Journal of Pioneering Medical Sciences
Received: January 30, 2026 | Accepted: June 10, 2026 | Published: July 05, 2026
Volume 15, Issue 06, Pages 186-192

DOI https://doi.org/10.47310/jpms2026150624

Phenotypic and Molecular Characterization of Multidrug Resistant *Staphylococcus Aureus* Isolates from Clinical Samples in Diyala, Iraq

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Abstract A total of 250 clinical specimens (burns, wounds, urine, blood, nasal swabs, middle-ear discharge, throat swabs and sputum were collected from inpatients and outpatients of both sexes and all age groups at Al-Batool Maternity and Children Hospital and Baqubah Teaching Hospital, Diyala Governorate, between November 2024 and March 2025. Eighty isolates were identified as *Staphylococcus aureus* on the basis of phenotypic, cultural and biochemical characteristics and identification was confirmed using the VITEK-2-Compact system. Susceptibility to 12 antimicrobial agents was determined by the Kirby-Bauer disc diffusion method and inhibition zones were interpreted according to current Clinical and Laboratory Standards Institute (CLSI) criteria. The highest resistance was recorded against oxacillin (90%), followed by amoxicillin and ceftriaxone (86.25% each) and ampicillin (78.75%); resistance to erythromycin and azithromycin was 67.5%, to levofloxacin 42.5%, to ciprofloxacin and trimethoprim-sulfamethoxazole 40% and to amikacin and gentamicin 38.75% and 37.5%, respectively. The lowest resistance was observed against vancomycin (12.5%). Applying the international criteria of Maiorano's *et al.* (2012), 61 isolates (76.25%) were classified as multidrug-resistant (MDR), 7 (8.75%) as extensively drug resistant (XDR) and the remaining 12 (15%) as non-MDR. Phenotypic screening showed that all isolates produced β-haemolysis, coagulase and gelatinase (100%), whereas extended-spectrum β-lactamase (ESβL), metalo-β-lactamase (MβL) and class C β-lactamase (AmpC) were detected in 34 (42.5%), 51 (63.75%) and 62 (77.5%) of isolates, respectively. To explore the genetic basis of sulphonamide resistance, the DNA of 16 representative MDR isolates was screened by polymerase chain reaction (PCR); *sul1* was detected in 6 isolates (37.5%) and *sul2* in 7 isolates (43.75%). Because the molecular analysis was restricted to this purposively selected subset, the genotypic findings should be interpreted as indicative rather than representative of all isolates. Overall, the study documents a high burden of MDR *S. aureus* in clinical settings in Diyala and provides preliminary evidence linking phenotypic sulphonamide resistance to the carriage of *sul1* and *sul2*, supporting the need for continued antimicrobial surveillance and stewardship.

Key Words *Staphylococcus Aureus*, Antimicrobial Resistance, Multidrug Resistance, Virulence Factors, Sulphonamide Resistance Genes

INTRODUCTION

Staphylococcus aureus is among the most clinically important human pathogens and a leading cause of both hospital-acquired and community-acquired infections [1]. Although it commonly colonizes the skin and mucosae as part of the normal flora, it readily behaves as an opportunistic pathogen and invades virtually any tissue when host barriers are breached [2]. Clinically, isolates are broadly categorized as methicillin-susceptible (MSSA) or methicillin-resistant (MRSA), the latter representing a particularly serious therapeutic challenge [3]. Globally, MRSA has shown the largest increase in attributable mortality of any resistant pathogen, being associated with approximately 130,000 deaths in 2021 more than double the figure recorded three decades earlier which underscores the urgency of regional surveillance [4].

The pathogenic success of *S. aureus* reflects an extensive armamentarium of virulence factors, including toxins and

extracellular enzymes that promote tissue invasion and immune evasion, together with a marked capacity to acquire resistance to multiple antimicrobial classes, particularly β-lactams and aminoglycosides [5]. The organism also forms structured biofilms of extracellular polymeric substances that shield embedded cells from desiccation, nutrient limitation and, critically, antibacterial agents, thereby facilitating persistence and recurrent infection [6]. The progressive erosion of treatment options extending even to vancomycin, the mainstay against MRSA has transformed staphylococcal resistance into a pressing public-health problem [7].

Sulphonamide resistance determinants, notably *sul1* and *sul2*, encode drug-insensitive dihydropteroate synthases and are among the most widely disseminated, horizontally transferable resistance genes. They are frequently integron associated and co-selected with other resistance determinants, which makes them informative markers of mobile resistance. While these genes have been characterized extensively in Gram-negative bacteria, their distribution in clinical *S. aureus*, especially in relation to phenotypic resistance against trimethoprim-sulfamethoxazole remains comparatively underexplored and local data from Diyala are scarce. Addressing this gap, rather than re-documenting overall resistance rates already reported in regional surveillance, constitutes the principal contribution of the present work.

Accordingly, this study was designed to characterize the phenotypic antimicrobial-resistance patterns of *S. aureus* recovered from diverse clinical sources and to determine the prevalence of MDR and XDR phenotypes among the isolates. A further objective was to screen a representative subset of MDR isolates for the sulphonamide resistance genes *sul1* and *sul2* and to assess their association with the observed phenotypic resistance to trimethoprim-sulfamethoxazole. We hypothesized that clinical *S. aureus* isolates in this setting display a high prevalence of MDR phenotypes and that phenotypic sulphonamide resistance is accompanied by carriage of *sul1* and/or *sul2*.

METHODS

Study Design, Setting and Ethical Approval

This cross-sectional study was carried out at Al-Batool Maternity and Children Hospital and Baqubah Teaching Hospital, Diyala Governorate, Iraq, between November 2024 and March 2025. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the institutional ethics committee; oral informed agreement was obtained from participants prior to specimen collection.

Sample Collection and Inclusion/Exclusion Criteria

A total of 250 clinical specimens (burns, wounds, urine, blood, nasal swabs, middle ear discharge, throat swabs and sputum) were collected from inpatients and outpatients of both sexes and all age groups with clinically suspected bacterial infection during the study period. Specimens yielding no growth, mixed or contaminated cultures and duplicate isolates obtained from the same patient were excluded; only the first *S. aureus* isolate per patient was maintained for analysis. Because all eligible serial specimens during the defined period were registered, sampling was repeated.

Identification of Isolates

Isolates were presumptively identified as *S. aureus* on the basis of colonial morphology on blood agar and mannitol salt agar, Gram staining and conventional biochemical tests (catalase, oxidase, coagulase and motility). Identification was subsequently confirmed using the VITEK-2 Compact automated system (bioMérieux, France).

Antimicrobial Susceptibility Testing

Susceptibility to 12 antimicrobial agents was determined by the Kirby Bauer disc diffusion method on Mueller-Hinton agar [8]. Commercially prepared discs were applied at the following potencies: amikacin (30µg), gentamicin (10µg), ciprofloxacin (5µg), levofloxacin (5µg), amoxicillin (25µg), oxacillin (1µg), ampicillin (10µg), ceftriaxone (30µg), vancomycin (30µg), erythromycin (15µg), azithromycin (15µg) and trimethoprim-sulfamethoxazole (1.25/23.75µg). Inhibition-zone diameters were measured after 16-24 h of incubation at 35±2 °C and interpreted as susceptible, intermediate or resistant according to the current breakpoints of the Clinical and Laboratory Standards Institute [9].

Definition of MDR and XDR

Isolates were classified according to the internationally accepted criteria of Magiorakos *et al.* [10]. Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories and extensive drug resistance (XDR) as non-susceptibility to at least one agent in all but two or fewer categories. Isolates that did not meet the MDR criterion (non-susceptible to ≤2 categories or susceptible to all agents tested) were designated non-MDR. These definitions were applied consistently throughout the analysis.

Phenotypic Detection of Virulence Factors and Resistance Enzymes

All 80 isolates were screened phenotypically for production of β-haemolysis, coagulase and gelatinase and for the elaboration of extended-spectrum β-lactamase (ESβL), metalo-β-lactamase (MβL) and class C β-lactamase (AmpC) using standard plate- and inhibitor-based assays.

Bacterial DNA extraction

Sixteen isolates were purposively selected from the MDR group to represent the range of clinical sources and resistance profiles, with the aim of examining the carriage of the sulphonamide resistance determinants *sul1* and *sul2*. Genomic DNA was extracted using the Presto™ Mini gDNA Bacterial Kit for Gram-positive bacteria following the manufacturer's instructions (Bioneer, Korea). DNA concentration

Table 1: Composition of the PCR reaction mixture.

No.	Reaction component	Volume (µL)
1	Forward primer	1.0
2	Reverse primer	1.0
3	Template DNA	1.5
4	Nuclease-free deionized water	16.5
5	GoTaq Green Master Mix	5.0
Total volume		25.0

Table 2: Nucleotide primer sequences used in the study.

Primer	Sequence (5′→3′)	Product (bp)	Annealing (°C)	Reference
sul1-F	TGGTGACGGTGTTCGGCATTC	789	55	Ferri *et al.* [35]
sul1-R	GCGAGGGTTTCCGAGAAGGTG	-	-	
sul2-F	TCAACATAACCTCGGACAGT	707	53	Peng *et al.* [36]
sul2-R	GATGAAGTCAGCTCCACCT	-	-	

Table 3: Thermal-cycling conditions for uniplex PCR amplification.

Gene	Initial denat.	Cycles	Denaturation	Annealing	Elongation	Final ext.
sul1	94 °C / 5 min	35	94 °C / 50 s	55 °C / 50 s	72 °C / 1 min	72 °C / 7 min
sul2	94 °C / 5 min	35	94 °C / 1 min	53 °C / 45 s	72 °C / 1 min	72 °C / 7 min

and purity were assessed with a NanoDrop spectrophotometer (ACTGene, Taiwan); an A260/280 ratio of 1.6-1.9 was adopted as the criterion for high-purity DNA suitable for amplification.

Detection of Target Genes by PCR

The extracted DNA was used to detect the target genes in a 25-µL reaction (Table 1), with gene specific primers (Table 2) and thermal-cycling conditions (Table 3). Following amplification, 5 µL of each product was resolved by agarose-gel electrophoresis and visualized under ultraviolet illumination.

Statistical Analysis

Data were analysed using SPSS (version 2019). The chi-square (χ^2) test was used to compare proportions among qualitative variables, with statistical significance set at $p \leq 0.05$ and $p \leq 0.01$.

RESULTS AND DISCUSSION

Identification and Isolation

Of the 250 specimens cultured, 229 (91.6%) were culture-positive and 21 (8.4%) were culture-negative. *Staphylococcus aureus* accounted for 80 isolates (32%), while 149 specimens (59.6%) yielded other bacterial species. Presumptive identification was based on colonial, microscopic and biochemical characteristics following growth on blood agar and mannitol salt agar under aerobic conditions at 37°C for 24hrs. On blood agar, colonies were convex, glistening, smooth-edged and whitish to golden-yellow, with complete β-haemolysis, consistent with the findings of Delost *et al.* [11] (Table 4).

Definitive identification of all 80 isolates was achieved with the VITEK 2 Compact system, which confirms presumptive culture-, microscopy- and biochemistry-based diagnoses with reported accuracy up to 99%. The number of infections was higher among females (45 isolates, 56.25%) than males (35 isolates, 43.75%), although the difference was not statistically significant (Table 5).

Table 4: Preliminary diagnostic test results for S. aureus.

No.	Test	Result
1	Gram stain	+
2	Cell shape and arrangement	Grape-like clusters
3	Mannitol salt agar	Golden-yellow colonies
4	Catalase	+
5	Oxidase	–
6	Coagulase	+
7	Motility	–
8	Haemolysin	β-haemolysis

Table 5: Distribution of *S. aureus* isolates by sex.

Sex	Number	Percentage
Males	35	43.75
Females	45	56.25
Total	80	100
χ^2 (p-value)	—	1.250 NS (0.263)

NS = not significant

Table 6: Distribution of *S. aureus* isolates according to source of infection.

Source	Number	Percentage
Wounds	20	25
Urine	15	18.75
Burns	12	15
Blood	12	15
Nasal swab	9	11.25
Otitis media	6	7.5
Throat	5	6.25
Sputum	1	1.25
Total	80	100
χ^2 (p-value)	8.218 ** (0.0072)	-

** P ≤ 0.01

This pattern is consistent with Al-Fayyad [12], who, working on *S. aureus* from clinical samples in Diyala, reported isolation rates of 56.36% in females and 43.64% in males. The slightly higher recovery from females may reflect differences in physiological, immunological and genetic predisposition, unequal sample sizes and variation in sampling time and site, as well as the larger number of female attendees during the collection period [13]. Greater occupational exposure of women to detergents and chemicals and differences in the composition of the resident skin flora between sexes, have also been proposed as contributing factors [14].

Table 7: Antimicrobial susceptibility profile of the 80 *S. aureus* isolates.

Antimicrobial class	Agent (disc potency)	Resistant N (%)	Intermediate N (%)	Sensitive N (%)	χ^2, df (P-value)
Aminoglycosides	Amikacin (30 µg)	31 (38.75)	18 (22.5)	31 (38.75)	4.275, 2 (0.117) NS
	Gentamicin (10 µg)	30 (37.5)	3 (3.75)	47 (58.75)	34.741, 2 (<0.0001) **
Fluoroquinolones	Ciprofloxacin (5 µg)	32 (40)	13 (16.25)	35 (43.75)	10.675, 2 (<0.0001) **
	Levofloxacin (5 µg)	34 (42.5)	8 (10)	38 (47.5)	19.920, 2 (<0.0001) **
β-Lactams (penicillins)	Amoxicillin (25 µg)	69 (86.25)	4 (5)	7 (8.75)	100.98, 2 (<0.0001) **
	Oxacillin (1 µg)	72 (90)	0 (0)	8 (10)	116.8, 2 (<0.0001) **
	Ampicillin (10 µg)	63 (78.75)	6 (7.5)	11 (13.75)	74.725, 2 (<0.0001) **
β-Lactams (cephalosporins)	Ceftriaxone (30 µg)	69 (86.25)	3 (3.75)	8 (10)	100.98, 2 (<0.0001) **
Glycopeptides	Vancomycin (30 µg) †	10 (12.5)	0 (0)	70 (87.5)	107.5, 2 (<0.0001) **
Macrolides	Erythromycin (15 µg)	54 (67.5)	19 (23.75)	7 (8.75)	44.725, 2 (<0.0001) **
	Azithromycin (15 µg)	54 (67.5)	6 (7.5)	20 (25)	45.700, 2 (<0.0001) **
Folate-pathway antagonist	Trimethoprim-sulfamethoxazole (25 µg)	32 (40)	1 (1.25)	47 (58.75)	41.275, 2 (<0.0001) **

*p≤0.05, **p≤0.01, NS = not significant. Vancomycin was tested by disc diffusion; CLSI recommends confirmation by a minimum inhibitory-concentration method.

Table 8: Resistance patterns among the 80 *S. aureus* isolates.

Resistance pattern	N (%)
Multidrug-resistant (MDR)	61 (76.25)
Extensively drug-resistant (XDR)	7 (8.75)
Non-MDR	12 (15)
χ^2 (p-value)	67.457 ** (0.0001)

**p≤0.01

Distribution of Isolates by Source of Infection

The largest proportion of *S. aureus* isolates originated from wounds (n=20, 25%), followed by urine (n=15, 18.75%), burns and blood (n=12, 15%), nasal swabs (n=9, 11.25%), middle-ear discharge (n=6, 7.5%), throat (n=5, 6.25%) and sputum (n=1, 1.25%). The distribution was statistically significant ($\chi^2 = 8.218$, p≤0.01) (Table 6).

The predominance of *S. aureus* in wound infections is consistent with its status as a skin commensal capable of breaching the cutaneous barrier and colonizing wound beds. It is the organism most frequently recovered from post-operative wound infections, reflecting its abundant invasive and virulence determinants [15]. The present finding aligns with Hatem *et al.* [16], who reported the highest isolation rate from wounds (45.83%) and with Al-Hayali [17], who recorded 57.1% from wound samples. It contrasts, however, with Yassin *et al.* [18] and Ahmed [19], who found burns to be the principal source (29.67% and 30%, respectively) a deviation likely attributable to differences in patient populations, ward case mix and local infection control practices across centres.

Antimicrobial Susceptibility

The isolates displayed heterogeneous resistance across the 12 agents tested (Table 7). These agents were selected because they are commonly used against staphylococcal infections, allowing the extent and potential dissemination of resistance to be assessed.

Resistance was highest against oxacillin (90%), followed by amoxicillin and ceftriaxone (86.25% each), ampicillin (78.75%), erythromycin and azithromycin (67.5% each), levofloxacin (42.5%), ciprofloxacin and trimethoprim-sulfamethoxazole (40% each), amikacin (38.75%) and gentamicin (37.5%). Vancomycin retained the greatest activity, with only 12.5% of isolates classified as resistant. The marked β-lactam resistance is consistent with the high prevalence of MRSA-associated phenotypes observed in this collection.

The therapeutic history of *S. aureus* has been one of successive antibiotic introduction followed by rapid resistance emergence since the advent of penicillin, generating sustained clinical challenges [20]. The 90% oxacillin resistance recorded here is comparable with Al-Taey [21] and Fitranda *et al.* [22], who reported 92.5% and 90%, respectively, but exceeds the rates of Jabr [23] in Diyala (75%) and Ahmed [19] in Baghdad (76%) and the 65.7% reported from Iran by Ghari *et al.* [24]. Such variation between geographically proximate studies likely reflects differences in patient populations, antibiotic-prescribing pressure and the local circulation of MRSA clones.

Variable aminoglycoside resistance was also observed. In *S. aureus*, resistance to amikacin and gentamicin is typically mediated by aminoglycoside-modifying enzymes (acetyltransferases, phosphotransferases and nucleotidyl-transferases) encoded on mobile genetic elements, supplemented by reduced membrane permeability, efflux and target-site mutation [25]. The co-occurrence of these mechanisms with β-lactam resistance is consistent with the multidrug-resistant phenotype that predominated in this collection.

Multidrug Resistance in *Staphylococcus Aureus*

Most isolates were non-susceptible to at least one agent in three or more antimicrobial categories. Applying the criteria of Magiorakos *et al.* [10], 61 of the 80 isolates (76.25%) were classified as MDR and 7 (8.75%) as XDR (non-susceptible to all but ≤2 categories); the remaining 12 (15%) were non-MDR (resistant to ≤2 categories or fully susceptible). The distribution was highly significant ($\chi^2 = 67.457$, p≤0.01) (Table 8).

These results are consistent with two earlier Baqubah studies: Mohammed [26] reported MDR in 96% and XDR in 10% of *S. aureus* isolates, while Jasim and Alzubaidy [27] found that 82% were resistant to all tested antibiotics, with 14% meeting the XDR criterion. The emergence and intramural spread of MDR *S. aureus* is a major concern because it narrows therapeutic options and is associated with increased mortality, particularly among critically ill patients. The principal drivers are repeated and unregulated antibiotic use, including community self-medication without susceptibility testing, which selects for resistant subpopulations [28]. From a clinical standpoint, the 76.25%

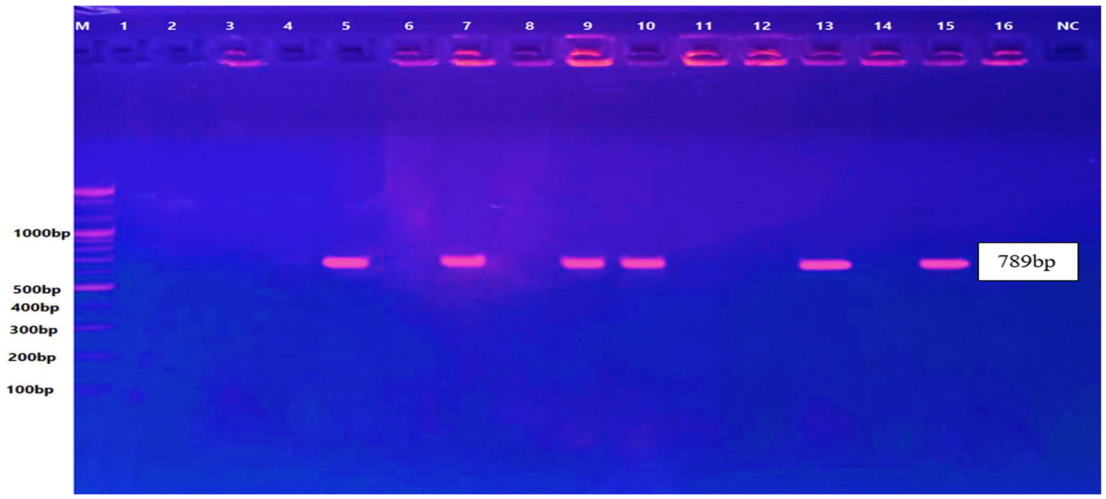

Figure 1: Agarose-gel electrophoresis (1.5% agarose, 7 V cm⁻¹, 60 min) for the *sul1* gene. Lane M, molecular-size marker (100–1500 bp); lanes 5, 7, 9, 10, 13 and 15, isolates positive for *sul1* (789 bp); lane N.C., negative control.

Table 9: Phenotypic detection of virulence factors and resistance enzymes in the 80 *S. aureus* isolates.

Result	Gelatin hydrolysis (%)	β-Haemolysis (%)	Coagulase (%)	AmpC β-lactamase (%)	Metallo-β-lactamase (%)	Extended-spectrum β-lactamase (%)
Producer	80 (100)	80 (100)	80 (100)	62 (77.5)	51 (63.75)	34 (42.5)
Non-producer	0	0	0	18 (22.5)	29 (36.25)	46 (57.5)
Total	80	80	80	80	80	80
χ^2 (P-value)	62.750 ** (0.0001)	62.750 ** (0.0001)	62.750 ** (0.0001)	24.20 ** (0.0001)	6.050 * (0.0136)	1.80 NS (0.179)

*p≤0.05, **p≤0.01, NS = not significant

MDR prevalence reported here implies that empirical β-lactam therapy is likely to fail in most cases, reinforcing the value of local antibiograms, vancomycin stewardship and strict infection-control measures.

Phenotypic Detection of Virulence Factors and Resistance Enzymes

All 80 isolates were assessed for haemolysin, coagulase and gelatinase production and for ESβL, MβL and AmpC activity (Table 9). β-Haemolysis, coagulase and gelatinase were produced by all isolates (100%), whereas AmpC, MβL and ESβL were detected in 62 (77.5%), 51 (63.75%) and 34 (42.5%) isolates, respectively. Differences were significant for all enzymes except ESβL.

DNA Extraction and Molecular Screening

Genomic DNA was successfully extracted from the *S. aureus* isolates using the Presto™ Mini gDNA Bacterial Kit, yielding high-purity preparations with A260/280 ratios of 1.6-1.9. Integrity was verified by electrophoresis on 1.5% agarose at 70 V for 60 min, followed by ultraviolet visualization (Figure 1). Sixteen isolates (SA1, SA6, SA13, SA18, SA25, SA33, SA45, SA48, SA54, SA62, SA65, SA67, SA69, SA71, SA75 and SA78) were selected for molecular analysis on the basis of representative clinical sources, pronounced multidrug resistance and the production of multiple virulence factors and were screened for the sulphonamide resistance genes *sul1* and *sul2* using gene specific primers.

Detection of the Sulphonamide Resistance Genes *Sul1* and *Sul2*

PCR screening of the 16 MDR isolates detected *sul1* in 6 isolates (37.5%) and *sul2* in 7 isolates (43.75%), with amplicons of the expected sizes (789 bp and 707 bp, respectively; Figure 1). The differences were not statistically significant ($p = 0.317$ and $p = 0.617$, respectively).

The carriage frequencies recorded here fall within the broad range reported internationally. A local study in Najaf [29] detected *sul1* in 15% and *sul2* in 50% of isolates, while studies in Pakistan (49.6% and 42.1%) [30] and Bangladesh (47.06% and 32.35%) [31] reported values comparable with the present findings. In contrast, much lower frequencies were observed in Nigeria (5.66%) [32] and China 28.21% [33] for *sul1* and 10.26% for *sul2*), differences that probably reflect divergent sulphonamide-prescribing practices and the differing mobile-genetic-element repertoires circulating in each setting.

Collectively, with the phenotypic data, the molecular results offer a partial explanation for the observed sulphonamide resistance. Phenotypic resistance to trimethoprim-sulfamethoxazole reached 40% across the whole collection and *sul1* and/or *sul2* were present in a substantial fraction of the molecularly screened MDR isolates, indicating that these determinants contribute to the resistance phenotype. The concordance is, however, incomplete: *sul* genes confer resistance only to the sulfamethoxazole component, so resistance to the trimethoprim moiety mediated by *dfr* genes (e.g. *dfrA*, *dfrG*, *dfrK*) and other mechanisms are likely to operate in parallel. Consequently, *sul*-positive isolates may not invariably be

phenotypically resistant and some phenotypically resistant isolates may lack *sul* genes. These observations argue for broader genotypic screening, including *dfr* determinants, to fully resolve the genotype-phenotype relationship.

The *sul1* and *sul2* genes are commonly found in staphylococci, including multidrug-resistant *S. aureus*. Their presence complicates therapy because the same isolates frequently co-harbour resistance to other classes such as β-lactams and, occasionally, glycopeptides [34].

CONCLUSIONS

This study showed a high prevalence of multidrug resistance among clinical *S. aureus* isolates in Diyala, with wounds the most frequent source of isolation. Phenotypic testing revealed substantial resistance to β-lactams and several other commonly prescribed agents, while vancomycin retained comparatively greater activity. Molecular screening of a representative subset of MDR isolates detected the sulphonamide resistance genes *sul1* and *sul2*, providing preliminary genetic support for the observed phenotypic resistance to trimethoprim-sulfamethoxazole. Given that this genotypic analysis was limited to 16 isolates, the molecular findings are best regarded as indicative and warrant confirmation in larger, multi-centre cohorts. Collectively, the results emphasize the growing burden of antimicrobial resistance in *S. aureus* and the need for continued surveillance, rational antibiotic use and effective infection-control strategies to limit the spread of resistant strains.

Limitations

Several limitations should be acknowledged. First, the study was confined to two hospitals within a single governorate, which may limit the generalizability of the findings. Second, sampling was consecutive and no a priori sample-size or power calculation was performed. Third, the cross-sectional design captures resistance at a single point in time and cannot describe temporal trends. Fourth, molecular characterization was restricted to a purposively selected subset of 16 MDR isolates and to two sulphonamide determinants (*sul1* and *sul2*); methicillin resistance markers (e.g. *mecA*/*mecC*) and trimethoprim (*dfr*) determinants were not assessed, so the genotypic findings should not be extrapolated to the full collection. Finally, vancomycin susceptibility was evaluated by disc diffusion; because CLSI recommends MIC testing for this antibiotic, the reported vancomycin results should be interpreted with caution and confirmed by an MIC-based method in future work.

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