

Molecular Detection of *Aph-3* and *ArmA* Genes in Aminoglycoside-Resistant *Staphylococcus aureus* Clinical Isolates from Khartoum State

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Abstract

Background and Objective: *Staphylococcus aureus* is a major human pathogen responsible for a wide range of clinical infections. While aminoglycoside antibiotics are vital for treating staphylococcal infections, resistance to these agents is an increasing global concern. This study aimed to determine the prevalence of aminoglycoside-modifying enzyme genes [*aph-3* and *armA*] in clinical isolates of *S. aureus* from various hospitals in Khartoum State. **Materials and Methods:** A cross-sectional study was conducted between May and August 2022. Seventy-two [72] *S. aureus* isolates were obtained from clinical specimens, including wound swabs, blood, and aspirated fluids. The isolates were identified using standard biochemical tests. Antimicrobial susceptibility testing for amikacin and gentamicin was performed using the disk diffusion method. All 72 isolates were analyzed via multiplex polymerase chain reaction [PCR] to detect the presence of the *aph-3* and *armA* resistance genes. **Results:** Of the 72 *S. aureus* isolates, 35 [48.6%] were positive for at least one resistance gene. Specifically, the *aph-3* gene was detected in 34 [47.2%] isolates [including one isolate carrying both genes], while the *armA* gene was detected in 2 [2.8%] isolates. Phenotypic resistance was found to be higher for amikacin than for gentamicin. Statistical analysis showed no significant association between the presence of these genes and patient gender, age, or specimen type [$p > 0.05$]. **Conclusion:** This study concludes that the *aph-3* gene is the predominant aminoglycoside resistance marker among *S. aureus* isolates in Khartoum State. The low prevalence of the *armA* gene suggests it is currently a less common mechanism in this region. The high frequency of resistance to amikacin underscores the need for continuous molecular surveillance to guide effective antimicrobial therapy.

Keywords: *Staphylococcus aureus*, aminoglycoside, amikacin, gentamicin, AMR, *aph-3*, *armA*.

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1. INTRODUCTION

Staphylococcus aureus is a major human pathogen and a common commensal that frequently colonizes the moist squamous epithelium of the anterior nares [1]. Approximately 20% of the population are persistent carriers, while 60% are intermittent carriers; notably, such colonization serves as a significant risk factor for invasive infections [1,2]. As a leading cause of both hospital-acquired and community-acquired infections, *S. aureus* is responsible for a wide clinical spectrum, ranging from minor skin and soft tissue infections to life-threatening conditions such as sepsis, endocarditis, and fatal necrotizing pneumonia [3-7].

Aminoglycoside antibiotics remain vital therapeutic agents for treating serious staphylococcal infections. However, the efficacy of these drugs is increasingly threatened by the global rise of antimicrobial resistance [AMR]. Resistance to aminoglycosides primarily arises through three mechanisms: reduced drug permeability, alteration of the ribosomal binding site, and most significantly the enzymatic inactivation of the drug [9-11].

The most frequent mechanism encountered in *Staphylococci* is the synthesis of aminoglycoside-modifying enzymes [AMEs], which include

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acetyltransferases [AACs], phosphotransferases [APHs], and nucleotidyltransferases [ANTs]. Among these, the *aph*[3']-IIIa [commonly referred to as *aph*-3] gene is of high clinical importance because it confers resistance to amikacin and kanamycin. Furthermore, high-level resistance can be mediated by 16S rRNA methylases, such as those encoded by the *armA* gene, which modify the antibiotic target site itself [12-17].

Despite the clinical significance of these resistance markers, their distribution and prevalence remain variable and are often insufficiently characterized across different geographical regions. In Sudan, the treatment of staphylococcal infections has become increasingly challenging due to emerging resistance, yet there is limited molecular data regarding the specific genes driving this trend in Khartoum State.

Therefore, this study aimed to determine the prevalence of the *aph*-3 and *armA* resistance genes among clinical isolates of *S. aureus* in Khartoum. By identifying these molecular determinants, this research seeks to provide essential data to guide local antimicrobial therapy and improve infection control strategies.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

A cross-sectional study was conducted using clinical isolates collected from various microbiology laboratories across Khartoum State between May and August 2022.

2.2 Ethical Considerations

Ethical approval for this study was obtained from the Ethical Committee of the University of Al-Butana.

2.3 Inclusion Criteria and Sample Size

The study included clinical isolates of *Staphylococcus aureus* obtained from various patient specimens. Only those isolates that demonstrated phenotypic resistance to aminoglycosides via initial antimicrobial susceptibility testing were included for further molecular analysis. While the initial study framework considered a larger pool, a final total of 72 confirmed aminoglycoside-resistant *S. aureus* isolates were successfully recovered and processed for this study.

2.4 Bacterial Isolates and Identification

The 72 *S. aureus* isolates were obtained from diverse clinical specimens, including wound swabs, blood, and aspirated fluids. Primary cultures were grown on nutrient agar and incubated overnight at 37°C. The identity of each isolate was re-confirmed using standard microbiological methods, which included the evaluation of colony morphology, Gram staining, and the catalase test.

2.5 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing [AST] was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar [MHA], in strict accordance with the Clinical and Laboratory Standards Institute [CLSI] guidelines. The antibiotics tested included gentamicin [GEN, 30 µg] and amikacin [AMK, 30 µg]. Prior to testing, bacterial suspensions were prepared in sterile saline and adjusted to a 0.5 McFarland turbidity standard to ensure a standardized inoculum.

2.6 DNA Extraction

Total genomic DNA was extracted from the *S. aureus* isolates using a boiling lysis method, as previously described [18]. Briefly, three to five pure colonies were suspended in 200 µl of sterile distilled water within a microcentrifuge tube and homogenized thoroughly. The suspension was incubated in a water bath at 100°C for 10 minutes, vortexed briefly, and subjected to a second 10-minute boiling cycle at 100°C. The resulting lysates were immediately chilled at -20°C for 10 minutes to facilitate the precipitation of cellular debris. Following centrifugation at 10,000 rpm for 10 minutes, the supernatant containing the DNA template was carefully collected, transferred to a new sterile labeled tube, and stored for subsequent PCR analysis.

2.7 Multiplex PCR [Polymerase Chain Reaction]

2.7.1 Confirmation of *S. aureus*

To confirm the identity of the isolates, PCR was performed using primers targeting a highly conserved region of the 16S rRNA gene of *S. aureus* [Forward: 5'-AGTTTGATCCTGGCTCAG-3'; Reverse: 5'-AGGCCCGGAACGTATTAC-3']. The amplification was conducted in a final volume of 25 µL, containing 8 µL of Blue Master Mix [Intron Biotechnology, Korea]—which includes Taq polymerase, reaction buffer, and dNTPs—2 µL of each primer [10 pmol], 5 µL of DNA template, and 13 µL of double-distilled water [ddH₂O]. The thermal cycling program consisted of an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes [19,21].

2.7.2 Detection of *aph*-3 and *armA* Genes

A multiplex PCR assay was employed to detect the aminoglycoside resistance genes *aph*-3 and *armA*. The 25 µL reaction mixture contained 5 µL of DNA template, 2 µL [10 pmol] of each specific primer [Forward *aph*-3: 5'-GGCTAAATGAGAATATCACCGG-3'; Reverse *aph*-3: 5'-CTTTAAAAAATCATACAGCTCGCG-3' and Forward *armA*: 5'-CCGAAATGACAGTTCCTATC-3'; Reverse *armA*: 5'-GAAAATGACTGCCTTGGAGG-3'], 5 µL of 2 mM dNTP mix, 2 µL of 25 mM MgCl₂, and 2.5 U of Taq DNA Polymerase [Intron Biotechnology, Korea] in 1x

reaction buffer. Amplification was performed using a thermal cycler [Techne, England] with the following conditions: initial denaturation at 94°C for 5 minutes; 40 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and elongation at 72°C for 2 minutes; and a final elongation step at 72°C for 5 minutes.

2.7.3 Electrophoresis and Visualization

The resulting PCR products were analyzed via 2% agarose gel electrophoresis. The gel was prepared by dissolving agarose in 1x TBE [Tris-Borate-EDTA] buffer and staining with 2 µL of ethidium bromide. Samples [5 µL] and a 100 bp DNA ladder [3 µL] were loaded into the wells. Electrophoresis was carried out at 130 V for 60 minutes. The amplified DNA fragments were visualized under UV light using a gel documentation system.

2.8 Study Limitations

A primary limitation of this research was the relatively small sample size of 72 clinical isolates. This constraint was largely dictated by the high operational costs associated with molecular detection techniques and the restricted duration of the study period. Consequently, while the findings provide important regional insights, a larger, multi-center study conducted over a longer duration would be beneficial to further validate the prevalence trends of the *aph-3* and *armA* genes in Khartoum State.

2.9 Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences [SPSS] software, version 23.0 [IBM Corp., Armonk, NY]. Initially, all raw data were compiled into a master sheet and subsequently coded into discrete variables for processing. Descriptive statistics were employed to summarize the characteristics of the study population. For inferential analysis, Fisher's exact test was used to evaluate associations between the presence of resistance genes and categorical clinical variables [e.g., gender, age, and specimen type]. Furthermore, binary logistic regression was performed to identify potential predictors of aminoglycoside resistance gene carriage. For all statistical tests, a *p*-value of less than 0.05 was considered to be statistically significant.

3.0 RESULTS

3.1 Demographic Characteristics of Clinical Isolates

A total of 72 aminoglycoside-resistant *S. aureus* isolates were analyzed in this study. The demographic distribution showed a higher frequency of isolates from male patients [*n*=43, 59.7%] compared to female patients [*n*=29, 40.3%], as shown in Figure 1 and Table 1.

The isolates were distributed across five age categories, with the elderly group [46–75 years] representing the largest segment at 30.6% [*n*=22], followed by young adults [16–30 years] at 26.4% [*n*=19]. The lowest prevalence was observed in the neonate group [0–3 years] at 6.9% [*n*=5]. These findings are summarized in Figure 2 and Table 1.

Table 1: Demographic and molecular characteristics of the study population [*n*=72]. The table summarizes the absolute counts and percentages for patient gender, age group, specimen origin, and the presence of the *aph-3* and *armA* resistance genes

Variables	Number	Percent
Gender		
Male	43	59.7%
Female	29	40.3%
Age groups		
Neonate [0-3 years]	5	6.9%
Children [3-15 years]	12	16.7%
Young Adults [16-30 years]	19	26.4%
Adults [31- 45] years	14	19.4%
Elderly [46-75 years]	22	30.6%
Type of specimen		
Dialysis fluid	1	1.4%
Ascitic fluid	2	2.8%
CSF	3	4.2%
Blood	12	16.7%
Wound swab	54	75%
Gene result		
Negative	37	51.4%
Positive	35	48.6%
Gene result		
Negative	37	51.4%
<i>aph-3</i>	33	45.8%

<i>armA</i>	1	1.4%
<i>aph-3+armA</i>	1	1.4%
The resistance gene [n=35]		
<i>aph-3</i>	33	94.3%
<i>armA</i>	1	2.9%
<i>aph-3+armA</i>	1	2.9%
n=72		

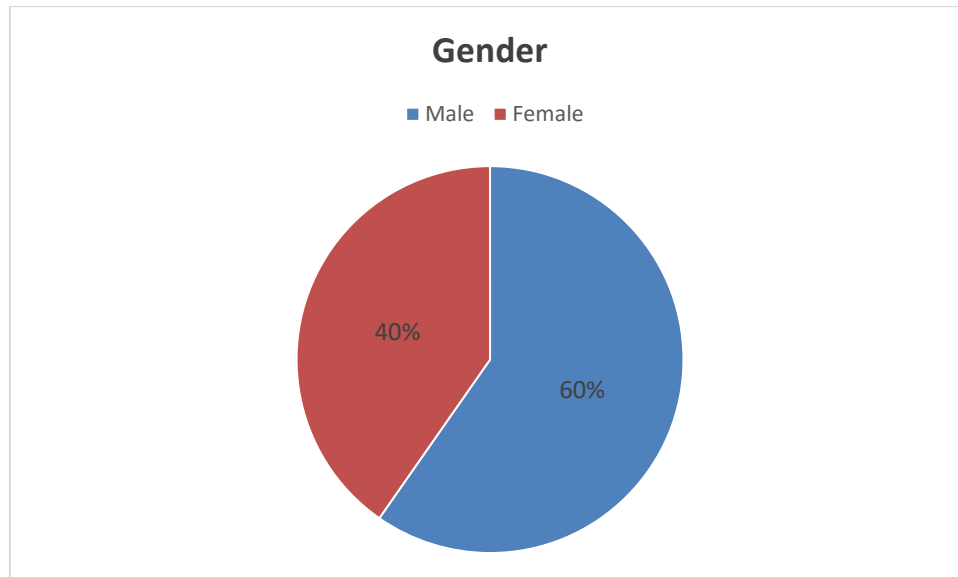


Figure 1: Gender distribution of patients yielding aminoglycoside-resistant *Staphylococcus aureus* isolates. The chart illustrates the proportion of isolates recovered from male [59.7%] and female [40.3%] patients among the 72 clinical samples collected in Khartoum State.

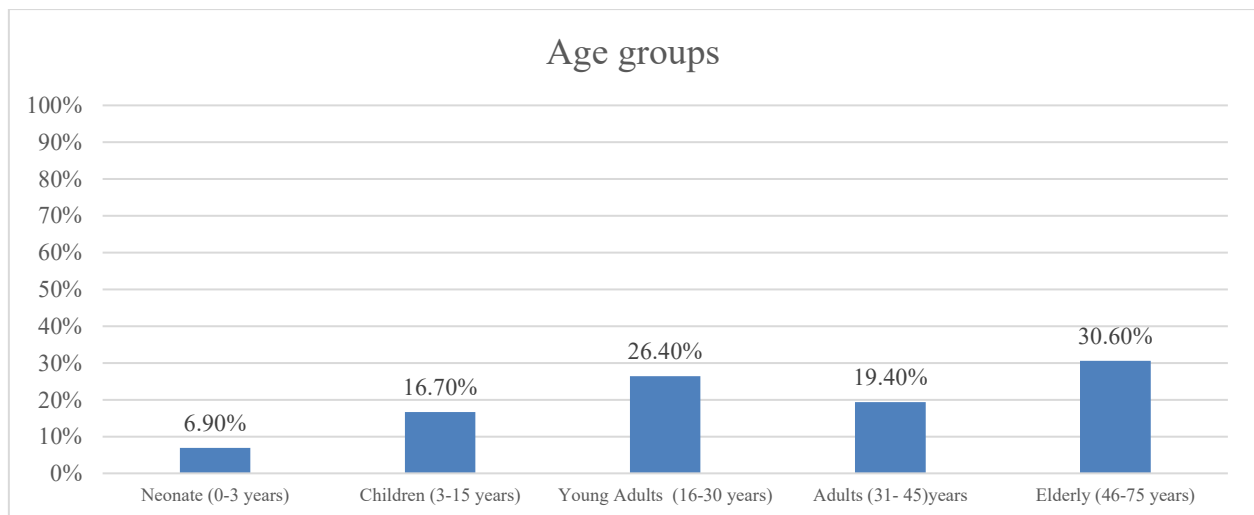


Figure 2: Distribution of *Staphylococcus aureus* isolates across patient age categories. The study population was stratified into five groups: neonates [0–3 years, 6.9%], children [3–15 years, 16.7%], young adults [16–30 years, 26.4%], adults [31–45 years, 19.4%], and the elderly [46–75 years, 30.6%]

3.2 Source and Distribution of Isolates

Isolates were recovered from five distinct clinical specimen types. Wound swabs were the most common source, accounting for 75% [n=54] of the isolates. Other sources included blood [16.7%, n=12],

cerebrospinal fluid [CSF] [4.2%, n=3], ascitic fluid [2.8%, n=2], and dialysis fluid [1.4%, n=1]. This distribution is visualized in Figure 3 and detailed in Table 1.

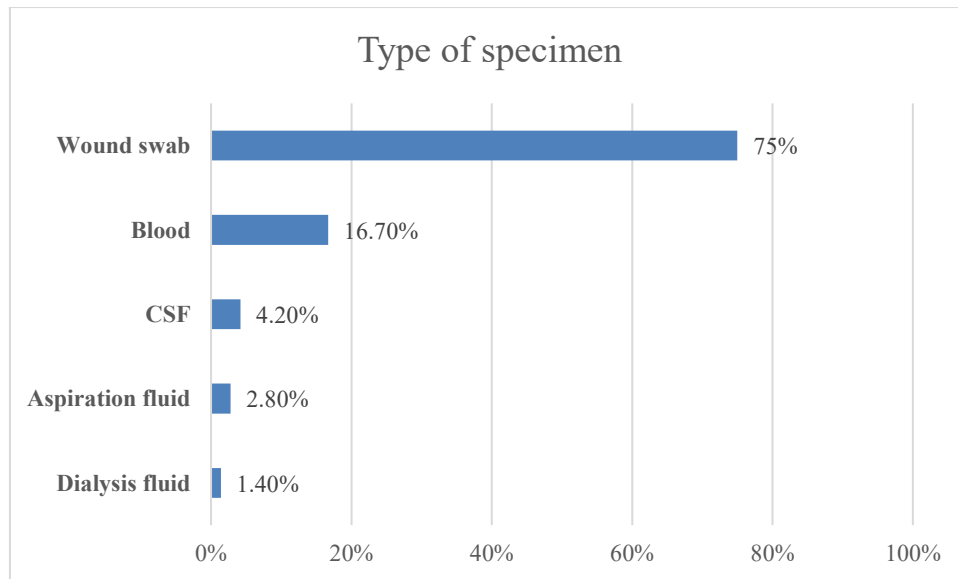


Figure 3: Clinical source distribution of *Staphylococcus aureus* isolates. The majority of resistant isolates were recovered from wound swabs [75%], with blood [16.7%] and other fluids [CSF, ascitic, and dialysis] comprising the remainder of the clinical specimens

3.3 Molecular Detection of *aph-3* and *armA* Genes

Multiplex PCR analysis confirmed the presence of at least one resistance gene in 35 [48.6%] of the 72 isolates [Table 1]. The *aph-3* gene was the predominant marker, identified in 34 [47.2%] isolates total [33 as a single gene and 1 in combination with *armA*]. The *armA*

gene was detected in only 2 [2.8%] isolates [1 alone and 1 combined]. No target genes were detected in 37 [51.4%] of the phenotypically resistant isolates. The electrophoretic separation of these products is depicted in Figure 4.

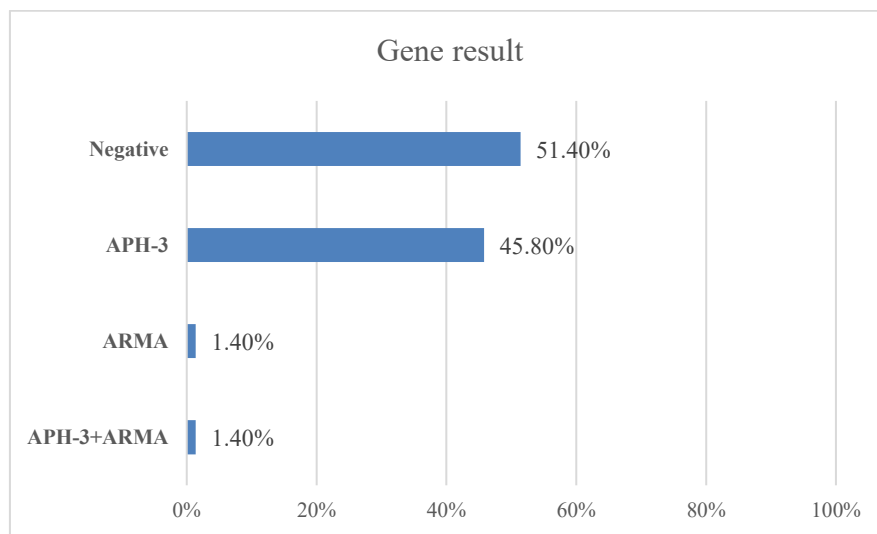


Figure 4: Frequency of resistance genes among *S. aureus* isolates detected by multiplex PCR. Representative gel electrophoresis and frequency data showing the prevalence of the *aph-3* [47.2%] and *armA* [2.8%] genes. Lane M: 100 bp DNA ladder; target bands correspond to the conserved 16S rRNA region and specific resistance determinants

3.4. Correlation Between Genotype and Phenotypic Resistance

The relationship between the presence of molecular resistance determinants and phenotypic antimicrobial susceptibility was evaluated for gentamicin [GEN] and amikacin [AMK]. Among the 33 isolates harboring the *aph-3* gene alone, 100%

demonstrated resistance to amikacin, while 28 [84.85%] were resistant to gentamicin.

Furthermore, high-level co-resistance was observed in isolates carrying rarer genotypes; both the strain harboring *armA* alone and the strain carrying the *aph-3+armA* combination exhibited 100% resistance to

both tested aminoglycosides. These findings, detailed in Table 2, highlight a perfect correlation between the

presence of these genes and amikacin resistance in this cohort.

Table 2: Correlation between resistance genotypes and phenotypic susceptibility to gentamicin and amikacin. Susceptibility was determined using the Kirby-Bauer disk diffusion method. The table highlights that 100% of isolates carrying the *aph-3* gene were resistant to amikacin, while varying degrees of co-resistance were observed for gentamicin

Variables		GEN		AMI	
Aminoglycoside transferees gene	Number of strain	S	R	S	R
<i>aph-3</i>	33	5[15.15%]	28[84.85%]	0[0%]	33[100%]
<i>armA</i>	1	0[0%]	1[100%]	0[0%]	1[100%]
<i>aph-3</i> + <i>armA</i>	1	0[0%]	1[100%]	0[0%]	1[100%]

3.5. Association Between Clinical Variables and Resistance Genes

Fisher's exact test was utilized to evaluate the association between clinical variables and the presence of aminoglycoside resistance genes. The analysis demonstrated that there was no statistically significant association between the carriage of these genes and patient gender [$p = 0.810$], age group [$p = 0.807$], or specimen type [$p = 0.186$], as all p -values were well above the 0.05 significance threshold [Table 3]. When

examining the specific distribution of the *aph-3* and *armA* genes across these variables, the findings remained statistically insignificant. There was no significant difference in the specific gene types found between males and females [$p = 0.686$] or across different age groups [$p = 0.537$]. Furthermore, the clinical source of the isolate—whether from wound swabs or other fluids—did not significantly correlate with the presence of either specific gene [$p = 0.999$], as detailed in Table 3.

Table 3: Statistical association [Fisher's Exact Test] between clinical variables and the presence of resistance genes. This table summarizes the relationship between demographic data and gene results, confirming that all associations were statistically insignificant [$p > 0.05$]

Variables		Gene result		Fisher's Exact Test P value
		Negative	Positive	
Gender	Male	23	20	0.810*
		53.50%	46.50%	
	Female	14	15	
		48.30%	51.70%	
Age groups	Neonate [0-3 years]	3	2	0.807*
		60.00%	40.00%	
	Children [3-15 years]	7	5	
		58.30%	41.70%	
	Young Adults [16-30 years]	11	8	
		57.90%	42.10%	
	Adults [31- 45] years	7	7	
		50.00%	50.00%	
Type of specimen	Elderly [46-75 years]	9	13	0.186*
		40.90%	59.10%	
	Wound swab	29	25	
		53.70%	46.30%	
	Blood	6	6	
		50.00%	50.00%	
	CSF	0	3	
		0.00%	100.00%	
Type of specimen	Ascetic fluid	2	0	0.590*
		100.00%	0.00%	
	Dialysis fluid	0	1	
		0.00%	100.00%	
	Wound swab	29	25	
		53.70%	46.30%	
	Others	8	10	
		44.40%	55.60%	

3.6. Predictors of Aminoglycoside Resistance Gene Carriage

Binary logistic regression analysis was performed to identify independent predictors for the carriage of aminoglycoside resistance genes [*aph-3* and *armA*] within the study population. While the model identified specific trends where certain groups showed higher odds of harboring resistance genes, no demographic or clinical variables were found to be statistically significant predictors [$p > 0.05$].

- Gender: Female patients demonstrated a higher likelihood of carrying resistance genes compared to males [AOR: 1.320], although this finding was not statistically significant [$p = 0.583$; 95% CI: 0.490–3.556].

- Age: Increased age was identified as a positive predictor for gene presence [AOR: 1.339], but the association did not reach statistical significance [$p = 0.145$; 95% CI: 0.905–1.980].
- Specimen Type: Isolates recovered from clinical specimens other than wound swabs ["Others"] were 1.691 times more likely to harbor resistance genes than those from wound swabs, yet this relationship was also statistically insignificant [$p = 0.368$; 95% CI: 0.539–5.312].

These findings indicate that while gender, age, and clinical source contribute to the overall resistance profile, they are not independent significant markers of gene carriage in this specific cohort [Table 4]

Table 4 Binary logistic regression for the prediction of aminoglycoside resistance gene presence

Variables	<i>aph-3</i> [n=33]	<i>armA</i> [n=1]	<i>aph-3+armA</i> [n=1]	p-value
Gender				0.686*
Male [n=20]	19 [95.0%]	0 [0.0%]	1 [5.0%]	
Female [n=15]	14 [93.3%]	1 [6.7%]	0 [0.0%]	
Age Groups				0.537*
Neonate [0–3 yr]	2 [100%]	0 [0.0%]	0 [0.0%]	
Children [3–15 yr]	4 [80.0%]	0 [0.0%]	1 [20.0%]	
Young Adult [16–30 yr]	8 [100%]	0 [0.0%]	0 [0.0%]	
Adult [31–45 yr]	7 [100%]	0 [0.0%]	0 [0.0%]	
Elderly [46–75 yr]	12 [92.3%]	1 [7.7%]	0 [0.0%]	
Specimen Type				0.999*
Wound swab [n=25]	23 [92.0%]	1 [4.0%]	1 [4.0%]	
Others [n=10]	10 [100%]	0 [0.0%]	0 [0.0%]	

4. DISCUSSION

In this study, 72 aminoglycoside-resistant clinical isolates of *Staphylococcus aureus* were analyzed to determine the prevalence of the *aph-3* and *armA* resistance genes. These molecular determinants are critical factors in the enzymatic inactivation of aminoglycosides, which remain a vital component of the therapeutic Drugs of choice against staphylococcal infections.

4.1. Prevalence and Comparative Analysis of *aph-3*

The prevalence of the *aph-3* gene in this study was found to be 45.8% [n=33]. This finding is in close agreement with the results reported by Al-Mayahi [22] in Iraq, who detected *aph-3* in 45% of isolates, and Al-khateeb *et al.*, [23], who identified the gene in 44% of strains. However, our results differ significantly from other regional studies. For instance, Khoramrooz *et al.*, [24] reported much higher frequencies in Iran, ranging from 61.1% to 68.4%. In the other Side, lower prevalence rates were observed by Perumal *et al.*, [25] in India [32.3%] and Shokravi *et al.*, [26], who reported only 19.0% for *aph-3*. These variations in genetic prevalence likely reflect differences in local antibiotic prescribing patterns and the specific clonal lineages of *S. aureus* circulating in these diverse geographical regions.

4.2. Low Frequency of the *armA* Gene

The *armA* gene, which encodes a 16S rRNA methylase, was detected in only 1.4% [n=1] of our isolates [plus one additional isolate in combination with *aph-3*]. This low frequency nearly like the findings of Ghafoor *et al.*, [27], who reported a significantly higher *armA* prevalence of 15.6%.

As noted in the guidelines for discussing discrepancies, these differences may be attributed to variations in sample size, the specific clinical source of the isolates, or broader geographical variations in the pathogenicity and resistance evolution of *S. aureus*. The low presence of *armA* in our Khartoum based cohort suggests that other mechanisms, such as aminoglycoside-modifying enzymes [AMEs], remain the dominant drivers of resistance in this study area.

4.3. Phenotypic Resistance and Genotypic Correlation

Phenotypic susceptibility testing revealed high levels of resistance to gentamicin [76.4%] and amikacin [70.8%]. Our gentamicin resistance rate is comparable to the 71.7% reported by Khoramrooz *et al.*, [24]. Similarly, our amikacin resistance level closely matches the 70% resistance found by Al-Mayahi [22].

Discrepancies were noted when comparing our data to Oyeboode *et al.*, [28], who found a lower gentamicin resistance rate of 55.8%, and Shokravi *et al.*, [26], who reported 51.7%. Furthermore, Ghafoor *et al.*, [27] reported an even distribution of 50% resistance for both gentamicin and amikacin, which differs from the higher resistance levels observed in our clinical samples. These differences emphasize the necessity of local surveillance to guide empirical therapy, as resistance profiles can vary significantly even between neighboring healthcare settings.

5. CONCLUSION

This study concludes that the *aph-3* resistance gene is significantly more prevalent [45.8%] among clinical isolates of *Staphylococcus aureus* in Khartoum State than the *armA* gene [1.4%]. Furthermore, phenotypic analysis demonstrated high rates of resistance, appearing in 76.4% of isolates for gentamicin and 70.8% for amikacin.

The high prevalence of *aph-3* confirms that aminoglycoside-modifying enzymes [AMEs] remain the primary molecular drivers of resistance in the region. To support the development of effective empirical therapy for staphylococcal infections, future research should focus on broader geographical surveillance and the investigation of additional AME families to further characterize evolving resistance patterns

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