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Prevalence and Molecular Characterization of Multidrug-Resistant *Staphylococcus* Species in a Healthcare Setting

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Abstract
Infection control within healthcare settings is a matter of great importance, as the emergence and spread of multidrug-resistant bacteria pose a significant threat to patient and healthcare workers' safety. *Staphylococcus* species are notorious for their ability to develop resistance to multiple antibiotics, making them formidable nosocomial pathogens. Hence, this study was carried out to investigate the prevalence and molecular characteristics of multidrug-resistant *Staphylococcus* species in a healthcare environment. A total of 80 skin swab specimens were collected from different healthcare workers across different units at Federal Medical Centre, Yenagoa, and were analyzed by culture, gram staining, and biochemical tests, after which 27 isolates were obtained. The *Staphylococcus* species isolated include *Staphylococcus epidermidis* 13(48.2%), *Staphylococcus aureus* 12(44.4%), and *Staphylococcus scuri* 2(7.4%). Antibiotic susceptibility test revealed that the isolated *Staphylococcus* species were most resistant to cefotaxime, ceftriaxone, cefexime, imipenem/cilastatin, and cefuroxime, and most sensitive to amoxicillin-clavulanate, levofloxacin, ofloxacin, erythromycin, gentamycin, and azithromycin. There was a significant difference ($p \leq 0.05$) in the antibiotic susceptibility profile of the isolated *Staphylococcus* species. Phylogenetic analysis using 16s rRNA sequences identified *Staphylococcus scuri*. Biofilm formation analysis indicated that *Staphylococcus scuri* and one strain of *Staphylococcus epidermidis* were biofilm producers. Plasmid profiling through gel electrophoresis indicated the presence of a plasmid molecular weight above 23130bp, suggesting the possibility of horizontal transmission of resistance genes. Plasmid curing of multidrug-resistant isolates resulted in improved antibiotic susceptibility. Continuous surveillance and molecular characterization of multi-drug resistant *Staphylococcus* species are necessary to mitigate the spread of these resilient pathogens.

Keywords: Infection Control, Multidrug-Resistant *Staphylococcus*, Healthcare Environment, Plasmid Profiling

Introduction

In recent years, the emergence and spread of multidrug-resistant (MDR) bacterial strains have posed a significant threat to public health, particularly in healthcare settings (Terreni et al., 2021). Among the implicated pathogens, *Staphylococcus* species have gained considerable attention due to their capability to induce a diverse array of infections and their propensity to acquire resistance to various types of antimicrobial agents (Cheung et al., 2021). *Staphylococcus* species, particularly *S. aureus* and coagulase-negative staphylococci (CoNS) are notorious for their ability to cause various infections, varying from minor dermis infection and cellulitis to fatal life-threatening conditions like bloodstream infections and valvular heart disease (Cheung et al., 2021; Terreni et al., 2021). The overuse and misuse of antibiotics in healthcare settings have led to the development of antibiotic-resistant strains, complicating the treatment of Staphylococcus infections (Cheung et al., 2021). Staphylococci, known for their ability to adapt quickly to changing environments, have exhibited remarkable resilience in the face of antibiotic exposure, leading to the emergence of multidrug resistance (Kumar et al., 2024). Multidrug-resistant *Staphylococcus* species infections are associated with a wide array of illnesses, spanning from minor skin and soft tissue infections to severe, bloodstream infections and pneumonia with life-threatening implications (Asante et al., 2021). The ability of *Staphylococcus* species to develop resistance to various types of antibiotics is attributed to a variety of molecular mechanisms. One key mechanism involves the acquisition of resistant genes through horizontal gene transfer, facilitated by mobile genetic elements such as plasmids and transposons (Souza-Silva et al., 2022). These genes encode enzymes that modify or degrade antibiotics, rendering them ineffective. Additionally, *Staphylococcus*

species can upregulate efflux pumps, reducing the intracellular concentration of antibiotics, and undergo mutations that alter the target sites of antimicrobial agents (Rodríguez-Beltrán et al., 2021).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a Gram-positive bacterium that lacks motility non-motile and is coagulase-positive. It belongs to the Firmicutes phylum that has been recognized as a significant pathogen posing a substantial risk due to its development of multidrug resistance (Turner et al., 2019). Over time, MRSA has become well-recognized as a public health problem throughout the world. Previous research has highlighted its prevalence in various countries; Tunisia ranging from 16% to 41%, South Africa (24% to 36%), Botswana (23% to 44%), Egypt (45% to 52%) and Nigeria (55% and 39%) both in Northern and Southern parts respectively (Falagas et al., 2013; Schaumburg et al., 2014). This clear variation across different parts of the world can be attributed to various environmental influences, diversity among strains, and differences in the use or misuse of antibiotics. The impact of MRSA can be seen in the significant rise in mortality and morbidity resulting from the infections it causes, and the significant financial strain it places on limited healthcare resources (Turner et al., 2019). Coagulase-negative staphylococci (CoNS), once considered contaminants or commensals, have emerged as facultative disease-causing microorganisms, notably in nosocomial settings (Becker et al., 2014). They are prevalent invader of the dermis and mucosa, and have been implicated in various clinically relevant infections, such as urinary tract infections, endocarditis, bloodstream infections (including neonatal sepsis), and infections related to medical devices (Asante et al., 2020). The abundant presence of CoNS species on the dermis and mucosa of the hosts, serves as a significant reservoir for endogenous CoNS infections, with transmission often facilitated through medical procedures (Becker et al., 2014).

These bacteria commonly exhibit resistance to multiple antibiotics, including methicillin, making them challenging to treat. Their capability to create biofilms on medical equipment contributes to persistent diseases and complicates therapeutic interventions (Asante et al., 2021). Clinical manifestations of multidrug-resistant *Staphylococcus* species include persistent and severe infections, often challenging to treat due to antibiotic resistance (Asante et al., 2021). Patients may experience prolonged illness, increased healthcare costs, and a heightened risk of complications (Lozano et al., 2020). Multidrug-resistant *Staphylococcus* species can survive on surfaces like doorknobs, medical equipment, gym equipment, and shared personal items (towels, razors) for extended periods, providing opportunities for transmission to individuals who encounter them (Balamohan et al., 2021). Risk factors for acquiring or transmitting these pathogens include prolonged hospitalization, previous antibiotic use, invasive medical procedures, and close contact with infected individuals (Mao et al., 2019). Prevention involves rigorous infection control measures in healthcare settings, emphasizing prudent antibiotic use, strict adherence to hygiene protocols, and surveillance for early detection (Kumar et al., 2024). Detecting and identifying MRSA early in nosocomial infections, along with comprehending their antibiotic resistance profiles, are crucial for successful diagnosis and determining optimal antibiotic treatment regimens. Thus, this study aimed to investigate the prevalence and molecular characterization of multidrug-resistant *Staphylococcus* species in a healthcare setting using 16S rRNA gene sequencing analysis.

Materials and Method

The area of study is Federal Medical Centre (FMC), Yenagoa, Bayelsa State, Nigeria, which provides quality health services to patients within Bayelsa State, Nigeria. Federal Medical Centre, Yenagoa is a federally owned medical centre located at Hospital Road, Ovum, Yenagoa, at a longitude 6.268198^{o}E and latitude 4.938862^{o}N. This research involved a prevalence study where skin swabs were collected from 80 healthcare workers across 5 different healthcare units at Federal Medical Centre, Yenagoa, Bayelsa State, for two months. Study participants provided explicit compliance following a thorough explanation of the investigation orally and a guarantee of privacy. Before commencing the study, ethical approval was acquired from the Directorate of Research and Quality Assurance at Federal University Otuoke. Subsequently, the approval was submitted to the Ethical Board of Federal Medical Centre, Yenagoa, Bayelsa State. Eighty (80) skin swab specimens were collected from healthcare workers across the parasitology lab, theatre, surgery, ENT, and obstetrics and gynaecology units at Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria. The specimens were transported to the Microbiology Laboratory of Federal University Otuoke, Bayelsa State, for further microbiological analysis. The specimens were cultured on Mannitol salt agar (Chaitanya Agro Biotech, India), and incubated at 37^{o}C for 24-48 hours to allow for growth of the organisms. Observed distinct colonies were then subcultured on Nutrient agar (Chaitanya Agro Biotech, India) and incubated for 24 hours to obtain pure cultures. Pure isolates obtained were characterized using gram staining techniques and biochemical tests which include catalase and coagulase.

A standardized Kirby–Bauer disk diffusion technique employing Muller–Hinton agar (Hi Flown Biotech, Berkshire, UK), plate was conducted according to the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI) (Humphries et al., 2021). A bacterial suspension equivalent to 0.5 McFarland turbidity standard was prepared for inoculation. A sterile swab stick was immersed in the prepared bacterial suspension and then used to inoculate the Mueller-Hinton agar plate by swabbing the suspension evenly across the surface of the plate. Sterile forceps were subsequently utilized to position the Gram-positive antibiotic discs (Celtech diagnostic, Belgium), onto the inoculated agar plate. The antibiotics contained in the discs include amoxicillin clavulanate (30µg), cefotaxime (25µg), ceftriaxone sulbactam (45µg), cefexime (5µg), levofloxacin (5µg), ciprofloxacin (5µg), imipenem/cilastatin (10/10µg), cefuroxime (30µg), ofloxacin (5µg), erythromycin (15µg), gentamycin (10µg), and azithromycin (15µg). The discs were gently pressed down to ensure contact with the agar. The plates were then incubated at 37°C for 18-24 hours to allow for the growth of the bacteria. After incubation, the plates were observed. The clear zones (zones of inhibition) around each antibiotic disk indicating the effectiveness of the antibiotics against the bacteria, were measured using a meter rule, and recorded in millimetres. The zone diameters were then compared to the standard interpretive criteria provided by the Clinical and Laboratory Standards Institute (CLSI), and the results were classified as susceptible, intermediate, or resistant based on the zone sizes and the specific antibiotic (Humphries *et al*., 2021).

Extraction of plasmid from bacteria isolates was carried out using the TENS-miniprep method (Zhou et al., 1990). An overnight bacterial culture on media plates was harvested utilizing a sterile inoculating loop and transferred into 100µl of nutrient broth medium. Each Eppendorf tube was vortexed using a vortex machine (Sci Finetech vortex mixer microfield) at high speed to resuspend the cells completely. 300µl of TENS buffer was added and the tubes were mixed by inverting them 3-5 times until the mixture became sticky ensuring to prevent chromosomal DNA degradation which may affect the plasmid DNA. A solution containing 150µl of 3.0M sodium acetate (pH 5.2) was introduced into the tubes respectively that contained the isolates harvested cells, all the tubes were vortexed to mix the cells and the reagents completely. After vortexing, the tubes were spun using a microcentrifuge (Beckman Coulter Microfuge) for 5 minutes at the highest revolution per minute (14,000 rpm) to pellet the cell debris and chromosomal DNA. After spinning, the supernatant was transferred into a fresh labelled sterile Eppendorf tube, where it was mixed with 900µl of 100% ethanol (absolute)which was pre-cooled to -20ºC. Then spun for two (2) minutes at 1000rpm to precipitate the plasmid DNA (white pellet is observed) from the supernatant. The supernatant was discarded; the pellet was rinsed twice with 500µl of 70% ethanol, mixed by vortexing, and spun for two (2) minutes at 14,000rpm. The supernatant was discarded by decanting, blotted, and dried for 3hours in a safe and sterile atmosphere. The plasmids extracted were kept inside the freezer for further use.

The plasmid and DNA were analyzed through gel electrophoresis. To prepare the gel, 1.5g of agarose was dissolved in 100 ml of 1 X TBE (Tris borate EDTA) buffer, resulting in a 1.5% agarose gel concentration. The slurry was heated in an electric cooker to dissolve the agarose, and then cooled to approximately 50°C before use. Two (2) drops of ethidium bromide (EtBr) as an intercalating agent were added to the solution and gently swirled for an even mixture. The solution was carefully poured into the horizontal gel casting tray of which two (2) combs were inserted before the pouring and the gel was left to set at room temperature. The combs were carefully removed and 4µl each of the plasmid samples was mixed with 1µl of the loading dye giving 5µl each for nineteen (19) samples. The DNA ladder was placed in the initial well, followed by the loading of the samples into each well using a micropipette, beginning from the second well. The gel was submerged in the electrophoresis tank, and the tank was filled with 0.5 X TBE buffer to cover the surface of the gel. The electrodes were connected to the power source and operated at 80 volts for 45 minutes. Plasmids were visualized on ultraviolet (UV-Transilluminator) and the bands were photographed using gel documentation. Twelve bands were pictured from the nineteen samples run using gel documentation.

An 18 h multidrug-resistant bacterial isolate from the previous susceptibility test was utilized before the curing. The bacterial isolates were swabbed with a sterile swab stick on Mueller-Hinton agar plates. Antibiotic susceptibility testing was performed by using the Kirby-Bauer disc diffusion method according to the recommended standard of the Clinical Laboratory Standard Institute (CLSI). The discs (Maxicare Medical Lab) contained the following antibiotics for gram-positive: chloramphenicol C (30µg), cefotaxime CTX (30µg), amoxicillin AUG (30 µg), tetracycline TE (30µg), erythromycin E (15µg), linezolid LNZ (30µg), vancomycin VA (30µg), azithromycin AZM (15µg). The results were documented following 24 hours of incubation, and measurement was taken using the diameter of the inhibition zone (mm) around each disc and interpreted as sensitive, intermediate, and resistant using CLSI guidelines (CLSI, 2020; Hudzicki, 2009).

After gel documentation, the two (2) bacterial isolates that produced bands were subjected to curing by treatment with acridine orange. The preserved bacterial isolates were subcultured by streaking on nutrient agar plates and incubated at 37ºC for 24 hours. The overnight bacteria cultures were harvested in 1ml of lysogeny broth, each labelled and incubated at 37ºC for 24 hours. 85ml of nutrient agar was prepared into a conical flask and it was supplemented with 0.043g of acridine orange. The solution was carefully mixed by swirling and a reaction (colour change) was observed. The overnight broth culture was vortexed for 1 minute to mix completely and the micro-centrifuge was used for spinning at 10,000rpm for 5 minutes to pellet cell debris. After spinning, the supernatant was discarded by decanting leaving the cell debris. 1ml of the acridine orange broth was suspended in each of the Eppendorf tubes, mixed by vortexing and each tube was wrapped with aluminium foil because acridine orange is light sensitive. The tubes were put in the incubator at 37ºC for 24 h in a rotary incubator.

Following curing, the three (3) bacterial isolates in acridine orange broth incubated in a shaking/rotary incubator were brought out and each was swabbed with a sterile swab stick on Mueller-Hinton agar plates. Antibiotic sensitivity testing was conducted utilizing the Kirby-Bauer disc diffusion method in accordance with the recommended guidelines of the Clinical Laboratory Standard Institute (CLSI, 2020). The discs (Maxicare Medical Lab) contained the following antibiotics for positive: chloramphenicol C (30µg), cefotaxime CTX (30µg), amoxicillin AUG (30 µg), tetracycline TE (30µg), erythromycin E (15µg), linezolid LNZ (30µg), vancomycin VA (30µg), azithromycin AZM (15µg). The outcomes were recorded following 24 h of incubation and measurement was taken using the diameter of the inhibition zone (mm) around each disc and interpreted as sensitive, intermediate, and resistant using CLSI guidelines (CLSI, 2020; Hudzicki, 2009). The qualitative method for the detection of biofilm production was described by Freeman et al. (1989). The method of Congo Red Agar (CRA) consisting of Brain heart infusion broth (37 gms/L), sucrose (50 gms/L), agar no.1 (10 gms/L) and congo red stain (0.8 gms/L, Himedia). Positive results were indicated by the formation of black colonies with a dry crystalline texture, signifying biofilm producers, while negative results were indicated by pink-coloured colonies for non-biofilm producers.

Extraction was carried out using the ZR fungal/bacterial DNA mini-prep extraction kit provided by Inqaba South Africa. Heavy growth of the pure culture of the suspected isolates was suspended in 200 microliters of isotonic buffer within a ZR Bashing Bead Lysis tube, followed by the addition of 750 microliters of lysis solution. These tubes were securely placed in a bead beater equipped with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. Subsequently, the ZR bashing bead lysis tube was centrifuged at 10,000x*g* for 1 minute. Following centrifugation, 400 microliters of supernatant were carefully transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000x*g* for 1 minute. Then, 1200 microliters of fungal/bacterial DNA binding buffer were added to the filtrate in the collection tubes bringing the final volume to 1600 microliters. Of this volume, 800 microliters were moved to a Zymo-Spin IIC column in a collection vial and spun at 10,000x*g* for 1 minute, after which the flow-through was emptied. The residual volume was centrifuged using the same Zymo-spin. 200 microliters of the DNA Pre-Wash buffer were added to the Zymo-spin IIC in a new collection vial and spun at 10,000x*g* for 1 minute, then the addition of 500 microliters of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000x*g* for 1 minute. The Zymo-spin IIC column was then moved to a clean 1.5 microliter centrifuge tube, and 100 microliters of DNA elution buffer was included in the column matrix. After centrifugation at 10,000x*g* for 30 seconds, the DNA was eluted. Finally, the ultra-pure DNA was stored at -20ºC for subsequent downstream reactions. The genomic DNA obtained was assessed for quantity utilizing the Nanodrop 1000 spectrophotometer. To initiate the equipment's software, the Nanodrop icon was double-clicked. The spectrophotometer was primed with 2 ul of sterile distilled water and blanked with normal saline. Following this, 2 microliters of the DNA extracted were applied to the base, and the higher base was lowered to make contact with the extracted DNA. The concentration of DNA was determined by selecting the "measure" button.

The ribosomal RNA genes from the isolates in the 16s rRNA region underwent amplification utilizing the 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3') primers, conducted on an ABI 9700 Applied Biosystems thermal cycler in a final volume of 50 microliters over 35 cycles. The PCR mixture consisted of X2 Dream Taq Master mix from Inqaba, South Africa (containing Taq polymerase, DNTPs, MgCl), primers at a concentration of 0.4M, and the DNA extracted as the matrix. PCR conditions utilized were set as follows: Initial denaturation at 95ºC for 5 mins, followed by denaturation at 95ºC for 30 sec, annealing at 52ºC for 30 sec extension at 72ºC for 30 sec for 35 cycles, and a final extension at 72ºC for 5 mins. Subsequently, the resulting by-product was separated on a 1% agarose gel at 120V for 15 mins and observed under a UV transilluminator.

Sequencing was performed using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological in Pretoria, South Africa. The sequencing was conducted in a final volume of 10 µl, comprising 0.25 µl of BigDye® terminator v1.1/v3.1, 2.25 µl of 5x BigDye sequencing buffer, 10 µM of PCR primer, and 2-10 ng of PCR template per 100 bp. The sequencing conditions consisted of 32 cycles of denaturation at 96°C for 10 seconds, annealing at 55°C for 5 seconds, and extension at 60°C for 4 minutes. The derived sequences underwent editing utilizing the bioinformatics algorithm Trace edit, and comparable sequences were retrieved from the National Center for Biotechnology Information (NCBI) database using BLASTN. Subsequently, alignment of these sequences was performed utilizing ClustalX. The phylogeny was then deduced using the Neighbor-Joining method within MEGA 6.0 (Saitou & Nei, 1987). A resampling support tree derived from 500 resamples (Felsenstein, 1985) was employed to depict the phylogeny of the examined taxonomic group. Phylogenetic distances were calculated utilizing the Jukes-Cantor method (Jukes & Cantor 1969). The data obtained from the results were subjected to an Analysis of Variance (ANOVA) test to ascertain the significant difference at a 95% confidence level.

Results

Out of the total 80 skin swab specimens studied, 27 isolates were obtained. The bacterial strains identified with their ratio of occurrence are as outlined; *S. epidermidis* 13(48.2%), *S. aureus* 12(44.4%), and *S. scuri* 2(7.4%). The highest number of identified isolates was *S. epidermidis* 13(48.2%) while the lowest was *S. scuri* 2(7.4%). There was a significant difference ($P = 0.000645$, $P \leq 0.05$) between the number of isolates and the percentage occurrence of the *Staphylococcus* species as presented in Table 1.

Table 1: Percentage Occurrence of *Staphylococcus* species isolated from the skin of healthcare workers at Federal Medical Centre, Yenagoa

Organisms	Number of isolates (%)
S. aureus	12(44.4)
S. epidermidis	13(48.2)
S. scuri	2(7.4)
Total	27(100)

Figure 1 illustrates the frequency distribution of *Staphylococcus* species isolated from the skin of healthcare workers at different units in Federal Medical Centre, Yenagoa. The distribution of the *S. aureus* for the various units was Parasitology Lab, Theatre, Obstetrics & Gynaecology (O&G); 1(3.7%) respectively, Surgical 4(14.8%) and Ear, Nose & Throat (ENT); 5(18.5%). For *S. epidermidis*; Parasitology Lab 1(3.7%), Theatre 3(11.1%), Surgical and ENT 2(7.4%) respectively, and O&G 5(18.5%). *S. scuri*; Parasitology Lab, Theatre and O&G had no isolate while Surgical and ENT had 1(3.7%) isolate respectively. The highest number of total isolates was obtained from the ENT unit, with 8(29.6%) while the least number of isolates obtained was from the Parasitology Lab with 2(7.4%). There was a significant difference ($P \leq 0.05$) between the number of isolates obtained and the different units at Federal Medical Centre, Yenagoa.

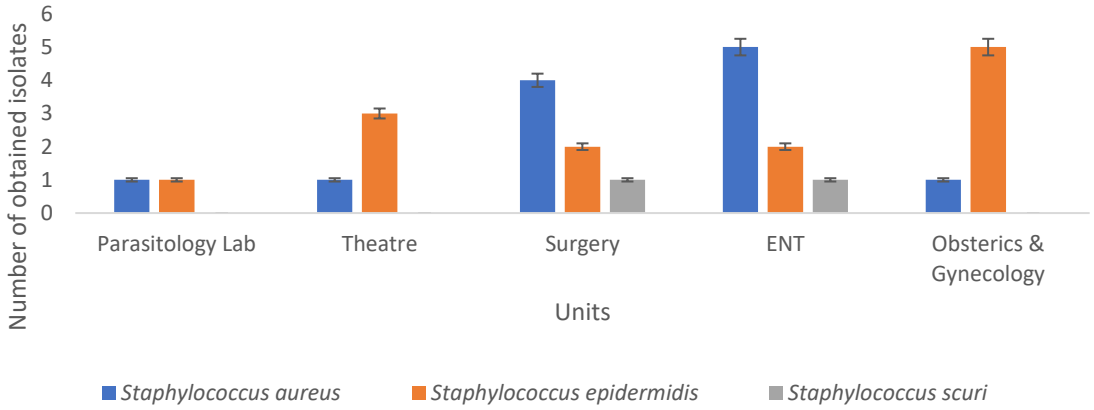

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Fig 1: Frequency distribution of *Staphylococcus* species isolated from the skin of healthcare workers at different units in Federal Medical Centre, Yenagoa.

The distribution of isolates obtained from healthcare workers at Federal Medical Centre, Yenagoa by age is shown in **Figure 2**. The age group with the highest isolates was 21-30 years with 19(70.4%). Statistically, there was a significant difference (P = 0.000621, P ≤ 0.05) between the isolates obtained, and the age of the healthcare workers.

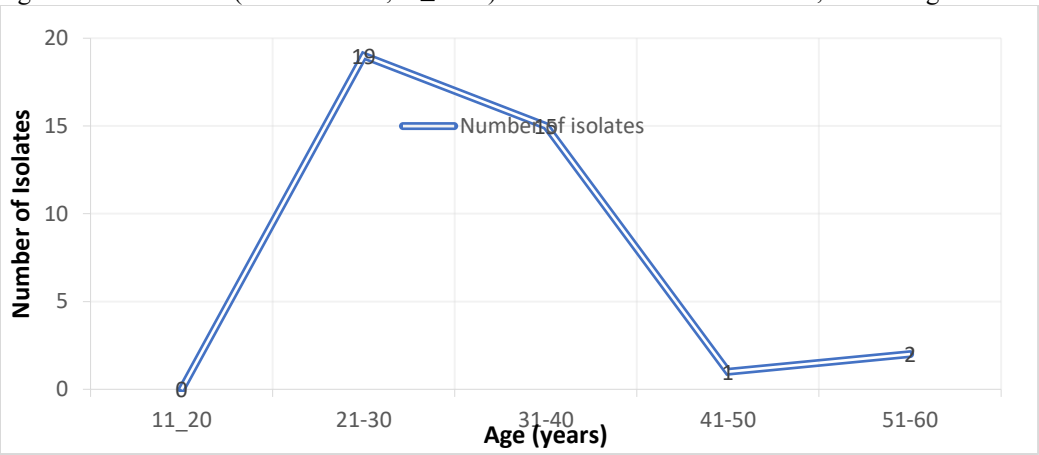

Fig 2: Distribution of isolates obtained from healthcare workers at Federal Medical Centre, Yenagoa by age.

The distribution of isolates by gender among the healthcare workers is indicated in **Table 2**. The gender with the highest number of isolates was female with 18(66.7%) while the least was male with 9(33.3%).

Table 2: Distribution of isolates obtained from healthcare workers at Federal Medical Centre, Yenagoa by gender.

Age interval (Years)	Male	Female	Total number of specimen	Number of isolates Male	Female	Total number of isolates
11-20	0	0	0	0	0	0
21-30	15	36	51	5	14	19
31-40	5	10	15	2	3	5
41-50	2	5	7	1	0	1
51-60	4	3	7	1	1	2
Total	26	54	80	9	18	27

Table 3 shows the antibiotic sensitivity pattern of *Staphylococcus* species obtained from the skin of healthcare workers in the Federal Medical Centre, Yenagoa. Notably, all the isolated *Staphylococcus* species were susceptible to amoxicillin-clavulanate but high sensitivity was observed with amoxicillin clavulanate, levofloxacin, ofloxacin, erythromycin, gentamycin, and azithromycin. High resistance of the *Staphylococcus* species to cefotaxime, ceftriaxone, cefixime, imipenem/cilastatin, and cefuroxime was also observed. *S. aureus* was most susceptible to levofloxacin and azithromycin with 12(100%) respectively, followed by gentamicin at 11(91.7%). *S. eperdimidis* was most susceptible to levofloxacin and azithromycin with 13(100%) respectively, followed by ofloxacin and erythromycin 11(84.6%) respectively. *S. scuri* was mostly susceptible to amoxicillin-clavulanate, levofloxacin, erythromycin, gentamicin an d azithromycin with 2(100%) respectively, followed by ofloxacin 1(50%). The antibiotic susceptibility profile of the isolated *Staphylococcus* species showed a significant difference at (p ≤ 0.05).

Table 3: Antibiotic susceptibility profile of *Staphylococcus* species isolated from skin of healthcare workers in Federal Medical Centre, Yenagoa

Antibiotics	*S. aureus* n=12 (%)			*S. epidermidis* n=13 (%)			*S. scuri* n=2 (%)		
	NS	NI	NR	NS	NI	NR	NS	NI	NR
AUG	8(66.7)	0(0.0)	4(33.3)	9(69.2)	0(0.0)	4(30.8)	2(100)	0(0.0)	0(0.0)

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CTX	0(0.0) 0(0.0) 12(100)	0(0.0) 0(0.0) 13(100)	0(0.0) 0(0.0) 2(100)
CRO	0(0.0) 0(0.0) 12(100)	0(0.0) 0(0.0) 13(100)	0(0.0) 0(0.0) 2(100)
ZEM	0(0.0) 0(0.0) 12(100)	0(0.0) 0(0.0) 13(100)	0(0.0) 0(0.0) 2(100)
LBC	12(100) 0(0.0) 0(0.0)	13(100) 0(0.0) 0(0.0)	2(100) 0(0.0) 0(0.0)
CIP	0(0.0) 12(100) 0(0.0)	0(0.0) 13(100) 0(0.0)	0(0.0) 2(100) 0(0.0)
IMP	0(0.0) 0(0.0) 12(100)	0(0.0) 0(0.0) 13(100)	0(0.0) 0(0.0) 2(100)
CXM	0(0.0) 0(0.0) 12(100)	0(0.0) 0(0.0) 13(100)	0(0.0) 0(0.0) 2(100)
OFX	9(75) 1(8.3) 2(16.7)	11(84.6) 0(0.0) 2(15.4)	1(50) 1(50) 0(0.0)
ERY	9(75) 2(16.7) 1(8.3)	11(84.6) 2(15.4) 0(0.0)	2(100) 0(0.0) 0(0.0)
GN	11(91.7) 0(0.0) 1(8.3)	10(76.9) 1(7.7) 2(15.4)	2(100) 0(0.0) 0(0.0)
AZN	12(100) 0(0.0) 0(0.0)	13(100) 0(0.0) 0(0.0)	2(100) 0(0.0) 0(0.0)

*NS-number of sensitive, NI-number of intermediate, NR-number of resistant, %-percentage.

Key: AUG: amoxicillin clavulanate(30µg), CTX: cefotaxime(25µg), CRO: ceftriaxone sulbactam(45µg), ZEM: cefexime(5µg), LBC: levofloxacin(5µg), CIP: ciprofloxacin(5µg), IMP: imipenem/cilastatin(10/10µg), CXM: cefuroxime(30µg), OFX: ofloxacin(5µg), ERY: erythromycin(15µg), GN: gentamycin(10µg), and AZN: azithromycin (15µg). The plasmid profile using gel electrophoresis indicates the presence of plasmid DNA bands of *Staphylococcus* species at molecular weight above the DNA molecular weight of 23130bp respectively as shown in **Figure 3**. Lane 1, DNA ladder; Lane 2, *S. scuri*; Lane 3, *S. epidermidis*; Lane 4, *S. aureus*; Lane 5, *S. epidermidis.*

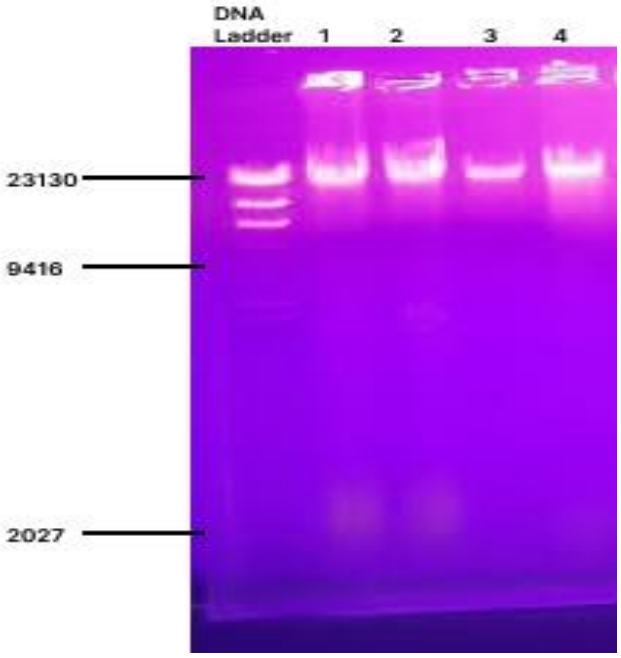

Figure 3: Plasmid DNA of *Staphylococcus* species on agarose gel stained with ethidium bromide. Lane M; marker- 23130bp DNA ladder Lane 1, *S. scuri*; Lane 2, *S. epidermidis*; Lane 3, *S. aureus*; Lane 4, *S. epidermidis.*

Table 4: Antibiotic Susceptibility test before Plasmid curing

Isolates	AUG (mm)	CTX (mm)	C (mm)	TE (mm)	E (mm)	LNZ (mm)	VA (mm)	AZN (mm)
S. scuri	0	0	10	5	0	8	8	5
S. epidermidis	6	11	10	4	0	5	0	3
S. aureus	0	0	8	9	7	8	8	0
S. epidermidis	0	7	12	6	0	10	8	2

Key: AUG= amoxicillin clavulanate,CTX= cefotaxime,C= chloramphenicol, TE= tetracycline, E= erythromycin, LNZ= linezolid, VA=vancomycin, AZN= azithromycin,

The antibiotic susceptibility test before plasmid curing is shown on **Table 4.** Before curing, all the *Staphylococcus* species exhibited resistance to all the antibiotics. The multidrug-resistant *Staphylococcus* species that were subjected to plasmid curing showed notable improvement in susceptibility to some of the antibiotics as indicated in **Table 5**. *S. scuri* was susceptible to cefotaxime and chloramphenicol with inhibition zones of 27mm and 24mm respectively. *S. epidermidis* was susceptible to chloramphenicol and vancomycin with 19mm and 13mm respectively while *S. aureus* was susceptible to cefotaxime and vancomycin with inhibition zone of 24mm and 16mm respectively.

Table 5: Antibiotic Susceptibility test after Plasmid curing

Isolates	AUG (mm)	CTX (mm)	C (mm)	TE (mm)	E (mm)	LNZ (mm)	VA (mm)	AZN (mm)
S. scuri	6	27	24	18	6	14	6	7
S. epidermidis	6	22	19	18	6	13	13	7
S. aureus	6	24	7	19	6	15	16	8
S. epidermidis	6	20	8	7	6	12	6	8

Key: AUG= amoxicillin clavulanate, CTX= cefotaxime, C= chloramphenicol, TE= tetracycline, E= erythromycin, LNZ= linezolid, VA=vancomycin, AZN= azithromycin

The biofilm formation analysis is presented in Table 6. *S. scuri* and one variant of *S. epidermidis* were found to be biofilm producers while the other strain of *S. epidermidis* and *S.aureus* was found not to produce biofilm.

Table 6: Biofilm formation analysis

Isolates	Description	Remark
S. scuri	The colour of the medium turns from red to black after 24 hours of incubation	Positive
S. epidermidis	The colour of the medium remains the same after 24hrs incubation	Negative
S. epidermidis	The color of the medium turns from red to black after 24 hours of incubation	Positive

The phylogenetic tree showing the evolutionary relationship between the *Staphylococcus* isolates is shown in Figure 4. The evolutionary distances computed using the Jukes-Cantor method agreed with the phylogenetic placement of the 16S rRNA of the isolates within the *Staphylococcus* species and revealed a close relatedness to *S. scuri*.

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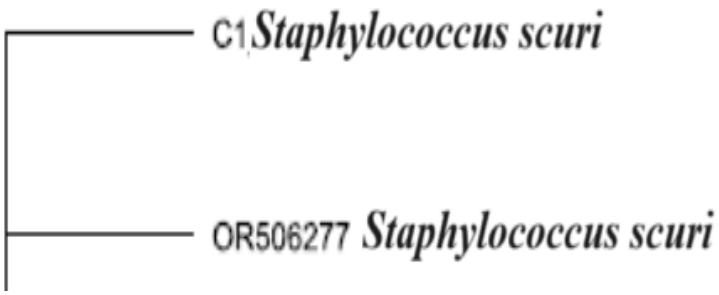

Fig 4: Phylogenetic tree of the evolutionary relationship between the *Staphylococcus* species.

Discussion

In this study, the *Staphylococcus* species identified were *S. epidermidis, S. aureus,* and *S. scuri,* among which *Staphylococcus epidermidis* 13(48.2%), had the highest percentage of occurrence, followed by *Staphylococcus aureus* 12(44.4%), while *Staphylococcus scuri* 2(7.4%) had the lowest occurrence. Our result corroborates the findings of Côrtes et al. (2022), who reported *S. epidermidis* and *S. aureus* to be the most isolated *Staphylococcus* species in a healthcare setting, and *Staphylococcus epidermidis* as the most prevalent species of coagulase-negative staphylococci involved in human infections. Furthermore, the finding by Laux et al. (2019) reported *S. aureus* as the leading cause of healthcare-associated infections that is known to colonize the dermis and mucosa of individuals. Its high occurrence in this study could be attributed to the likelihood of cross-contamination from infected patients to healthcare workers who are predominantly more exposed to this bacterium due to the nature of their work (Moremi et al., 2019). Also, due to the capability of *Staphylococcus epidermidis* to produce bacterial films, it may have had more opportunities for bacterial biofilms production on medical instruments used by healthcare workers, thereby leading to its increased occurrence and resistance (de Vor et al., 2020). The age group displaying the highest number of isolates consisted of individuals aged 21-30 years with 19(70.4%). The highest obtained isolates were seen in female healthcare workers with 18(66.7%) while the least was male with 9(33.3%). A significant difference ($p \leq 0.05$) was observed between the isolates obtained, the age and the gender of the healthcare workers.

The antibiogram profile revealed the *Staphylococcus* species were multidrug drug resistant. Most resistance was observed with cefotaxime, ceftriaxone, cefexime, imipenem/cilastatin, and cefuroxime. The elevated resistance levels could potentially stem from inadequate hygiene practices and improper handling of medical procedures and devices by healthcare workers, creating an environment conducive to organism proliferation, leading to the formation of biofilms. Additionally, misuse and non-compliance with antibiotic treatment courses by patients could exacerbate the situation (Anyiam & Udoegbulam, 2024). The *Staphylococcus* species were also highly sensitive to amoxicillin clavulanate, levofloxacin, ofloxacin, erythromycin, gentamycin, and azithromycin. The increased susceptibility could be as a result non-abuse and strict compliance of these drugs by the patients. The findings in our study can be compared to the research conducted by Akani et al. (2021) who reported that the isolated *Staphylococcus* species were most sensitive and resistant to the same antibiotics used in this study.

In this study, the plasmid analysis showed the presence of plasmid DNA bands of the *Staphylococcus* species at a molecular weight above 23130bp of the DNA ladder. The presence of the plasmids is an indication of the existence of specific antibiotic resistance genes with potential for lateral gene exchange. The consistency of the single plasmid size type above 23130bp across the *Staphylococcus* species that were profiled indicates that their resistance is not chromosomal-borne. As reported in a study by Souza-Silva et al. (2022), genes located on plasmids are typically expressed at higher levels compared to genes present in singular copies on the chromosome. Consequently, this elevated expression often confers increased resistance to corresponding drugs at higher levels. As described in a review by Rodríguez-Beltrán et al. (2021), it is conceivable that antimicrobial-resistant genes may move between the plasmid and the chromosome, conversely suggesting the reason the resistant genes in the isolates became susceptible after plasmid curing. Furthermore, the observed plasmid bands appearing higher than the molecular weight of the DNA ladder could be because of changes in EDTA concentration or type, which can impact the migration pattern of plasmid DNAs on the gel. A study by Sanderson et al. (2014) demonstrated that changes in EDTA concentration or type could potentially lead to plasmid bands migrating differently on the gel, causing them to appear higher or lower

than expected relative to the molecular weight markers in the DNA ladder. The study also suggested that plasmid bands appearing higher than the molecular weight of the DNA ladder could be due to the specific characteristics of the plasmid DNA itself. Plasmids are often supercoiled or circular molecules, which can affect their mobility during gel electrophoresis compared to linear DNA molecules present in the DNA ladder. Supercoiled plasmids, due to their compact structure, may experience different frictional forces and interactions with the gel matrix compared to linear DNA molecules, which could result in altered migration rates, causing supercoiled plasmid bands to migrate slower or appear higher on the gel than expected based on their actual molecular weight (Smrek et al., 2021).

Before plasmid curing, all the *Staphylococcus* species exhibited resistance to all the antibiotics. On subjected to curing, the multidrug resistant *Staphylococcus* species showed notable improvement in susceptibility to some of the antibiotics, which indicates a positive impact of plasmid curing in reducing antibiotic resistance in the multidrug resistant *Staphylococcus* species. Complete and permanent curing of resistance may be challenging due to the adaptability and resilience of bacteria in response to selective pressures, resulting from the complex interplay of genetic, environmental, and evolutionary factors that contribute to the variability observed in the outcomes of efforts to eliminate antibiotic resistance in bacterial populations (Hernando-Amado et al., 2019; Chinemerem et al., 2022). Nonetheless, *S. scuri* was susceptible to cefotaxime and chloramphenicol with inhibition zones of 27mm and 24mm respectively. *S. epidermidis* was susceptible to chloramphenicol and vancomycin with 19mm and 13mm respectively while *S. aureus* was susceptible to cefotaxime and vancomycin with inhibition zone of 24mm and 16mm respectively.

Out of the multidrug resistant isolates that were analyzed for biofilm formation, *S. scuri* and one strain of *S. epidermidis* were seen to be producers of biofilm. This agrees with a study by Al-Jubory and Essa (2021), where they compared three biofilm detection methods in coagulase negative *Staphylococci* species and found *S. scuri* and *S. epidermidis* to produce biofilms. Although numerous studies have found *S. epidermidis* to be a biofilm producer, its other strain isolated in this study was observed not to produce biofilm, which could be because of genetic variability among bacterial strains with some lacking the ability to exhibit strong biofilm-forming capabilities (Lianou et al., 2020). The isolate's 16S rRNA sequence, upon phylogenetic analysis, demonstrated an exact match with *S. scuri* during the megablast search of highly similar sequences within the NCBI non-redundant nucleotide (nr/nt) database. Furthermore, the evolutionary distances was computed using the Jukes-Cantor method, corroborated the phylogenetic placement of the isolate's 16S rRNA within the *Staphylococcus* species, indicating a significant proximity to *S. scuri* (Jukes and Cantor, 1969).

Conclusion

The rising prevalence of multidrug-resistant *Staphylococcus* species, especially within a healthcare environment, poses a significant concern due to their heightened potential for transmission. According to this study, *S. epidermidis* and *S. aureus* emerge as the most prevalent species of coagulase-negative and coagulase-positive staphylococci, respectively, implicated in human infections within healthcare settings. To prevent cross-contamination of these resistant pathogens, it is important to strengthen infection control practices, emphasizing strict adherence to hand hygiene, proper disinfection, and sterilization procedures. Prioritizing continuous monitoring and molecular characterization of multi-drug resistant *Staphylococcus* species is essential in both human and animal sectors to curb the dissemination of these resistant pathogens. This study identified genetic variability among the *Staphylococcus epidermidis* strains, leading to differences in biofilm-forming capabilities; therefore, further genomic studies should be conducted to elucidate the genetic factors contributing to this variability. Whole-genome sequencing or genetic profiling could provide a more comprehensive understanding of the genetic diversity within *Staphylococcus* strains and its implications for biofilm formation. Efforts should be made to advocate for the operation of strong management systems to continuously observe and track the prevalence of multidrug-resistant *Staphylococcus* species in a healthcare setting. While plasmid curing showed improvement in antibiotic susceptibility in this study, there was low feasibility of completely and permanently curing resistance due to bacterial adaptability. Further research could focus on understanding the long-term efficacy of plasmid-curing interventions and identifying factors that influence the sustainability of reduced antibiotic resistance over time.

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