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Virulence and Resistance Co-occurrence in *Staphylococcus aureus*: Insights from A Pediatric Cohort from Egypt

Rasha Hassan Hassan[1], Maysaa El Sayed Zaki[2]*, Eman Hosney Mohammad Salem[3], Lobna Abdelaziz Kassem[4], Dina Mohammed Abdel-Hady[1] and Omnia A. Salem[1]

[1]Pediatric Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[2]Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[3]Medical Microbiology and Immunology Department, Faculty of Medicine, Menoufia University, Menoufia, Egypt.
[4]Clinical Pathology Department, King Khalid Hospital and Prince Sultan Centre, Al-Kharj, Saudi Ministry of Health, Kingdom of Saudi Arabia.

Abstract

Staphylococcus aureus* (*S. aureus*) is a leading cause of infections in children. The emergence of multidrug-resistant (MDR) strains, especially methicillin-resistant *S. aureus* (MRSA), presents significant therapeutic challenges. Data on molecular resistance and virulence determinants in pediatric isolates from Egypt remain limited. This retrospective study analysed 120 non-duplicate *S. aureus* isolates recovered from pediatric patients. Antimicrobial susceptibility testing (AST) was performed using the CLSI disk diffusion method. Conventional PCR detected resistance genes (*mecA, blaZ, ermA/B/C, msrA, mphC, tetK, tetM*, aminoglycoside-modifying enzyme (AME) genes, and *qacA/B*) and the Panton–Valentine leukocidin (*PVL*) gene. Logistic regression assessed associations with MDR. Of the 120 isolates examined, 55% were from hospital-acquired infections (HAI). The *blaZ* gene was present in 83.3% of isolates, and *mecA* in 36.7%. Genes *tetK, tetM, ermC,* and *mphC* were significantly associated with MDR. The *PVL* gene was detected in 18.3% of isolates. Approximately one-third of isolates met the definition of MDR, showing resistance to three or more antimicrobial categories. MDR in pediatric *S. aureus* in Egypt is primarily driven by genetic determinants, underscoring the significance of molecular surveillance and antimicrobial stewardship (AMS).

Keywords: *S. aureus*, MRSA, Tetracycline Resistance, Macrolide Resistance, *PVL*, Molecular Surveillance

***Correspondence:** may_s@mans.edu.eg

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) infection remains a major cause of pediatric infections, ranging from skin and soft tissue infections to severe invasive diseases such as bloodstream infections, pneumonia, and sepsis. The increasing emergence of multidrug-resistant (MDR) strains has further amplified the clinical and public health burden of these infections in children.[1,2]

The pathogenic success of *Staphylococcus aureus* is largely attributed to its remarkable genetic adaptability, which enables the acquisition of antimicrobial resistance (AMR) and virulence determinants through mobile genetic elements, including plasmids, transposons, and the staphylococcal cassette chromosome mec (SCC*mec*).[3,4] The *mecA* gene, carried by SCC*mec*, confers methicillin resistance by producing the altered penicillin-binding protein PBP2a.[5] In addition, several resistance genes confer resistance to multiple antimicrobial classes, including β-lactams, macrolides, tetracyclines, aminoglycosides, and antiseptics/disinfectants.[6-8] Virulence determinants, particularly the Panton–Valentine leukocidin (*PVL*) genes (*lukS/F-PV*), are associated with severe skin infections and necrotising pneumonia in pediatric patients.[9,10]

Although previous studies have investigated selected resistance markers in *S. aureus*, most reports from pediatric populations have focused on limited gene targets or phenotypic resistance patterns alone. Comprehensive molecular characterisation integrating a broad spectrum of resistance determinants and virulence-associated genes in pediatric isolates remains scarce, particularly in low- and middle-income countries such as Egypt.[11-13] Furthermore, regional epidemiological variations in AMR and virulence profiles necessitate continuous local surveillance to guide empirical therapy and infection control strategies.

The novelty of the present study lies in the simultaneous molecular investigation of an extensive panel of antimicrobial resistance genes together with *PVL* virulence genes among pediatric *S. aureus* isolates collected from a tertiary care children's hospital in Egypt. By combining resistance and virulence profiling, this study provides updated local epidemiological data and deeper insight into the molecular characteristics of circulating pediatric isolates, which may contribute to improved antimicrobial stewardship and infection prevention policies.

Therefore, this study aimed to characterize the molecular profiles of antimicrobial resistance and virulence genes among pediatric *S. aureus* isolates using PCR-based detection of *mecA*, *blaZ, femA, ermA, ermB, ermC, msrA, mphC, tetK, tetM, aac(6')-Ie-aph(2")-Ia, aph(3")-IIIa, ant(4")-Ia, qacA/B*, and *lukS/F-PV* genes.

MATERIALS AND METHODS

Study design

This was a prospective, laboratory-based study of children conducted at Mansoura University Children's Hospital (MUCH), Egypt, from January 2024 to July 2025. The study enrolled 120 non-duplicate clinical isolates of *S. aureus.* This study was approved by the Research Ethics Committee, Faculty of Medicine, Mansoura University, Egypt (Approval No.: R.25.11.3455). Written informed consent was obtained from the legal guardians of the contributing children before sample collection.

Clinico-demographic characteristics were retrieved from laboratory and hospital records. These included patient age, sex, hospital ward, specimen source, and outcome. Isolates were classified as community-acquired (CAI) if infection occurred within 48 hours of admission without prior hospitalisation. Hospital-acquired infections (HAI) developed 2 or more days after admission or within 30 days of discharge, as defined by Magill et al.[14] for healthcare-associated infections.

Inclusion and exclusion criteria

Pediatric patients aged <18 years who had clinically significant *Staphylococcus aureus* isolates recovered during the study period were eligible for inclusion. Only cases with available clinical and demographic data and preserved bacterial isolates suitable for laboratory confirmation and molecular analysis were included.

Duplicate isolates obtained from the same patient were excluded to avoid bias from repeated sampling. When multiple isolates were recovered from the same patient, only the first

isolate was included in the analysis. Isolates with incomplete clinical information or unavailable/non-viable stored samples were also excluded.

Sample size

The sample size was calculated to estimate the prevalence of antimicrobial resistance gene carriage among pediatric *Staphylococcus aureus* isolates using the single-proportion formula:

$$n = (Z^2 \times p \times (1 - p)) / d^2$$

where Z = 1.96 for a 95% confidence interval, d = 0.09 (absolute precision), and P = 0.5 (expected prevalence).

A prevalence estimate of 50% was selected because no prior local data were available regarding the molecular prevalence of resistance genes among pediatric *S. aureus* isolates in the study setting. Using P = 0.5 is considered the most conservative approach in sample size estimation, as it yields the maximum required sample size and ensures adequate study power when prevalence is uncertain.

Accordingly

$n = (1.96^2 \times 0.5 \times (1 - 0.5)) / 0.09^2$

$n = (3.8416 \times 0.25) / 0.0081$

$n = 0.9604 / 0.0081$

$n \approx 118.57$

The calculated sample size was rounded to 120 isolates. This sample size was considered sufficient to achieve the study objective, assuming simple random sampling (design effect = 1). The calculation was based on World Health Organisation (WHO) recommendations[15] and standard biostatistical methods.[16]

Isolation and identification of *S. aureus*

Clinical specimens—including blood, wound swabs, urine, cerebrospinal fluid (CSF), and other sterile body fluids—were inoculated onto mannitol salt agar and blood agar plates, followed by aerobic incubation at 37 °C for one to two days.

Colonies displaying characteristic morphology were subjected to Gram staining and microscopic examination and assessed for catalase and tube coagulase activity. Molecular identification was confirmed by PCR amplification of the *femA* and 16S rDNA genes.[17]

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed utilising the Kirby–Bauer disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2024) guidelines.[18]

The antimicrobial agents tested in this study included oxacillin, penicillin, erythromycin, clindamycin, tetracycline, gentamicin, trimethoprim-sulfamethoxazole, fusidic acid, and linezolid.

Cefoxitin (30 µg) disk diffusion testing was additionally conducted as a surrogate marker for methicillin resistance, following Clinical and Laboratory Standards Institute recommendations. Cefoxitin is recognised as a more reliable inducer of *mecA*-mediated resistance than oxacillin. Isolates exhibiting resistance to cefoxitin were classified as methicillin-resistant *Staphylococcus aureus* (MRSA) per CLSI interpretive criteria.

Isolates exhibiting linezolid resistance by disk diffusion were further confirmed by broth microdilution minimum inhibitory concentration (MIC) testing, performed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Resistance was defined as an MIC $\geq$8 µg/mL, based on CLSI breakpoints.

MDR isolates are those that exhibit resistance to at least 3 antimicrobial classes.

DNA extraction

Genomic DNA was extracted using the boiling lysis method. Two to three colonies were suspended in 200 µL sterilized distilled water, heated at 100 degrees Celsius for ten minutes (min), and centrifuged at 12,000 rpm for five min. The PCR template was made from the DNA-containing supernatant.

Detection of resistance and virulence genes by conventional PCR

Conventional PCR was performed to detect antimicrobial resistance and virulence genes in *Staphylococcus aureus* isolates using previously validated primers (Table 1).

Each PCR reaction mixture (25 µL) contained 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.5 µM of each primer, 1 U Taq DNA polymerase, and 2 µL of DNA template.

Table 1. Conventional PCR Primers for *Staphylococcus aureus*

Gene (target)	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon (bp)	Ref.
mecA	AAAATCGATGGTAAAGGTTGGC	AGTTCTGCAGTACCGGATTTGC	533	(19)
blaZ	ACTTCAACACCTGCTGCTTTC	TGACCACTTTTATCAGCAACC	173	(20)
femA	AAAAAAGCACATAACAAGCG	GATAAAGAAGAAACCAGCAG	132	(20)
16S rDNA	CAGCTCGTGTCGTGAGATGT	AATCATTTGTCCCCACCTTCG	420	(20)
ermA	AAGCGGTAAACCCCTCTGA	TTCGCAAATCCCTTCTCAAC	190	(20)
ermB	CTATCTGATTGTTGAAGAAGGATT	GTTTACTCTTGGTTTAGGATGAAA	142	(20)
ermC	AATCGTCAATTCCTGCATGT	TAATCGTGGAATACGGGTTTG	299	(20)
msrA	TCCAATCATTGCACAAAATC	AATTCCCTCTATTTGGTGGT	163	(20)
mphC	GAGACTACCAAGAAGACCTGACG	CATACGCCGATTCTCCTGAT	722	(1)
tetK	GTAGCGACAATAGGTAATAGT	GTAGTGACATAAACCTCCTA	360	(20)
tetM	AGTGGAGCGATTACAGAA	CATATGTCCTGGCGTGTCTA	158	(20)
aac(6')-Ie-aph(2")-Ia	GAAGTACGCAGAAGAGA	ACATGGCAAGCTCTAGGA	491	(20)
aph(3')-IIIa	AAATACCGCTGCGTA	CATACTCTTCCGAGCAA	242	(20)
ant(4')-Ia	AATCGGTAGAAGCCCAA	GCACCTGCCATTGCTA	135	(20)

PCR amplification was performed under optimised cycling conditions based on the melting temperatures of the respective primers. The cycling protocol generally included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at gene-specific temperatures for 30 sec, and extension at 72 °C for 45 sec, with a final extension step at 72 °C for 5 min. The annealing temperatures used were optimised for each target gene and ranged from 52-60 °C (55 °C for *mecA* and *blaZ*, 56 °C *femA*, 52 °C for *ermA, ermB,* and *tetK*, 54 °C for *ermC*, *msrA,* and *tetK*, 58 °C for *mphC*), according to previously published protocols and primer characteristics (Table 1). PCR products were visualised by electrophoresis on 1.5% agarose gels stained with ethidium bromide and examined under ultraviolet illumination.[20,21]

Quality control

Reference strains *S. aureus* ATCC 25923 (methicillin-susceptible) and *S. aureus* ATCC 43300 (MRSA) were utilised as positive controls in all susceptibility and PCR assays to ensure accuracy and reproducibility.

All collected data were anonymised, and no patient identifiers were used.

Statistical analysis

Data entry was conducted using Microsoft Excel 365. Statistical analyses were performed using IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA). Categorical variables, including distribution of antibiotic resistance genes, multidrug-resistance (MDR) status, infection source, and isolate type, were summarised as frequencies and percentages. Continuous variables, such as the number of resistance genes per isolate, were expressed as mean ± standard deviation (SD).

Associations between categorical variables were evaluated using the Chi-square (χ^2) test, while Fisher's exact test was applied when expected cell counts were less than five. The relationship between the presence of individual resistance genes and MDR status was also assessed using the Chi-square test.

To identify factors independently associated with MDR, multivariable logistic regression analysis was performed. Variables with potential clinical or statistical relevance identified in univariate analysis were included in the regression model, including infection source (hospital- versus community-acquired) and isolation source (e.g., CSF, urine, and other clinical specimens). The strength of associations was expressed as odds ratios (ORs) with corresponding 95% confidence intervals (CIs). Model adequacy was assessed using the Hosmer–Lemeshow goodness-of-fit test, and predictive performance was evaluated using classification accuracy. A

Table 2. Clinico-demographic Characteristics of *Staphylococcus aureus* Isolates (N = 120)

Variable	Category	Hospital-acquired n (%)	Community-acquired n (%)	P-value
Age (years)	Mean ± SD	8.82 ± 3.20	9.00 ± 3.10	
Sex	Male	38 (57.6%)	31 (57.4%)	1.000
	Female	28 (42.4%)	23 (42.6%)	
Source of sample	Blood	33 (27.5%)	18 (15.0%)	0.0003
	CSF	11 (9.2%)	0 (0.0%)	
	Other	7 (5.8%)	4 (3.3%)	
	Urine	5 (4.2%)	9 (7.5%)	
	Wound	10 (8.3%)	23 (19.2%)	

Chi-square tests were utilised to compare HAI and CAI. Age is presented as mean ± SD. P < 0.05 was deemed significant

two-tailed P < 0.05 was considered statistically significant.

RESULTS

Table 2. The analysis revealed that *Staphylococcus aureus* isolates were more frequently associated with HAI (55%) than with CAI (45%). Hospital-acquired isolates were predominantly recovered from neonatal intensive care unit (NICU) and pediatric intensive care unit (PICU) wards, whereas community-acquired isolates were mainly obtained from outpatient samples.

Blood and wound specimens were the most common clinical sources of infection, together accounting for more than two-thirds of all isolates. There was a significant association between the source of the sample and hospital acquisition (P < 0.05), suggesting that invasive samples, such as blood and CSF, were more likely to be collected in hospital settings.

The sex distribution didn't differ significantly between hospital and community infections (P > 0.05). The mean age of pediatric patients spanned infancy and childhood, consistent with prior epidemiological reports of pediatric *S. aureus* infection.

Table 3 summarises the distributions of significant AMR and virulence genes among the *S. aureus* isolates. The β-lactamase gene (*blaZ*) was the most predominant resistance determinant, identified in 83.3% of isolates, indicating widespread penicillin resistance among CAI and HAI. The methicillin-resistance gene (*mecA*) was identified in 36.7% of isolates, confirming a substantial proportion of MRSA within the cohort.

Table 3. Frequency of resistance genes

Gene	Positive Isolates	Percentage (%)
mecA	44	36.7
blaZ	100	83.3
ermA	17	14.2
ermB	13	10.8
ermC	30	25.0
msrA	22	18.3
mphC	22	18.3
tetK	36	30.0
tetM	33	27.5
aac(6')-Ie-aph(2")-Ia	35	29.2
aph(3')-IIIa	23	19.2
ant(4')-Ia	16	13.3
qacA	17	14.2
qacB	8	6.7
PVL	22	18.3

Among the macrolide-lincosamide-streptogramin (MLS) resistance genes, *ermC* was the most frequent (25.0%), followed by *ermA* (14.2%) and *ermB* (10.8%). In comparison, efflux-mediated resistance genes (*msrA* and *mphC*) were each present in 18.3% of isolates. This pattern suggests heterogeneous mechanisms of macrolide resistance, combining ribosomal methylation and active efflux pathways.

Tetracycline resistance was also common, with *tetK* (30.0%) and *tetM* (27.5%) found at comparable rates, indicating the coexistence of plasmid-mediated efflux and ribosomal protection

Table 4. Frequency of Antibiotic Resistance among *S. aureus* Isolates (N = 120)

Antibiotic	Resistant (n)	Resistant (%)
Oxacillin	27	22.5
Penicillin	25	20.8
Erythromycin	23	19.2
Clindamycin	22	18.3
Tetracycline	26	21.7
Gentamicin	19	15.8
Trimethoprim-sulfamethoxazole	26	21.7
Fusidic acid	23	19.2
Linezolid	26	21.7

genes. In addition, AME genes, including *aac(6')-Ie-aph(2")-Ia* (29.2%), *aph(3')-IIIa* (19.2%), and *ant(4')-Ia* (13.3%), were determined at moderate frequencies, consistent with multidrug-resistant phenotypes observed in hospital isolates.

Notably, *qacA* (14.2%) and *qacB* (6.7%) were identified, representing biocide resistance genes associated with reduced susceptibility to antiseptics, such as chlorhexidine—an emerging concern for infection control in healthcare environments. The *PVL* gene, a main virulence factor associated with SSTIs, was identified in 18.3% of isolates, denoting that a subset of strains retains high virulence potential.

Table 4. The resistance profile of the *Staphylococcus aureus* isolates displayed variable susceptibility across antimicrobial categories. Increased resistance ratios to beta-lactams, particularly penicillin and oxacillin, were observed, consistent with the widespread presence of β-lactamases and *mecA*-mediated resistance. Moderate resistance frequencies were recorded for macrolides (erythromycin) and tetracyclines, while lower resistance levels were observed for gentamicin and trimethoprim-sulfamethoxazole. Fusidic acid and linezolid maintained the highest activity and the lowest rates of resistance among the tested agents. Overall, these data reflect a trend toward multidrug-resistance among clinical *S. aureus* isolates, emphasising the need for ongoing antimicrobial stewardship (AMS) in pediatric settings.

Figure illustrates the distributions of MDR and non-MDR *S. aureus* isolates obtained from pediatric patients. "Multidrug-resistance (MDR), defined as resistance to three or more antimicrobial classes, was identified in 47 of 120 *S. aureus* isolates, corresponding to a prevalence of 39.2%, while 73 isolates (60.8%) were classified as non-MDR".

Table 5. Among the genes examined, *tetK*, *tetM, PVL, ermC,* and *mphC* showed significant associations with MDR ($P < 0.05$). The genes *tetK* and *tetM* showed the strongest associations (OR ≈ 7.9 and 8.1, respectively), indicating that isolates carrying these genes were approximately eight times more likely to exhibit MDR. Similarly, *PVL*, *ermC*, and *mphC* exhibited moderate but significant associations (ORs ranging from 4.1-6.5), suggesting their co-occurrence with MDR phenotypes.

In contrast, other tested genes demonstrated odds ratios near zero, with no statistically significant association with MDR, indicating that their presence was not predictive of multidrug-resistance. Overall, the data suggest that tetracycline resistance genes (*tetK*, *tetM*)

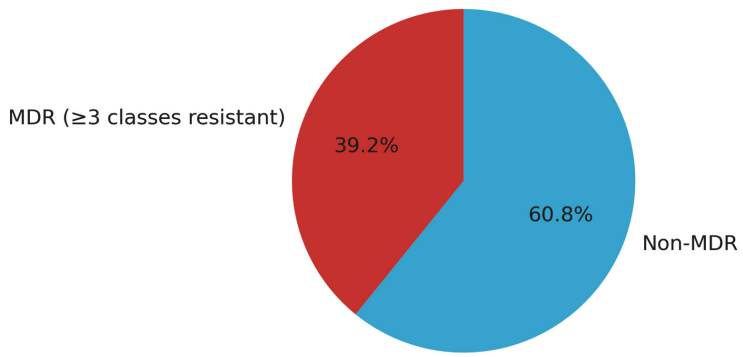

Figure. Proportion of Multidrug-resistant (MDR) *S. aureus* Isolates

Table 5. Association between antimicrobial resistance genes and multidrug-resistance (MDR) among *S. aureus* isolates using logistic regression analysis

Resistance Gene	Odds Ratio (OR)	95% Confidence Interval (CI)	P-value	Interpretation
tetM	7.39	2.45-22.31	<0.001	Significant association with MDR
tetK	6.82	2.21-21.04	0.001	Significant association with MDR
PVL	5.12	1.78-14.74	0.002	Significant association with MDR
mphC	3.94	1.42-10.93	0.008	Significant association with MDR
ermA	3.61	1.26-10.31	0.017	Significant association with MDR
ermC	3.42	1.20-9.74	0.021	Significant association with MDR
aac(6')-Ie-aph(2")-Ia	2.81	1.04-7.57	0.041	Significant association with MDR
aph(3')-IIIa	2.63	1.01-6.89	0.048	Significant association with MDR
mecA	2.41	0.94-6.17	0.067	Not statistically significant
blaZ	1.95	0.76-5.02	0.165	Not statistically significant
ant(4')-Ia	1.42	0.51-3.95	0.503	Not statistically significant
ermB	1.36	0.49-3.80	0.556	Not statistically significant
msrA	1.28	0.46-3.54	0.639	Not statistically significant
qacA	0.72	0.19-2.65	0.621	Not statistically significant
qacB	0.51	0.08-3.12	0.471	Not statistically significant

Table 6. Multivariable logistic regression analysis between MDR and infection source and isolate type

Variable	Coefficient (β)	Std. Error	z	P-value	95% CI
Intercept	-0.162	0.386	-0.42	0.674	(-0.918, 0.594)
(Hospital_Acquisition) [Hospital]	0.434	0.409	1.06	0.288	(-0.367, 1.235)
(Source)[CSF]	-1.253	0.748	-1.68	0.094	(-2.718, 0.213)
(Source)[Other]	0.070	0.671	0.10	0.917	(-1.245, 1.385)
(Source)[Urine]	0.298	0.624	0.48	0.633	(-0.925, 1.522)
(Source)[Wound]	-0.277	0.472	-0.59	0.557	(-1.203, 0.649)

Chi-square test was utilized to assess the relationship between MDR status and predictor variables. No significant relationships were detected (P > 0.05)

and macrolide resistance genes (*ermC*, *mphC*) are major contributors to the MDR phenotype. At the same time, *PVL*-primarily a virulence determinant may be linked to MDR through co-selection or genetic linkage within mobile genetic elements.

Table 6 displays the findings of a multivariable logistic regression assessing the relationship between multidrug-resistance (MDR) and two predictor variables: infection source (hospital- or community-acquired) and isolate source (e.g., CSF, urine, or other clinical specimens). The regression model used the Chi-square test to evaluate statistical significance.

The findings indicate no statistically significant associations between MDR status and either infection source or isolate type (P > 0.05). Although the coefficient for hospital-acquired infections (β = 0.43, P = 0.29) suggested slightly higher odds of MDR, this trend was not significant. Similarly, CSF isolates showed a non-significant tendency toward reduced MDR odds (β = –1.25, P = 0.09). In contrast, isolates from urine and other sources showed no meaningful difference compared to the reference group.

Overall, the regression analysis suggests that neither infection source nor isolate type independently predicts MDR status in this dataset. This implies that MDR occurrence may be primarily driven by genetic determinants of resistance rather than by the clinical source or infection setting.

DISCUSSION

S. aureus remains an important pathogen causing a broad spectrum of pediatric infections, ranging from skin and soft tissue infections to severe invasive diseases such as bacteremia, pneumonia, and meningitis.[22,23] The emergence of MDR and MRSA strains further increases the clinical and epidemiological burden of these infections.[24,25]

In the present study, hospital-acquired infections were more frequent than community-acquired infections, consistent with previous reports identifying healthcare settings, particularly intensive care units, as important reservoirs for MDR *S. aureus* because of selective antibiotic pressure and cross-transmission.[26-28] Blood and wound specimens represented the most common sources of isolates, in agreement with previous studies reporting *S. aureus* as a leading cause of bacteremia and wound infections.[29,30]

blood and CSF, were significantly associated with hospital-acquired infections, supporting earlier findings linking invasive *S. aureus* infections to healthcare exposure and invasive procedures.[31,32] These findings underscore the importance of continuous surveillance, infection-control measures, and antimicrobial stewardship programs in pediatric healthcare settings.[33,34]

The high prevalence of the *blaZ* gene (83.3%) indicates widespread β-lactamase-mediated resistance among pediatric isolates, while the detection of *mecA* in 36.7% confirms a substantial burden of MRSA.[35-37] Although *blaZ* was highly prevalent, phenotypic penicillin resistance was observed less frequently, which may reflect differences between gene carriage and actual gene expression under laboratory conditions. Similar genotype–phenotype discrepancies have previously been described in *S. aureus* isolates. These findings suggest that empirical penicillin therapy may be unreliable and emphasize the importance of local resistance surveillance and appropriate antimicrobial selection.[38-40]

Among macrolide resistance determinants, *ermC* was the most prevalent, followed by *ermA* and *ermB*, consistent with previous studies identifying *ermC* as the dominant MLS resistance determinant in clinical *S. aureus* isolates.[6,41] The presence of *msrA* and *mphC* suggests coexistence of multiple macrolide resistance mechanisms, including active efflux and ribosomal methylation.[42] Tetracycline resistance genes (*tetK* and *tetM*) and aminoglycoside-modifying enzyme genes were also frequently detected, similar to findings reported in Egyptian and international studies.[43,44] In addition, detection of *qacA/qacB* may indicate reduced susceptibility to antiseptics such as chlorhexidine, while the presence of *PVL*-positive isolates reflects persistence of virulent pediatric strains.[45,46]

Notably, *tetK* and *tetM* demonstrated the strongest association with MDR, followed by *ermC*, *mphC*, and *PVL*. Similar findings have been reported in pediatric and clinical *S. aureus* studies, where tetracycline- and macrolide-resistance genes were frequently associated with MDR phenotypes and mobile genetic elements facilitating resistance dissemination. Previous clinical studies have identified *tetK*, *tetM*, and *ermC* as common resistance determinants among MRSA isolates and important contributors to multidrug resistance.[47-49] These findings highlight the importance of molecular surveillance and targeted antimicrobial stewardship programs for controlling MDR *S. aureus* in pediatric settings. In contrast, infection source and isolate type were not independent predictors of MDR, suggesting that resistance is driven primarily by specific genetic determinants rather than by clinical origin alone.[48-50] These findings highlight the importance of molecular surveillance and targeted antimicrobial stewardship to control the spread of MDR *S. aureus*.

The observed linezolid resistance rate was higher than that reported in many previous pediatric studies and should therefore be interpreted cautiously. Although susceptibility testing and broth microdilution confirmation were performed according to CLSI recommendations, further molecular investigations targeting resistance determinants such as *cfr* and *optrA* are warranted.

Limitations

The present study has several limitations that should be acknowledged. First, the D-test for inducible clindamycin resistance was not performed despite the analysis of MLS resistance

genes. Consequently, phenotypic confirmation of inducible MLS_B resistance could not be assessed. Future studies should incorporate D-zone testing according to CLSI recommendations to provide a more comprehensive evaluation of clindamycin resistance and improve genotype–phenotype correlation.

Second, the study was conducted at a single tertiary-care center with a relatively limited sample size, which may restrict the generalizability of the findings to other pediatric populations and healthcare settings. In addition, molecular characterization was limited to selected resistance and virulence genes, while other clinically relevant resistance determinants, including linezolid resistance genes such as *cfr* and *optrA*, were not investigated. Finally, the cross-sectional design prevented assessment of temporal trends in antimicrobial resistance and transmission dynamics of *S. aureus* isolates over time.

CONCLUSION

In summary, *S. aureus* remains an important pediatric pathogen, with hospital-acquired infections, particularly in NICU and PICU settings, representing a considerable proportion of cases in the present study. A high prevalence of β-lactamase-associated (*blaZ*) and methicillin-resistance (*mecA*) genes was identified, together with diverse resistance determinants including tetracycline (*tetK*, *tetM*), macrolide (*ermC*, *mphC*), and aminoglycoside-modifying enzyme genes. *PVL*-positive isolates were also detected, indicating the coexistence of resistance and virulence characteristics among some isolates.

Tetracycline and macrolide resistance genes showed significant associations with multidrug-resistance (MDR), whereas infection source and isolate type were not independently associated with MDR in the regression analysis. These findings support the value of continued antimicrobial resistance surveillance and infection-control measures in pediatric healthcare settings.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

RHH conceptualized and designed the study. OAS and DMAH performed clinical data collection. LAK, EHMS and MESZ performed laboratory work. EHMS, MESZ, DMAH and RHH performed data analysis. OAS, RHH, MESZ, EHMS, LAK and DMAH wrote the manuscript. MESZ, DMAH and RHH reviewed and revised the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Research Ethics Committee, Faculty of Medicine, Mansoura University, Egypt (Approval No.: R.25.11.3455).

INFORMED CONSENT

Written informed consent was obtained from the participants' legal guardians before enrollment in the study.

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