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Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Staphylococcus aureus* (VRSA) among admitted patients at Baqubah Teaching Hospital in Diyala

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Abstract

Background: Antibiotic resistance in *Staphylococcus aureus* has emerged as a critical global public health threat, driven primarily by the rising prevalence of methicillin-resistant strains (MRSA) and their diminishing susceptibility to conventional antimicrobials.

Objective: To determine the prevalence of methicillin-resistant (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) and to evaluate their antimicrobial susceptibility patterns across various clinical isolates.

Patients and Methods: A cross-sectional study was conducted at Baqubah Teaching Hospital in Diyala, Iraq, from March 2025 to April 2026. A total of 385 samples collected from various clinical specimens, including urine, wound, vaginal, and ear swabs. Include 110 *S. aureus* isolates were consecutively Antimicrobial susceptibility testing was performed using standard methods. Data were analyzed using SPSS version 24, and Chi-square tests were applied to assess the associations between resistance patterns and specimen sources. P-values less than 0.05 were considered statistically significant.

Result: The isolated *S. aureus* strains exhibited substantial resistance to several commonly used antibiotics. The most prominent resistance rates were observed against benzylpenicillin (95.45%) and oxacillin (80.91%), indicating a high phenotypic prevalence of methicillin-resistant *S. aureus* (MRSA). Moderate resistance profiles were recorded for moxifloxacin (65.45%), erythromycin (66.36%), gentamicin (51.82%), levofloxacin (51.82%), tobramycin (50.91%), and rifampicin (50.00%). Conversely, the isolates retained relatively higher susceptibility to teicoplanin (73.64%), trimethoprim/sulfamethoxazole (62.73%), vancomycin (56.36%), and tetracycline (56.36%). Notably, the distribution of antimicrobial resistance varied significantly based on the specimen source; isolates recovered from urine samples demonstrated the highest resistance rates, followed by wound and vaginal swabs, whereas ear swabs exhibited the lowest. Statistical analysis confirmed significant variations in susceptibility patterns ($P \leq 0.01$), underscoring a non-random distribution of resistance among the tested pathogens.

Conclusion: In conclusion, this study demonstrates a high prevalence of multidrug-resistant *S. aureus*, including a substantial proportion of methicillin-resistant (MRSA) strains, at Baqubah Teaching Hospital. The significant variations in susceptibility patterns across different antibiotics and anatomical infection sites emphasize the necessity of tailored empirical therapy. These alarming resistance rates warrant the immediate implementation of strict antimicrobial stewardship programs and regular local surveillance to curb the dissemination of these refractory pathogens.

Keywords: *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VRSA), Antibiotic resistance, Antimicrobial susceptibility,

1. Introduction

S. aureus is a gram-positive bacterium that colonizes the skin and nasal passages of humans and animals, acting as both a commensal organism and a potent pathogen capable of causing a wide range of diseases [1]. The high adaptability of *S. aureus,* coupled with its ability to form biofilms on medical devices and persist in hospital environments, further complicates eradication efforts and contributes to recurrent infections [2]. The emergence of methicillin resistance in *S. aureus* strains is primarily mediated by the acquisition of the *mecA* gene, which encodes a penicillin-binding protein (PBP2a) with reduced affinity for beta-lactam antibiotics, rendering standard methicillin and related beta-lactam therapies ineffective [3]. Consequently, MRSA infections pose substantial clinical and economic burdens due to prolonged hospital stays, increased morbidity and mortality rates, and higher healthcare costs associated with the use of advanced antimicrobial agents [4]. In parallel, the emergence of vancomycin-resistant *S. aureus* (VRSA) has raised additional concerns regarding therapeutic options, as vancomycin has long been considered the antibiotic of last resort for severe MRSA infections. Vancomycin resistance in *S. aureus* is typically mediated by the acquisition of *vanA* gene clusters from vancomycin-resistant enterococci (VRE), resulting in altered peptidoglycan synthesis that reduces vancomycin binding and efficacy [5,6]. Although VRSA remains relatively rare compared to MRSA, documented cases have been reported in multiple countries, underscoring the potential for rapid dissemination in healthcare settings and the urgent need for robust surveillance systems. VRSA infections often arise in patients with chronic medical conditions, prior exposure to vancomycin, or prolonged hospitalization, highlighting the interplay between antimicrobial pressure and bacterial evolution [7]. Infection control measures, including hand hygiene, environmental cleaning, contact precautions, and screening of high-risk patients, have demonstrated variable efficacy in limiting the spread of resistant strains [8]. In this context, antimicrobial stewardship programs play a pivotal role in mitigating selective pressure by optimizing the use of antibiotics, reducing inappropriate prescriptions, and promoting adherence to evidence-based guidelines. Moreover, public health education and awareness campaigns targeting healthcare workers, patients, and the general population are essential to curtail the dissemination of resistant *S. aureus* strains and reduce the burden of infections [9]. This study aimed to determine the prevalence of methicillin- and vancomycin-resistant *S. aureus* (MRSA and VRSA) and to evaluate the antibiotic susceptibility patterns of these isolates obtained from various clinical specimens.

2. Patients and Methods

2.1 Study Design and Setting

This cross-sectional analytical study was conducted to evaluate the prevalence and antimicrobial resistance patterns of methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) at Baqubah Teaching Hospital in Diyala Governorate, Iraq. The study was carried from March 2025 to April 2026 in Baqubah Teaching Hospital was selected as the study site due to its high patient turnover and diverse clinical presentations, and because it features a fully equipped microbiology laboratory staffed by trained personnel capable of performing standardized bacterial isolation and phenotypic characterization. A total of 385 samples collected from various clinical specimens, including urine, wound, vaginal, and ear swabs. Include only 110 *S. aureus.*

2.2 Study Population and Sampling Strategy

Non-probability consecutive sampling was employed, recruiting all patients who presented with suspected bacterial infections and met the selection criteria until the target sample size was achieved.

Inclusion Criteria: Patients of all age groups and both sexes with microbiologically confirmed *S. aureus* infections.
Exclusion Criteria: Patients who had received antibiotic therapy within 72 hours prior to sample collection, individuals with mixed bacterial infections, immunocompromised status with systemic infections, or those with incomplete medical and clinical records.

2.3 Ethical Considerations

The study protocol was reviewed and officially approved by the Research Ethics Committee of the College of Medicine, University of Diyala. Prior to specimen collection, informed written consent was obtained from all adult participants or from the legal guardians of minors. Participants were briefed on the study objectives, procedures, and their right to withdraw at any stage without compromising their medical care. Data confidentiality was strictly maintained by converting all personal data into anonymized alphanumeric codes. All procedures aligned with the ethical standards established in the Declaration of Helsinki.

2.4 Data Collection and Specimen Processing

Demographic information and clinical data were documented using a structured data collection sheet. Clinical specimens were gathered under strict aseptic conditions by trained healthcare professionals following standardized institutional protocols. All specimens were immediately placed in appropriate transport media and transferred to the microbiology laboratory to ensure bacterial viability and minimize pre-analytical errors.

2.5 Bacterial Isolation and Phenotypic Identification

Specimens were inoculated onto selective and differential media, including Mannitol Salt Agar and Blood Agar plates, and incubated aerobically at 37°C for 24–48 hours [10]. Golden-yellow colonies exhibiting β-hemolysis on blood agar and mannitol fermentation on salt agar were provisionally identified as *S. aureus* [11]. Microscopic confirmation was performed using Gram staining to observe Gram-positive cocci in clusters. Biochemical confirmation was achieved via positive catalase and coagulase (both slide and tube methods) tests. Verified isolates were preserved in nutrient broth supplemented with 15% glycerol and stored at −20°C for subsequent testing [12].

2.6 Antimicrobial Susceptibility Testing (AST)

In vitro phenotypic susceptibility testing was performed utilizing the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, strictly adhering to the Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. Bacterial inocula were adjusted to a 0.5 McFarland turbidity standard (1.5×10^8 CFU/mL) to ensure uniform lawn growth. The panel of antibiotic disks tested included: Benzylpenicillin, Oxacillin, Gentamicin, Tobramycin, Levofloxacin, Moxifloxacin, Erythromycin, Clindamycin, Teicoplanin, Vancomycin, Tetracycline, Tigecycline, Nitrofurantoin, Fusidic acid, Rifampicin, and Trimethoprim/Sulfamethoxazole. Following incubation at 37°C for 24 hours, zones of inhibition were measured in millimeters. Isolates were categorized as Susceptible (S), Intermediate (I), or Resistant (R) based on the specific CLSI interpretive breakpoints. Multidrug resistance (MDR) was defined as acquired resistance to at least one agent in three or more antimicrobial categories.

2.7 Quality Control

To guarantee the accuracy, reliability, and reproducibility of the laboratory procedures, *S. aureus* ATCC 25923 was utilized as a reference quality control strain for all phenotypic and susceptibility assays. All prepared culture media and chemical reagents underwent routine sterility and performance checks prior to use.

2.8 Statistical Analysis

Data management and statistical evaluations were performed using SPSS software (version 24.0, IBM Corp., Armonk, NY, USA). Categorical variables (frequencies and percentages of susceptibility profiles across different specimen sources) were summarized in descriptive tables. The Chi-square (χ^2) test (or Fisher's exact test where applicable) was utilized to determine the statistical significance of associations between antibiotic resistance patterns and clinical specimen sources. Continuous variables were expressed as mean $\pm$ standard deviation. A p-value less than 0.05 was considered statistically significant for all analyses.

3. Results

3.1. Antibiotic Susceptibility and Resistance Profiling

The antimicrobial susceptibility profiles of the *S. aureus* isolates (n=110) revealed high resistance rates toward several conventional antibiotics. The highest resistance rate was observed against Benzylpenicillin (95.45%, n=105), then Oxacillin (80.91%, n=89). Moderate resistance patterns were observed for Levofloxacin (51.82%), Gentamicin (51.82%), and Tobramycin (50.91%). Furthermore, resistance to Moxifloxacin and Erythromycin remained relatively high at 65.45% and 66.36%, respectively. Conversely, comparatively lower resistance rates—and thus better sensitivity profiles—were exhibited toward Nitrofurantoin (46.36%), Tigecycline (45.45%), Fusidic acid (45.45%), Clindamycin (44.55%), Vancomycin (43.64%), and Tetracycline (43.64%). Remarkably, Teicoplanin emerged as the most effective agent, demonstrating the lowest resistance rate (26.36%) and the highest susceptibility rate (73.64%). Trimethoprim/Sulfamethoxazole also maintained favorable clinical utility with a sensitivity rate of 62.73%. Statistical evaluation demonstrated a significant association between the specific antibiotic type and the resulting susceptibility patterns (Chi-square = 9.71, P=0.001), as showed in table (1).

Table (1): Antibiotic Susceptibility Profile of *S. aureus* Isolates

Antibiotics	Resistance No.	%	Sensitive No.	%	P. value & Chi-square
Benzylpenicillin	105	95.45	5	4.55	P. value = 0.001* Chi-square = 9.71
Oxacillin	89	80.91	21	19.09	
Gentamicin	57	51.82	53	48.18	
Tobramycin	56	50.91	54	49.09	
Levofloxacin	57	51.82	53	48.18	
Moxifloxacin	72	65.45	38	34.55	
Erythromycin	73	66.36	37	33.64	
Clindamycin	49	44.55	61	55.45	
Teicoplanin	29	26.36	81	73.64	
Vancomycin	48	43.64	62	56.36	
Tetracycline	48	43.64	62	56.36	
Tigecycline	50	45.45	60	54.55	
Nitrofurantoin	51	46.36	59	53.64	
Fusidic acid	50	45.45	60	54.55	
Rifampicin	55	50.00	50	45.45	
Trimethoprim/Sulfamethoxazole	41	37.27	69	62.73	

*Statistically significant ($p < 0.05$).

3.2. Distribution of Resistance Profiles Across Clinical Specimen Sources

The distribution of *S. aureus* isolates varied significantly based on the anatomical site of the clinical sample. Overall, urine samples were the primary source of *S. aureus* strains, yielding 53 isolates (48.2%), followed by wound swabs (38 isolates; 34.5%) and ear swabs (17 isolates; 15.5%), whereas vaginal swabs yielded only two isolates (1.8%). Statistical analysis revealed a significant association between the clinical sample source and the number of *S. aureus* positive isolates (P. value = < 0.0001), indicating that the clinical source of the isolate is a critical factor, as shown in Table (2).

Table (2): Distribution of *S. aureus* isolates according to clinical specimen source

Specimen source	Positive for *S. aureus* NO.	(%)	P. value
Ear swab	17	(15.5%)	< 0.0001
Wounds	38	(34.5%)	
Vaginal swab	2	(1.8%)	
Urine	53	(48.2%)	
Total	110	(100%)	

*Statistically significant ($p < 0.05$).

4. Discussion

The emergence of antibiotic-resistant *S. aureus* poses a significant challenge to public health worldwide. The increasing prevalence of methicillin-resistant and multidrug-resistant (MDR) strains complicates treatment

strategies, particularly in hospital and community settings. Understanding the patterns of antimicrobial susceptibility and resistance is essential to guide rational antibiotic use and improve clinical outcomes. The analysis of antimicrobial susceptibility in this study revealed that β-lactam antibiotics, particularly Benzylpenicillin and Oxacillin, were largely ineffective against the isolates, indicating a high prevalence of methicillin-resistant *S. aureus* (MRSA) strains. These findings align with a previous study reporting high beta-lactam resistance among nasal *S. aureus* isolates [14], attributing this resistance to β-lactamase production or the presence of the $mecA$ gene. In contrast, another study observed greater susceptibility to fluoroquinolones in isolates from medical students—a variation likely reflecting differences in prior antibiotic exposure and the traditional epidemiological distinction between community-acquired (CA-MRSA) and hospital-acquired (HA-MRSA) methicillin-resistant *S. aureus* strains [15]. This comparison emphasizes how population characteristics and local antibiotic prescribing patterns heavily influence contemporary resistance trends. Furthermore, the statistical evaluation of resistance patterns revealed significant variation among the tested antibiotics. This variation aligns with a 2021 study that documented significant differences in antimicrobial activity between methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) strains, underscoring the need for susceptibility-guided therapy rather than empirical protocols [16].

These differences also reflect the complex biological mechanisms driving resistance—including genetic diversity, horizontal gene transfer, and divergent selective pressures. This aligns with molecular insights from previous studies demonstrating that community-associated and hospital-associated strains harbor distinct resistance determinants; this directly contributes to variations in treatment outcomes and confirms that the clinical source and strain type dictate susceptibility patterns [17].

Analysis based on sample source revealed a critical epidemiological pattern: urine samples accounted for the majority of resistant bacterial isolates, whereas ear and vaginal swabs showed the lowest levels of resistance (reflected in a smaller number of isolates) suggesting that the specific anatomical site, local microbial environment, and environment-specific antibiotic pressures accelerate the development of resistance [18]. In contrast, Babić et al. (2016) [19] indicated that beta-lactam resistance operates largely independently of sample type. This discrepancy highlights the complex interplay between systemic clonal expansion and local antibiotic selective pressure, underscoring the urgent need for source-specific surveillance to improve infection control and the rational use of antibiotics.

5. Conclusion

This study demonstrates a widespread prevalence of antibiotic resistance among clinical *S. aureus* isolates in Baqubah. The observed resistance to beta-lactams—specifically benzylpenicillin and oxacillin—highlights a significant burden of methicillin-resistant *S. aureus* (MRSA) in the region. Furthermore, the resistance rates observed against fluoroquinolones and macrolides underscore the emergence of multidrug-resistant (MDR) strains. In contrast, vancomycin, teicoplanin, trimethoprim/sulfamethoxazole, and tetracycline showed high efficacy, making them more effective therapeutic alternatives. Notably, the distribution of isolates was significantly influenced by the clinical source; urine samples yielded the highest proportion of isolates, followed by wound and ear swabs, while vaginal swabs recorded the lowest. Collectively, these findings emphasize the critical need for rigorous antibiotic surveillance, rational use programs, and enhanced infection control protocols to curb the spread of resistant *S. aureus* strains in clinical settings.

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