Research Article

Nasal Carriage of *Staphylococcus aureus* as a Potential Source of Surgical Site Infections: Phenotypic and Molecular Evidence Using 16S rRNA PCR-RFLP Typing

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Abstract | Surgical site infections (SSIs) represent one of the most common complications after surgery. Nasal colonization with *Staphylococcus aureus* (*Staph. aureus*) is a well-recognized risk factor for SSIs. Colonizing *Staph. aureus* may serve as a reservoir for subsequent infection. This study aimed to evaluate the prevalence of *Staph. aureus* nasal carriage and to assess its association with the development of endogenous SSIs. This cross-sectional observational study enrolled 250 patients scheduled for clean elective surgery. Pre-operative nasal swabs were collected. *Staph. aureus* isolates were identified conventionally and tested for *in vitro* antimicrobial susceptibility using disc diffusion, E-test, and VITEK system. Patients were followed postoperatively for the occurrence of *Staph. aureus* SSIs. Relatedness between colonizing and infecting strains was assessed using antibiotic-susceptibility profiling and polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) of 16S rRNA using XhoI, EcoRI, BamHI, SacI, and HindIII restriction enzymes. *Staph. aureus* nasal carriage was detected in 10 % of patients (25/250). Carrier patients showed a significantly higher incidence of SSIs ($p < 0.001$). All *Staph. aureus* isolates (100%) were unsusceptible to penicillin, amoxicillin–clavulanic acid, and ampicillin–sulbactam, whereas all isolates (100%) were susceptible to vancomycin and linezolid. Methicillin-resistant *Staph. aureus* (MRSA) was identified in 44% (11/25) of the colonized patients. Out of the 25 colonized patients, 7 (28%) developed postoperative *Staph. aureus* SSIs, of which 3 (42.9%) patients were confirmed to be endogenously infected, and all the three cases were caused by MRSA. Nasal carriage of MRSA represents an important source of endogenous SSIs. The PCR-RFLP method efficiently revealed genetic diversity and relatedness among the bacterial isolates, supporting its usefulness as a potentially reliable molecular tool for assessing these traits. Pre-operative screening and targeted MRSA decolonization may reduce the risk of infections and improve the surgical outcomes.

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Introduction

Surgical site infections (SSIs) are considered one of the most critical and common obstacles after surgery, resulting in increased risk of morbidity and mortality. Accordingly, prevention of these infections has been recommended (Khan *et al*., 2023). One of the most frequent pathogens associated with SSIs is *Staphylococcus aureus* (*Staph. aureus*) (Mohammed *et al*., 2020). *Staph. aureus* is both a normal inhabitant of human skin and mucosa and a pathogen responsible for many types of infections, including life-threatening infections, contributing to remarkable morbidity, mortality, and healthcare costs (Bashabsheh *et al*., 2024). In humans, the most frequent carriage site is the anterior nares, which serves as a reservoir for the spread of this pathogen (Sakr *et al*., 2018). *Staph. aureus* permanently colonizes the anterior nares of 12% to 30% of the human population (Piewngam and Otto, 2024).

Nasal carriage of *Staph. aureus* has been demonstrated to be an important risk factor for SSIs (Tai *et al*., 2013; Bode *et al*., 2016; Pongbangli *et al*., 2021). Antibiotic resistance has become a serious global health threat in both community and hospital settings, particularly among the ESKAPE (*Enterococcus faecium, Staph. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.) pathogens (Alkhawagah *et al*., 2025). *Staph. aureus is* classified as a member of the ESKAPE pathogens, which have a major clinical concern due to their high antimicrobial resistance. Nasal colonization with resistant isolates, especially with methicillin-resistant *Staph. aureus* (MRSA) and vancomycin-resistant *Staph. aureus* (VRSA), poses a considerable risk for postoperative SSIs. In patients with *Staph. aureus* nasal carriage, decolonization using mupirocin ointment could reduce the incidence of SSIs by 42%–58% (Bode *et al*., 2016). Regarding this, it is recommended to perform a pre-operative nasal screening for *Staph. aureus* carriage to treat carriers with an intranasal mupirocin ointment alone or in combination with chlorhexidine bathing (Pongbangli *et al*., 2021).

Despite the clinical importance of SSIs, the contribution of endogenous *Staph. aureus* colonization to their development remains insufficiently characterized, largely because establishing epidemiological relatedness between colonizing and infecting isolates requires reliable molecular typing (Mehndiratta and Bhalla, 2012). High-resolution typing methods, including Pulsed-Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), *Staph. aureus* protein A gene typing (*spa* typing), and whole-genome sequencing (WGS) are widely regarded as the reference approaches for epidemiological investigations because of their superior discriminatory power. However, their routine implementation is often constrained under resource-limited settings by high costs, specialized instrumentation, and the technical expertise required for data generation and analysis (Mehndiratta and Bhalla, 2012). In contrast, 16S rRNA PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) is a simple, rapid, and relatively inexpensive molecular method that can be readily implemented in routine microbiology laboratories. Although its discriminatory capacity is inherently limited by the highly conserved nature of the 16S rRNA gene, making it difficult to distinguish among the closely related strains, PCR-RFLP can still provide a practical preliminary assessment of isolate-relatedness. Moreover, the genetically distinct isolates may yield identical restriction patterns, and their performance depends on the restriction enzyme(s) selected, meaning that some sequence polymorphisms may remain undetected. Consequently, 16S rRNA PCR-RFLP should be regarded as a cost-effective screening tool that complements, rather than replaces, high-resolution typing methods such as MLST or WGS (Mitani *et al*., 2005). Within this context, the present study aimed to investigate the potential association between nasal carriage of *Staph. aureus* and SSIs by combining conventional phenotypic characterization with 16S rRNA PCR-RFLP as a cost-effective preliminary approach for assessing the relatedness of the colonizing and infecting isolates.

Materials And Methods

Estimation of sample size

The minimum sample size was calculated to be 203 based on the Raosoft® online statistical software (Raosoft, 2004). The parameters used were a margin of error of 5%, a confidence level of 95%, and an expected response of 15.6% (Wolde *et al*., 2023). To increase the power of the study, 250 participants were enrolled.

Study populations

The present study was conducted on 250 patients admitted to the surgery department at Al-Zahraa

University Hospital, Cairo, Egypt, for elective surgery from October 2024 to July 2025. From all participants, informed consent was taken after clarifying the aim of the work. The study included patients who could be followed up for at least 30 d after surgery and excluded those with evidence of active infection at the time of surgery, those receiving systemic antibiotic therapy within 2 weeks prior to surgery, and those undergoing urgent surgery.

Specimens collection and transportation

Using single-use sterile cotton swabs, 250 nasal swabs were collected from patients pre-operatively by performing 5 complete rotations of the swab in each nostril of the same patient (Flouchi *et al*., 2021). Wound swabs were collected from the infected surgical sites that developed later among the studied patients. All specimens were transported to the laboratory within 1 hour and processed immediately upon arrival to confirm their viability.

Staphylococcus aureus isolation and identification

All specimens (*i.e*., nasal and wound swabs) were initially inoculated and streaked onto Mannitol Salt Agar (MSA) (Oxoid, UK) plates, and incubated for 24 h at 37 ˚C. After incubation, the obtained suspected colonies were identified based on their culture characteristics on blood and nutrient agar (NA) plates, Gram reaction, in addition to a set of biochemical assays, including catalase, coagulase, and DNase were conducted (Forbes *et al*., 2007).

Antibiotic susceptibility testing

All *Staph. aureus* isolates obtained from nasal swabs and surgical site specimens were subjected to an *in vitro* antimicrobial susceptibility testing. Three complementary methods were employed to ensure accurate assessment of resistance, mainly disc diffusion (Kirby-Bauer), E-test, and the automated VITEK system. In the Kirby–Bauer disk diffusion method, a bacterial suspension equivalent to a 0.5 McFarland standard was prepared and uniformly inoculated onto the Muller-Hinton agar (MHA) surface using a sterile cotton swab. Antibiotic disks were placed aseptically on the surface of the seeded plates, followed by aerobic incubation at 35 ± 2˚C for 16–18 h. After incubation, the developed inhibition zone diameters were measured (mm) and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2024) guidelines. The Kirby-Bauer method was carried out using 15 antibiotic discs (Oxoid, UK), including: penicillin (10 µg); ampicillin-sulbactam (10 µg); amoxicillin + clavulanic acid (10 µg); cephalexin (30 µg); cefoxitin (30 µg); imipenem (10 µg); ciprofloxacin (5 µg); tetracycline (30 µg); doxycycline (30 µg); erythromycin (15 µg); gentamycin (10 µg); clindamycin (5 µg); trimethoprim + sulfamethoxazole (12.5 -23.75 µg); linezolid (30 µg) and ceftaroline (30 µg). All *Staph. aureus* isolates that showed resistance to the cefoxitin antibiotic disc (with an inhibition zone ≤ 21mm) were classified as MRSA according to CLSI guidelines (CLSI, 2024). The obtained MRSA isolates were further evaluated for vancomycin susceptibility based on E-test strips (BioMérieux, France). The automated VITEK® 2 Compact system with AST-GP67 cards (bioMérieux, France) was used to determine the minimum inhibitory concentrations (MICs) of the tested antibiotics for paired *Staph. aureus* isolates recovered from pre-operative nasal swabs and postoperative SSIs in those patients with pre-operative nasal carriage.

Detection of endogenous infections

The endogenous origin of SSIs was assessed by comparing bacterial isolates obtained from patients' colonizing sites with those recovered from their SSI, using both phenotypic (antimicrobial susceptibility profiling) and genotypic (PCR-RFLP) methods.

Antibiotic susceptibility profiling

For patients from whom both nasal and surgical site *Staph. aureus* isolates were recovered; paired isolates were analyzed. For each patient, the antimicrobial susceptibility profiling of the nasal isolate was compared with that of the corresponding surgical site isolate. Antimicrobial susceptibility profiles were considered identical when paired isolates demonstrated the same susceptibility category (susceptible, intermediate, or resistant) for all the tested antimicrobial agents (Mehndiratta and Bhalla, 2012). Identical resistance patterns were considered concordant and suggestive of an endogenous source of infection, whereas differing patterns were considered discordant and indicative of an exogenous source of infection. PCR–RFLP analysis was performed to confirm this conclusion.

16S rRNA gene PCR-RFLP

DNA preparation and 16s rRNA PCR amplification

DNA was extracted and purified from each bacterial isolate using GeneJET Kit (Thermo Scientific™, USA) based on the manufacturer's recommended

protocol. Amplification of the 16S rRNA gene was performed using two bacterial primers, namely 27F (forward) and 1429R (reverse). The primer sequences were 5`-AGAGTTTGATCMTGGCTCAG -3` for the forward and 5`-TACGGYTACCTTGTTACGACTT-3` for the reverse (López and Alippi, 2019). The PCR was conducted in a 50 µl reaction volume, containing 25 µl of 2× EmeraldAmp® GT PCR Master Mix, 2 µl of each primer (10 pmol), and 4 µl of template DNA. To complete a 50 µl reaction volume, nuclease-free water was added. The initial denaturation step of the PCR technique was at 95 ˚C for 3 min. A total of 35 cycles was then followed of 95 ˚C for 30 s, 50 ˚C for 30 s, and 72 ˚C for 90 s, with a final extension at 72 ˚C for 10 min using a Bio-Rad T100 thermal cycler (Hercules, CA, USA). Electrophoresis on ethidium bromide (EB)-stained agarose gel was used to visualize the amplified PCR products. Target bands were excised and purified using the Gel Extraction Kit (GeneJET, Thermo Scientific™, USA) prior to RFLP analysis.

Restriction fragment length polymorphism (PCR-RFLP) analysis of the 16S rRNA gene

The genetic relatedness between pre-operative nasal and postoperative *Staph. aureus* isolates were assessed by PCR-RFLP analysis of the *16S rRNA* gene. Briefly, 1,500 bp PCR amplicons were purified to remove residual primers, nucleotides, enzymes, and other reaction components, thereby ensuring high-quality DNA for restriction digestion (Scheidegger *et al*., 2009; Chakraborty *et al*., 2025). Purified PCR products were digested using three restriction enzyme systems: XhoI, EcoRI/BamHI, and SacI/HindIII (New England Biolabs, USA). The paired enzymes were selected based on their compatibility with the same reaction buffer, according to the manufacturer's recommendations to ensure an efficient and complete digestion (Lai *et al*., 2023). To verify the reproducibility of the restriction profiles, each isolate was independently amplified, digested, and analyzed in triplicate under identical experimental conditions. Only banding patterns that were consistently reproduced across all three replicate were considered for subsequent analysis. The resulting restriction fragments were separated by agarose gel electrophoresis, stained with ethidium bromide (EB), and visualized under ultraviolet illumination. A 100 bp DNA ladder was included in each gel to estimate the fragment sizes. Gel images were captured using the XR+ Gel Documentation System (Bio-Rad, USA) and the restriction profiles were compared visually. Isolates exhibiting identical restriction banding patterns across all enzyme systems were considered genetically related, whereas differences in one or more bands were interpreted as distinct PCR-RFLP profiles.

Statistical analysis

Data analysis was performed using SPSS software (Version 28, IBM Corp., USA). The mean and median measures were used to describe the quantitative data. In addition, count (no.) and percent (%) were used to summarize the categorical data. The association between categorical data was assessed using the chi-square (χ^2) test. The strength of the association between pre-operative nasal *Staph. aureus* carriage and SSIs was estimated by calculating the odds ratio with its 95% confidence interval (CI). A statistically significant result was considered when $p < 0.05$.

Results

Demographic and clinical data of the patients

The enrolled patients were 137 females (54.8%) compared to 113 males (45.2%), with an age range of 18 to 77 years, and a mean of 49.78 ± 13.12 (Table 1). Regarding types of operations involved in this study, hernia was the most common with 47 cases (18.8 %), followed by cholecystectomy with 45 cases (18 %), and appendectomy with 33 cases (13.2 %) (Table 2).

Table 1: *Demographic data of the enrolled patients.*

Demographic data		All patients (n=250)	
		Count	%
Sex	Male	113	45.2
	Female	137	54.8
Age (year)	Mean ± SD	49.78 ± 13.12	
	Min-Max	18.0 – 77.0	

Where; SD: Standard deviation; Min: Minimum age; Max: Maximum age.

Microbiological identification of Staphylococcus aureus

Identification of all *Staph. aureus* isolates recovered from nasal and wound swabs was conducted conventionally based on colony morphology, Gram-staining, and biochemical tests, including catalase, coagulase, and DNase (Figure 1).

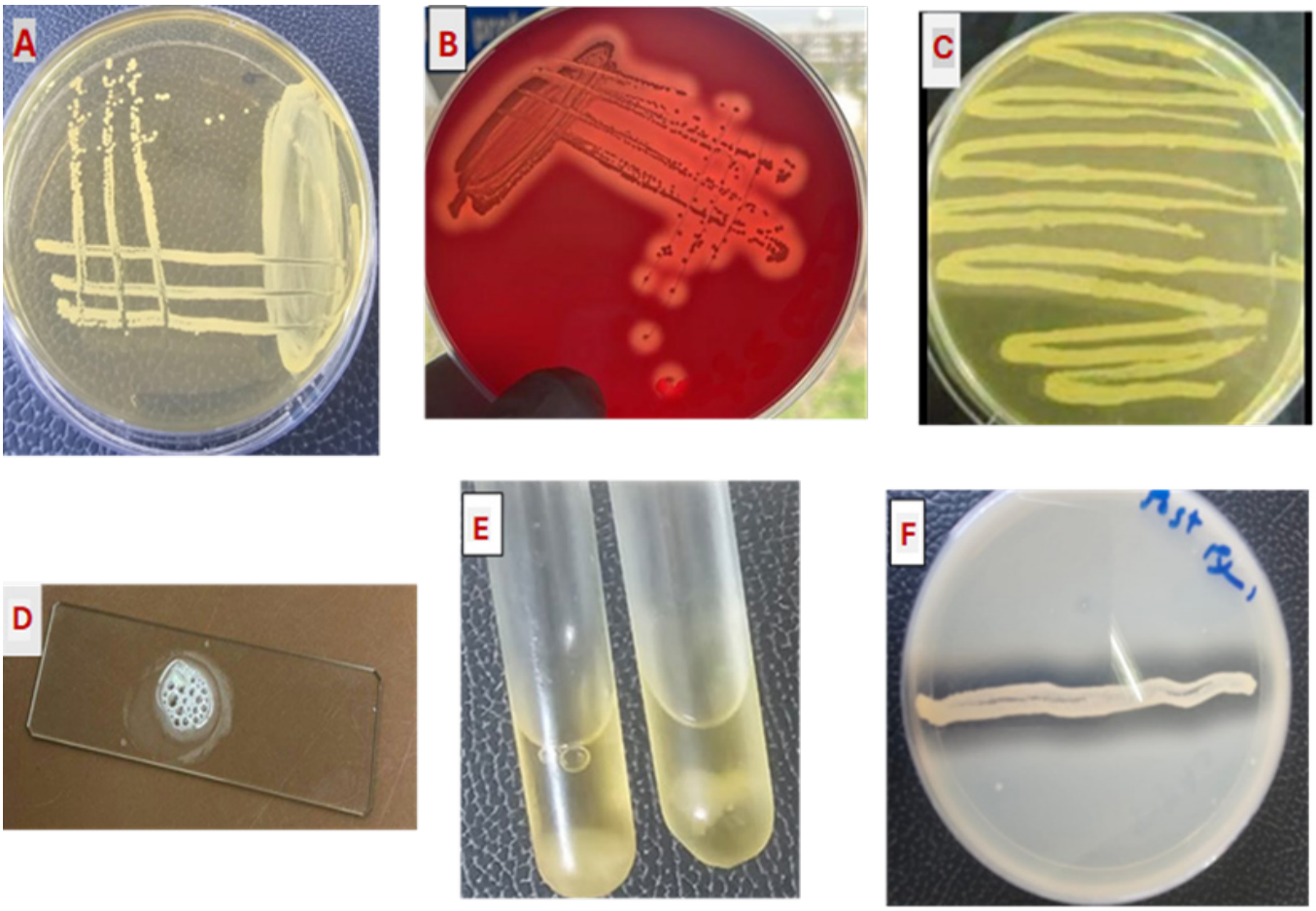

Figure 1: *Conventional identification of Staph. aureus showing: golden yellow colonies on nutrient agar (A), β hemolysis on blood agar (B), yellow colonies on MSA (C), positive catalase, coagulase, and DNase tests (D-F, respectively).*

Table 2: *Types of operations conducted among the study patients.*

Type of operation	Patients (n=250)	
	Count	%
Hernia	47	18.8
Cholecystectomy	45	18.0
Appendectomy	33	13.2
Piles	30	12.0
Bowel surgery	30	12.0
Thyroidectomy	24	9.6
Cyst removal	16	6.4
Plastic surgery	13	5.2
Exploration	5	2.0
Fistula	5	2.0
Mastectomy	2	0.8
Total	250	100

Staphylococcus aureus pre-operative nasal colonization and postoperative surgical site infections

Among the 250 patients included in this study, pre-operative nasal colonization with *Staph. aureus* was detected in 25 (10%) patients. Postoperative SSIs caused by *Staph. aureus* occurred in 11 cases (4.4%). Of these 11 patients, seven were *Staph. aureus* nasal carriers, and four were non-carriers. A statistically significant association between nasal colonization and the development of postoperative surgical site infections was observed (OR= 21.49, 95% CI: 5.75-80.36; $p < 0.001$) (Table 3).

Table 3: *Association between pre-operative Staphylococcus aureus nasal colonization and post-operative Staphylococcus aureus SSIs.*

Nasal colonization (n)	SSI (%)	No. SSI (%)	Odds ratio	95% CI	p value
Positive (n= 25)	7 (28.0)	18 (72.0)	21.49	(5.75-80.36)	$p < 0.001$
Negative (n= 225)	4 (1.8)	221 (98.2)			
Total (250)	11 (4.4)	239 (95.6)			

Where; CI: Confidence interval.

Antibiotic susceptibility pattern of the Staphylococcus aureus isolates

Antibiotic susceptibility testing revealed that all *Staph. aureus* isolates (100%) obtained from both pre-operative nasal swabs and postoperative SSIs were

resistant to penicillin, amoxicillin–clavulanic acid, and ampicillin–sulbactam. On the other hand, all isolates (100%) were fully susceptible to vancomycin and linezolid (Table 4, Figure 2). MRSA was identified in 44% (11/25) of the pre-operative nasal isolates and 36.4% (4/11) of the postoperative wound isolates. MIC determination using the VITEK® 2 Compact system was performed for the paired pre-operative nasal and postoperative *Staph. aureus* isolates. The MIC results of the tested antibiotics are presented in Table 5.

Occurrence of Staphylococcus aureus SSIs and phenotypic detection of endogenous infections

Among the 25 patients who were pre-operatively colonized with *Staph. aureus*, 7 patients (28%) subsequently developed postoperative *Staph. aureus* SSIs. The relationship between the pre-operative nasal colonizing isolates and the postoperative SSI isolates was evaluated using both phenotypic and genotypic methods. Phenotypic matching was performed by comparing the antibiotic susceptibility profiles and revealed that, among the 7 colonized patients who experienced postoperative *Staph. aureus* SSIs, 3 (42.9%) were suspected to be endogenously infected, and all were colonized and infected with MRSA isolates (Figure 3).

Table 4: *Antibiotic resistance profile of nasal and wound Staphylococcus aureus isolates.*

Antibiotic	Nasal isolates (n=25)		Wound isolates (n=11)	
	S	R	S	R
Penicillin	—	25(100%)	—	11(100%)
Cefoxitin*	14 (56%)	11 (44%)*	7 (63.6%)	4 (36.4%)*
Doxycycline	23 (92%)	2 (8%)	11 (100%)	—
Tetracycline	21 (84%)	4 (16%)	9 (82%)	2 (18%)
Cephalexin	11 (44%)	14 (56%)	6 (54.5%)	5 (45.5%)
Imipenem	14 (56%)	11 (44%)	7 (63.6%)	4(36.4%)
Amoxicillin + Clavulanic acid	—	25(100%)	—	11(100%)
Ampicillin Sulbactam	—	25 (100%)	—	11(100%)
Ciprofloxacin	18 (72%)	7 (28%)	9 (81.8%)	2(18.2%)
Erythromycin	15 (60%)	10 (40%)	7 (63.6%)	4 (36.4%)
Gentamicin	16 (64%)	9 (36%)	8 (72%)	3 (28%)
Clindamycin	15 (60%)	10 (40%)	8 (72%)	3 (28%)
Linezolid	25 (100%)	—	11 (100%)	—
Vancomycin	25 (100%)	—	11 (100%)	—
Ceftaroline	19 (76%)	6 (24%)	10 (91%)	1(9%)
Trimethoprim + Sulfamethoxazole	19 (76%)	6 (24%)	9 (81.8%)	2(18.2%)

*Where; S: Sensitive, R: Resistant, *Cefoxitin resistance was used as an indicator of methicillin-resistant Staphylococcus aureus (MRSA) infection.*

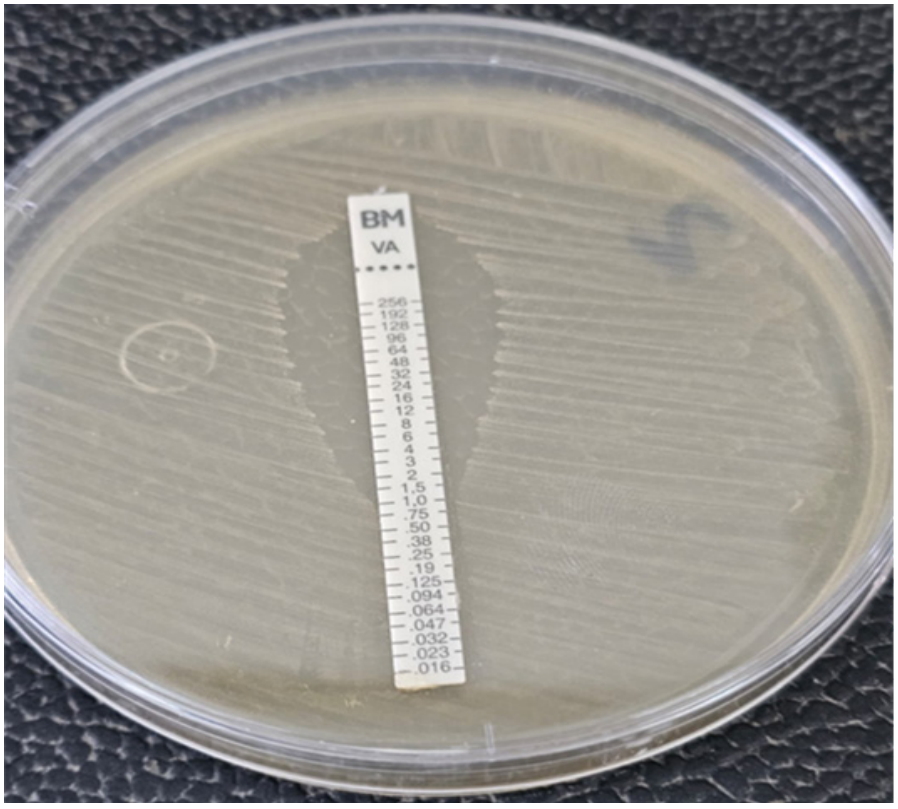

Figure 2: *Vancomycin E-test demonstrating vancomycin-sensitive Staph. aureus (MIC ≤ 2 µg/ml) according to CLSI guidelines.*

Table 5: *Minimum inhibitory concentration (MIC) results of paired pre-operative nasal and postoperative Staphylococcus aureus isolates.*

Antimicrobial agent	Pair 1*		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6		Pair 7	
	Nasal	SSI	Nasal	SSI	Nasal	SSI	Nasal	SSI	Nasal	SSI	Nasal	SSI	Nasal	SSI
Penicillin G	≥0.5	≥0.5	≥0.5	≥0.5	≥0.5	≥0.5	≥0.5	≥0.5	≥0.5	≥0.5	≥0.5	≥0.5	≥0.5	≥0.5
Oxacillin	2	2	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4
Gentamicin	≥16	≥16	8	8	8	8	≤0.5	8	≥16	≤0.5	≤0.5	8	≤0.5	8
Ciprofloxacin	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Levofloxacin	0.25	0.25	0.25	0.25	≤0.12	≤0.12	0.5	≤0.12	≤0.12	0.25	0.25	≤0.12	0.5	≤0.12
Moxifloxacin	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	1	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	1	≤0.25
Erythromycin	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Clindamycin	≤0.25	≤0.25	≤0.25	≤0.25	4	4	≥8	4	≤0.25	4	≤0.25	4	≥8	4
Quinupristin/ Dalfopristin	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	1	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	1	≤0.25
Linezolid	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Vancomycin	1	1	1	1	2	2	1	2	1	1	2	1	2	1
Tetracycline	≥16	≥16	≤1	≤1	≥16	≥16	≤1	≥16	≥16	≤1	≤1	≥16	≤1	≥16
Tigecycline	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12
Nitrofurantoin	≤16	≤16	≤16	≤16	≤16	≤16	≤16	≤16	≤16	≤16	≤16	≤16	≤16	≤16
Rifampicin	≤0.5	≤0.5	≤0.5	≤0.5	1	1	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Trimethoprim/ Sulfamethoxazole	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10

*Where; *Each pair represents a pre-operative nasal isolate and its corresponding postoperative surgical site infection (SSI) isolate from the same patient.*

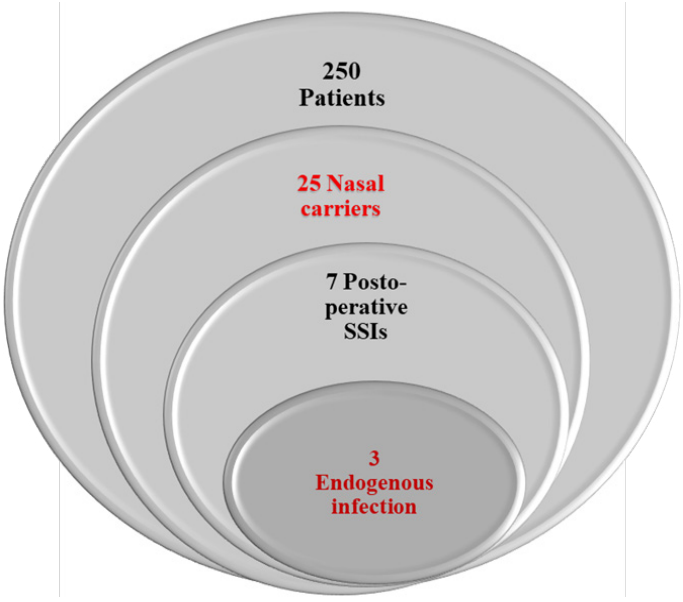

Figure 3: *Concentric circle diagram illustrating the stepwise stratification of the study population. The outermost circle represents the total number of recruited patients (n =250). The second circle shows patients colonized by Staph. aureus (n = 25, 10% of total patients). The third circle displays colonized patients who had postoperative Staph. aureus SSIs (n=7). The innermost circle demonstrates patients who acquired endogenous SSIs (n = 3; 42.9% of colonized patients with SSIs).*

Molecular evidence of endogenous infection

Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis of the *16S rRNA* gene was performed on three paired *Staph. aureus* isolates obtained pre-operatively (pre) and postoperatively (post) from three patients suspected of having endogenous infections. The molecular findings are limited to the three paired isolates analyzed, which may limit the broader generalizability of the findings. Initially, a single PCR product of approximately 1500 bp was successfully amplified from all the six isolates, confirming amplification of the target region. The amplicons were then subjected to restriction digestion using the pre-mentioned endonucleases.

As illustrated in Figure 4, digestion with XhoI produced identical patterns in the pre- and post-operative isolates from each patient. No digestion was observed in the isolates obtained from patients 1 and 2, where a single undigested band of approximately 1500 bp remained. In contrast, the pre- and post-operative isolates from patient 3 showed XhoI digestion fragments of approximately 1300 bp and 200 bp (Figure 5).

Digestion with the combined restriction enzymes

SacI and HindIII generated consistent and clearly distinguishable profiles among the tested isolates. In patients 1 and 2, both pre-operative and postoperative isolates exhibited identical restriction patterns, consisting of two fragments of approximately 1100 bp and 400 bp, indicating conserved restriction sites within the amplified *16S rRNA* gene regions (Figure 4). A similar digestion pattern was also observed between the pre- and postoperative isolates from patient 3 (Figure 5).

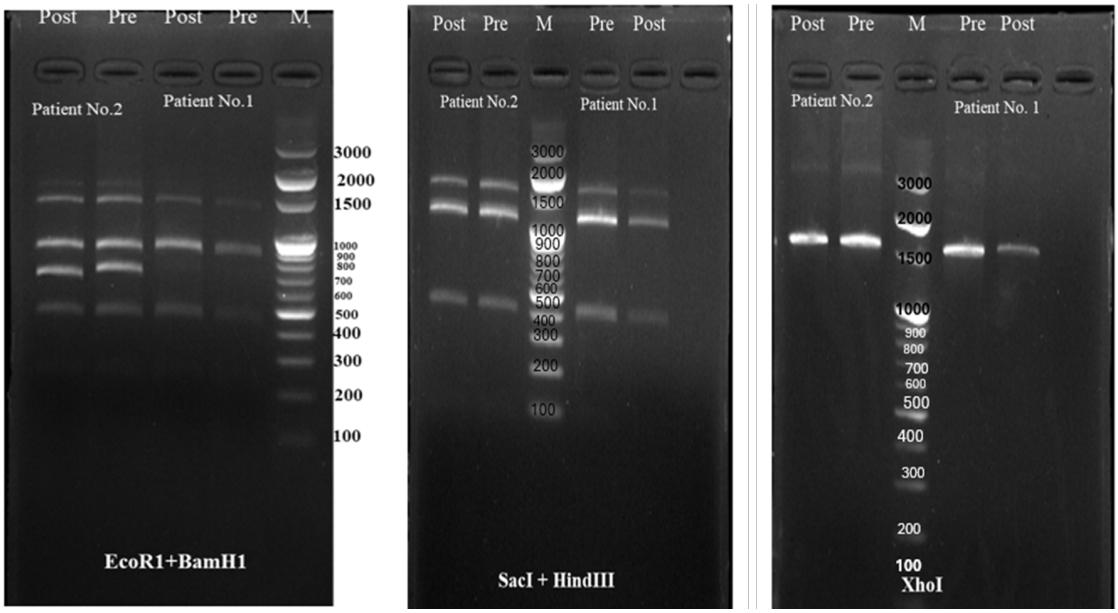

Figure 4: *PCR–RFLP patterns of the 16S rRNA gene from the pre-operative and post-operative isolates obtained from two patients following digestion with restriction enzymes XhoI, SacI, HindIII, EcoRI, and BamHI. M indicates the 100 bp DNA ladder (Enzynomics, USA) used as a molecular size marker. DNA ladder fragment sizes (bp) are indicated on the right side of the ladder.*

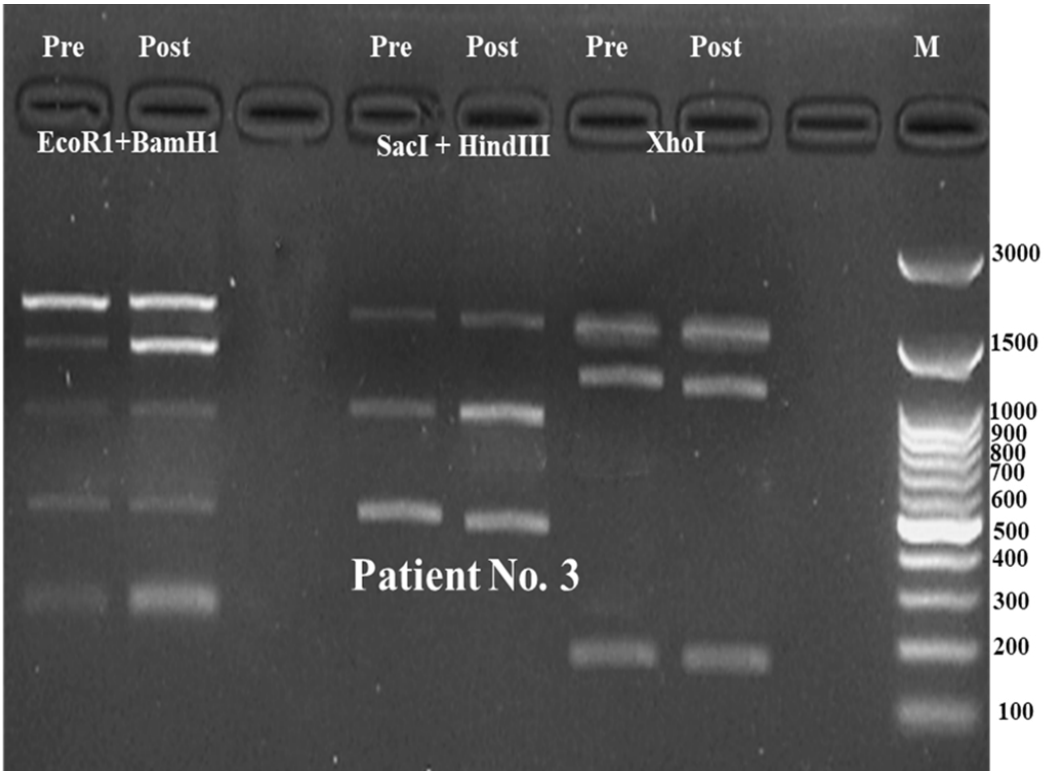

Figure 5: *PCR–RFLP patterns of the 16S rRNA gene from the pre-operative and post-operative isolates obtained from the third patient following digestion with restriction enzymes XhoI, SacI, HindIII, EcoRI, and BamHI. M indicates 100 bp DNA ladder (H3 RTU; GeneDirex Inc., Las Vegas, USA). DNA ladder fragment sizes (bp) are indicated on the right side of the ladder.*

A clear and distinguishable digestion pattern was observed following treatment with the combined restriction enzymes EcoRI and BamHI. This pattern revealed the molecular variation within the *16S rRNA* gene fragments among the isolates obtained from the three patients. For each patient, the pre- and post-samples displayed identical digestion profiles, indicating stability within the individual pairs; however, distinct patterns were observed between the different patients. In patient one, the restriction pattern revealed two resolved bands of 1000 bp and 500 bp. In patient two, a similar pattern was observed, with the addition of an 800 bp band, while a faint 700 bp band was also detected but was not clearly visible under the UV transillumination (Figure 4). In patient three, both pre- and post-samples shared the same digestion profile, which consisted of two bands at 1000 bp and 500 bp, along with two additional bands at 1200 bp and 300 bp (Figure 5). Overall, these results demonstrated an inter-patient variation in the restriction profiles while maintaining consistent intra-patient patterns between the pre- and post-isolates.

Discussion

Surgical Site Infection represents a remarkable cause of morbidity and mortality in patients undergoing all types of operations (Gomaa *et al*., 2021). This cross-sectional study was conducted on 250 patients admitted to the surgery department for elective surgeries at Al-Zahraa University Hospital to evaluate the prevalence of *Staph. aureus* nasal carriage and to assess its association with the development of endogenous SSIs. Our study revealed that the prevalence of *Staph. aureus* pre-operative nasal colonization was 10% (25/250), which was lower than that reported in several previous studies. For example, Kapoor *et al*. (2014) in the USA, Bouza *et al*. (2020) in Spain, and Gallouche *et al*. (2026) in France, reported higher prevalence of *Staph. aureus* pre-operative nasal colonization at the rates of 29.5%, 28.5%, and 29.4%, respectively. The lower prevalence observed in this study may be attributed to differences in patient demographics, sample size, local epidemiology, and prior antibiotic exposure.

In the current study, all *Staph. aureus* isolates obtained from both pre-operative nasal swabs and postoperative SSIs were 100% resistant to penicillin, amoxicillin–clavulanic acid, and ampicillin–sulbactam. Conversely, all isolates were fully susceptible to linezolid and vancomycin (100%). Similar results were reported by another study that showed 100% resistance to penicillin and 100% sensitivity to vancomycin (Tashakori *et al*., 2014). In addition, a previous study conducted in India reported that all *Staph. aureus* isolates obtained from postoperative patients showed 100% sensitivity to linezolid (Singh *et al*., 2025).

In the present study, MRSA was detected in 44% (11/25) of nasal colonized patients. Meanwhile, another study conducted in Mogadishu reported a MRSA nasal carriage of 51.87% (222/428) (Hassan *et al*., 2026). In addition, a much higher rate was shown by another Egyptian study that reported that MRSA was detected in 62.1% (59/95) of the nasal *Staph. aureus* isolates obtained from both healthcare workers and community members in Minia City (Mohamed *et al*., 2025). A much lower rate was displayed by a study that reported that MRSA was detected in 1.1% (7/122) of patients with *Staph. aureus* nasal colonization (Panhotra *et al*., 2005).

In this study, 28% of the colonized patients (7/25) developed postoperative SSIs, showing a statistically significant association between nasal colonization and the development of surgical site infections ($p < 0.001$). Among the seven colonized patients who subsequently developed postoperative *Staph. aureus* SSIs, 3 (42.9%) were infected by MRSA isolates that matched their nasal isolates, which was confirmed by both antibiotic susceptibility profiling and PCR-RFLP. These findings support the role of pre-operative nasal carriage of *Staph. aureus*, particularly by MRSA, as a source of endogenous SSIs. Several previous studies have reported similar findings, indicating that *Staph. aureus* nasal carriers, particularly MRSA carriers, are at an increased risk of SSIs (Lu *et al*., 2023; Ahmann *et al*., 2025). In 2020, a meta-analysis study demonstrated that MRSA nasal carriers have a significantly higher risk of SSIs than non-carriers ($p < 0.001$) (Ning *et al*., 2020). In addition, a 2023 meta-analysis examined the association between *Staph. aureus* or MRSA nasal colonization and the risk of SSI following spinal surgeries. The obtained results revealed that the SSI rate was not significantly different between the *Staph. aureus* carriers and the non-carrier patients (p=0.21), but only MRSA nasal carriage increased the risk of SSI (p=0.08) (Lu *et al*., 2023).

In the present study, the endogenous origin of SSIs was investigated through a stepwise approach combining phenotypic and molecular methods. Initial screening was performed using *in vitro* antimicrobial susceptibility profiling to assess the similarity between nasal and wound isolates. This was followed by PCR–RFLP analysis of the *16S rRNA* gene, which provided complementary molecular evidence for assessing the relatedness of the isolates recovered from the same patient. Although this combined approach strengthened the preliminary assessment of the isolate similarity, it was not intended to replace the high-resolution molecular epidemiological typing methods.

The PCR-RFLP targeting the 16S rRNA gene has been used as a rapid and economical molecular approach for differentiating *Staphylococcus* isolates, particularly at the intra- and interspecies levels (Lai *et al*., 2023). The method combines amplification of the 16S rRNA gene with restriction enzyme digestion to generate banding patterns that reflect the underlying sequence polymorphisms within the amplified region (Scheidegger *et al*., 2009). Compared to DNA sequencing, PCR-RFLP is technically simple, requires only conventional PCR and gel electrophoresis, and can be implemented without sequencing platforms or advanced bioinformatics infrastructure, making it particularly attractive for routine laboratories and resource-limited settings (Aggarwal *et al*., 2020). Nevertheless, the discriminatory power of 16S rRNA PCR-RFLP is inherently constrained by the highly conserved nature of the 16S rRNA gene. Consequently, the genetically distinct isolates may occasionally produce identical restriction profiles, limiting their ability to resolve the closely related strains or accurately reconstruct the transmission events. Therefore, PCR-RFLP should be regarded as a cost-effective preliminary typing tool rather than a substitute for modern molecular epidemiological approaches such as *spa* typing, MLST, or WGS, which provide substantially higher discriminatory power for outbreak investigations and transmission analysis.

The restriction enzyme digestion exposes the polymorphic sites within the amplified region, producing fragment profiles that may assist in differentiating bacterial isolates. Previous studies have demonstrated that RFLP analysis of the *16S rRNA* gene or the 16S-ITS region_had successfully differentiated the multiple *Staphylococcus* species and the generated strain-specific haplotypes (Sudagidan *et al*., 2005). In the present study, digestion with XhoI, SacI/HindIII, and EcoRI/BamHI generated reproducible restriction profiles, with the EcoRI/BamHI combination providing the greatest apparent discrimination among the analyzed isolates, particularly between the pre-operative and postoperative isolates recovered from the third patient. However, because only a limited number of paired isolates were subjected to molecular characterization, these findings should be interpreted cautiously and cannot be generalized to broader *Staph. aureus* populations. Thus, further detailed studies are required to determine the reproducibility and discriminatory performance of these enzyme combinations across the genetically diverse clinical isolates.

Our findings are consistent with a previous study has shown that PCR-RFLP of the clinical *Staph. aureus* isolates can generate reproducible restriction patterns that may support epidemiological investigations (Hookey *et al*., 1998). Similar PCR-RFLP approaches have also been successfully applied to the coagulase gene for differentiating *Staph. aureus* subspecies (Hookey *et al*., 1998). Furthermore, PCR-RFLP has shown a diagnostic value in culture-negative infections by detecting bacterial DNA even after prior antibiotic exposure (Rohit *et al*., 2016). For example, in neonatal sepsis, PCR-RFLP targeting the 16S rRNA gene identified pathogens, including *Staph. aureus*, more frequently than conventional culture methods (Rohit *et al*., 2016), highlighting its potential utility when viable microorganisms are difficult to recover. However, these applications should be viewed as complementary to, rather than replacements for, high-resolution genomic typing methods when precise epidemiological discrimination is required. Although variability in restriction profiles may facilitate differentiation among the bacterial isolates and provide useful epidemiological information (Figueras *et al*., 2012; Liu *et al*., 2016), the level of discrimination achieved by 16S rRNA PCR-RFLP remains lower than that provided by *spa* typing, MLST, or WGS. Consequently, the observed similarity between nasal and wound isolates in this study should be interpreted as supportive rather than definitive evidence of endogenous transmission.

Limitations

Although this study was conducted at a single healthcare center and molecular characterization was

performed on only three paired isolates, the combined phenotypic and molecular approach provided supportive evidence for assessing the isolate relatedness within the study setting. In this study, the perioperative variables known to influence the risk of SSIs were not evaluated; therefore, their potential confounding effects could not be assessed. While 16S rRNA PCR-RFLP offered a practical and a cost-effective molecular typing approach, its discriminatory capacity was lower than that of high-resolution methods such as spa typing, MLST, and WGS. Consequently, the observed similarity between the nasal and wound isolates should be interpreted as supportive rather than definitive evidence of endogenous transmission. Future multicenter studies including larger numbers of paired isolates and complementary high-resolution molecular typing methods would further strengthen and extend the present findings.

Conclusions and Recommendations

This study highlights the potential contribution of the pre-operative nasal colonization, particularly with methicillin-resistant *Staphylococcus aureus* (MRSA) to the endogenous postoperative surgical site infections (SSIs). The complete susceptibility of the recovered isolates to linezolid and vancomycin, along with the observed resistance to penicillin, amoxicillin-clavulanic acid, and ampicillin-sulbactam, underscores the importance of continuous local antimicrobial surveillance to support an appropriate empirical therapy. The current findings also support the implementation of infection prevention strategies, including targeted screening and, where appropriate, nasal MRSA decolonization, to help reduce the risk of postoperative SSIs. In addition, 16S rRNA PCR-RFLP provided a rapid and a cost-effective approach for the preliminary assessment of genetic relatedness among the analyzed *Staph. aureus* isolates, complementing the conventional phenotypic characterization. Although the 16S rRNA PCR-RFLP demonstrated its applicability within the present study, further validation in larger, multicenter cohorts, and direct comparison with high-resolution molecular typing methods, such as *spa* typing, MLST, or WGS is recommended to further establish its epidemiological utility.

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Novelty Statement

To assess the potential relationship between *Staph. aureus* nasal carriage and endogenous SSIs, this study provided a low-cost combined phenotypic approach and 16S rRNA PCR-RFLP typing. In contrast to several previous studies that relied on high-resolution, more expensive molecular techniques such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *spa* typing, and WGS, this study explored the availability of simpler, more affordable methods for a preliminary epidemiological assessment under a resource-limited setting. In addition, the possibility of an endogenous origin of SSIs was assessed by analyzing the paired nasal and wound isolates, an area that is underreported in the local context.

Authors' Contributions

AHE: Investigation, data curation, formal analysis, writing–original draft. SEA: Conceptualization, methodology, supervision, writing–review and editing. GME: Investigation, data curation, formal analysis, writing–original draft. SMA: Conceptualization, methodology, supervision, validation, writing–review and editing. All authors have read and approved the final version of the manuscript.

Ethical approval
The Institutional Research Ethics Committee of the Faculty of Medicine, Al-Azhar University, reviewed and approved the study protocol with an approval number 2024072432. Written consent was provided by the participating patients.

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Generative AI and AI-assisted technology statement
The authors declare that neither generative AI nor AI-assisted technology has been used in this study.

Conflict of interests
The authors declare that they have no conflicts of interest.

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