman SH, Ksou MFA. Production, Purification and Determination Molecular Weight of Coagulase Enzyme from *Staphylococcus Aureus* Isolated from Clinical Samples. European Journal of Medical Genetics and Clinical Biology 2024;1(8).
14. Fusco A, Savio V, Perfetto B, Mattina R, Donnarumma G. Antimicrobial peptide human β-defensin-2 improves in vitro cellular viability and reduces pro-inflammatory effects induced by enteroinvasive Escherichia coli in Caco-2 cells by inhibiting invasion and virulence factors' expression. Frontiers in Cellular and Infection Microbiology. 2022;12:1009415.
15. Alwash SJ, Aburesha RA. Detection of toxin-associated genes in seven spa-types of *Staphylococcus aureus* in Iraq. Indian Journal of Forensic Medicine & Toxicology. 2021;15(3):4157.
16. Jiao F, Wang P, Zeng D, Bao Y, Zhang Y, Tao J, et al. Identification of Potential PBP2a Inhibitors Against Methicillin-Resistant *Staphylococcus aureus* via Drug Repurposing and Combination Therapy. Chemical Biology & Drug Design. 2025;105(3):e70088.
17. Gazi RF, Al-shukri MS, Chabuck ZAG, Jasim SA. Staphylococcal variable number tandem repeat (VNTR)-*spa* genotyping and their role in phylogenic study. Journal of Applied and Natural Science. 2023;15(1):429-34.
18. Tsouklidis N, Kumar R, Heindl SE, Soni R, Khan S. Understanding the fight against resistance: hospital-acquired methicillin-resistant *Staphylococcus aureus* vs. community-acquired methicillin-resistant Staphylococcus aureus. Cureus. 2020;12(6).
19. Wang L, Gu B, Zhang L, Zhu Z. Recent Advances in Bacterial Biofilm Studies: Formation,

Regulation, and Eradication in Human Infections. 2024.

20. Singh A, Amod A, Pandey P, Bose P, Pingali MS, Shivalkar S, et al. Bacterial biofilm infections, their resistance to antibiotics therapy and current treatment strategies. Biomedical Materials. 2022;17(2):022003.
21. Nguyen ANX, Thirapanmethee K, Audshasai T, Khuntayaporn P, Chomnawang MT. Insights into molecular mechanisms of phytochemicals in quorum sensing modulation for bacterial biofilm control. Archives of Microbiology. 2024;206(12):459.
22. Sharifi A, Mahmoudi P, Sobhani K, Ashengroph M. The Prevalence and Comparative Analysis of Adhesion and Biofilm-Related Genes in *Staphylococcus aureus* Isolates: A Network Meta-Analysis. Microbiology and Immunology. 2025;69(2):104-13.
23. Javanmard Z, Pourhajibagher M, Bahador A. Advancing anti-biofilm strategies: Innovations to combat biofilm-related challenges and enhance efficacy. Journal of Basic Microbiology. 2024;64(12):e2400271.
24. Abd Al-Mogadami HM, Al-Taai HRR, Al-Dolaymi AAF. MOLECULAR DETECTION OF QNR GENE IN CIPROFLOXACIN RESISTANCE ENTEROBACTER CLOACAE IN BAQUBAH CITY, IRAQ.
25. Lan T, Zhang B, Liu JL, Jia Q, Gao J, Cao L, et al. Prevalence and Antibiotic Resistance Patterns of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in a Hospital Setting: A Retrospective Study from 2018 to 2022. Indian Journal of Microbiology. 2024:1-9.
26. Jiang N, Wyres KL, Li J, Feßler AT, Krüger H, Wang Y, et al. Evolution and genomic insight into methicillin-resistant *Staphylococcus aureus* ST9 in China. Journal of Antimicrobial Chemotherapy. 2021;76(7):1703-11.
27. McClure J, Zhang K. The Evolution of *Staphylococcus aureus*. Genetics and Evolution of Infectious Diseases: Elsevier; 2024. p. 773-809.
28. Aminshahidi M, Arastehfar A, Pouladfar G, Arman E, Fani F. Diarrheagenic Escherichia coli and Shigella with high rate of extended-spectrum Beta-lactamase production: two predominant etiological agents of acute diarrhea in Shiraz, Iran. Microbial Drug Resistance. 2017;23(8):1037-44.
29. Taha AE, Badr MF, El-Morsy FE, Hammad E. Prevalence and Antimicrobial Susceptibility of Methicillin-Resistant *Staphylococcus aureus* in an Egyptian University Hospital. Journal of Pure & Applied Microbiology. 2019;13(4).
30. Smith EM, Needs PF, Manley G, Green LE. Global distribution and diversity of ovine-associated Staphylococcus aureus. Infection, Genetics and Evolution. 2014;22:208-15.
31. Yousaf Z, Rehman SU, Iqbal Z, Ismail R, Haseeb U, Abdullah M. DRUG PATTERN OF HOSPITAL AND COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS. The Research of Medical Science Review. 2025;3(1):656-62.
32. Sonbol FI, Abdelaziz AA, El-banna TE-S, Farag O. Detection and characterization of *staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in ear infections in Tanta, Egypt. Journal of Advanced Medical and Pharmaceutical Research. 2022;3(2):36-44.
33. Goudarzi M, Kobayashi N, Dadashi M, Pantůček R, Nasiri MJ, Fazeli M, et al. Prevalence, genetic diversity, and temporary shifts of inducible clindamycin resistance *Staphylococcus aureus* clones in Tehran, Iran: a molecular–epidemiological analysis from 2013 to 2018. Frontiers in microbiology. 2020;11:663.
34. Jaafar SS, Shareef HK. Relationship Between *Staphylococcus aureus* Biofilm Formation and Antibiotic Resistance that Isolated from Infections of the Urinary Tract in Babylon Province, Iraq. Medical Journal of Babylon. 2025;22(1):123-8.
35. Fajer ZB, Al-Ezzy AIA, AL-Zuhairi AH. Molecular prevalence of *mec*A and Blaz Genes with phenotypic analysis of Antibiotic Sensitivity Pattern for *S. aureus* Isolated From Dermal lesions of Sheep Breeders In Diyala Governorate–Iraq. Diyala Journal of Medicine. 2023;25(1):12-26.