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Molecular profiling of methicillin and vancomycin resistant *Staphylococcus aureus* isolates from Baghdad hospitals

Tabark Asim Qader*, Ali Haider Alsakini, Munim Radwan Ali

Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

***Correspondence to**
Tabark Asim Qader, Email: alzahaweytk@gmail.com, tabark.asim@uomustansiriyah.edu.iq

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Abstract

Introduction: Oxacillin and vancomycin are commonly used to treat *Staphylococcus aureus* infections. Molecular typing of *S. aureus* is essential for understanding resistance patterns.

Objectives: This study aims to determine the phenotypic and genotypic features of *S. aureus* (methicillin-resistant *S. aureus* [MRSA] and vancomycin-resistant *S. aureus* [VRSA]) isolates obtained from various sources, including wounds, skin, urine, vaginal swabs, ear swabs, and body fluids, from samples of different outpatients and hospitalized patients in several key hospitals and medical laboratories in Baghdad governorate, Iraq.

Patients and Methods: In this cross-sectional study, biochemical diagnostic tests were conducted on the isolates, coagulase-positive isolates were collected. These isolates were confirmed through ordinary microbiological and molecular testing. Antibiotic screening test was determined. A phenotypic and genotypic biofilm forming ability and other characteristics were investigated. Molecular typing of the isolates was conducted using polymerase chain reaction (PCR).

Results: Out of 130 coagulase-positive cocci, 72 were identified as MRSA and 19 as VRSA. Biofilm assay results showed that 22 (18.92%) isolates had strong biofilm formation, 24 (18.46%) had moderate formation, 26 (20.00%) had weak formation, and 58 (44.62%) had no biofilm formation. The MRSA biofilm-associated gene *mecA* was detected in 37 (80.4%) of the isolates, while in VRSA, *mecA* was detected in 13 (68.42%). Additionally, the *cna* gene was present in five cases (26.32%) of the VRSA isolates. Genes were characterized based on P-value into true-false and presence-absence counts. Antimicrobial screening showed that most isolates were sensitive to gentamicin, with higher resistance observed for oxacillin and rifampin. The staphylococcal cassette chromosome mec *(SCCmec)* patterns for MRSA and VRSA were determined using PCR results.

Conclusion: This study indicates that *S. aureus,* including MRSA and VRSA, should be considered significant opportunistic pathogens across all age groups and clinical units. Gentamicin and tigecycline are recommended for treating certain *S. aureus* infections.

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Introduction

Staphylococcus aureus is a gram-positive, coagulase-positive cocci from the Staphylococcus family and an opportunistic pathogen in humans (1). It is a leading human pathogen known for its ability to evade the immune system and cause a variety of infections (2). Many *S. aureus* infections are iatrogenic, often associated with the colonization of indwelling medical devices (3). During an infection, an immune response is initiated by macrophages, which release cytokines to recruit neutrophils (4). *S. aureus* commonly causes skin and skin structure infections, osteoarticular infections, septicemia, infective endocarditis, pneumonia, ocular infections, and central nervous system infections (5). *S. aureus* bacteremia can spread to virtually any body site, leading to severe disease, significant morbidity, and death, placing a substantial burden on healthcare systems with high mortality and morbidity rates (6-8). *S. aureus* is a major etiological agent of both hospital-acquired and community-acquired infections, presenting a constant therapeutic challenge due to its increasing antimicrobial resistance and status as a leading cause of nosocomial infections worldwide (9,10). Antibiotic resistance in *S. aureus* is carried through chromosomes and plasmids, exacerbated by indiscriminate antibiotic use (11). Contact with infected individuals or their belongs can spread *S. aureus*, and asymptomatic colonized individuals facilitate human-to-human transmission (12,13). Vancomycin has been an effective treatment for methicillin-resistant *S. aureus* (MRSA); however, the increased administration of vancomycin has led to selective pressure for resistance development (14). The high

Key point

Staphylococcus aureus is a major pathogen in both hospital and community settings, posing a significant global public health concern. The rates of methicillin-resistant *S. aureus* (MRSA), coupled with the extensive use of various antibiotic classes, presents a considerable therapeutic challenge. The frequent use of vancomycin to treat MRSA has led to an increase in strains resistant to this antibiotic, known as vancomycin-resistant *S. aureus* (VRSA). Genetic tools, such as polymerase chain reaction are effective methods for assessing bacterial resistance.

prevalence of MRSA and vancomycin-resistant *S. aureus* (VRSA) in hospitals underscores the need for stringent infection control practices and investigation of underlying risk factors to prevent further spread (15). Regulation of virulence genes in *S. aureus* follows a designing plan starting with the staffing of the bacterium in the host and subsequent attacks on the host defenses. *S. aureus* initially upregulates genes coding for surface proteins involved in adhesion and defense versus the host immune system, followed by the upregulation of toxin production to facilitate tissue spread later in infection. One of the two-component regulatory systems in *S. aureus* is the accessory gene regulator (agr) system, which regulates over 70 genes, including 23 related to virulence. It is responsible for upregulating many exoproteins and downregulating the synthesis of cell wall-associated proteins (16,17).

Nowadays, molecular typing is vastly utilized to reconnoitering the transmission routes of bacterial infections, especially nosocomial infections. Polymerase chain reaction (PCR)-based molecular typing is determined because of its highly expedite, specificity simplicity, and low outlays (18).

Objectives

In this study, MRSA and VRSA isolates of S. *aureus* were investigated using routine microbiological protocols, including antimicrobial susceptibility tests to evaluate resistance to various commonly used antibiotics. PCR was employed as a tool for gene content analysis and typing. Statistical analysis systems were applied to comprehensively understand bacterial virulence relationships, including biofilm formation ability, antibiotic resistance, sample type, medical sources, and gene content.

Patients and Methods

In this cross-sectional study, 130 coagulase-positive staphylococci were collected from various sample cultures of outpatients and inpatients hospitalized in different substantial hospitals and medical laboratories in Baghdad governorate from July 2023 to January 2024. Identification of *S. aureus* was performed using standard microbiological tests and a biochemical characterization procedure (19). *S. aureus* isolates were thoroughly emphasized by PCR using the 16S rRNA primer (20). A dendrogram (Figure 1) with isolates configuration was generated by using UPGMA algorithm similarity index by Jaccard (21).

Antibiotic susceptibility of *S. aureus* isolates was conducted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (22) using commercially available discs (Liofilchem® S.r.l. Via Scozia, Italy) for 17 antimicrobial agents from 14 different classes by the Kirby–Bauer disk diffusion method. The antibiotic panel included; gentamicin (10 µg/disk), rifampin (5 µg/disk), ceftaroline (30 µg/disk), oxacillin (5 µg/disk), ciprofloxacin (5 µg/disk), moxifloxacin (5 µg/disk), Trimethoprim-sulphamethoxazole (1.25/23.75 µg/disk), Fusidic acid (FU:10 µg/disk), vancomycin (30 µg/disk), teicoplanin (30 µg/disk), clindamycin (2 µg/disk), erythromycin (15 µg/disk), linezolid (30 µg/disk), chloramphenicol (30 µg/disk), tetracycline (30 µg/disk), doxycycline (30 µg/disk), and tigecycline (15 µg/disk).

Figure 1. UPGMA dendrograms with genetic Jaccard similarity coefficients analysis based on antibiotic susceptibility test results for whole *S. aureus* isolates.

Quality control was maintained using *S. aureus* ATCC 25923, and test and antibiogram analysis results were interpreted according to the CLSI 2022 (22).

Biofilm formation of *S. aureus* isolates was revealed by using a microtiter plate (96-well plate) with crystal violet dye. *S. aureus* biofilm quantitation was proceeded with a spectrophotometric method as previously described (23). All conducted experiments were performed in triplicate. Genomic DNA of *S. aureus* was extracted using the boiling method (24,25). DNA was extracted after centrifugation for five minutes at 12,000 RPM. Presence of *S. aureus* biofilm-related genes such as *mecA* and other virulence-related genes was detected by PCR with a suitable annealing gradient. Specific primers for *S. aureus* were designed using bioinformatic software, Geneious Prime. The target genes included those encoding surface adhesion protein (*Cna*) (55 °C), ferric uptake regulator protein (*SdrD*) (55 °C), regulating iron availability (*Fur*) (55 °C), *agr (I, II, III, IV*) (55°C), *SCCmec* (55 °C) systems gene, *Pvl* (53 °C), and *mecA* (53°C), based on NCBI references (26). To obtain specific primer sequences, the desired gene sequences were downloaded from NCBI and aligned using multiple alignment on Geneious Prime.

The PCR reaction admixture consisted of an overall volume of 25 µL. Thermal cycling was applied with the following conditions: 95°C for five minutes, followed by 30 cycles of 94 °C for one minute, 52 °C for one minute, 72 °C for one minute, and a final elongation step at 72 °C for ten minutes. Electrophoresis of products was performed using 1.5% agarose gel, and bands were conceived under ultraviolet transilluminator light (25,27).

Statistical analysis

The R studio ggplot2 package was utilized to perform the correlation and display the results. The Chart Builder tool in R studio with the ggplot2 package was conducted to create stacked charts within the use the pchisq function in R to determine the *P* value within significance ratio of 0.05 that corresponds to the chi-square test statistic to measure the significancy between variables (28).

Results

Out of 130 coagulase-positive cocci, 58 isolates (44.62%) showed negative phenotypic biofilm formation. Weak biofilm formation was observed in 26 isolates (20.00%), moderate formation in 24 isolates (18.46%), and strong biofilm formation in 22 isolates (16.92%). The chi-square test with *P* value showed a range of 0.7669, indicating no significant association between biofilm formation and the groups (Figure 2).

Another factor investigated was the biofilm-forming ability of *S. aureus* isolates. Biofilm formation on hospital superficies and human tissues is a critical survival feature of *S. aureus*. Most isolates in this study were biofilm procreator. Statistical analysis revealed a significant relationship between biofilm formation ability and multi-extended drug resistance and multidrug sensitivity capacities (Figure 3).

Statistical analysis revealed that 87 samples were from female patients. Among these, 43 isolates were multidrug-resistant, 14 were extended-resistant, and 30 were multidrug-sensitive ($P=0.0006$). For male patients, there were 43 samples, with 23 multidrug-resistant isolates, 4

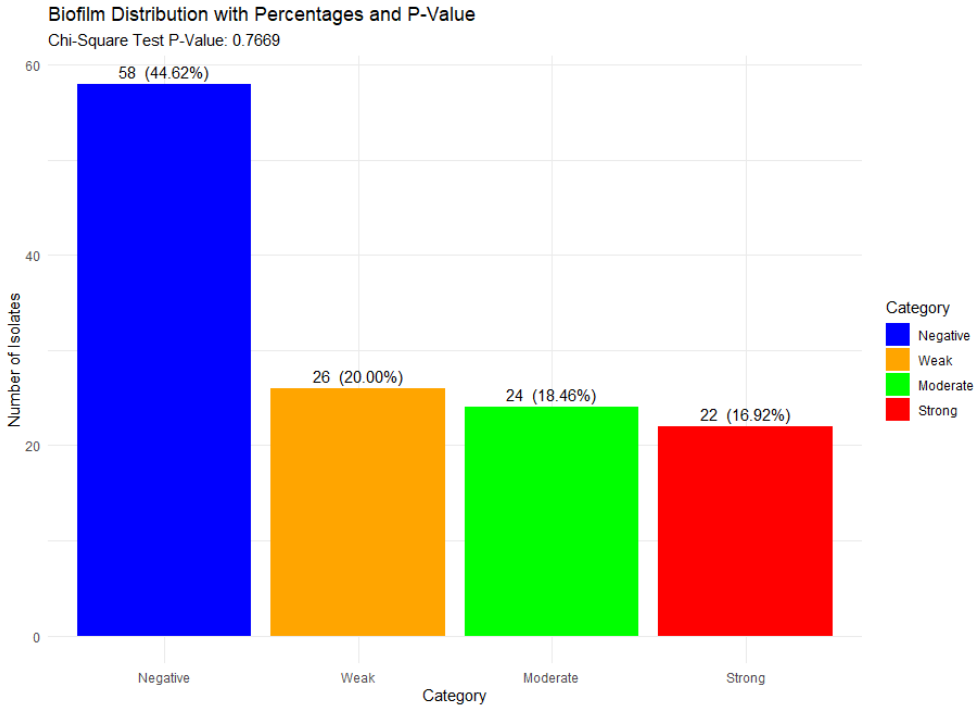

Figure 2. *Staphylococcus aureus* biofilm distribution according chi-square test and *P* value computations.

extended-resistant isolates, and 16 multidrug-sensitive isolates ($P=0.0015$). The overall P value for gender and antibiotic resistance was 0.5734, indicating no significant relationship between gender and antibiotic resistance.

Regarding age, the highest frequency of antibiotic resistance was observed in patients aged 20-70 years, with 70 isolates. Patients under 20 years had 11 resistant isolates, and those over 71 years had 4 resistant isolates. The total P-value was 0.9836, indicating no significant relationship between age and antibiotic resistance.

Based on the sample types, wound swabs had the highest count with 53 isolates and a significant P value of 0.0075. However, the total P value for all sample types was 0.0798, indicating no significant relationship between sample type and antibiotic resistance.

The total significance P value for the sources of isolates in hospitals in Baghdad showed that the predominant hospital was Al-Kindy hospital with 50 isolates and a total P value of 0.0028.

MRSA antimicrobial susceptibility analysis showed high resistance to rifampin. Fusidic acid and linezolid showed similar resistance levels, while minimal resistance was observed for moxifloxacin and gentamicin (Figure 4).

Moreover, VRSA antimicrobial susceptibility analysis showed a high resistance for clindamycin and teicoplanin, minimal resistance was shown in gentamicin and ceftaroline (Figure 5).

PCR results analysis showed variable different sizes of the amplicons ranged from 310 bp to >659bp, *Cna, SdrD*, and *Fur* designed genes, appearance categorizations were inconstant between MRSA and VRSA, through concluded analysis of the results, the genetic variety among MRSA and VRSA strains were spotted (Figures 6 and 7).

PCR analysis *SCCmec* pattern revealed peculiar types and percentages in MRSA and VRSA in according to significances P value ranges, moreover, they could be divided into 4 common types in addition to unknown one (Figure 8).

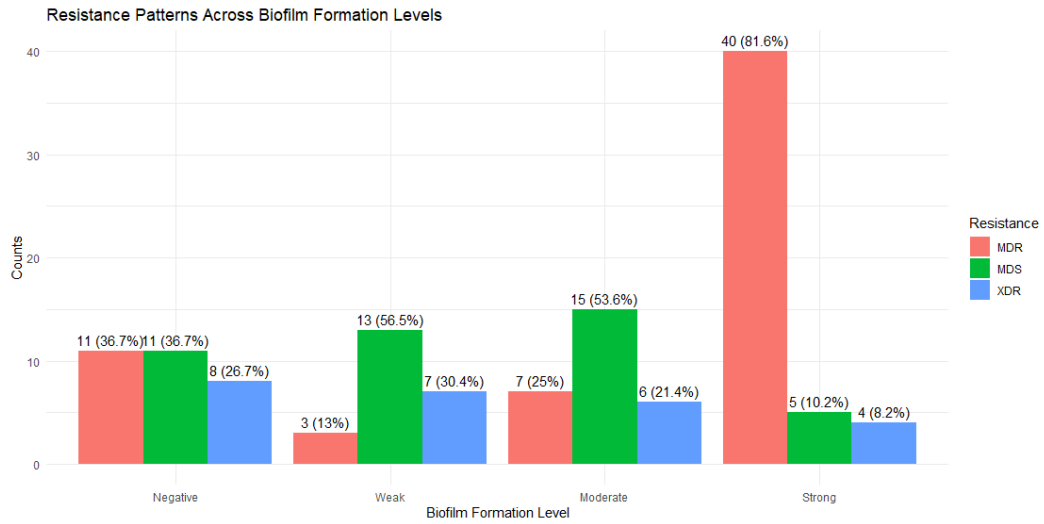

Figure 3. Resistance patterns across biofilm formation levels.

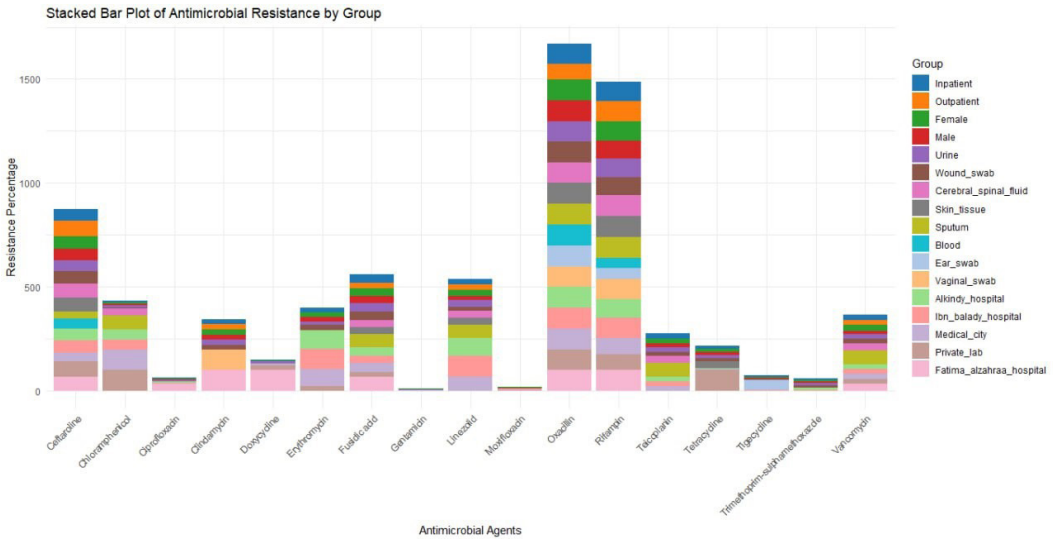

Figure 4. MRSA stacked bar plot of antimicrobial resistance with source groups.

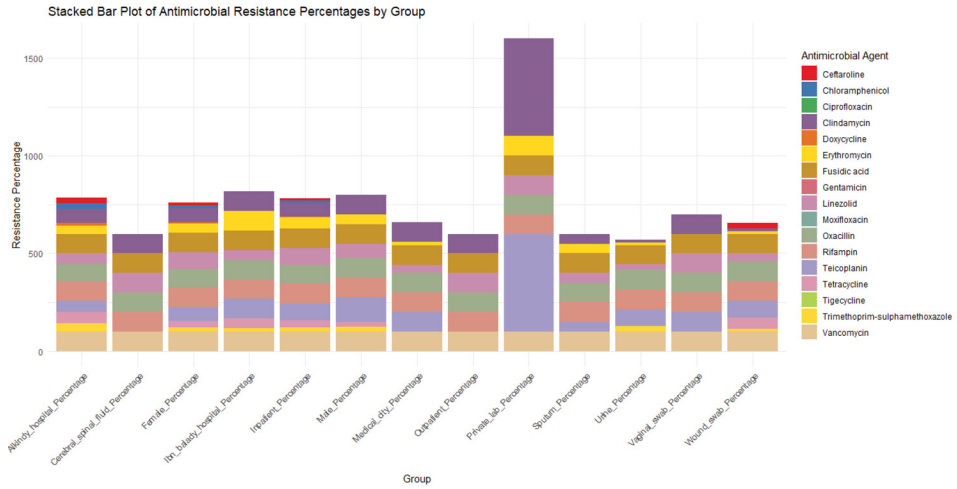

Figure 5. VRSA stacked bar plot of antimicrobial resistance with source groups.

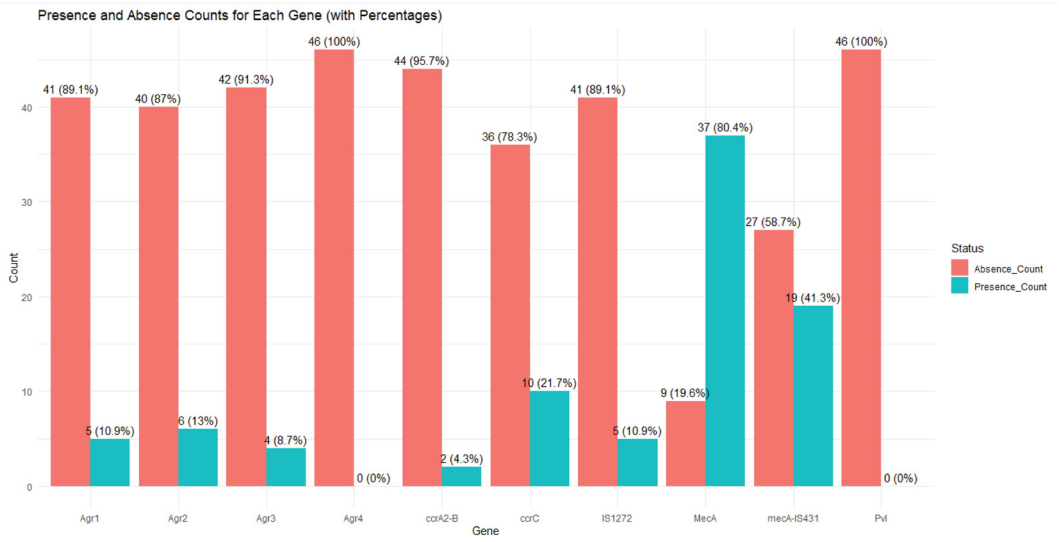

Figure 6. Prevalence of Agr genes associated with other detectable virulence genes percentages in MRSA isolates.

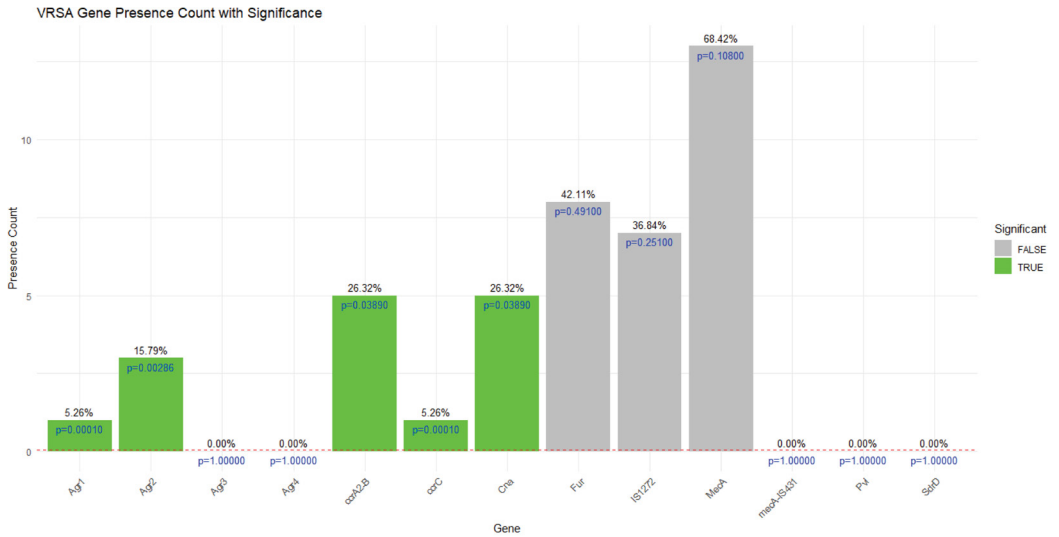

Figure 7. VRSA genes presence count with significance ratios. Green bars (True) denote genes significantly associated with a specific phenotype or condition (*P* value < 0.05), while grey bars (False) represent genes without significant association

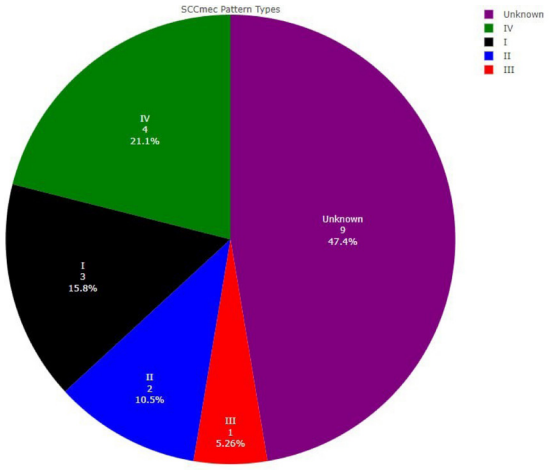

Figure 8. Prevalence of MRSA and VRSA *SCCmec* typing patterns.

The variability between isolates mainly related to their disproportion in biofilm formation potency, antibiotic resistance, and genomic content.

Discussion

In this study explores, the predominance of *S. aureus* isolated from several key hospitals in the Baghdad governorate presented different gene distributions compared to other studies, despite potentially lower overall counts compared to other hospital-acquired bacteria. This is significant due to *S. aureus's* intrinsic resistance to common antimicrobial agents, appearance of virulence genes, and biofilm formation capability.

Several reports on the expansion of *S. aureus* in the Baghdad governorate indicate a higher distribution of clinical samples compared to a previous study in 2015. The current results show that the distribution of *S. aureus* was as follows; urine (n = 44), blood (n = 9), cerebral spinal fluid (n = 7), skin tissues (n = 6), sputum (n = 5), vaginal swab (n = 2), wound swab (n = 53), and ear swab (n = 4) (29).

The isolates were confirmed by the principle biochemical protocols. This study, in line with others, found that the domination of *S. aureus* was higher in females than in males. Females had 43 multidrug-resistant (MDR) isolates and 14 extended drug-resistant (XDR) isolates ($P = 0.00069$), while males had 23 MDR isolates and 4 XDR isolates ($P = 0.00159$). *S. aureus* infections are important to study due to their potential impact on gender-specific health issues, such as male sperm activity and various phases of a woman's reproductive life, including pre-pregnancy, fertilization, pregnancy, and reproduction (30).

Significant infections with multi-extended drug resistance of *S. aureus* were most common in the age group 20-70 years. According to various studies, hospitalized infants, and children, as well as other age groups, can have varying susceptibility to *S. aureus* infections depending on gender, historical profile, and other key factors (31-33).

Trimethoprim-sulphamethoxazole and vancomycin are considered effective antibiotics for treating *S. aureus* infections. However, recent reports of resistance have raised concerns over their continued efficiency. substitutional antibiotics such as gentamicin and linezolid have been found efficacious against invasive *S. aureus* infections (34,35).

About this study findings, gentamicin and tigecycline were found to be effective against *S. aureus*, while rifampin was deemed unsuitable. The efficacy of quinupristin-dalfopristin was not studied due to its rare or unavailability in Iraqi hospitals. Different antimicrobial susceptibility results have been reported from Iraq and elsewhere.

Previous studies in several Iraqi hospitals found high resistance of *S. aureus* isolates to ampicillin and amoxicillin. The overall assessment of antibiotics used for urinary tract infections revealed that amikacin was effective (36,37).

Consistent with these findings, a study at Baghdad University reported that 40 (69%) of *S. aureus* isolates were resistant to gentamicin and cefoxitin. Vancomycin susceptibility testing using the Vitek-2 compact system showed that 57 (98%) of *S. aureus* isolates were sensitive, with only one isolate (2%) exhibiting intermediate resistance. The study identified 58 *S. aureus* isolates from different clinical specimens (38).

The findings of this study are consistent with other reports that demonstrate a proportional range of resistance to rifampin, which arises from chromosome mutations. This suggests that rifampin may be inappropriate for treating advanced hospital-acquired *S. aureus* infections (39,40).

MRSA is an important hospital pathogen, with its incidence increasing annually, especially in high-risk groups. MRSA is notable for causing both nosocomial infections and community-onset infections, with a widespread distribution over the last 40 years (41-43).

While vancomycin remains a first-line drug for MRSA treatment, complete resistance has emerged in recent years, mediated by the *vanA* gene cluster transferred from vancomycin-resistant *Enterococcus*. The first clinical VRSA isolate was reported in the United States in 2002 (44,45).

This study's findings align with other research indicating that antibiotic resistance development in developing countries is strongly linked to irrational antibiotic use due to ease of obtainability without prescription, imprudent use in hospitals, and unhindered employ in agriculture, animal frugality, and fisheries (46).

MRSA and VRSA were identified using standard microbiological methods, and their genetic diversity was quickly and inexpensively verified using PCR with specifically designed primers. The PCR results classified 46 MRSA isolates into different *SCCmec* types, as illustrated in (Figures 5 and 6), providing a discriminative pattern.

Our PCR results showed that MRSA and VRSA have varied genetic content, which may be attributed to genetic diversity based on the collection environment or stress

pressures that lead to mutations. Our findings regarding *Agr* dysfunction align with a Korean study (47) but differ from (48) study that reported a higher *Agr* system appearance ratio. Additionally, no MRSA or VRSA isolates in this study contained the *Pvl* gene, which contrasts with a study of (31), that found 14 out of 24 isolates positive for the *Pvl* gene. The absence of the *Pvl* gene could suggest lower virulence, different epidemiological origins, or reliance on other resistance mechanisms and virulence factors.

By demonstrated analyzing of the environmental samples and employing molecular investigational typing, we identified and concluded the environmental gene variability of MRSA and VRSA isolates in central medical spots in the Baghdad governorate.

Conclusion

This study dissected the prevalence of *Staphylococcus aureus* in Baghdad hospitals, revealing significant multidrug and extended drug resistance, with higher resistance rates in female patients. Biofilm formation was a major challenge, contributing to drug resistance and persistence in hospital environments and human tissues. The genetic variability included the lack of the *Pvl* gene in MRSA and VRSA isolates. Despite noted antibiotic resistance, gentamicin and linezolid were effective. Continuous surveillance, antibiotic stewardship, and phenotypic and genotypic studies for virulence capacities are recommended to control the spread of infections.

Limitations of the study

The variability in gene content is challenging to assess due to the limited sample collection. Further collaboration with medical centers treating patients with *S. aureus* infections is necessary to enhance the study's scope and accuracy.

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Authors' contribution

Conceptualization: Ali Haider Alsakini, Munim Radwan Ali, Tabark Asim Qader.
Data curation: Tabark Asim Qader.
Formal analysis: Ali Haider Alsakini, Munim Radwan Ali, Tabark Asim Qader.
Investigation: Ali Haider Alsakini, Munim Radwan Ali, Tabark Asim Qader.
Methodology: Ali Haider Alsakini.
Project administration: Ali Haider Alsakini, Munim Radwan Ali.
Resources: Ali Haider Alsakini, Munim Radwan Ali, Tabark Asim Qader.
Software: Munim Radwan Ali.
Supervision: Ali Haider Alsakini, Munim Radwan Ali.
Validation: Ali Haider Alsakini, Munim Radwan Ali, Tabark Asim Qader.
Visualization: Munim Radwan Ali, Tabark Asim Qader.
Writing–original draft: Tabark Asim Qader.
Writing–review & editing: Tabark Asim Qader.

Study Highlights

- *Staphylococcus aureus* included MRSA and VRSA were detected in variable type of pathological samples from different medical spots.
- Detection of the relation between *S. aureus* biofilm ability, antibiotic resistance, and genes content.
- Biofilm screening resulted appearance with negative formation (44.62%) for 58 isolate, weak (20.00%) for 26 isolate, moderate (18.46%) for 24 isolate and strong (16.92%) for 22 staphylococci.
- MRSA within complete resistance to vancomycin have emerged in recent years.
- Molecular pattern investigation for MRSA and VRSA within intended gene prevalence.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

This research adhered to the principles outlined in the Helsinki Declaration and was approved by the Ethics Committee of Mustansiriyah University college of Sciences (Ref No. BCSMU/0923/0007B). The study was designed and conducted with the participants' well-being in mind. Participants were fully informed about the research and its implications before agreeing to participate. Participation was voluntary, and participants could withdraw at any time. This study was extracted from M.Sc. thesis of Tabark Asim Qader (thesis #61377) at the department of biological science of Mustansiriyah university. Besides, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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References

1. Harris LG, Foster SJ, Richards RG. An introduction to *Staphylococcus aureus,* and techniques for identifying and quantifying S. aureus adhesins in relation to adhesion to biomaterials: review. Eur Cell Mater. 2002;4:39-60. doi: 10.22203/ecm.v004a0.
2. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med. 1998;339:520-32. doi: 10.1056/NEJM199808203390806.
3. Pietrocola G, Campoccia D, Motta C, Montanaro L, Arciola CR, Speziale P. Colonization and Infection of Indwelling Medical Devices by *Staphylococcus aureus* with an Emphasis on Orthopedic Implants. Int J Mol Sci. 2022;23:5958. doi: 10.3390/ijms23115958.
4. Cheng AG, DeDent AC, Schneewind O, Missiakas D. A play in four acts: *Staphylococcus aureus* abscess formation. Trends Microbiol. 2011;19:225-32. doi: 10.1016/j.tim.2011.01.007.
5. Ondusko DS, Nolt D. *Staphylococcus aureus*. Pediatr Rev. 2018;39:287-298. doi: 10.1542/pir.2017-0224.
6. Keynan Y, Rubinstein E. *Staphylococcus aureus* bacteremia, risk factors, complications, and management. Crit Care Clin. 2013;29:547-62. doi: 10.1016/j.ccc.2013.03.008.
7. Steinberg JP, Clark CC, Hackman BO. Nosocomial and community-acquired *Staphylococcus aureus* bacteremias from 1980 to 1993: impact of intravascular devices and methicillin resistance. Clin Infect Dis. 1996;23:255-9. doi: 10.1093/clinids/23.2.255.
8. Kern WV. Management of *Staphylococcus aureus* bacteremia

and endocarditis: progresses and challenges. Curr Opin Infect Dis. 2010;23:346-58. doi: 10.1097/QCO.0b013e32833bcc8a.
9. Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE. The molecular evolution of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect. 2007;13:222-35. doi: 10.1111/j.1469-0691.2006.01573.x.
10. Alsolami A, ALGhasab NS, Alharbi MSM, Bashir AI, Saleem M, Syed Khaja AS, et al; Ha'il COM Research Unit Group. Community-Acquired Methicillin-Resistant *Staphylococcus aureus* in Hospitals: Age-Specificity and Potential Zoonotic-Zooanthroponotic Transmission Dynamics. Diagnostics (Basel). 2023 Jun 16;13:2089. doi: 10.3390/diagnostics13122089.
11. Espinal MA, Laszlo A, Simonsen L, Boulahbal F, Kim SJ, Reniero A, et al. Global trends in resistance to antituberculosis drugs. World Health Organization-International Union against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. N Engl J Med. 2001 Apr 26;344:1294-303. doi: 10.1056/NEJM200104263441706.
12. Archer GL, Climo MW. Antimicrobial susceptibility of coagulase-negative staphylococci. Antimicrob Agents Chemother. 1994;38:2231–7.
13. Morell EA, Balkin DM. Methicillin-resistant *Staphylococcus aureus:* a pervasive pathogen highlights the need for new antimicrobial development. Yale J Biol Med. 2010;83:223.
14. Al-Amery K, Elhariri M, Elsayed A, El-Moghazy G, Elhelw R, El-Mahallawy H, et al. Vancomycin-resistant *Staphylococcus aureus* isolated from camel meat and slaughterhouse workers in Egypt. Antimicrob Resist Infect Control. 2019;8:129. doi: 10.1186/s13756-019-0585-4.
15. Kejela T, Dekosa F. High prevalence of MRSA and VRSA among inpatients of Mettu Karl Referral Hospital, Southwest Ethiopia. Trop Med Int Health. 2022;27:735-741. doi: 10.1111/tmi.13789.
16. Bien J, Sokolova O, Bozko P. Characterization of Virulence Factors of *Staphylococcus aureus:* Novel Function of Known Virulence Factors That Are Implicated in Activation of Airway Epithelial Proinflammatory Response. J Pathog. 2011;2011:601905. doi: 10.4061/2011/601905.
17. Novick RP, Ross HF, Projan SJ, Kornblum J, Kreiswirth B, Moghazeh S. Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. EMBO J. 1993;12:3967-75. doi: 10.1002/j.1460-2075.1993.tb06074.x.
18. Liu HY, Hopping GC, Vaidyanathan U, Ronquillo YC, Hoopes PC, Moshirfar M. Polymerase Chain Reaction and Its Application in the Diagnosis of Infectious Keratitis. Med Hypothesis Discov Innov Ophthalmol. 2019;8:152-155.
19. Ghayyib AA, Ahmed IA, Ahmed HK. Isolation, Molecular Identification, and Antimicrobial Susceptibility Testing of *Staphylococcus aureus* Isolates. HIV Nurs. 2022;22:278–83. doi: 10.31838/hiv22.02.56.
20. Gumaa MA, Idris AB, Bilal NE, Hassan MA. First insights into molecular basis identification of 16 s ribosomal RNA gene of *Staphylococcus aureus* isolated from Sudan. BMC Res Notes. 2021;14:240. doi: 10.1186/s13104-021-05569-w.
21. Garcia-Vallvé S, Puigbo P. DendroUPGMA: a dendrogram construction utility. Univ Rovira i Virgili. 2009;1–14.
22. CLSI. Perform Stand Antimicrob Susceptibility Test. 2022; Available from: https://clsi.org/standards/products/microbiology/documents/m100/.
23. Al-kafaween MA, Mohd Hilmi AB, Jaffar N, Al-Jamal HAN, Zahri MK. Determination of optimum incubation time for formation of Pseudomonas aeruginosa and *Streptococcus pyogenes* biofilms in microtiter plate. Bull Natl Res Cent. 2019;43:1–5. doi: 10.1186/s42269-019-0131-9.
24. Nasif S, Alsakini Ah, Ali MR. Prevalence of integrons and antibiogram typing among *Escherichia coli* causing community-acquired urinary tract infection. Chinese J Med Genet. 2022;32: 269-277.
25. Mohsin AS, Alsakini AH, Ali MR. Molecular characterization of *Dr/Afa* genes prevalent among multi drug resistant *Escherichia coli* isolated from urinary tract infections. Biomedicine. 2022;42:523–9. doi: 10.51248/.v42i3.1632.
26. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol. 2005;43:5026-33. doi: 10.1128/JCM.43.10.5026-5033.2005.
27. Yehia HM, Al-Masoud AH, Alarjani KM, Alamri MS. Prevalence of methicillin-resistant (*mecA* gene) and heat-resistant *Staphylococcus aureus* strains in pasteurized camel milk. J Dairy Sci. 2020;103:5947-5963. doi: 10.3168/jds.2019-17631.
28. Morgado LN, Noordeloos ME, Hausknecht A. *Clitopilus reticulosporus,* a new species with unique spore ornamentation, its phylogenetic affinities and implications on the spore evolution theory. Mycol Prog. 2016;15:1–8. doi 10.1007/s11557-016-1165-0.
29. Kareem SM, Al-Jubori SS, Ali MR. Prevalence of pvl gene among methicillin resistance S. aureus isolates in Baghdad city. World J Pharm Res. 2015;4:455–71.
30. Shi L, Wang H, Lu Z. Staphylococcal infection and infertility. In: Darwish AM, ed. Genital Infections and Infertility. InTech Rijeka, Croat; 2016. p. 159–77. doi: 10.5772/62663.
31. Al-Ghazal SA, Al-Hassnawi HH. Molecular detection of pore-forming leuko toxin in methicillin resistant Staphylococcus aureus isolated from skin infection. Medical Journal of Babylon. 2024;21:186-90.doi: 10.4103/MJBL.MJBL_842_23.
32. Ahmed RZT, Abdullah RM. Prevalence of Multidrug Resistant *Staphylococcus aureus* and their Pathogenic Toxins Genes in Iraqi Patients, 2022-2023. AIDS. 2023;3:4. doi: 10.30699/ijmm.17.5.559.
33. M'Aiber S, Maamari K, Williams A, Albakry Z, Taher AQM, Hossain F, et al. The challenge of antibiotic resistance in post-war Mosul, Iraq: an analysis of 20 months of microbiological samples from a tertiary orthopaedic care centre. J Glob Antimicrob Resist. 2022;30:311-318. doi: 10.1016/j.jgar.2022.06.022.
34. Mal P, Dutta S, Bandyopadhyay D, Dutta K, Basu A, Bishayi B. Gentamicin in combination with ascorbic acid regulates the severity of *Staphylococcus aureus* infection-induced septic arthritis in mice. Scand J Immunol. 2012;76:528-40. doi: 10.1111/j.1365-3083.2012.02766.x.
35. Pintado V, Pazos R, Jiménez-Mejías ME, Rodríguez-Guardado A, Díaz-Pollán B, Cabellos C, et al. Linezolid for therapy of *Staphylococcus aureus* meningitis: a cohort study of 26 patients. Infect Dis (Lond). 2020;52:808-815. doi: 10.1080/23744235.2020.1789212. PMID: 32648796.
36. Alwatar WMA, Suha A. Al Fakhar, Saad Hasan Mohammed Ali, Khalil Ismail Abid Mohammed, Jenan M. Mousa (2023). Prevalence of Antibiotics Resistance among Patients in Iraqi Hospitals. Haya Saudi J Life Sci. 2023;8:118–26. doi: 10.36348/sjls.2023.v08i07.005.
37. Bengtsson-Palme J, Larsson DG. Antibiotic resistance genes in the environment: prioritizing risks. Nat Rev Microbiol. 2015;13:396. doi: 10.1038/nrmicro3399-c1.
38. Alkhafajy RTA, Al-Mathkhury HJF. Gentamicin Upregulates the Gene Expression of hla and nuc in *Staphylococcus aureus*. Iraqi J Sci. 2023;1079–92. doi: 10.24996/ijs.2023.64.3.5.
39. Jafar Alwash S, Abed Aburesha R. The Differences in Antibiotic-resistance among Several *Staphylococcus aureus* strains in Iraq. Medico-legal Updat. 2021;21: 476-485.

40. Al-Saadi DAA, Abd Al-Mayahi FS. Antibiogram susceptibility patterns of *Staphylococcus aureus* harboring of MecA gene and prevalence aminoglycoside modifying enzymes (AMEs) genes in Iraq. In: IOP Conference Series: Earth and Environmental Science. IOP Publishing; 2021. p. 12049. doi: 10.1088/1755-1315/923/1/012049.
41. Vidhani S, Mehndiratta PL, Mathur MD. Study of methicillin resistant S. aureus (MRSA) isolates from high risk patients. Indian J Med Microbiol. 2001;19:13-6.
42. Azeez-Akande O. Global trend of methicillin-resistant Staphlococcus aureus and emerging challenges for control. African J Clin Exp Microbiol. 2010;11: 150-158.
43. Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clin Microbiol Infect. 2016;22:416-22. doi: 10.1016/j.cmi.2015.12.002.
44. Cong Y, Yang S, Rao X. Vancomycin resistant *Staphylococcus aureus* infections: A review of case updating and clinical features. J Adv Res. 2019;21:169-176. doi: 10.1016/j.jare.2019.10.005.
45. Centers for Disease Control and Prevention (CDC). *Staphylococcus aureus* resistant to vancomycin--United States, 2002. MMWR Morb Mortal Wkly Rep. 2002;51:565-7.
46. Holloway K. Antimicrobial resistance: the facts. Essent Drug Monit WHO. 2000;28:7–8. doi:10.1016/S1473-3099(11)70054-8.
47. Chong YP, Kim ES, Park SJ, Park KH, Kim T, Kim MN, et al. Accessory gene regulator (agr) dysfunction in *Staphylococcus aureus* bloodstream isolates from South Korean patients. Antimicrob Agents Chemother. 2013;57:1509-12. doi: 10.1128/AAC.01260-12.
48. Rasheed NA, Hussein NR. Characterization of different virulent factors in methicillin-resistant *Staphylococcus aureus* isolates recovered from Iraqis and Syrian refugees in Duhok city, Iraq. PLoS One. 2020;15:e0237714. doi: 10.1371/journal.pone.0237714.