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Detection of Virulence Genes of Staphylococcus Aureus from Wound Infections of Patients Attending Some Selected Hospitals in Maiduguri, Borno State

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Abstract **Original Research Article**

Staphylococcus aureus is a major bacterial pathogen responsible for a wide range of infections, from minor skin conditions to severe, life-threatening diseases such as sepsis, pneumonia, and endocarditis. Its pathogenicity is attributed to various virulence factors, including toxins, enzymes, and surface proteins, which aid in tissue invasion, immune evasion, and antimicrobial resistance. This study aimed to isolate S. aureus from wound infections and detect the virulence genes contributing to its pathogenicity. A total of 180 wound swabs were collected from patients with suspected bacterial infections at healthcare facilities. Conventional bacteriological methods, including Gram staining, biochemical testing, and culture on selective media, were employed for bacterial identification, while conventional PCR was used to detect the virulence genes hlaA (alpha-hemolysin), fnbA (fibronectin-binding protein A), and icaA (intercellular adhesion protein). Out of the 180 samples analysed, 20 tested positives for S. aureus, yielding a prevalence rate of 11.1%. The positive isolates were subjected to antibiotic susceptibility testing to determine their resistance profiles. The majority of isolates exhibited multidrug resistance (MDR), with 9 out of 20 showing resistance to multiple antibiotics. High resistance rates were observed against ceftriaxone-sulbactam (100%), imipenem/cilastatin (100%), cefixime (100%), and amoxicillin-clavulanate (100%), while levofloxacin demonstrated the lowest resistance (5%), suggesting its potential as a preferred treatment option. Molecular analysis revealed the presence of virulence genes, with hlaA detected in 53.9% of isolates, indicating its major role in pathogenicity. The icaA and fnbA genes were identified in 23.1% of the isolates, emphasizing the importance of biofilm formation and tissue adhesion in some strains. These findings highlight the significant role of S. aureus in wound infections and the growing concern of antibiotic resistance.

Keywords: Staphylococcus aureus, Bacterial (wound) infections, multi-drugs resistance, Virulence genes, ceftriaxone-sulbactam, levofloxacin.

INTRODUCTION

The word "*Staphylococcus*" is derived from Greek words that mean "a bunch of grapes" (staphyle) and "berry" (kokkos) (Licitra, 2013). This genus contains a number of pathogenic bacteria, but one of the most important in the medical field is *Staphylococcus aureus*, which can be a short-term resident, a temporary contaminant, or a long-lasting colonizer and can cause a wide range of diseases. It is a common, gram-positive, facultatively anaerobic bacterium that is non-motile (does not move) and does not form spores. It is also known to cause diseases that can be passed from animals to humans and is a major cause of food poisoning cases (Rasul *et al.*, 2022). In humans, *S. aureus* frequently causes severe

infections, particularly in hospital environments, where it can initiate minor skin infections and progress to life-threatening conditions like bacteremia and endocarditis. Studies have identified *S. aureus* as a major contributor to hospital-acquired infections worldwide, responsible for illnesses such as endocarditis and osteomyelitis (Noor-E-Jannat *et al.,* 2021). A virulence factor that aids in the organism's identification, the coagulase enzyme is produced by almost all *Staphylococcus aureus* isolates (Brown *et al.,* 2005). With a mannitol-salt agar medium containing 7-5% sodium chloride, it can thrive and is salt tolerant, but it does not produce spores or flagella; instead, it has a capsule (Brown *et al.,* 2005; Tayeb-Fligelmen *et al.,* 2017). The organism has oxidase-negative and catalase-positive traits. Lactose fermentation and deoxyribonuclease have also been discovered to be positive in *Staphylococcus aureus* (Chino *et al.,* 2017). 20% of humans are permanently colonized by *Staphylococcus aureus*, a major human pathogen, and 60% are transiently colonized by it (Noskin *et al.,* 2005). These bacteria are accountable for a wide range of diseases. including cellulitis, folliculitis, impetigo, and abscesses, including skin and soft tissue infections (SSTIs), (Olaniyi *et al.,* 2016) to more invasive diseases such as sepsis, pneumonia, osteomyelitis and endocarditis (Wertheim *et al.*,2005). There are numerous virulence factors that *Staphylococcus aureus* possesses. These elements allowed the organism to thrive as a pathogen, infecting both humans and animals in a variety of ways. The induction of toxin-mediated syndrome, tissue invasion, attachment to host cells, lysis of the host immune system, and sepsis are all made possible by virulence factors. Han and Cho (2021). *Staphylococcus aureus* virulence genes linked to various wound infections include intracellular adhesion A (*icaA*), enterotoxins (sea), fibronectin-binding proteins A and B (*fnbA),* and human leukocyte antigen (*hla*). A considerable number of infections are caused by *Staphylococcus aureus* because of its virulence factors and high resistance to the majority of antibacterial drugs, as stated by Putnam *et al.* (2015). Controlling microbial populations on the skin's surface and preventing diseases from spreading to the underlying tissue are the main goals of the skin (Ndip *et al.,* 2017).

MATERIALS AND METHODS

Study Area

The study was conducted at State Specialist Hospital Maiduguri (SSHM), Umaru Shehu Ultra-Modern Hospital (USUMH), and Muhammed Shuwa Memorial Hospital (MSMH) in Maiduguri, Borno State. Borno State is located in northeastern Nigeria, with Maiduguri as its capital. The state was created in 1976 from the division of the northeastern states. It is situated at latitude 11° 50' North and longitude 13° 09' East, covering a total area of 61,435 square kilometers. The city of Maiduguri spans four local government areas: Maiduguri Metropolitan, Jere, Konduga, and Mafa. Borno State occupies most of the Chad Basin and shares international borders with the Republics of Niger to the north, Chad to the northeast, and Cameroon to the east. Within Nigeria, it is bordered by Adamawa to the south, Yobe to the west, and Gombe to the southwest. The climate of Maiduguri is characterized by a long dry season from October to May and a short rainy season for the rest of the year (Jime *et al.,* 2016). According to the 2006 census, the city has an estimated population of 1.275 million, with an annual growth rate of about 3.5% and a population density of 1,145 people per square kilometer, making it the most densely populated city in the Northeast (National Population Commission NPC, 2006).

Ethical Consideration

Ethical clearance/approval was obtained from the Research and Ethics Committees of the Borno State Ministry of Health and Human Services before commencing the study.

Sample Collection

A total of one hundred and eighty (180) wound infection samples (inpatient and outpatient) were collected at State Specialist Hospital Maiduguri (SSHM), Umaru Shehu Ultra-Modern Hospital (USUMH) and Muhammed Shuwa Memorial Hospital (MSMH), Maiduguri, Borno State. Sterile swabs that were labeled accordingly were used to collect the samples aseptically. All collected samples were carefully handled and aseptically transported to the Microbiology Laboratory, University of Maiduguri.

Determination of sample size

The desired minimum sample size was calculated to be 180 using the sample size formula described by Araoye. (2004) as follows for cross-sectional studies with a population of ≤ 10,000;

$$n_t = \frac{Nn}{N+n}$$

but can only be calculated from a population ≥10,000; given by

$n = \frac{z^2pq}{d^2}$ (Desired sample size when the population is greater than 10,000)

z = standard normal deviate at 95% confidence level usually set at 1.96

p= prevalence of MRSA reported in Maiduguri (13.55 %) (Gulani *et al.,*2016)
Where q= 1-p = 0.5
d= degree of precision or acceptable error margin (5% or 0.05 for this study).
This gives $n = \frac{1.96^2(0.135)(0.865)}{(0.05)^2}$
= 180

Isolation, Characterization and Identification of *Staphylococcus aureus*

The resulting samples were inoculated on Nutrient Agar (NA), sub cultured onto Mannitol Salt Agar (MSA) plates, and then incubated at 37°C for 24 to 48 hours. Different bacterial colonies and morphologies were examined on each plate. Colonies that showed clear hemolytic and tiny yellow traits on MSA and BA, respectively, were sub cultured on nutrient agar (NA) to produce pure colonies. For further studies, the pure colonies were maintained in NA slants. Based on the techniques used, isolates were examined (Akpudo *et al.,* 2023)
After streaking a suspect colony from each sample on nutrient agar, the bacterial colonies were subjected to a series of biochemical tests (catalase, coagulase and DNase test), in addition to morphological features, including Gram reaction, cell shape and arrangement assessed using Gram staining, to identify the organism.

Gram Staining

After placing a clean, heat-fixed smear slide on a staining rack, the smear was generously flooded with crystal violet and left to stand for one minute. The slide was then gently tilted and rinsed with distilled water using a wash bottle. After using Lugol's gram iodine to wash the smear, it was left to stand for one minute. After gently tilting the slide, distilled water was used to rinse it with a wash bottle. Using 95 percent ethyl alcohol, the smear was destained and showed up as purple on the slide. After applying the alcohol drop by drop for 10 seconds while tilting the slide, it was carefully counterstained with Safranin and left to stand for 45 seconds. A wash bottle was used to gently rinse the slide with distilled water while it was tilted slightly, and absorbent paper was used to pat dry oil immersion in objective lens of the microscope was used to examined the smeared slide at a magnification of 100x. *Staphylococcus aureus* has a purple appearance because it is a gram-positive organism (Leboffe, 2016).

Biochemical Tests

Catalase Test

This test aids in the differentiation of organisms that are catalase-positive, such as Staphylococci, from those that are catalase-negative, such as Streptococci. After transferring and streaking a small portion of the culture growth onto a sterile slide, a few drops of hydrogen peroxide H_2O_2 were added to the bacterial inoculum. The production of an oxygen bubble signified the presence of catalase and a positive result. The result is negative if there are no bubbles or only a few dispersed bubbles, which indicates that the

enzyme is not present. According to Reiner (2010), the glass slides were disposed of in a biohazard glass disposal container. This test is conducted using the tube method or the slide or drop method (Nguyen *et al.,* in 2023).

Coagulase Test

Coagulase, which is produced by *Staphylococcus aureus*, has the ability to agglutinate cocci on a slide or clot plasma into a gel in a tube. To differentiate *Staphylococcus aureus* from other coagulase-negative staphylococcal species, this test is crucial. Both free and bound coagulases are produced by the majority of *Staphylococcus aureus* strains. The tube coagulase test detects free coagulase, while the slide coagulase test detects bound coagulase. *Staphylococcus aureus* isolates can be screened using the slide coagulase test, and confirmation can be obtained using tube coagulase (Katz, 2010). Two ends of a sterile glass slide were coated with thick suspensions of cultured staphylococci in order to perform slide coagulase testing. The labels "test" and "control" are applied to both. After thoroughly mixing the test suspension, a drop of citrated plasma was added. Cocci clumping or agglutination within 5-10 seconds is regarded as positive (Katz, 2010).

DNase Test

The DNase test is used to assess a microorganism's capacity to produce deoxyribonuclease (DNase), an enzyme that breaks down DNA. This test is commonly employed to differentiate *Staphylococcus aureus* from other coagulase-negative Staphylococci. *Staphylococcus aureus* is typically DNase-positive, meaning it can hydrolyze DNA.

Procedure: DNase agar contains 2% tryptose, 0.2% deoxyribonucleic acid, 0.5% sodium chloride and a methyl green indicator was used to inoculate the plates. The surface of the DNase test agar plate was streaked with an inoculating loop using a well-isolated colony from a pure culture. The inoculated DNase test agar plates were placed in an incubator set at 37°C for 24 hours. Color changes were being observed in the colony growth *Staphylococcus aureus* typically produces a clear zone around the colonies due to the hydrolysis of DNA in the medium which indicates the colonies of ***Staphylococcus aureus*** (CLSI, 2020).

Antibiotic Susceptibility Test

The modified Kirby-Bauer disc diffusion method (CLSI). (2023). used to perform the susceptibility test. Commercially manufactured multidisc containing ten different antibiotics (POLY-TEST LABS; ENUGU, NIGERIA) were used in the study. Streptomycin (20 mg/ml), Ciprofloxacin (10 mg/ml), Erythromycin (30 mg/ml), Gentamicin (10 mg/ml), Levofloxacin (30 mg/ml), Norfloxacin (10 mg/ml), Rifampicin (10 mg/ml), Amoxicillin (30 mg/ml), Ampiclox (30 mg/ml), and Chloramphenicol (30 mg/ml) are all included on a single disc. A nutrient agar plate was covered with a very small volume (0-1 ml) of the bacterial suspension, surface that had been thoroughly dried, and it was tilted to distribute it uniformly across the plate surface. After draining the extra fluid, the mixture was dried in an incubator for ten minutes. After that, antibiotic discs were placed in the inoculation plates, refrigerated to enable the antibiotics to properly diffuse, and then aerobically incubated at 37°C for 24 hours. In every single case two distinct readings were obtained, including standard measurement of Zone Inhibition Diameter (ZID) using a meter scale and the routine sensitive or resistant reading. According to the manufacturer's manual guidelines, the results were interpreted as either sensitive or resistant. Multi-drug resistance (MDR) is the ability of a bacterial strain to withstand at least three (3) different classes of antibiotics; Extensive drug resistance (XDR) is the ability to resist only one or two classes of antibiotics; and pan-drug resistance (PDR) is the ability to resist all subclasses of all antibiotic classes (CLSI, 2018).

MOLECULAR DETECTION OF POSITIVE ISOLATES

DNA Extraction

As explained by Onanuga *et al.,* (2020), the DNA of every isolate of *Staphylococcus aureus* that was biochemically confirmed was extracted by suspending a large portion of the isolates' colonies from their

overnight Culture on nutrient agar plates in 100 µL 1X Tris-EDTA buffer, vortex and boiled at 100 °C for 10 minutes and was immediately placed in a freezer set at −20 °C for 10 minutes, left at room temperature, vortexed, and centrifuged at 10,000 rpm for 10 minutes. The DNA of each isolate was extracted from the resulting supernatant, which was then stored at 4°C and used as a template for PCR assays.

Extracted Isolates

Following biochemical tests and Gram staining, the isolates confirmed as positive for *Staphylococcus aureus* were:

62, 143, 16, 146, 72, 54, 6, 48, 15, 32, 18, 150, 140, 107, 113, 99, 77, 19, 149, 147

PCR Detection of Virulence genes (*hla, IcaA* and *fnbA)* **of the isolates (Table 4.8)**

***Hla* gene**

PCR analysis was used to determine whether the isolates carried the virulence gene, as previously reported (Ferreira *et al.,*2016). Each primer contains 10 pmol in 4 µL of 5X FIREPol Master Mix (Solis Biodyne, Estonia) (Genewiz, UK), 2 ¼L of the DNA template and nuclease free water (Bioconcept, Switzerland) were all included in a 20 µl reaction mixture. A thermocycler (Applied Biosystems, Germany) PCR samples were amplified using the following protocol: first denaturation at 95°C for 15 minutes, then 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, 72°C for 50 seconds, and a final extension at 72°C for 10 minutes.

***IcaA* gene**

The presence of the virulence gene in the isolates was assessed by PCR analysis as previously described (Monteiro *et al.,*2019). A 20 µl reaction mixture contained 4µL of 5X FIREPol Master mix (Solis Biodyne, Estonia), 10 pmol each of the primers (Genewiz, UK), 2.4 µl of the DNA template, and was made up with Nuclease Free Water (Bioconcept, Switzerland). In accordance with the following program, the PCR samples were amplified using a thermocycler (Applied Biosystems, Germany): initial denaturation at 95°C for 15 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, 72°C for 50 seconds, and a final extension at 72°C for 10 minutes.

***fnbA* gene**

The presence of the virulence gene in the isolates was assessed by PCR analysis as previously described (Monteiro *et al.,*2019). A 20 µl reaction mixture contained 4µL of 5X FIREPol Master mix (Solis Biodyne, Estonia), 10 pmol each of the primers (Genewiz, UK), 2.4 µl of the DNA template, and was made up with Nuclease Free Water (Bioconcept, Switzerland). The PCR samples were amplified using a thermocycler (Applied Biosystems, Germany) in accordance with the following protocol: 15 minutes of initial denaturation at 95°C, 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, 72°C for 50 s, and extension at 72°C for 10 min.

Gel Electrophoresis of the Amplicons

Amplicons were electrophoresed utilizing 1 X TAE (40 mM Tris (pH 7.6), 20 mM acetic acid, and 1 mM EDTA) on a 1 percent (w/v) agarose gel containing 0.001µg/ml ethidium bromide at 100V for 45 minutes. An Imaging System for Maestrogen (MaestroGen Inc. Taiwan) was employed to take the picture. A 100 bp molecular weight marker (BioLab, New England) was present in every gel.

Table 1 presents the primers used for the amplification of the *hla, icaA,* and *fnbA* genes. The *hla* gene, encoding haemolysin, was amplified using primers that produced a 209 bp fragment. The *icaA* gene, responsible for intracellular adhesion, was targeted with primers generating a 770 bp product. Similarly, the *fnbA* gene, which encodes the fibronectin-binding protein, was amplified with primers yielding a 128 bp fragment. These primers are essential for detecting and characterizing virulence factors in *Staphylococcus aureus*.

Table 1 Primers Used for Amplification of the hla, icaA, fnbA Genes

Gene	Primer sequence (5'-3')	Size (bp)
hla (Haemolysin)	F: CTGATTACTATCCAAGAAATTCGATTG R: CTTTCCAGCCTACTTTTTTATCAGT	209
icaA (*Intracellular adhesion)*	F: GATTATGTAATGTGCTTGGA R: ACTACTGCTGCGTTAATAAT	770
fnbA (Fibronectin binding protein)	F: CATAAATTGGGAGCAGCATCA R: ATCAGCAGCTGAATTCCCATT	128

Statistical Analysis

The IBM-SPSS version (23.0) was used to analyze the data from this study and a chi- squared test of association was performed to determine whether there was a statistically significant difference (coincidence level of 95%) between *Staphylococcus aureus* isolates and the relevant variables in this study, a p-value <0.05 was deemed significant.

RESULTS

Isolation of *Staphylococcus aureus*

Table 2 shows the isolation rate of *Staphylococcus aureus* from State Specialist Hospital, Umaru Shehu Ultra-Modern Hospital, and Muhammed Shuwa Memorial Hospital (SSHM, USUMH, MSMH) Maiduguri, with no significant difference ($p > 0.05$) observed among Patients from the Selected Maiduguri Hospitals. The parameters with no significant differences were identified ($\chi 2 = 2.999$, $p = 0.083$). A total of 180 samples were cultured, yielding 20 isolates. This study found a prevalence of 26.36%.

Table 2. Isolation rates of *Staphylococcus aureus* in Relation to Samples from Patients at State Specialist Hospital, Umaru Shehu Ultra-Modern Hospital and Muhammed Shuwa Memorial Hospital Maiduguri

Hospitals	Number of Samples (%)	Number (%) of *S. aureus*	χ^2	p-value
		Positive (%)		
State Specialist Hospital	105	15(14.29)	2.99	0.083
Umaru Shehu Ultra-Modern Hospital	47	4(8.51)		
Muhammed Shuwa Memorial Hospital	28	1(3.57)		
Total	**180**	**20(11.11%)**		

($p<0.05$), χ^2 = Chi square

Antimicrobial Susceptibility Testing

Table 3. shows the antimicrobial susceptibility pattern of twenty (20) *Staphylococcus aureus* isolated from clinical samples collected at State Specialist Hospital Maiduguri, Umaru Shehu Ultra-Modern Hospital, and Muhammed Shuwa Memorial Hospital Maiduguri, using 12 antibiotics. Amoxicillin, Cefotaxime, Ceftriaxone Sulbactam, Imipenem/Cilastain, and Cefexime had the highest resistance with 20 (100%) positive samples, while Levofloxacin had the lowest resistance with 1 (5%) positive sample.

Then, Levofloxacin demonstrated the highest susceptibility with 17 (85%) samples, and also Azithromycin had the lowest with 1 (5%) positive sample Table 3

PLATE:- A show *Staphylococcus aureus* multi-drug Resistance, **PLATE:- B** show *Staphylococcus aureus* Susceptibility.
Figure 1: Antimicrobial confirmation of *S. aureus.* **Plate: - A** MDR of *S. aureus.*
Plate: - B Susceptibility of *Staphylococcus aureus* Photo credit: M.S Mala**.**

Table 3. Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* Isolated from Clinical Samples in Some Selected Hospital Maiduguri

S/N	Antibiotics	Symbol	S No (%)	R No (%) n=20
1	Amoxicillin Clavulanate	AUG (30µg)	0(0%)	20(100%)
2	Cefotaxime	CTX (25µg)	0(0%)	20(100%)
3	Ceftriaxone Sulbactam	CRO (45µg)	0(0%)	20(100%)
4	Imipenem/Cilastain	IMP (10/10µg)	0(0%)	20(100%)
5	Cefuroxime	CXM (30µg)	0(0%)	18(90%)
6	Ofloxacin	OFX (5µg)	14(70%)	2(10%)
7	Cefexime	ZEM (5µg)	0(0%)	20(100%)
8	Levofloxacin	LBC (5µg)	17(85%)	1(5%)
9	Ciprofloxacin	CIP (5µg)	3(15%)	5(25%)
10	Erythromycin	ERY (15µg)	0(0%)	15(75%)
11	Gentamycin	GN (10µg)	9(45%)	10(50%)
12	Azithromycin	AZN (15µg)	1(5%)	17(85%)

S= Sensitive, **R**= Resistant, **n**= Number of positive isolates **(µg)** = Micro gram

Detection of Virulence Genes

The virulence genes of the isolates in this study are shown in Table 4.8, where *hla, icaA,* and *fnbA* were identified by PCR amplification. *hla* was the most prevalent type, accounting for 30% of the isolates. Fifteen percent (15%) of the isolates had *fnbA* and *icaA*. The amplicon sizes of **209 bp for *hla*, 770 bp for *icaA*, and 128 bp for *fnbA*** were determined using PCR amplification with gene-specific primers. DNA extracted from the isolates was amplified, and the resulting PCR products were analyzed using agarose gel electrophoresis alongside a 100 bp DNA ladder for size comparison. The presence of bands at the expected sizes confirmed the genes' presence, with positive (*Staphylococcus aureus*) and negative (*Escherichia coli* ATCC 25922) controls ensuring specificity. These sizes were established based on prior genetic studies and validated through PCR and electrophoresis analysis (Table 4).

Table 4.: List of *hla*, *icaA* and *fnbA* Positive Isolates

Isolates	Genes
62	*hla*
143	-
16	*fnbA hla*
146	*hla*
54	*IcaA*
6	-
48	-
15	-
72	-
32	*IcaA*
18	*hla*
150	-
140	*fnbA*
107	*hla*
113	*hla*
99	-
77	*fnbA*
19	*IcaA*
149	-
147	-

key;
***fnbA;* -** fibronectin binding protein A, ***hla*: -** hemolysin adhesin
***icaA*: -** Intracellular adhesin

Virulence Genes Distribution of *Staphylococcus aureus*

Table 5. shows the virulence genes of the isolates in this study. *hla, icaA* and *fnbA* were detected by PCR amplification, showing that *hla* was the most predominant in 53.85% of the isolates. *icaA* was found in 23.08% of isolates. Amplicon sizes of 128, 209, and 770 bp were considered positive for the presence of *hla, icaA,* and *fnbA*, respectively. And the gel electrophoresis image confirms the presence of *hla*, *icaA*, and *fnbA* genes in various *Staphylococcus aureus* isolates. Lanes 2-4, representing positive controls, verify successful amplification, while lane 5 (negative control, *Escherichia coli* ATCC 25922) ensures specificity. The *icaA* gene, associated with biofilm formation, is detected in isolates 32, 54, and 19 (lanes 6, 7, and 14), while the *hla* gene, linked to haemolysis, is present in isolates 18, 107, and 113 (lanes 8, 12, and 15). The *fnbA* gene, involved in bacterial adhesion, appears in isolates 140 and 77 (lanes 10 and

13). Isolates 48 and 72 (lanes 9 and 11) show no amplification, indicating the absence of these genes. These findings provide insights into the virulence potential of the isolates (Plate 1).

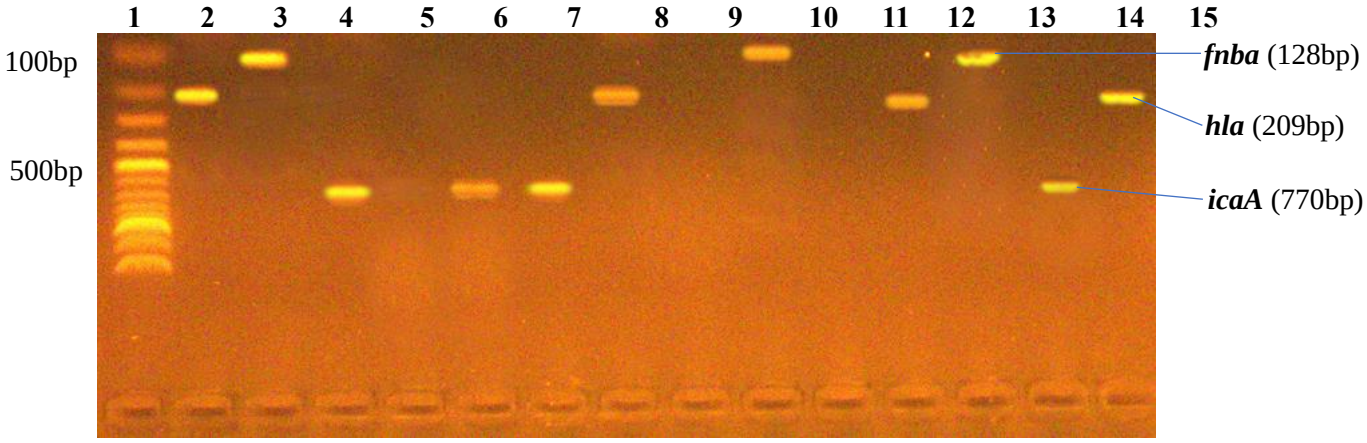

Plate 1: A gel picture showing the bands of the amplified *hla, icaA* and *fnba* genes
Lane 1: 100 bp ladder; Lane 2: positive control (*Staphylococcus aureus);* Lane 3: positive control (Staphylococcus aureus); Lane 4: positive control (*Staphylococcus aureus*); Lane 5: negative control (Escherichia coli ATCC 25922); Lane 6: isolate 32 (*icaA*); Lane 7: isolate 54 (*icaA*); Lane 8: isolate 18 (*hla*); lane 9; isolate 48 (negative); lane 10; isolate 140 (*fnba*); Lane 11: Isolate 72 (Negative); Lane 12: isolate 107 (*hla*); Lane 13: isolate 77 (*fnba*); Lane 14: isolate 19 (*icaA*); Lane 15: Isolate 113 (*hla*)

Keys:
***fnbA;* -** fibronectin binding protein A
***hla*: -** hemolysin adhesin A
***icaA*: -** Intracellular adhesin A
bp: - base pairs

Virulence Genes Distribution of *S. aureus* Causing Wound Infections

S/N	Virulence genes	Total No. = 12 (%)	MDR No. =9 (%)
1	*Hla*	7 (53.9)	5 (55.6)
2	*icaA*	3 (23.1)	3 (33.3)
3	*fnbA*	3 (23.2)	1 (11.1)
TOTAL		**13**	**9**

Key:
hla= alpha-hemolysin, *icaA*= intercellular adhesion gene A, *fnbA* = fibronectin-binding protein A, MDR = multi-resistance

DISCUSSION CONCLUSION AND RECOMMENDATION
DISCUSSION

Hospitals face a serious clinical problem with wound infections, especially in developing nations where adequate healthcare is impeded by a lack of funding (Tatah *et al.,* 2014). *Staphylococcus aureus* is an important pathogen with significant infectious potential due to its high pathogenicity and involvement in causing a wide range of infections. It is also the most common pathogenic bacterium present in various wound infections (Chen *et al.,* 2012; Bessa *et al.,* 2015). The prevalence of *Staphylococcus aureus* observed in this study is 26.36% of all clinical samples from patients, which is lower than the results of Ahmed *et al.* (2017) in Sokoto Specialist Hospital, where 52.6% was isolated from wounds of patients.

Onanuga and Awhowho (2012) reported 33.6% prevalence in patients in Yenagoa, South-South Region, Nigeria. This study's prevalence rate of 26.36% aligns with findings from other parts of Nigeria. The lack of significant differences in *Staphylococcus aureus* isolation rates between some selected hospitals in Maiduguri (SSHM, USUMH, and MSMH) suggests that the pathogen's distribution is consistent with the results of other studies in Nigeria, which have shown uniform infection rates across hospitals (Iregbu *et al.,* 2021). Similar to my findings of the present study in Maiduguri, studies in Lagos have shown an even distribution of *Staphylococcus aureus* infections across hospitals. Research conducted in several hospitals in Lagos, including the University of Lagos Teaching Hospital and the Lagos State University Teaching Hospital, found no significant differences in *Staphylococcus aureus* isolation rates (Iregbu *et al.,* 2021). Studies in Kano have also demonstrated that *Staphylococcus aureus* infections are relatively evenly distributed across various hospitals, including Murtala Mohammed Specialist Hospital and Aminu Kano Teaching Hospital. No significant differences were found in *Staphylococcus aureus* isolation rates (Sadiya *et al.,* 2019), which is consistent with the findings of this research. This supports the conclusion that there is no significant disparity in isolation rates among patients from selected hospitals in Maiduguri.

CONCLUSION

Staphylococcus aureus is considered the major pathogen due to its high pathogenicity and ability to cause a wide range of infections, out of 180 samples collected from wound infections 20 were found to be positive *S. aureus* with prevalence of 11.1%. The positive isolate was subjected to antibiotic susceptibility test and 9 were multi-drug resistance Levofloxacin and Ofloxacin showed the highest susceptibility with 85% susceptibility. This study showed a high prevalence of virulence genes among *Staphylococcus aureus* isolates, and the presence of these genes contributed significantly to the severity and progression of wound infections, increasing the likelihood of complications and treatment failure and therefore the detection of *Staphylococcus aureus* virulence genes from the wound facilitated infections are associated with multidrug resistance (MDR), which provides important insights into the pathogenicity and treatment challenges of this bacterium within the Selected hospitals. According to the current study's limitations, the incidence of infections caused by MDR *Staphylococcus aureus* has increased as a result of the difficult, pervasive, and increasingly difficult bacterial resistance to antibiotics. In *Staphylococcus aureus* isolates, this study found several significant virulence genes, such as ***hla*, *icaA* and *fnbA*** were detected in 11 of the isolates indicating presence of potentially pathogenic *Staphylococcus aureus.* The existence of these genes emphasizes the bacterium's capacity to produce toxins, form biofilms, and adhere to host tissue in order to cause serious infections.

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