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*Research article*

# Detection and Antibiotic Resistance Pattern of Multidrug Resistant *Staphylococccus aureus* from Wound Infection at Tertiary Hospital in Yenagoa, Nigeria

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## Abstract

Multidrug resistant *Staphylococcus aureus* is increasingly prevalent worldwide, albeit with significant regional variations. The development of bacterial resistance to various antibacterial agents coupled with its virulence factors have significantly contributed to its pathogenicity. This investigation aimed to identify multidrug resistant *S. aureus* in wound infections. A total of 40 specimens from burn and cut wound specimens were examined utilizing culture, Gram staining, biochemical analysis, Kirby Bauer disc diffusion technique using ten multidisc antibiotics and plasmid profiling. Descriptive analysis was employed to determine the prevalence of *S. aureus* in specimens collected from individuals with open wounds, comprising 18 (45%) males and 22 (55%) females. Antibiogram profiles were used to determine the antibiotic resistance of the isolates. A total number of 19 (48%) *S. aureus* isolates were obtained in this study. Among patients with burn wounds, 12 (63.5%) exhibited the highest number of isolates, while those with cut wounds accounted for 7 (36.8%) representing the lowest count. The number of males with burn wounds was 7 (58.3%), higher than that of females 5 (41.7%). Females had a greater number of cut wounds with 5 (71.4%) compared to males with 2 (28.6%). The age group 16-25 years exhibited the highest number of isolates at 7 (37%), while the lowest was observed in the age group 66-75 years, with 1 (5%). Notably, these age intervals showed a statistically significant difference with $P<0.05$. The highest occurrence of isolate was recorded in males within the age group 16-25 years, totalling 5 (24%). In contrast, for females, the most prevalent isolate was found in the age 26-35 years, amounting to

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3 (16%). The susceptibility profile of *S. aureus* revealed sensitivity to gentamycin 7 (36.8%) and levofloxacin 11 (57.9%), while resistance to norfloxacin, chloramphenicol, erythromycin, amoxil and ampiclox was 100%. Plasmid profiling identified multiple plasmid bands in the obtained multidrug resistant *S. aureus,* with a molecular weight of 9466kbp. The susceptibility pattern of the multidrug *S. aureus* both before and after plasmid curing indicated that previously resisted antibiotics became susceptible after curing except for amoxicillin-clavulanic acid and cefpodoxime. Cefotaxime, with a 25 mm zone of inhibition, was the most sensitive antibiotic after the plasmid curing. The discovery of plasmid in this study may prove valuable for the effective monitoring of antibiotic resistance patterns in bacteria from wound infections and clinical settings.

## 1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), a bacterium resistant to multiple antibiotics, is responsible for contagious infection that can be readily spread from person to person through direct contact with wounds, abrasions and contaminated items [1]. MRSA infections are considered potentially fatal due to the bacterium's ease of spread and resistance to treatment. The emergence of multi-resistant strains has further made therapy options difficult [2].

The World Health Organization (WHO) recognizes antibiotic resistance as a significant global health threat [3, 4]. According to the Centers for Disease Control and Prevention, at least 2.8 million individuals worldwide get infected with antibiotic-resistant bacteria each year, resulting in more than 35,000 deaths [5]. *Staphylococcus aureus'* devastating impact on the human population, its abilty to limit treatment alternatives, and its potential to complicate healthcare administration pose a global danger to public health systems and the economies. Rise in healthcare costs and the increased disease burden further underscore the urgent need to identify and characterize drug-resistant bacteria, and to develop more effective treatment strategies.

The widespread presence of the genus *Staphylococcus* in the skin microbiome, in the absence of bacterial infections and the occurrence of skin infections associated with *Staphylococcus* spp. strongly suggests that these bacteria form part of the skin's bacterial communities alongside various other species. However, *S. aureus* can act both as a commensal and a human pathogen [6, 7]. It can lead to various infections if it enters blood stream, causing conditions like sepsis, but it does not cause infection in healthy skin.

Nonetheless, colonization by *S. aureus* is a prerequisite for clinical infections of tissues of the body [8]. *Staphylococcus aureus* is a human pathogen responsible for high morbidity and mortality rates, particularly in hospitals and rural areas. Invasive infections attributed to this pathogen include boils, carbuncles, and abscesses [9], septicemia, necrotizing fasciitis, endocarditis, osteomyelitis, meningitis, bacteremia, and pneumonia [8, 10]. A concerning aspect of these infections is the high incidence of poorly responsive and recurrent *S. aureus* illness in immunocompetent hosts, with specific high-risk populations such as children in daycare settings [9], sports teams [11-13] and prisoners [14-16]. *Staphylococcus aureus* colonization is associated with various identified risk factors, including previous hospitalization, intensive care unit stays, prolonged hospitalization, overcrowding, advanced age, invasive procedures, pre-existing wounds, and prolonged antibiotic use [17]. Moreover, the rapid emergence and spread of multidrug resistant *S. aureus* is placing a heavy burden on the healthcare system. The emergence and rapid dissemination of multiple drug resistance in *S. aureus* has been an unavoidable setback despite the great advancements in antibacterial chemotherapy [18, 19]. Some of these strains are resistant to methicilin, lincosamides, macrolides, aminoglycosamides, fluroquinolones, or combinations of these antibiotics. Over the last decade, regulatory approvals have been granted for the treatment of infections caused by drug-resistant Gram-positive pathogens with drugs such as linezolid,

daptomycin, telavancin and ceftaroline [20, 21]. Additional studies have reported the use of meropenem, gentamycin and amikacin [22] and imipenem [23, 24] in the treatment of wound infections caused by *S. aureus.*

According to a meta-analysis, *S. aureus* resistance to several antibiotics ranged from 13 to 82% in Nigeria. The results from a study revealed the resistance of *S. aureus* to routinely used antibiotics in Nigeria to be 82% [2]. Furthermore, the increase in drug resistance is of a great concern and requires urgent attention. Hence, our study was aimed to characterize multidrug resistant *S. aureus* from wound infections and the investigtion involved the use of plasmid profiling to identify multi-drug resistant *Staphylococcus aureus* from wound infections.

## 2. Materials and Methods

### 2.1 Description of the study area

The research site was the Federal Medical Center (FMC), Yenagoa, Bayelsa State, which is situated in southern Nigeria. It is situated in the Niger Delta region of Nigeria [25], which has a land mass of over 29,100 km2 and is made up of coastal lowlands, water marshlands, creeks, and lagoons. According to Adamu *et al*. [26] it is located between latitude 4.15$^{o}$N and 6.01$^{o}$N and longitude 5.05$^{o}$E and 7.35$^{o}$E.

### 2.2 Ethical approval

This study was carried out with written permission from the Department of Accidents and Emergencies/Injuries, FMC, Yenagoa, Bayelsa State. Prior to sampling, participants in this study gave their informed permission. Information obtained from research subjects was kept confidential.

### 2.3 Specimen collection

A total of 40 specimens were collected, with twenty samples obtained from burn wound patients and twenty from cut wound patients. Utilizing the Levine approach, which is the current best practice related to the Z-technique [27], standardized wound swabs were collected from each burn and cut wound before wound dressing. This entailed spinning a wound swab across a 1cm$^2$ region of the wound [28]. As quickly as was feasible, specimens were collected and brought to the laboratory for culture and sensitivity testing.

### 2.4 Culturing of specimens and identification

The cross-streak technique was used to aseptically inoculate the obtained clinical specimens onto the bases of blood agar (Oxoid, England) and mannitol salt agar (Oxoid, England). After 24 h of aerobic incubation at 37°C, the plates were checked for growth. Bacterial colonies that exhibited the usual morphology and features of *S. aureus*, such as colonies that were hemolytic on 5% sheep blood agar and colonies that were golden yellow in color on mannitol salt agar [29] were sub-cultured on basal media and incubated aerobically at 37°C for 24 h. The method of Dilnessa and Bitew [29] with a slight modification was adopted for the Gram stain and biochemical tests (catalase, oxidase, urease, citrate, coagulase, and voges Proskauer) to confirm the presence of *S. aureus* [30].

## 2.5 Antibiotic susceptibility test of isolates

The Kirby Bauer disc diffusion technique was employed in compliance with Clinical and Laboratory Standard Institute standards [31] on Muller Hinton Agar to ascertain the antibiotic susceptibility pattern of the bacterial isolates. The bacterial inoculum was generated using nutrient broth (HI Media, India) and the turbidity was adjusted to 0.5 McFarland standard. A sterile swab stick dipped in the solution was used to uniformly cover freshly prepared Muller Hinton Agar. Using sterile forceps, an appropriate antibiotic disc containing ten different antibiotics; ciprofloxacin-10 µg, norfloxacin-10 µg, gentamycin-10 µg, amoxil-20 µg, streptomycin-30 µg, rifampicin-20 µg, erythromycin-30 µg, chloramphenicol-30 µg, ampiclox-20 µg, and levofloxacin-20 µg was put on the surface of the agar plate seeded with each isolate and softly pressed down to ensure contact with the agar plate. After that, the plate was inverted and incubated at 37°C for 24 h. The test plates were inspected, and the zone of inhibition in millimeters was measured (mm). An antibiogram profile was created using the method described by Clinical and Laboratory Standard Institute [31], and interpreted the results based on the diameter of the zones of inhibition demonstrated by the individual drugs in mm, with the test results classed as Sensitive (S), Intermediate, or Resistant (R) using the Clinical Laboratory Standards Institute guidelines [32].

## 2.6 Molecular characterization of *Staphylococcus aureus*

### 2.6.1 Plasmid extraction

The TENS-miniprep approach was used to extract plasmids from bacterial isolates [33]. A sterile inoculating loop was used to collect each overnight bacterial culture on agar plates and transfer it to each Eppendorf tube containing 100 µL of nutrient broth medium. Each sample in Eppendorf tube (5 replication) was vortexed at high speed using a vortex machine (Sci Finetech vortex mixer microfield) to thoroughly resuspend the cells. TENS buffer (300 µL) was added to each tube, and the tubes were inverted 3-5 times until the mixture became sticky, preventing chromosomal DNA degradation that may have harmed the plasmid DNA. One hundred and fifty µL of 3.0 mL of sodium acetate (pH 5.2) was added to the tubes containing the isolated collected cells, and all tubes were vortexed to mix the cells and reagents. Following vortexing, the tubes were spun for 5 min at the maximum revolution per minute (14,000 rpm) on a microcentrifuge (Beckman Coulter Microfuge) to pelletize the cell debris and chromosomal DNA. Following spinning, the supernatant was transferred to a newly labeled sterile Eppendorf tube and mixed with 9 mL of pre-cooled to -20$^{o}$C 100% ethanol (absolute). The supernatant was then spun for 2 min at 1,000 rpm to precipitate the plasmid DNA (a white pellet was noticed). The supernatant was discarded, and the pellet was washed twice with 500 µL of 70% ethanol, vortexed, and spun at 14,000 rpm for 2 min. The supernatant was decanted, blotted, and dried for 3 h in a safe and sterile atmosphere. The isolated plasmids were stored in a Ziploc bag in the freezer for future use.

### 2.6.2 Agarose gel electrophoresis (AGE)

The plasmid and DNA samples were analyzed by gel electrophoresis. Agarose gel (1.5%) was prepared by dissolving 1.5 g agarose in 100 mL of 1X TBE (Tris borate EDTA) buffer. The slurry was heated on an electric cooker to dissolve the agarose and the solution was allowed to cool to about 50$^{o}$C. Two drops of ethidium bromide (EtBr) as an intercalating agent were added to the solution and was gently swirled to get an even mixture. The solution was carefully poured into a horizontal gel casting tray of which two combs had been inserted prior to the pouring and the gel was left to set at room temperature. The combs were carefully removed and 4 µL of each plasmid

samples was mixed with 1 µL of the loading dye giving a volume of 5 µL for each of the nineteen samples. A DNA ladder was loaded in the first well and the samples were loaded using a micropipette into each well starting from the second well. The gel was submerged in the electrophoresis tank and 0.5X TBE buffer was poured into the tank until the buffer covered the surface of the gel. The electrodes were connected to the power source and run at 80 volts for 45 min. Plasmids were visualized on ultraviolet (UV-Transilluminator) and the bands were photographed using gel documentation. Twelve bands were pictured from the 19 samples that were run using gel documentation.

### 2.6.3 Plasmid curing with acridine orange

Plasmid curing was carried out by treatment with acridine orange. After the gel documentation, one bacterial isolate that produced a band was subjected to curing. The preserved bacterial isolate was subcultured by streaking on nutrient agar and incubated at 37°C for 24 h. Eacg overnight bacterial culture was harvested in 1 mL of lysogeny broth, labelled and incubated at 37°C for 24 h. Nutrient agar (85 mL) was prepared into a conical flask and was supplemented with 0.043g of acridine orange. The solution was carefully mixed by swirling and a reaction (change in colour) was observed. The overnight broth culture was vortexed for 1 min to mix completely and a micro-centrifuge was used for spinning at 10,000 rpm for 5 min to pelletize cell debris. After spinning, the supernatant was discarded by decanting, leaving the cell debris. The acridine orange broth (1 mL) was suspended in each of the Eppendorf tubes, mixed by vortexing and each tube was wrapped in aluminum foil to prevent the light because acridine orange is light sensitive. The tubes were incubated at 37°C for 24 h in a rotary incubator.

### 2.6.4 Application of antibiotic sensitivity disc

Two isolates in acridine orange broth cultured in a rotary incubator were pulled out and swabbed on Mueller-Hinton Agar plates with a sterile swab stick. The Kirby-Bauer disc diffusion method was used to evaluate antibiotic susceptibility according to the Clinical and Laboratory Standard Institute (CLSI) established standard [31]. The eight different discs (Maxicare Medical Lab) included the following antibiotics for Gram positive bacteria: gentamicin CN (10µg), azithromycin AZM (15 µg), chloramphenicol CH (30 g), cefpodoxime CPD (10 µg), amoxacillin-clavulanic acid AUG (30 µg), cefotaxime CTX (30 µg), ciprofloxacin CPX (10 µg), and tetracycline TE (30 µg). The findings were recorded after 24 h of incubation, and the diameter of the inhibitory zone surrounding each disc was measured and interpreted as sensitive, intermediate and resistant using CLSI guideline [32].

## 2.7 Statistical analysis

Descriptive analysis was used to describe the prevalence of *S. aureus* in specimens obtained from patients with wounds. Antibiotic resistance of isolates was described using an anti-biogram profile [31].

# 3. Results and Discussion

## 3.1 Result

In this study, 40 wound specimens were investigated, with 18 (45%) being male and 22 (55%) being female. *Staphylococcus aureus* isolates comprised 19 (47.5%) of the total isolates. Patients with

burn wounds accounted for 12 (63.5%) of the highest isolates, while those with cut wounds had 7 (36.8%) isolates, the lowest count. The percentage of males with burn wounds (7, 58.3%) exceeded that of females (5, 41.7%). Moreover, females exhibited a higher number of cut wounds (5, 71.4%) compared to males (2, 28.6%). The distribution of patients with wound infection by age and sex is presented in Table 1.

**Table 1.** Age and sex distribution of patients with wound infections attending the Federal Medical Center, Yenagoa

| Age Group (years) | Number of Specimens | Male (%) | Female (%) | Total (%) |
|---|---|---|---|---|
| 16-25 | 12 | 8 (20) | 4(10) | 12(30) |
| 26-35 | 9 | 3 (7.5) | 6(15) | 9(22.5) |
| 36-45 | 7 | 3 (7.5) | 4(10) | 7(17.5) |
| 46-55 | 3 | 3 (7.5) | 2(5) | 3(7.5) |
| 56- 65 | 7 | 3 (7.5) | 4(10) | 7(17.5) |
| 66-75 | 2 | 0 (0) | 2(5) | 2(5) |
| Total | 40 | 18 (45) | 22(55) | 40(100) |

Table 2 shows the frequency distribution of *S. aureus* from patients with wound infection. The highest number of isolates was observed in the age group 16-25 years comprising 7(37%), while the lowest count was in the age group 66-75 years with only 1 (5%) isolate. Statistically, there was a significant difference between the age interval with P=0.01, confirming statistical significance P<0.05. Furthermore, the most prevalent isolate among males was in the age group 16-25 years accounting for 5 (24%). Among females, the highest occurrence of the isolate was seen in the age group 26-35 years with 3 (16%).

Table 3 shows the antibiotic susceptibility pattern of *S. aureus* from patients with wound infection. Levofloxacin and gentamycin exhibited high effectiveness against *S. aureus*, with 11 (57.9%) and 7 (36.8%) susceptibility rates, respectively while resistance was observed with amoxil, rifampicin, norfloxacin, chloramphenicol, erythromycin and ampiclox. The intermediate resistant pattern was also observed in streptomycin 3 (15.8%), ciprofloxacin 8 (42.1%) and levofloxacin 4 (21.1%).

**Table 2.** Frequency distribution of *S. aureus* from patients with wound infections obtained from the Federal Medical Center Asaba and Federal Medical Center Yenogoa

| Age Group (years) | Male (%) | Female (%) | Total (%) |
|---|---|---|---|
| 16-25 | 5(26) | 2(11) | 7(37) |
| 26-35 | 1(5) | 3(16) | 4(21) |
| 36-45 | 2(11) | 1(5) | 3(16) |
| 46-55 | 0(0) | 2(11) | 2(11) |
| 56- 65 | 1(5) | 1(5) | 2(11) |
| 66-75 | 0(0) | 1(5) | 1(5) |
| Total | 9(47) | 10(53) | 19(100) |

**Table 3.** Antibiotic susceptibility pattern of *S. aureus* with wound infections obtained from the Federal Medical Center Asaba and the Federal Medical Center Yenogoa

| | ***Staphylococcus aureus*** **n= 19** | | |
|---|---|---|---|
| **Antibiotics** | **NS** | **NI** | **NR** |
| Amoxil | 0 (0) | 0 (0) | 19 (100) |
| Rifampicin | 0 (0) | 0 (0) | 19 (100) |
| Streptomycin | 0 (0) | 3(15.8) | 16 (84.2) |
| Norfloxacin | 0 (0) | 0 (0) | 19 (100) |
| Chloramphenicol | 0 (0) | 0 (0) | 19 (100) |
| Ciprofloxacin | 0 (0) | 8 (42.1) | 11 (57.9) |
| Erythromycin | 0 (0) | 0 (0) | 19 (100) |
| Levofloxacin | 11(57.9) | 4 (21.1) | 4(21.1) |
| Gentamycin | 7 (36.8) | 0 (0) | 12 (63.1) |
| Ampiclox | 0 (0) | 0(0) | 19 (100) |

Key: NS= Number Sensitive, NI= Number Intermediate, NR= Number Resistant
Ciprofloxacin-10µg, Norfloxacin-10µg, Gentamycin-10µg, Amoxil-20µg, Streptomycin-30µg, Rifampicin-20µg, Erythromycin-30µg, Chloramphenicol-30µg, Ampiclox-20µg, and Levofloxacin -20µg. P=0.01 and P<0.05. Statistically, there was a significant difference between the antibiotics used.

The susceptibility pattern of the multidrug resistant *S. aureus* before and after curing is shown in Table 4. Following curing, most antibiotics to which the bacteria had previously exhibited resistance showed sensitivity except for amoxicillin-clavulanic acid and cefpodoxime. The majority of antibiotics that had previously shown resistance were discovered to be sensitive after curing. Cefotaxime, with a substantial 25mm zone of inhibition, was the antibiotic shown to be the most sensitive antibiotic.

Figure 1 illustrates the multiple bands and the molecular weight of plasmid DNA found in multidrug resistant *S. aureus* on agarose gel stained with ethidium bromide. Lane M displays the marker DNA, a 23130bp DNA ladder, while lanes 1, 2, and 3 show the absence of plasmid.

**Table 4.** Susceptibility test before and after curing

| **Antibiotics** | **Susceptibility Test before Curing** | | | | **Susceptibility Test after Curing** | | | |
|---|---|---|---|---|---|---|---|---|
| | | **S** | **I** | **R** | | **S** | **I** | **R** |
| Gentamicin | 11 | - | - | 11 | 16 | 16 | - | - |
| Azithromycin | 13 | - | - | 13 | 19 | 19 | - | - |
| Chloramphenicol | 9 | - | 9 | - | 18 | 18 | - | - |
| Cefpodoxime | - | - | - | - | - | - | - | - |
| Amoxicillin-clavulanic acid | - | - | - | - | - | - | - | - |
| Cefotaxime | 12 | - | 12 | - | 25 | 25 | - | - |
| Ciprofloxacin | 10 | - | - | 10 | 24 | 24 | - | - |
| Tetracycline | 12 | - | - | 12 | 20 | 20 | - | - |

All measurement were taken in millimeters (mm). TE, tetracycline, AZM, azithromycin, C, chloramphenicol, CIP, ciprofloxacin, CN, gentamicin, CTX, cefotaxime, AUG, amoxicillin-clavulanic acid, CPD, cefpodoxime

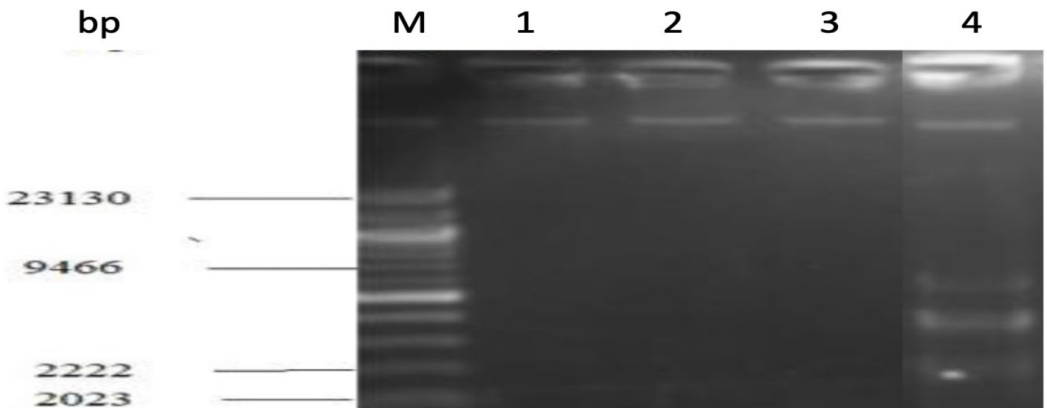


**Figure 1.** Molecular weight of plasmid DNA on agarose gel stained with ethidium bromide. Lane (M) marker DNA - 23130bp DNA ladder, lanes 1, 2, and 3: no plasmid band, lane 4: 9466kbp multiple plasmid DNA bands of multidrug resistant *S. aureus*

## 3.2 Discussion

The involvement of *S. aureus* in cut and burn wound infections, as demonstrated in the current study, raises significant health concerns. The study comprised 22 female and 18 male subjects with ages ranging from 16-70 years and a mean age of 39.2 years. The implications of *S. aureus* in this finding and its diverse clinical manifestations underscore its epidemiological importance. The prevalence of *S. aureus* in this study was 47.5%, in contrast to the findings of Biadglegne *et al*. [34] and Upreti *et al*. [35], with prevalence rates of 49% and 56.9%, respectively. It is crucial to emphasize that notable regional differences should be taken into consideration. This possibility of S. *aureus* being the most prevalent bacterium in most wound infections may be attributed to its presence as indigenous flora on human skin, facilitating easy entry into wounds, or as a result of cross-contamination during hospital procedures and by health workers. This predominance aligns with a previous study conducted by Guo *et al*. [36]. Notably, patients with burn wounds exhibited a higher prevalence of *S. aureus* compared to those with cut wounds. Burn wounds represent a significant global health challenge [37], as such wounds provide an ideal environment for bacterial proliferation and serve as persistent reservoirs of infection in comparison to surgical wounds [38]. In comparison, among patients with burn wounds, males accounted for 7 (58.3%), while females comprised 5 (41.7%). In the case of cut wounds, males represented 2 (28.6%), while females accounted for 5 (71.4%). Wound infections were observed to be most common in the age group of 16-25 years, in contrast to the findings of Upreti *et al*. [35], and were the least seen in the age group 66- 75 years, The majority of isolates were found in males in the 16 to 25-year range. This variation could have been attributed to gender-related differences in immune responses to various microorganisms [39], reduced wound healing capacity in men [40], and unhealthy lifestyle factors such as smoking and excessive alcohol intake. The most frequently occurring isolates among females were also observed in the 26-35 years age group, accounting for 3 (15.7%).

The antibiotic susceptibility pattern of *S. aureus* isolates from patients with wound infections revealed the effectiveness of levofloxacin and gentamycin, accounting for 11 (57.9%) and 7 (36.8%) of the total isolates, respectively. This finding was consistent with the research reported by Onwubiko *et al*. [41], who observed that MRSA exhibited a 93.7% sensitivity to levofloxacin. The susceptibility of *S. aureus* to levofloxacin and gentamycin in this study may have been attributed

to strict compliance to prescribed dosages (indicating responsible use of antibiotics by the individuals), or it may have been because the pathogen had not yet developed resistance to these particular antibiotics within the studied subjects. In contrast, *S. aureus* was resistant to amoxil, rifampicin, norfloxacin, chloramphenicol, erythromycin, and ampiclox. Notably, all 19 (100%) isolates exhibited resistance to more than two antibiotics, indicating that *S. aureus* was a multidrug resistant microorganism. An intermediate resistant pattern was observed in S 3(15.8%), CPX 8 (42.1%) and LEV 4 (21.1%), and this indicates that these three observed drugs posed a treatment risk. This finding was consistent with previous research by Yusuf *et al*. [42] as well as earlier investigations conducted in Ghana by Ozumba [43] and Wolters *et al*. [44]. The high percentage of resistant isolates suggests that some of the resistance may be attributed to antibiotics misuse and indiscriminate prescription of antibiotics, a lack of infection prevention and control practices, and the potential spread or acquisition of resistant genes from the environment. MRSA can contribute significantly to society's overall financial burden as transmission in health care settings continues to increase. Costs should be reduced if transmission and infection incidence decrease. Moreover, detecting MRSA infections early and treating them appropriately may result in significant cost savings for both patient, healthcare providers and vulnerable individuals.

The transfer and acquisition of antibiotic resistant genes in *S. aureus*, typically encoded by transposons, integrative conjugative elements (ICEs), staphylococcal chromosomal cassettes (SCCs), and SaPI, is highly dependent on plasmids [45]. Plasmids are typically actively assimilated from the environment by transformation or are transferred between cells via conjugation [46] or phage transduction. This transfer, particularly through phage transduction, is crucial for plasmid transmission in staphylococci. Consequently, it is possible to predict that the spread of antibioitc resistance among microbes associated with humans, especially during antibiotic therapy, will frequently occur [47].

In this study, plasmid DNA with molecular weight was identified in multidrug resistant *S. aureus*, although not in all the bacterial isolates studied. Nevertheless, the results of the susceptibility tests conducted both before and after the plasmid treatment, revealed that most of the previously identified resistant antibiotics demonstrated sensitivity, with the exceptions of cefpodoxime and amoxicillin-clavulanic acid, which remained resistant. Additionally, cefotaxime displayed the highest sensitivity, with a 25 mm zone of inhibition. The observed multidrug resistance to both antibiotics is likely not plasmid mediated but genomic-mediated; suggesting that chromosomal DNA could be responsible, or the plasmids were likely acquired as also suggested by Okoye *et al*. [48]. Consequently, our research elucidates the relationship between plasmids and various types of drug resistance, shedding light on how plasmids contribute to the spread of drug resistance among bacteria.

Addressing multidrug resistant *S. aureus* is extremely urgent given the limited number of available treatments. A comprehensive study encompassing various strategies, along with the development of alternative tools to address these public health issues, is essential for understanding the mechanisms behind the bacteria's resistance and the creation of new antimicrobial agents. The use of antibiotic combination therapy for treating infections caused by this pathogen may offer new therapeutic options. However, challenges persist, including medication shortages, exorbitant prices, and resistance issues [49]. Nevertheless, the burgeoning field of combinatorial approaches holds promise as a viable tool for exploring innovative alternatives and contributing to the fight against resistance mechanisms.

It is important to acknowledge several limitations in this study. Firstly, the research relies on data collected from a clinical setting with extremely limited information on antimicrobial resistance (AMR). Additionally, the study's constraints include the use of a small sample size, which was necessitated by the limited number of patients available during the research period. Key information, such as the duration of hospital stays and the history of antibiotic usage, was not

available for analysis. Moreover, the study did not involve molecular characterization of isolates or the detection of resistance genes.

## 4. Conclusions

Antimicrobial resistance presents a global problem that demands immediate attention. The implementation of preventative and control strategies is crucial, particularly because wound infections are often overlooked and often lead to chronic infections. While it is acknowledged that the study's sample size was small and may not have comprehensively represented the broader population or the region under investigation, the results are vital for understanding the dissemination of resistant *S. aureus* strains within the studied population. This understanding is pivotal for effective surveillance of antibiotic-resistant *S. aureus* and the accurate estimation of resistance burden. The mitigation of the resistance threat can be facilitated through infection prevention and control measures, effective medication dispensing protocols, and improved monitoring of the etiology and antimicrobial sensitivity of wound isolates.

## 5. Acknowledgements

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