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# Isolation and Characterization of Bacteria Isolated from Wound Infection

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**ABSTRACT**

Wound infections continue to pose a major public health challenge in many low- and middle-income countries, where delays in diagnosis and limited laboratory capacity contribute to preventable morbidity. Accurate characterization of the bacterial agents involved is essential for effective management; yet, routine diagnostics in many settings rely solely on phenotypic methods that may overlook emerging or clinically significant species. This study investigated the bacteriological profile of wound infections from a tertiary hospital in Southeastern Nigeria. A total of fifty patients presenting with traumatic (n = 13), surgical (n = 10), burn (n = 10), infected (n = 10), and diabetic foot wounds (n = 7) were recruited, of which 30% were male and 70% were female. The wound samples were analyzed for bacteria isolates usingstandard microbiological culture methods, biochemical, and PCR techniques for molecular identification. A total of six bacteria isolates were recovered, which includes *Staphylococcus aureus* as the predominant organism (34%), followed by *Klebsiella* spp. (22%), *Enterococcus* spp. (18%), *Escherichia coli* (10%), *Salmonella* spp. (8%), and *Proteus* spp. (4%). Traumatic and infected wounds yielded the highest number of isolates, which includes *Staphylococcus aureus*, *Klebsiella* spp., *Enterococcus* spp., *Escherichia coli*, *Salmonella* spp., and *Proteus* spp., while the least number of isolates were recovered from diabetic foot wounds and includes *Staphylococcus aureus*, *Klebsiella* spp., *Enterococcus* spp., and *Escherichia coli*. This study has highlighted some of the bacterial isolates normally associated with different wound infections, and this will assist in strengthening the epidemiological baseline for future surveillance, infection control planning, and antimicrobial stewardship initiatives.

***Keywords:*** wound infection, identification, characterization, and bacteria

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## 1. INTRODUCTION

Wound infections remain a significant clinical and public health concern because once the integrity of the skin is disrupted, microorganisms can infiltrate underlying tissues and initiate complex inflammatory processes. These infections contribute to prolonged hospital admission, increased healthcare expenditure, delayed wound healing, heightened morbidity and mortality. Both chronic and acute wounds, including surgical site infections (SSIs), traumatic injuries, burns, and pressure ulcers, are increasingly recognized as part of a growing global burden. Recent estimates place the pooled global incidence of SSIs at approximately 2.5% (95% CI: 1.6–3.7%), with substantially higher rates, reaching up to 7.2%, reported in parts of Africa (Mengistu *et al.,* 2023; Zuniga *et al.,* 2025). Even in high-income countries, an estimated 2% of the population may be affected by chronic non-healing wounds at any given time (Sen, 2021). The burden is more pronounced in low- and middle-income countries, where overcrowded facilities, inadequate infection control measures, and limited diagnostic capacity contribute to the persistence and spread of wound-associated microorganisms (WHO, 2023; Rezaei *et al.,* 2025). Fundamentally, wound infections arise when pathogenic microbes penetrate the protective skin barrier and proliferate within compromised tissues, triggering localized or systemic inflammatory responses (Agyapong *et al.,* 2023).

The microbial landscape of wound infections is diverse, dynamic, and often polymicrobial. Commonly implicated organisms include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus* spp., and various *Streptococcus* species (Bessa et *al.,* 2022; Puca et *al.,* 2021; Ibraheem et *al.,* 2025). These bacteria possess virulence traits that enhance their ability to persist within the wound environment, such as the production of enzymes, toxins, and adhesins that facilitate tissue invasion and survival. A particularly important feature of many wound pathogens is their capacity to form biofilms structured microbial communities encased in extracellular polymeric substances. Biofilms protect bacteria from environmental stressors, host immune responses, and physical clearance mechanisms, enabling them to establish long-lasting infections and significantly delay wound healing (Siddiqui and Bernstein, 2010; Guerra *et al.,* 2022).

The microbial composition of wounds varies according to several factors, including the type and depth of the wound, the duration before presentation, underlying host conditions such as diabetes and vascular insufficiency, and environmental exposure within healthcare settings (Yao *et al.,* 2020; Iyun et *al.,* 2024). These variations emphasize the need for precise bacteriological investigation, as the organisms present and their interactions with one another—directly influence the clinical course of the infection.

Given this complexity, the accurate isolation and characterization of bacteria from wound infections is essential for understanding the microbial ecology of wounds and supporting effective clinical management. Traditional bacteriological techniques, including microscopy, culture, and biochemical testing, remain fundamental for identifying pathogenic species. However, phenotypic similarities between closely related organisms, particularly within groups such as the *Klebsiella pneumoniae* species complex, can sometimes lead to diagnostic uncertainty. To address these limitations, recent studies recommend integrating phenotypic approaches with molecular tools, such as 16S rRNA sequencing, to achieve precise species-level identification, improve surveillance, and support clearer epidemiological mapping of wound pathogens (Reddy *et al.,* 2024; Guerra *et al.,* 2022).

Accurate knowledge of the local bacterial profile of wound infections is essential for diagnosis, wound-care planning, and the development of evidence-based infection-control measures. This study, therefore, focuses on isolating and characterizing bacteria associated with wound infections in a tertiary healthcare setting.

## 2. MATERIALS AND METHODS

### Study Design and Setting

This study employed a cross-sectional, laboratory-based design and was conducted at the Federal Medical Centre (FMC), Owerri, Imo State, Nigeria. FMC is a major tertiary referral hospital serving both urban and peri-urban communities across southeastern Nigeria. All microbiological procedures were carried out in the Department of Microbiology laboratory under standard quality-control and biosafety conditions.

### Sample Population and Eligibility Criteria

The study population consist of patients presenting with clinically diagnosed wound infections, including traumatic wounds, diabetic ulcers, burns, pressure sores, and post-surgical wounds. Only patients who had not received systemic antimicrobial therapy within the previous 72 hours were eligible. Individuals on active antimicrobial treatment or those who declined consent were excluded from the study.

### Sample Size and Sampling Technique

A total of 50 wound swab samples were collected. A consecutive sampling strategy was adopted, ensuring that all eligible and consenting patients who presented within the study period were included.

### Sample Collection and Transport

Wound exudates were collected aseptically using sterile cotton swabs. Before sampling, superficial contaminants were gently removed by cleansing the wound surface with sterile normal saline. Each swab was immediately placed into Amies transport medium and transported to the laboratory within 30 minutes for prompt microbiological processing.

### Culture and Isolation of Bacteria

Each specimen was inoculated onto blood agar, MacConkey agar, Mannitol Salt Agar (MSA), chocolate agar, Salmonella–Shigella (SS) agar, and nutrient agar. All culture media (Oxoid, UK) were prepared and quality-checked according to manufacturer instructions. Plates were incubated aerobically at 37°C for 18–24 hours. Chocolate agar plates were incubated in a $CO_2$-enriched atmosphere (5–10%). Distinct colonies were purified by sub-culturing to obtain discrete isolates for characterization.

### Phenotypic Identification of Isolates

Bacterial identification was performed based on colony morphology, Gram staining, and conventional biochemical tests following CLSI M35-A2 (2024) and standard diagnostic procedures: catalase, coagulase, citrate utilization, indole production, oxidase, Triple Sugar Iron (TSI), motility, and MR–VP tests. Interpretation of results was based on established biochemical profiles for clinically relevant bacteria. The most prevalent organisms were selected for molecular characterization.

**Molecular Identification of Bacterial Isolates**

**Genomic DNA Extraction**

Genomic DNA was extracted from pure cultures using the Zymo Research Fungal/Bacterial DNA MiniPrep Kit (Inqaba Biotech, South Africa) following the manufacturer's protocol. DNA was eluted in 100 µL and stored at –20°C.

**DNA Quality and Quantification**

DNA purity and concentration were assessed using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). Samples with A260/280 ratios of 1.7–2.0 were accepted for downstream analysis.

**PCR Amplification of 16S rRNA Gene**

Partial 16S rRNA gene amplification was carried out to confirm bacterial identity. Universal primers used were: **27F:** 5′-AGAGTTTGATCMTGGCTCAG-3′ and **1492R:** 5′-TACGGYTACCTTGTTACGACTT-3′. PCR reactions (25 µL) contained 12.5 µL of 2× Master Mix, 0.5 µM of each primer, and 5 µL of DNA template.

**Thermal cycling conditions:**

Initial denaturation: 95°C for 5 min; cycles of: 95°C for 30 s, 55°C for 30 s and 72°C for 1 min.

Final extension: 72°C for 7 min. PCR products were visualized on 1.5% agarose gel stained with ethidium bromide.

**Sequencing and Bioinformatics Analysis**

Amplicons were purified and sequenced bidirectionally. Resulting sequences were edited using BioEdit v7.2 and compared with NCBI GenBank entries using BLASTn. Species identity was assigned based on ≥98.5% sequence similarity. Sequences were deposited in GenBank and assigned accession numbers.

## 3. RESULTS

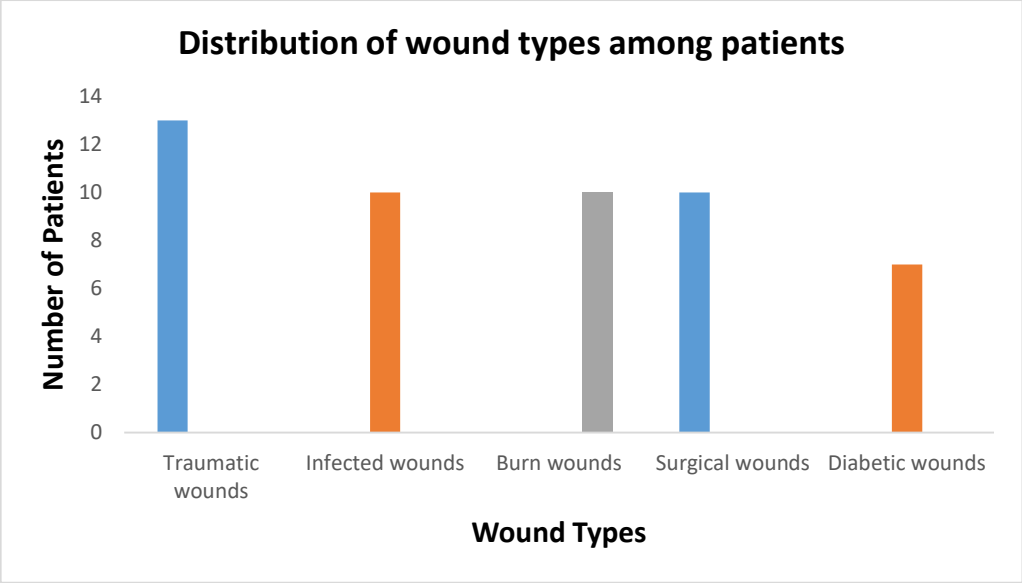


**Figure 1.** Distribution of Wound Types Among Patients.

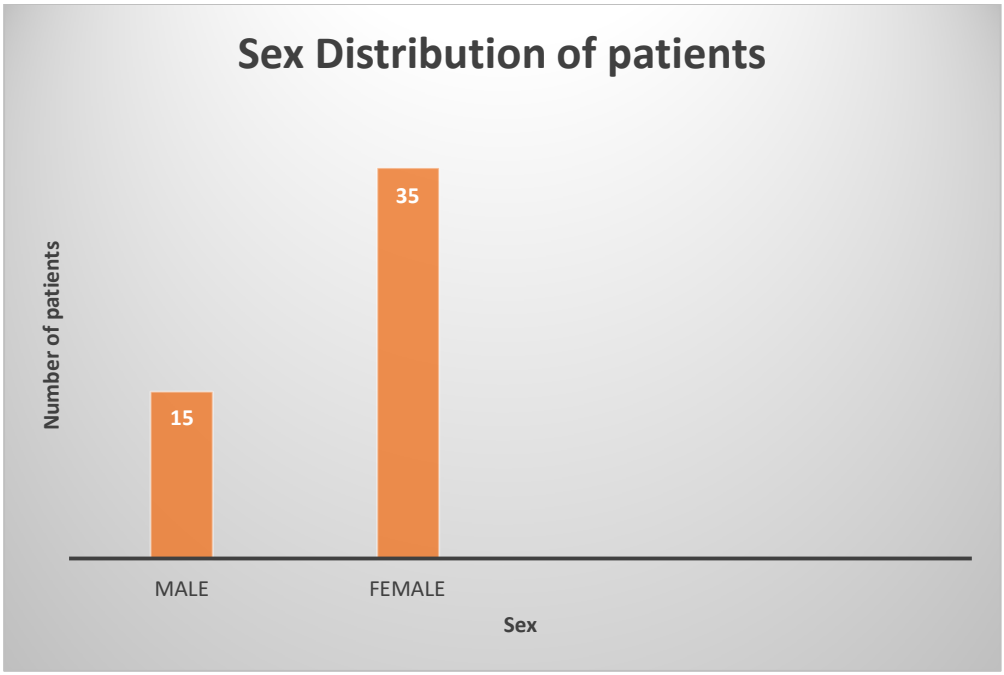


**Figure 2.** Sex Distribution of Patients.

**Table 1.** Colonial and Morphological Characteristics of Bacterial Isolates.

| Isolate | Colonial, Morphological Characteristics |
|---|---|
| **1** | Pink, Raised growth, Smooth and dry |
| **2** | Black, Raised growth and Smooth, |
| **3** | Pink, Raised growth and Slimy |
| **4** | Pink, Irregular and Smooth |
| **5** | Golden, Circular and Smooth |
| **6** | Creamy white, Circular and Smooth |

**Table 2.** Morphological and Biochemical Characteristics of Isolated Bacteria.

| Isolate | Gram | Arrangement | Motility | Cat | Cit | Ind | Oxi | Coag | VP | MR | Glu | Lac | Suc | Probable Organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | – | Rod | + | + | – | + | – | – | – | + | + | + | + | *Escherichia coli* |
| 2 | – | Rod | + | + | + | + | – | – | – | + | – | – | – | *Salmonella* spp. |
| 3 | – | Rod | + | + | + | – | – | – | + | – | + | + | + | *Klebsiella* spp. |
| 4 | – | Rod | + | + | + | – | – | – | + | – | – | – | – | *Proteus* spp. |
| 5 | + | Cocci | – | + | – | – | – | + | + | – | + | + | + | *Staphylococcus aureus* |
| 6 | + | Pairs | – | – | – | – | – | – | + | – | + | + | + | *Enterococcus* spp. |

**Key Cat- Catalase, Cit- Citrate, Ind- Indole, Oxi- Oxidase, Coag- Coagulase, VP- Voges Proskauer, MR- Methyl Red, Glu- Glucose, Lac- Lactose, Suc- Sucrose**

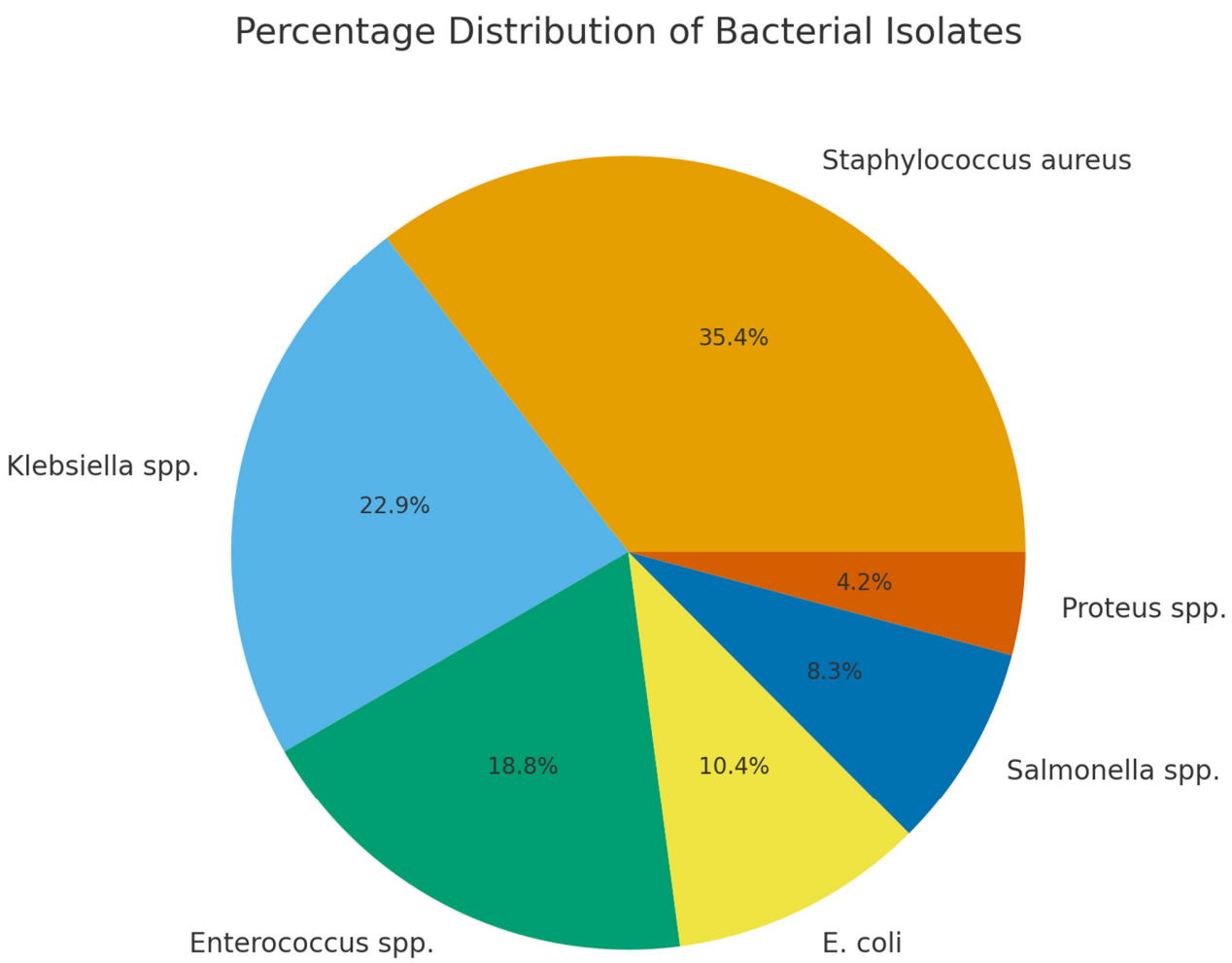


**Figure 3.** Percentage Distribution of Bacterial Isolates.

**Table 3.** Prevalence of Bacterial Isolates According to Wound Types.

| **Organisms** | **Traumatic Wounds (n=13)** | **Surgical Wounds (n=10)** | **Burn Wounds (n=10)** | **Infected Wounds (n=10)** | **Diabetic Foot Wounds (n=7)** | **Total (n)** | **Prevalence (%)** |
|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | 5 | 4 | 3 | 3 | 2 | 17 | 34.0% |
| Klebsiella spp. | 3 | 2 | 3 | 2 | 1 | 11 | 22.0% |
| Enterococcus spp. | 2 | 2 | 2 | 2 | 1 | 9 | 18.0% |
| *Escherichia coli* | 1 | 1 | 1 | 1 | 1 | 5 | 10.0% |
| Salmonella spp. | 1 | 1 | 1 | 1 | – | 4 | 8.0% |
| Proteus spp. | 1 | – | – | 1 | – | 2 | 4.0% |

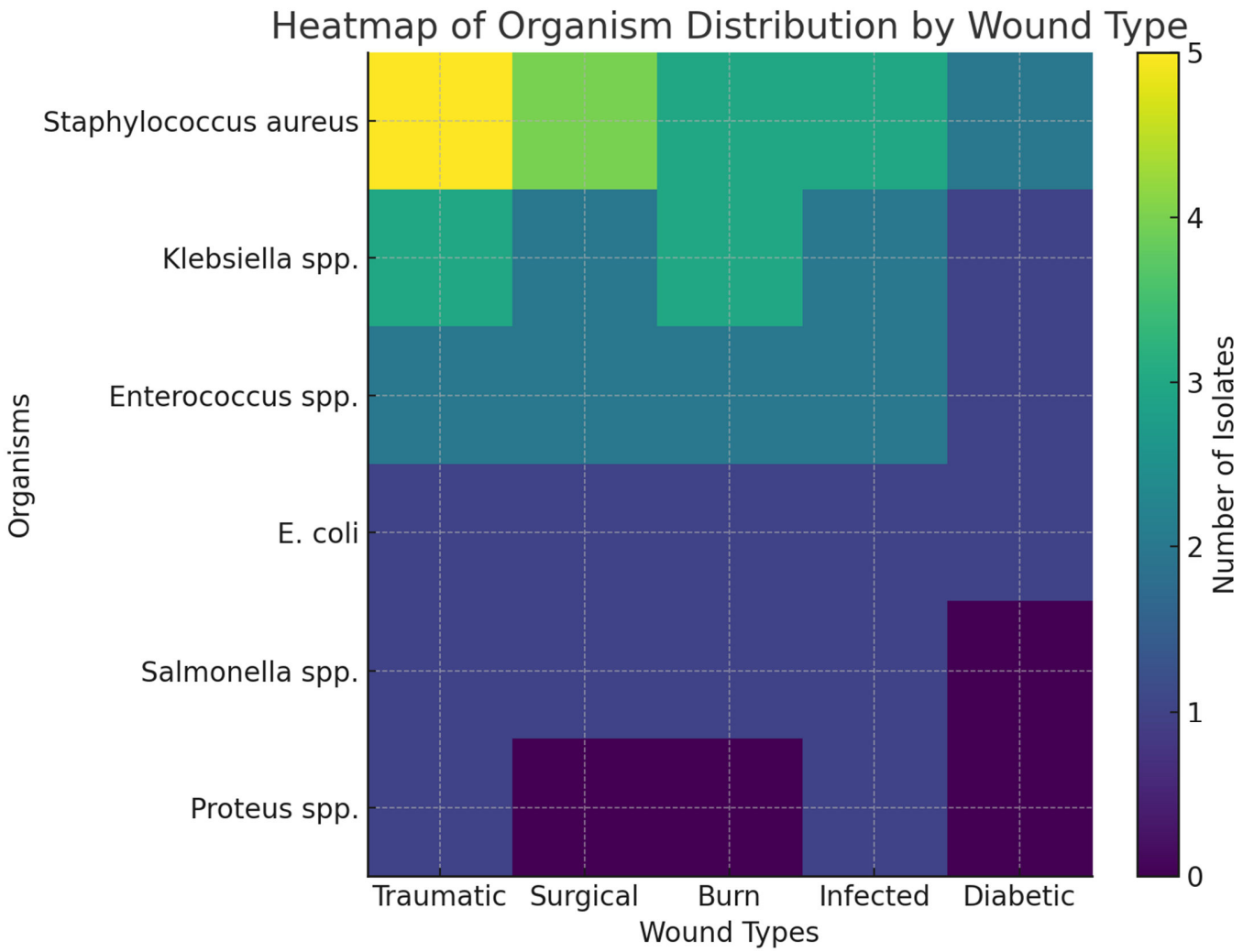


**Figure 4.** Heat map of Organism Distribution by Wound Type.

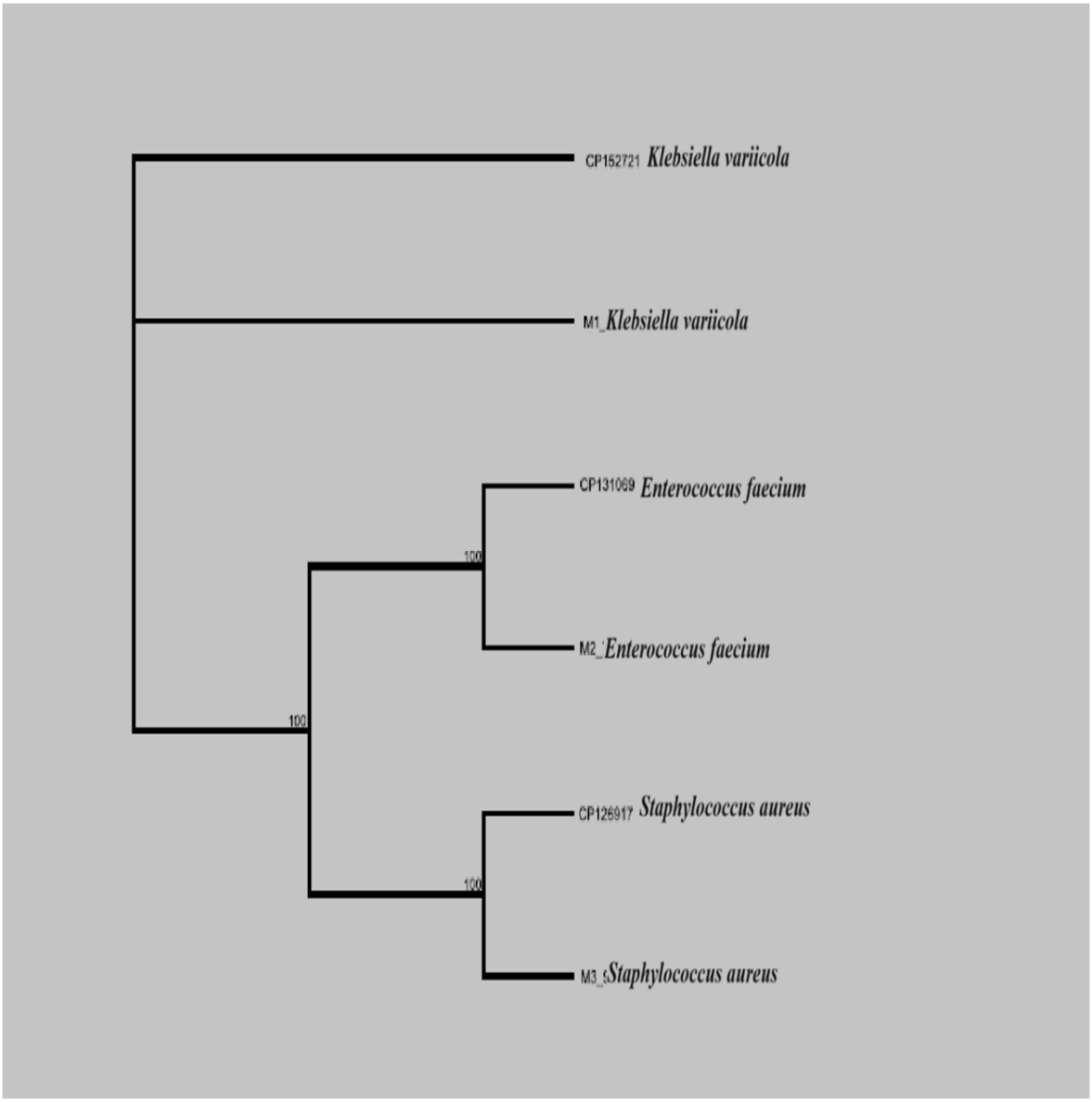


**Figure 6.** Molecular characterization of selected isolates.

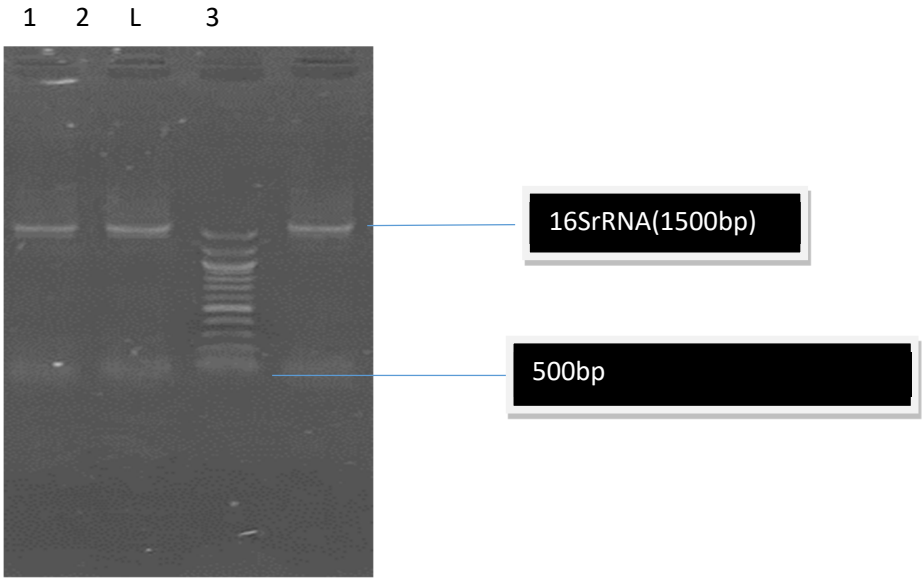


**Figure 7.** Agarose gel electrophoresis showing the amplified 16srRNA. Lanes 1-3 represent the amplified 16srRNA at 1500bp while lane L represents the 100bp DNA ladder**.**

## 4. DISCUSSION

This study provides an updated picture of the bacteriology of wound infections in a tertiary hospital in Southeastern Nigeria, combining classical culture techniques with molecular identification of key isolates. The organisms recovered *Staphylococcus aureus*, *Klebsiella* spp., *Enterococcus* spp., *Escherichia coli*, *Proteus* spp., and *Salmonella* spp. correspond with pathogens widely implicated in wound, skin, and soft-tissue infections globally, particularly within low- and middle-income countries where such infections contribute substantially to morbidity (Linz *et al.,* 2023; Bandy *et al.,* 2022).

Traumatic wounds constituted the largest proportion of samples, followed by surgical, burn, and clinically infected wounds, whereas diabetic foot wounds accounted for the smallest group. This distribution mirrors patterns observed in African hospitals, where trauma and post-operative wounds dominate clinical presentations due to high rates of road traffic injuries, domestic accidents, and surgical interventions (Oladeinde et *al.,* 2013; Bandy *et al.,* 2022). The lower number of diabetic wounds in the present study likely reflects institutional case mix rather than a genuinely reduced prevalence, as diabetic foot ulcers remain well-documented contributors to chronic wound burden in Nigeria and other African settings (Onwuezobe *et al.,* 2020).

Females accounted for 70% of the study population. Although based on a modest sample size, similar female predominance has been reported in some West African wound studies and may correspond to gender differences in healthcare-seeking behaviour, obstetric and gynecological procedures, domestic burns, and occupational exposure (Iruegbu *et al.,* 2013; Onwuezobe *et al.,* 2020).

The bacteriological findings reveal *Staphylococcus aureus* as the predominant pathogen, responsible for 34**%** of all isolates. This finding aligns with longstanding evidence identifying *S. aureus* as the major etiological agent of skin and soft-tissue infections across Africa and internationally. Studies from Nigeria, Ghana, and East Africa consistently report *S. aureus* as the leading isolate, especially in traumatic and accidental wounds where disruption of the skin barrier allows rapid invasion by this organism (Oladeinde *et al.,* 2013; Deku et *al.,* 2024; Ahmed *et al.,* 2023; Abdullahi and Lawal, 2024). Its predominance is also consistent with its colonization of the skin and anterior nares, enabling efficient translocation into compromised tissues.

*Klebsiella* spp. (22%) and *Enterococcus* spp. (18%) were the next most frequent isolates, highlighting the increasing contribution of Enterobacterales and enterococci to wound and surgical-site infections in low-resource settings. Their prominence in this study aligns with recent multicenter reports from Africa and the Middle East, where *Klebsiella* spp. and *E. coli* have emerged as major Gram-negative contributors to wound infections, often linked to antimicrobial pressure, environmental persistence, and hospital transmission (Monk *et al.,* 2024; Mansoor *et al.,* 2024; Bandy *et al.,* 2022). The recovery of *Enterococcus* spp., particularly *E. Faecium* reflects global trends indicating their transition from relatively benign commensals to significant hospital-associated pathogens capable of surviving harsh environments, contaminating surfaces, and spreading via healthcare contact (Zhou *et al.,* 2020; Wei *et al.,* 2024; Farsi *et al.,* 2023).

*Escherichia coli* accounted for **10%** of isolates, consistent with its role as an occasional opportunistic invader in wound infections rather than a dominant pathogen. Similarly, *Salmonella* spp. (8%) and *Proteus* spp. (4%) occurred at lower frequencies. These proportions correspond with other African studies, where both organisms appear primarily in mixed or polymicrobial infections rather than as principal wound pathogens (Pandukur et *al.,* 2020; Ahmed *et al.,* 2023).

Overall, the pattern observed here, dominated by *S. aureus*, followed by *Klebsiella* spp. and *Enterococcus* spp., fits within the broader regional and global epidemiological profile of wound infections. Variations in relative proportions across different studies are commonly attributed to local hospital flora, patient demographics, antimicrobial usage patterns, and infection-control practices (Bandy *et al.,* 2022; Yucens et *al.,* 2025).

The inclusion of molecular characterization adds important depth to these findings. The confirmation of *Klebsiella variicola* among the predominant isolates is particularly significant. Members of the *K. pneumoniae* complex *K. variicola*, *K. quasipneumoniae*, and *K. pneumonia* are often indistinguishable using routine biochemical methods, resulting in frequent misclassification (Rodríguez-Medina *et al.,* 2019; Fonseca *et al.,* 2017). Earlier studies estimate that 2.5–10% of isolates reported as *K. pneumoniae* are actually *K. variicola* (Fontana *et al.,* 2019). Increasing evidence shows that *K. Variicola* can cause invasive infections, including bloodstream, urinary, and soft-tissue infections, and may harbor clinically relevant virulence factors or acquire resistance determinants (Barrios-Camacho *et al.,* 2019; Imai *et al.,* 2019; Campos *et al.,* 2021; Legese et *al.,* 2025). Its detection in wound samples here underscores the need for molecular techniques to improve diagnostic accuracy and refine local epidemiological understanding.

The molecular identification of *Enterococcus faecium* reinforces its shifting role in modern hospital epidemiology. Once regarded as a low-virulence commensal, *E. faecium* has increasingly been implicated in hospital outbreaks, particularly in intensive care and postoperative settings (Dworniczek *et al.,* 2012; Rajkumari *et al.,* 2014; Farsi *et al.,* 2023). Its prominence in this study aligns with reports of its rising clinical relevance across Africa (Olawale *et al.,* 2011).

Furthermore, the partial overlap of organisms observed, particularly among *Staphylococcus Aureus*, *Klebsiella* spp., and *Enterococcus* spp., is compatible with polymicrobial and biofilm-associated models of wound infection described in contemporary literature. Chronic or long-standing wounds, in particular, are known to harbor multispecies communities that contribute to delayed healing and antimicrobial tolerance (Rubio-Canalejas *et al.,* 2022; Ahmed *et al.,* 2023).

Taken together, the findings of this study is consistent with national and international data and demonstrate that the microbiology of wound infections in this setting mirrors the evolving global landscape. The integration of molecular tools in this study enhances the precision of organism identification to specie level, as this will go a long way in strengthening the epidemiological baseline for future surveillance, infection control planning, and antimicrobial stewardship initiatives.

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