Molecular Study of the *Agr* Gene in Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) in the *S. aureus*

M.Sc. Student: Mays Ali Salim [1*],
Lect. Dr. Estabraq Ali Maklef [1]
and Prof. Dr. Montaha Abdul-Karim AL-Saffar[2]

1 Middle Technical University, College of Health & Medical Technology, Medical Lab. Tech. Department, Baghdad / Iraq
2 Middle Technical University, Institute of Medical Technology, Community Health Department, Baghdad / Iraq
* Corresponding author E-mail: mali37088@gmail.com

دراسة جزيئية لجين *Agr* في بكتريا المكورات العنقودية الذهبية ذات المقاومة المتعددة للادوية (MDR) والمقاومة الشديدة للادوية (XDR).

طالبة الماجستير: ميس علي سالم [1*]،
م. د. استبرق علي مكلف [1]
و أ. د. منتهى عبد الكريم الصفار[2]

1 الجامعة التقنية الوسطى، الكلية التقنية الصحية والطبية – قسم المختبرات الطبية، بغداد \ العراق
2 الجامعة التقنية الوسطى، المعهد الطبي التقني – قسم صحة المجتمع، بغداد \ العراق

Abstract

Staphylococcus aureus a gram-positive coccus and one of the most important opportunistic bacteria that can lead to nosocomial infections. The purpose of this study is to explore the distribution and prevalence of the *Agr* gene in multidrug-resistance (MDR) and extensively drug-resistant (XDR) *S. aureus*. 62 isolates performed antibiotic sensitivity test. The isolates showed 66.12% resistance to clindamycin with MIC at range≥4, erythromycin 77.41% with MIC at range≥8, ciprofloxacin 19.35% with MIC at range≥4, oxacillin 75.80% with MIC at range≥4, cefoxitin 45.16% with MIC at range≥8, gentamycin 29.03% with MIC at range≥16, nitrofurantoin 14.51% and MIC at range≥128, and tetracycline 75.80% MIC at range≥16. Following the extraction of genomic DNA from the bacterial isolates, the presence of the *Agr* gene was confirmed. Using specific primers, the *Agr* genes (*Agr*1, *Agr*2, *Agr*3, and *Agr*4) were successfully amplified and detected in *S. aureus*.

Keywords: *Staphylococcus aureus***, Agr gene, Multi-drug-resistant (MDR), Extensively drug-resistant (XDR), Antibiotic sensitivity test.**

المستخلص

تُعَدّ المكورات إلعنقودية من البكتيريا الانتهازية التي يمكن أن تؤدي إلى العدوى المكتسبة في المستشفيات. لذا تهدف هذه الدراسة إلى اكتشاف توزيع وانتشار جينAgr في السلالات المقاومة المتعددة للأدوية (MDR) والسلالات المقاومة للأدوية بشكل واسع (XDR) من المكورات العنقودية. تم أجراء اختبار حساسية المضادات الحيوية على 62 عزلة، حيث أظهرت العزلات مقاومة بنسبة 66.12% للكلايندامايسين وكان الحد الأدنى من التركيز المثبط (MIC) في نطاق ≤4، و77.41% للإريثروميسين مع (MIC) في نطاق ≤8، و19.35% للسيبروفلوكساسين مع (MIC) في نطاق ≤4، و75.80% للأوكساسلين مع (MIC) في نطاق ≤4، و45.16% للسيفوكسيتين مع (MIC) في نطاق ≤8، و29.03% للجنتاميسين مع (MIC) في نطاق ≤16، و14.51% للنيتروفورانتوين مع (MIC) في نطاق ≤128، و75.80% للتتراسايكلين مع (MIC)في نطاق ≤16. وتم عزل الحمض النووي الجيني من العزلات البكتيرية، وتم تاكيد وجود جين Agr باستخدام بادئات محددة، و أخيرا شخصت جينات Agr1 وAgr2 وAgr4 Agr3 بنجاح في المكورات العنقودية في هذه الدراسة.

الكلمات المفتاحية: المكورات العنقودية, جين Agr, مقاومة متعددة الادوية (MDR), مقاومة واسعة النطاق للادوية (XDR), اختبار الحساسية للمضادات الحيوية.

Introduction

The rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Staphylococcus aureus* (*S. aureus*) poses a significant global health challenge (Jiang *et al.*, 2024). This pathogen can cause severe infections, including skin abscesses and systemic diseases like bacteremia and endocarditis (Linz *et al.*, 2023). Antibiotic resistance in *S. aureus* complicates infection management, leading to higher treatment failures, prolonged hospitalizations, and increased mortality. MDR and XDR strains are particularly significant due to their resistance to most available antibiotics. MDR strains exhibit resistance to multiple antibiotic classes, while XDR strains are resistant to all available antimicrobial agents, leaving limited therapeutic options (Marciniak *et al.*, 2024). Genetic factors like horizontal gene transfer, chromosomal gene mutations, and virulence factor regulation contribute to this resistance (Mlynarczyk-Bonikowska *et al.*, 2022). The accessory gene regulator (*Agr*) system plays a crucial role in controlling virulence and antibiotic susceptibility in these resistant strains. The *Agr* gene in *S. aureus* regulates the expression of virulence factors, including hemolysins, proteases, and toxins. It is part of four genetic groups: *Agr*1, *Agr* 2, *Agr*3, and *Agr*4. The system produces and senses autoinducing peptides (AIPs) that trigger virulence gene expression when bacterial populations reach a certain threshold (Fang *et al.*, 2024). The study of the *Agr* gene in resistant *S. aureus* strains has revealed a complex link between quorum sensing, virulence regulation, and antibiotic resistance (Cella *et al.*, 2023). Mutations or polymorphisms in the *Agr* locus in MDR and XDR strains may alter resistance gene expression and enhance resistance evasion. This disruption could contribute to infection persistence and spread, as this system promotes

survival under antibiotic pressure (Raghuram *et al.*, 2022). Understanding the distribution of *Agr* types in MDR and XDR *S. aureus* is crucial for identifying genetic markers that predict resistance patterns and guiding therapeutic strategies (Raghuram *et al.*, 2022). Targeting the *Agr* system could reduce virulence and combat resistance. Studies on highly resistant strains have highlighted the need for molecular surveillance and deeper exploration of how regulatory mechanisms influence treatment outcomes (Horswill *et al.*, 2019). The study investigates the molecular characteristics of the *Agr* gene in MDR and XDR strains, focusing on the prevalence of *Agr* 1, *Agr* 2, *Agr* 3, and *Agr* 4 in resistant populations. It seeks to understand the role of *Agr* in MDR persistence and pathogenicity in *S. aureus.*

Ethical approval

This study was carried out in accordance with the principles outlined in the Declaration of Helsinki. The ethics committee of Health and Medical Techniques College (173/3) granted approval for the research before any interventions were made, as did the agreements of Ghazi Al-Hariri Hospital (No.2643), Al-Kindi Hospital (No.255/3), and Ibn Al-Baladi Hospital (No.850/3).

Materials and Methods

Bacterial isolation

During the period from January 2024 to April 2024, 62 *S. aureus* isolates were collected from various clinical sources, from patients in selected hospitals in Baghdad city, the bacterial identification was performed using

traditional methods including coagulase testing, catalase, mannitol fermentation assays and vitek 2 compact system assay.

Antibiotic Susceptibility Testing

The disc diffusion technique on Mueller-Hinton agar and the antibiotic resistance of the 62 isolated bacteria were performed according to CLSI criteria. Concerning clindamycin (CD) 2µg, oxacillin (OX) 1µg, ciprofloxacin (CIP) 5µg, gentamycin (CN) 10µg, tetracycline (TE) 10µg, cefoxitin (FOX) 30µg, nitrofurantoin (F) 300µg, and erythromycin (E) 15µg. Data analysis and interpretation were done based on CLSI, 2023 (Bhagaskara *et al.*, 2023).

DNA isolation

A genomic DNA extraction kit prepared by EasyPure® Genomic DNA Kit (TransGen, biotech. EE101-01) was used to extract DNA for the bacterial isolates under study according to the manufacturer's instructions.

DNA examination

In order to determine DNA purity, a nanodrop spectrophotometer was used to assess the concentration of DNA samples that demonstrated acceptable integrity at two wavelengths (260 and 280 nm) (Bruijns *et al.*, 2022).

Molecular diagnosis of the *Agr* genes in *Staphylococcus aureus*

PCR technology was used in this investigation to identify the *Agr* genes (*Agr*1, *Agr*2, *Agr*3, *Agr*4). As shown in Table (1).

Table 1. Oligonucleotide primer sequence and Size amplicon.

Target gene	Initial Sequence from 5′ to 3′	Product Size(bp)	Reference
Agr1	F-5′- GTCACAAGTACTATAAGCTGCGA-3 R-5′-ATGCACATGGTGCACATGC -3	441	(R. Z. T. Ahmed *et al.*, 2024)
Agr2	F-5′-TATTACTAATTGAAAAGTGGCCATAGC -3′ R-5′-ATGCACATGGTGCACATGC -3	575	(R. Z. T. Ahmed et al., 2024)
Agr3	F-5′- GTAATGTAATAGCTTGTATAATAATACCCAG -3	323	(R. Z. T. Ahmed et al., 2024)
Agr4	R-5′-ATGCACATGGTGCACATGC -3 F-5′-GTAATGTAATAGCTTGTATAATAATACCCAG -3 R-5′-CGATAATGCCGTAATACCCG -3	659	(R. Z. T. Ahmed et al., 2024)

As per the instructions provided by the manufacturer, 12.5 µl of 2x EasyTaq® PCR SuperMix, 1 µl each of forward and reverse primers, 4 µl of DNA template, and 6.5 µl of nuclease-free water were used to prepare 25 µl of the reaction mixture, as illustrated in Table (2), with the given reaction conditions, this combination was utilized to carry out the PCR for gene amplification.

Table 2. The optimum condition of detection *Agr* gene.

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	94°C	5 min.	1 cycle
2-	Denaturation -2	94°C	30 sec.	
3-	Annealing	57 °C	90 sec.	40 cycle
4-	Extension-1	72°C	60 sec.	
5-	Extension -2	72°C	7 min.	1 cycle

Agarose gel electrophoresis

Following ethidium bromide staining, the extracted DNA and amplified PCR fragments were separated on an agarose gel and seen under UV light.

Statistical analysis section

Every experiment was carried out and recorded in triplicate. The standard deviation numbers were provided alongside the average mean values. After confirming that the data was homogeneous and normal, the Chi-square test was performed to compare the means and determine the significance of the data (significant * <0.05; high significant ** <0.01; very high significant *** <0.001). R Studio 4.5 was used by OriginLab 2021Software for the correlations and the figures in the statistical study.

Results and Discussion

when culturing *S. aureus* on 7.5% NaCl-containing mannitol salt agar. Bacterial colonies are circular in shape, have a non-zigzag edge, and are golden in color (Socohou *et al.*, 2021). They undergo fermentation to mannitol, producing acidic byproducts that lower pH, which causes the material to turn yellow. This is regarded as a differential analysis between the *staphylococcus* species (Obanda *et al.*, 2022). In addition, The physical traits of the isolates grown on blood agar were used to make the identification (Abdulbaqi *et al.*, 2023). The bacterial colonies had a convex shape, were shiny, smooth, had rounded edges, and were white in appearance. They were also starting to turn golden yellow and totally β-hemolysis type (Tang *et al.*, 2024). Microscopic analysis of slides containing colonies stained with gram-stain revealed that gram-positive bacteria create non-spore-forming, dark purple clusters that resemble bunches of grapes (Cao *et al.*, 2021). Additionally, all isolates yielded negative results for the oxidase test, positive results for the catalase test, and initial positive results for the coagulase test, which was conducted using the tube method when a layer appeared inside the tube, indicating the ability of

bacteria to produce the plasma coagulant enzyme and work to convert fibrinogen to fibrin. These biochemical tests were approved for the diagnosis of *S. aureus* bacteria (Petrillo *et al.*, 2021). Because it may spread illness and has many drug resistances, *S. aureus* is one of the most harmful bacteria that can infect hospitals and the general public (Vittorakis *et al.*, 2023).

The results of the study demonstrated that among antibiotics, ciprofloxacin (79.03%), nitrofurantoin (59.67%), and cefoxitin (54.83%) have the greatest percentages of inhibitory activity against *S. aureus.* On the other hand, the lowest percentages were for tetracycline (24.19%) and erythromycin (12.90%). Among the antibiotics that *S. aureus* tested, erythromycin (77.41%), oxacillin (75.80%), and tetracycline (75.80%) exhibited the highest levels of resistance, while the lowest percentages were found for ciprofloxacin (19.35%) and nitrofurtoin (14.51%).

Penicillin-binding proteins, such as penicillin's and cephalosporins, are altered by the expression of the *mecA* gene, which causes methicillin resistance. Antibiotic resistance can also result from other processes, such as the synthesis of beta-lactamases, which degrade beta-lactam antibiotics (Ali *et al.*, 2021).Nevertheless, no statistically significant correlation was found between the types of samples and the antibiotic resistance pattern examined, indicating that the source of infection may not have an impact on the effectiveness of the antibiotic in this particular study, and the outcomes supports this research (Hanif *et al.*, 2019).Ciprofloxacin and nitrofurantoin were shown to have the lowest level of resistance to *S. aureus* in this specific study. In our investigation, the resistance rate to ciprofloxacin was 19.35%; this is in close agreement with the 14.6% found in another study (Patoli *et al.*, 2018). However, our study's 14.51% nitrofurantoin resistance rate deviates from another study's 8.3% reported resistance rate (Singh *et al.*,

2019). Conversely, *S. aureus* displayed high levels of resistance to antibiotics that target the formation of proteins and cell walls, such as erythromycin, tetracycline, and oxacillin. Multi-drug resistance (MDR) was found in 32 (51.61%) of the 62 *S. aureus* isolates, which is in good agreement with the 55% MDR found in earlier research (Suma *et al.*, 2023).

The percentage of *Agr*1, *Agr*2, *Agr*3, and *Agr*4 presence for *S. aureus* bacteria was 10 (21.27%), 1 (2.12%), 21 (44.68%), and 3 (6.38%) as presented in Table (3). According to the results of the electrophoresis, the generated bands had a molecular weight of 441, 575, 323, and 659 base pairs, according to a comparison of the multiplied bands and the volumetric index as demonstrated in Figure (1).

Table 3. The prevalence of genes among sample sources

Source	*Agr1*	*Agr*2	*Agr*3	*Agr*4	no *Agr*
Abscess	3	0	1	0	2
Aspiration	0	0	0	0	1
Blood	0	0	0	0	1
Burns	1	0	1	1	2
Csf	0	0	1	1	0
ear swab	0	0	3	0	0
Fluid	0	0	2	0	0
Nasal swab	0	0	1	0	0
Pus	2	0	0	0	2
Sputum	1	0	2	0	0
Tissue	1	0	2	0	0
Urine	2	0	3	0	1
vaginal swab	0	0	1	0	0
Wound	0	1	4	1	3
Total (N=47)	10	1	21	3	12
P-value	0.3NS	0.3NS	0.4NS	0.4NS	0.3NS

The statistical analysis evaluates the relationship between the sample sources and the different types of Agr genes (*Agr*1, *Agr*2, *Agr*3, and *Agr*4). The Chi-square test results indicate no statistically significant association between the sample sources. All p-values for the tests exceeded 0.05. And this result corroborates previous research (Xu *et al.*, 2021)

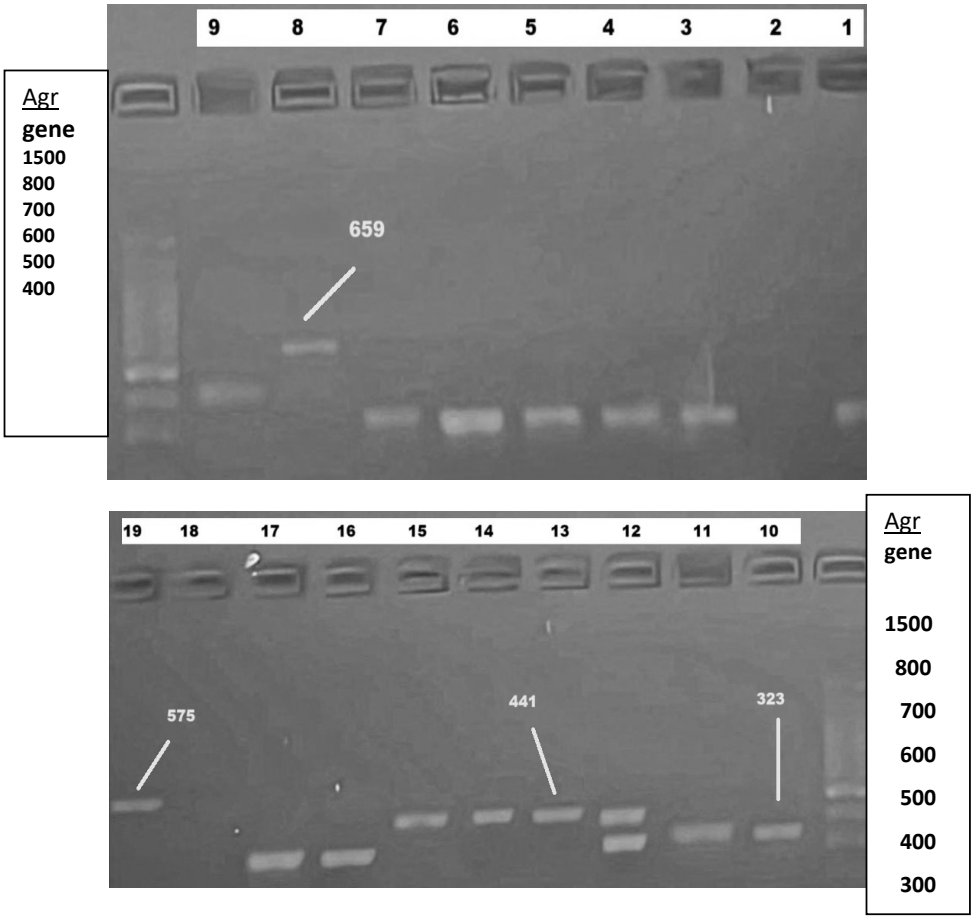

Figure 1: Electrophoresis of the *Agr*1, *Agr*2, *Agr*3, and *Agr*4 genes of *S. aureus* PCR reaction outcomes at a 441 bp, 575 bp, 323 bp, and bp product size. A DNA marker ladder (100-1500 bp) and a 2% agarose gel were utilized at 70 volts for 60 min.

The current study is closely agreed with the results of (Derakhshan *et al.*, 2021), who showed that the percentage of the *Agr*1 presence in *staphylococcal* bacteria was 20%, which is in close agreement with the 10 (21.27%). *Agr*2 was 1 (2.12%), but in another research the prevalence was found to be 53%, which is significantly higher. And the prevalence of *Agr*3 was 21 (44.68%), which is greater than the 35% reported in a different study (Ahmed *et al.*, 2024). This indicates a variability in the distribution of *Agr* system among different *S. aureus* isolates. *Agr*4 was 3 (6.36%), slightly lower than the 8.3% observed in the previous study (Maleki *et al.*, 2019). Some factors, like the regional variance and the sample size, influence the fluctuations (Yoon *et al.*, 2007). The classification levels (MDR and XDR) are substantially correlated with the presence of *Agr* gene types, according to the chi-square test, which yields highly statistical significance with an overall p-value of 0.01. Table (4) presented the correlation between the antibiotic classifications and gene type.

Table 4: The correlation between the antibiotic classifications and gene type.

Classification	*Agr*1	*Agr*2	*Agr*3	*Agr*4	no *Agr*
MDR	9	0	14	1	8
XDR	1	1	7	2	3
Total (N=46)	10	1	21	3	11
P-value	0.01**				

The study conducted by latifpour *et al* (Latifpour *et al.*, 2022) showed that *S. aureus* had *Agr*1 of 16 isolates (29.09%), *Agr*2 of 30 isolates (54.54%), *Agr*3 of 6 isolates (10.9%), and *Agr*4 of 3 isolates (5.45%) out of 55 isolates. Najafi Olya *et al* (Najafi Olya *et al.*, 2021) found that the rate of regulation of the virulence genes *Agr*1 in the resistance *S. aureus* was 14 (50%), *Agr*2 was

3 (10.7%), *Agr*3 was 10 (35.8%), and *Agr*4 was 1 (3.5%) of a total of 28 isolates. Furthermore, the current result differed from the research carried out by (Bibalan *et al.*, 2014), who showed that the percentage of *Agr*1 presence in bacteria was 5% and the percentage of *Agr*3 presence was 55%. But in the investigation of (Abbasian *et al.*, 2018) the presence rate of *Agr*1 was 131 isolates (78.4%), *Agr*2 was 17 isolates (10.2%), and *Agr*3 reached 8 isolates (4.8%). A finding from the study of (Ahmed *et al.*, 2022)2022 provide evidence that the presence rate of *Agr*1 reached 82 isolates (55%), *Agr*2 was 37 isolates (25%), *Agr*3 reached 10 isolates (7%) and *Agr*4 was 21 isolates (14%).

Multiple genes involved in *S. aureus* bacterium pathogenicity, including coagulase, lipase, toxic shock syndrome type T, leucocidin, a and β-hemolysin, and fibronectin-binding protein, have been linked to the *Agr* regulator, according to the study of (Enwuru *et al.*, 2023). Based on research findings, each group of Agr regulators mediates a number of diseases, including impetigo and exfoliative toxin (eta etb) encoded by *Agr*4, enterotoxins (Seg, Sei, Sem, and Seo) encoded by *Agr*1 and *Agr*2, leukotoxic-lethal genes LukF-PV and LukS-PV, and toxic shock syndrome genes (TST) regulated by *Agr*1, *Agr*2, and *Agr*3 (Horswill *et al.*, 2019). According to study, the typing method is now crucial for determining the genetic relationships between bacterial strains, categorizing bacteria according to their epidemiology, locating infection sources and methods, identifying high-virulence bacterial strains, and treating them to stop them from spreading (Furuya *et al.*, 2023). By using the *Agr* technique to type the *S. aureus* under investigation, the genetic link was discovered and recognized. The *Agr* method sequences were discovered to be distributed across several areas of the bacterial genome, including *Agr*1, *Agr*2, *Agr*3, and *Agr*4 (Pereira *et al.*, 2022). The present

study's findings demonstrated that bacterial isolates from various clinical sources had genetic relationships with one another.

Conclusions

This study emphasizes the serious threat that *S. aureus* poses due to its high level of antibiotic resistance and its widespread in hospitals and public settings. The study identifies the *Agr* gene types (*Agr1*, *Agr2*, *Agr3*, and *Agr4*) and provides insight into the molecular mechanisms underlying *S. aureus* pathogenicity. The research discovered no significant correlation between sample sources and antibiotic-resistant patterns, despite the fact that more than half of the isolates had high levels of multidrug resistance (MDR). This suggests that the origin of the illness may not have an effect on how well treatments work. The results highlight the significance of continuous monitoring of antibiotic resistance and the importance of molecular methods such as *Agr* typing to comprehend the genetic connections among bacterial strains and direct the creation of more potent treatment approaches to stop the spread of this infection.

Acknowledgments

The authors would like to thank all of the labs and hospitals who helped them finish this study. Furthermore, they would express gratitude to every volunteer who provided clinical samples.

References

- Abbasian, S., Farahani, N. N., Mir, Z., Alinejad, F., Haeili, M., Dahmardehei, M., . . . Darban-Sarokhalil, D. (2018). Abbasian. *New Microbes New Infect, 26*, 15-19. doi:10.1016/j.nmni.2018.08.001
- Abdulbaqi, A., & Ibrahim, A. S. (2023). Molecular analysis of *Staphylococcus aureus* Isolated from Clinical Samples and Natural Flora. *Cell Mol. Biol. (Noisy-le-grand), 69*(1), 145-149. doi:10.14715/cmb/2022.69.1.25
- Ahmed, R. Z. T., & Abdullah, R. M. (2024). Detection of Integron Classes and Agr Group in *Staphylococcus aureus* Isolated from Different Clinical Samples. *Ibn AL-Haitham Journal for Pure and Applied Sciences, 37*(2), 111-127. doi:doi.org/10.30526/37.2.3419
- Ahmed, Z. F., & Al-Daraghi, W. A. H. (2022). Molecular detection of medA virulence gene in *Staphylococcus aureus* isolated from Iraqi patients. *Iraqi Journal of Biotechnology,21*(1),8-18.doi:https://doi.org/10.32007/jfacmedbagdad.6622282
- Ali, T., Basit, A., Karim, A. M., Lee, J.-H., Jeon, J.-H., Rehman, S.U., & Lee, S.-H. (2021). Mutation-based antibiotic Resistance Mechanism in Methicillin-resistant *Staphylococcus aureus* Clinical Isolates. *Pharmaceuticals, 14*(5), 420. doi:https://www.mdpi.com/1424-8247/14/5/420#
- Bhagaskara, R. J., Ahmad, A. S., Prasetyawan, S. R., Amelia, D., Pratama, D. P., Tulloh, I. M. H., . . . Ismail, F. M. I., (2023), Antibiotic SusceptIbility Test of *Pseudomonas Aeruginosa* and *Staphylococcus Aupeus* with Disk Diffusion and Dilution Method. *Journal of Research in Pharmacy and Pharmaceutical Sciences, 2*(1), 29-37. doi: 10.3343/alm.2015.35.6.635.
- Bibalan, M. H., Shakeri, F., Javid, N., Ghaemi, A., & Ghaemi, E. A. (2014). Accessory Gene Regulator Types of *Staphylococcus aureus* Isolated in Gorgan, North of Iran. *J Clin Diagn Res, 8*(4), DC07-09. doi:10.7860/JCDR/2014/6971.4219
- Bruijns, B., Hoekema, T., Oomens, L., Tiggelaar, R., & Gardeniers, H. (2022). Performance of Spectrophotometric and Fluorometric DNA Quantification Methods. *Analytica, 3*(3), 371-384. doi:10.1186/1472-6750-3-20.
- Cao, Y., & Lin, H. (2021). Characterization and Function of Membrane Vesicles in Gram-positive Bacteria. *Applied Microbiology and Biotechnology, 105*, 1795-1801. doi:https://doi.org/10.1128/iai.74.3.1631-1642.2006
- Cella, M. A., Coulson, T., MacEachern, S., Badr, S., Ahmadi, A., Tabatabaei, M. S., . . . Griffiths, M. W. (2023). Probiotic Disruption of Quorum Sensing Reduces Virulence and Increases Cefoxitin Sensitivity in Methicillin-resistant *Staphylococcus aureus*. *Scientific Reports, 13*(1), 4373. doi: 10.1038/s41598-023-31474-2.

- Derakhshan, S., Navidinia, M., & Haghi, F. (2021). Antibiotic Susceptibility of Human-associated *Staphylococcus aureus* and Its Relation to Agr Typing, Virulence Genes, and Biofilm Formation. *BMC Infectious Diseases, 21*, 1-10. DOI:10.1186/s12879-021-06307-0
- Enwuru, N. V., Adesida, S. A., Enwuru, C. A., & Mendie, U. E. (2023). Accessory Gene Regulators and Virulence Genes Associated with the Pathogenicity of *Staphylococcus aureus* from Clinical and Community Settings in Lagos, Nigeria. *South African Journal of Science, 119*(9-10), 1-7. doi:https://orcid.org/0000-0003-2285-0682
- Fang, L., Cosgriff, C., & Alonzo III, F. (2024). Determinants ofMaturation of the *Staphylococcus aureus A*utoinducing Peptide. *Journal of Bacteriology, 206*(9), e00195-00124. doi: 10.1128/jb.00195-24. Epub 2024 Aug 23.
- Furuya, H., Ogura, K., Takemoto, N., Watanabe, S., Yamazaki, A., Ogai, K., . . . Okamoto, S. (2023). A multilocus sequence typing method of *Staphylococcus aureus* DNAs in a Sample from Human Skin. *Microbiol Immunol, 67*(10), 438-446. doi:10.1111/1348-0421.13094
- Hanif, E., & Hassan, S. A. (2019). Evaluation of Antibiotic Resistance Pattern in Clinical Isolates of *Staphylococcus aureus*. *Pak J Pharm Sci, 32*(4), 1749-1753. doi: 10.1016/j.otohns.2003.12.003.
- Horswill, A. R., & Gordon, C. P. (2019). Structure–activity Relationship Studies of Small Moleculer Modulators of the *Staphylococcal* Accessory Gene Regulator. *Journal of Medicinal Chemistry, 63*(6), 2705-2730. doi: 10.1021/acs.jmedchem.9b00798.
- Jiang, G., Liu, K., Qing, Y., Qin, L., Zou, Z., & Liu, Z. (2024). Global Trends of Antibiotic Resistance Genes in *Staphylococcus aureus*: A Comprehensive Genomic Analysis. *Foodborne Pathogens and Disease, 21(10)*, 653-661. doi:https://doi.org/10.1089/fpd.2024.0043
- Latifpour, M., Narimani, T., Sadeghi, A., & Niakan, M. (2022). Determination of Virulence Factors and Resistance Profile of Methicillin-Resistant *Staphylococcus aureus* Strains among Different Types of Spa, Agr, and SCCmec. *BioMed Research International, 2022*(1), 5863310. doi:https://doi.org/10.1155/2022/5863310
- Linz, M. S., Mattappallil, A., Finkel, D., & Parker, D. (2023). Clinical Impact of *Staphylococcus aureus* Skin and Soft Tissue Infections. *Antibiotics, 12*(3), 557. https://doi.org/10.3390/antibiotics12030557
- Maleki, D. T., Ghalavand, Z., Laabei, M., Nikmanesh, B., Houri, H., Kodori, M., . . . Eslami, G. (2019). Molecular analysis of accessory gene regulator functionality and virulence genes in *Staphylococcus aureus* Derived from Pediatric Wound Infections. *Infection, Genetics and Evolution, 73*, 255-260. doi:https://doi.org/10.1016/j.meegid.2019.05.013

- Marciniak, K., Tyczewska, A., & Grzywacz, K. (2024). Genetics of Antibiotic Resistance in Methicillin-Resistant *Staphylococcus aureus* (MRSA). *BioTechnologia, 105*(2), 169-177. doi:https://doi.org/10.5114/bta.2024.139756
- Mlynarczyk-Bonikowska, B., Kowalewski, C., Krolak-Ulinska, A., & Marusza, W. (2022). Molecular Mechanisms of Drug Resistance in *Staphylococcus aureus*. *International Journal of Molecular Sciences, 23*(15), 8088. https://doi.org/10.3390/ijms23158088
- Najafi Olya, Z., Najar-Peerayeh, S., Yadegar, A., & Bakhshi, B. (2021). Clonal Diversity and Genomic Characterization of Panton-valentine Leukocidin (PVL)-positive *Staphylococcus aureus* in Tehran, Iran. *BMC Infect Dis, 21*(1), 372. doi:10.1186/s12879-021-06060-4
- Obanda, B. A., Cook, E. A., Fèvre, E. M., Bebora, L., Ogara, W., Wang, S.-H., . . . Muyodi, J. (2022). Characteristics of *Staphylococcus aureus* Isolated from Patients in Busia County Referral Hospital, Kenya. *Pathogens, 11*(12), 1504. doi:https://www.mdpi.com/2076-0817/11/12/1504#
- Patoli, A. A., Patoli, B. B., Laghari, Z. A., & Maachi, T. M. (2018). Co-existence of Ciprofloxacin Resistance in Methicillin Resistant S*taphylococcus aureus* from Clinical Samples. *Pakistan Journal of Physiology, 14*(2), 24-27. doi:https://doi.org/10.69656/pjp.v14i2.224
- Pereira, G. d. N., Rosa, R. d. S., Dias, A. A., Gonçalves, D. J. S., Seribelli, A. A., Pinheiro-Hubinger, L., . . . Pereira, V. C. (2022). Characterization of the Virulence,Agr Typing and Antimicrobial Resistance Profile of *Staphylococcus aureus* Strains Isolated from Food Handlers in Brazil. *Brazilian Journal of Infectious Diseases, 26*(5), 102698. doi:https://doi.org/10.1016/j.bjid.2022.102698
- Petrillo, F., Pignataro, D., Di Lella, F. M., Reibaldi, M., Fallico, M., Castellino, N., . . . Santella, B. (2021). Antimicrobial Susceptibility Patterns and ResistanceTrends of S*taphylococcus aureus* and Coagulase-negative Staphylococci Strains Isolated from Ocular Infections. *Antibiotics, 10*(5), 527. doi:https://www.mdpi.com/2079-6382/10/5/527#
- Raghuram, V., Alexander, A. M., Loo, H. Q., Petit III, R. A., Goldberg, J. B., & Read, T. D. (2022). Species-wide phylogenomics of the *Staphylococcus aureus* Agr operon revealed convergent evolution of frameshift mutations. *Microbiology Spectrum, 10*(1), e01334-01321. doi: 10.1128/spectrum.01334-21.
- Singh, P., Malik, S., & Lal, V. (2019). Resistance Pattern of Nitrofurantoin of Uropathogens in Different Age Groups at Dr. Lal Path Labs, National Reference Laboratory, Rohini, Delhi. *Asian Journal of Medicine and Health, 16*(1), 1-8. DOI: 10.9734/AJMAH/2019/v16i130134

- Socohou, A., Sina, H., Degbey, C., Adjobimey, T., Sossou, E., Boya, B., . . . Baba-Moussa, L. (2021). Pathogenicity and Molecular Characterization of *Staphylococcus aureus* Strains Isolated from the Hospital Environment of CHU-Z Abomey-Calavi/Sô-Ava (Benin). *BioMed Research International, 2021*(1), 6637617. doi:https://doi.org/10.1155/2021/6637617
- Suma, T. A., Alam, N., Raihan, S. Z., Zahid, M. A., Mandal, S. C., Suchana, F. J., . . . Muhit, M. A. (2023). Association of Antibacterial Susceptibility Profile with the Prevalence of Genes Encoding Efflux Proteins in the Bangladeshi Clinical Isolates of *Staphylococcus aureus*. *Antibiotics (Basel), 12*(2), 305. doi:10.3390/antibiotics12020305
- Tang, W., Liu, Y., Li, X., Leng, G., Gao, J., Wang, Y., . . . Xu, Y. (2024). Microbiological Characteristics of Clinically Isolated *Staphylococcus aureus* with Different Hemolytic Phenotypes in China. *Infect Drug Resist, 17*, 3273-3287. doi:10.2147/IDR.S466416
- Vittorakis, E., Vică, M. L., Zervaki, C. O., Vittorakis, E., Maraki, S., Mavromanolaki, V. E., . . . Sinanis, T. (2023). Examining the Prevalence and Antibiotic Susceptibility of *S. aureus* Strains in Hospitals: An Analysis of the pvl Gene and Its Co-Occurrence with Other Virulence Factors. *Microorganisms, 11*(4), 841. doi:https://doi.org/10.3390/microorganisms11040841
- Xu, Y., Qian, S.-Y., Yao, K.-H., Dong, F., Song, W.-Q., Sun, C., . . . Lv, Z.-Y. (2021). Clinical and Molecular Characteristics of *Staphylococcus aureus I*solated from Chinese Children: Association among the Agr Groups and Genotypes, Virulence Genes and Disease Types. *World Journal of Pediatrics, 17*, 180-188. doi:10.1007/s12519-021-00421-4
- Yoon, H. J., Choi, J. Y., Lee, K., Yong, D., Kim, J. M., & Song, Y. G. (2007). Accessory Gene Regulator Group Polymorphisms in methicillin-resistant *Staphylococcus aureus*: an association with clinical significance. *Yonsei Medical Journal, 48*(2), 176-183. doi:https://doi.org/10.14715/cmb/2024.70.4.1