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Molecular Characterization and Detection of Some Virulent (Fnba, Hla and Cna) Genes of Staphylococcus aureus Isolated from Some Clinical Specimen in Madonna University Teaching Hospital (M.U.T.H), Elele.

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Abstract- ***Staphylococcus aureus (S. aureus) is a major human pathogen associated with diverse infections due to its array of virulence factors. This study aimed to isolate S. aureus from clinical samples at Madonna University Teaching Hospital (MUTH), Elele, Nigeria, and molecularly detect the virulence genes fibronectin-binding protein A (fnbA), collagen adhesin (cna), and alpha-haemolysin (hla). A total of 106 clinical samples (mainly urine, high vaginal swab, endocervical swab, wound swab, sputum, and skin swab) were collected from patients. S. aureus was isolated using standard bacteriological methods (blood agar, mannitol salt agar, Gram staining, catalase, and coagulase tests) and confirmed molecularly. Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method. Genomic DNA was extracted by the boiling method, and the target virulence genes were detected by polymerase chain reaction (PCR) followed by agarose gel electrophoresis. Out of 106 samples, 26 (24.5%) yielded S. aureus, with higher prevalence among females (69.2%) and in the 31–40 years age group (46.2%). Among 18 isolates tested, fnbA was detected in 13 (72.2%), hla in 5 (27.8%), while cna was absent in all isolates. No statistically significant associations were observed between the genes and demographic/clinical variables (p>0.05). This study provides the first molecular characterization of S. aureus virulence genes at MUTH and in the south-south geopolitical zone of Nigeria. The findings highlight the predominance of fnbA-mediated adhesion in local strains and the absence of the cna gene, suggesting regional clonal variations. Routine molecular surveillance is recommended for better management of S. aureus infections.***

Keywords: cna, disk diffusion, fnbA, genome, hla, PCR, virulent genes and Staphylococcus aureus,

I. INTRODUCTION

Staphylococcus aureus is a versatile Gram-positive coccus and facultative aero-anaerobic bacterium that functions both as a commensal and an opportunistic pathogen capable of causing a broad spectrum of human diseases (Getanah et al., 2021). It can infect almost any tissue and produce both acute and chronic infections ranging from superficial skin abscesses to deep-seated conditions such as osteomyelitis, endocarditis, and bacteraemia. Pathogenicity is driven primarily by a wide array of virulence factors, including surface adhesins, toxins, and immune-evasion strategies. Surface proteins such as fibronectin-binding protein A (FnBPA), clumping factor A (ClfA), and protein A mediate host-tissue adhesion and biofilm formation (Berry et al., 2022).

Toxins produced by S. aureus include alpha-toxin (encoded by hla), Panton-Valentine leukocidin (PVL), and enterotoxins; these contribute to host-cell damage and disease severity (Leistner et al., 2022). Biofilm formation further protects the organism from antibiotics and host immunity, facilitating persistence on medical devices and in chronic infections (Dancova et al., 2025). Immune-evasion mechanisms include protein A binding to the Fc region of antibodies, thereby inhibiting opsonization and phagocytosis (Schilcher and Horswill, 2020).

Methicillin-resistant S. aureus (MRSA) are mediated by mecA gene, which remains a major global health threat (Li et al., 2025; Kishita et al., 2023). Molecular

epidemiology studies employing multi-locus sequence typing (MLST) and whole-genome sequencing (WGS) have described clonal complexes (e.g., CC5, CC8, CC22) and sequence types associated with health-care or community associated infections (Monecke et al., 2023; Huang et al., 2024; Yu et al., 2025).

Despite the extensive data on the phenotypic and molecular from southwestern and northern Nigeria, the south-south geopolitical zone remains understudied. There is no published work that has systematically investigated the molecular epidemiology and virulence-gene profiles of S. aureus isolated from clinical specimen at Madonna University Teaching Hospital (MUTH), Elele. This knowledge gap is a major constraint in categorizing the risk, targeted therapy, and infection-control measures.

This present study therefore is aimed to isolate S. aureus from clinical samples at MUTH, identifying the isolates by standard bacteriological methods, and detecting the virulence genes fnbA, cna, and hla using molecular techniques, thereby providing the first baseline molecular data for the institution and the south-south region.

II. MATERIALS AND METHODS

Study Area, Study Site and Design

Madonna University Teaching Hospital (M.U.T.H) is a private tertiary healthcare institution located in Elele, Ikwerre Local Government Area, Rivers State, south-south Nigeria (latitude 5.1018°N, longitude 6.8190°E). Sample collection and bacteriological analysis was done in Microbiology Laboratory section of M.U.T.H, Elele while the molecular analyses were performed at Nucleometrix Research Laboratory, Bayelsa, all in Nigeria. This is a hospital-based cross-sectional study that lasted for six months.

Ethical consideration

Ethical approval was obtained from the University Ethical Committee (ref. no: MUN/VC/REC/MUN/PG/MLS/MSC/23/003).

Sample Collection and Processing

A total of 106 clinical specimens (urine, high vaginal swab [HVS], endocervical swab [ECS], wound swab, sputum, and skin swab) were collected from patients attending MUTH using sterile universal containers for urine and sputum samples while HVS, ECS, wound swab and skin swab were collected with a sterile swab stick. The samples were inoculated onto blood agar (0.001 mL calibrated loop) and incubated at 37°C for 24hr. Colonies were sub-cultured onto Mannitol Salt Agar (MSA) and incubated aerobically at 37°C for 24hr. Identification relied on colonial morphology, Gram staining, catalase test, and coagulase test (Wu et al., 2020; Thirunavukkarasu and Rathish, 2014). Credible S. aureus isolates were confirmed by 16S rRNA PCR.

Molecular Analysis

Genomic DNA was extracted by the boiling method. Overnight Luria-Bertani broth cultures were centrifuged, washed, resuspended in normal saline, heated at 95°C for 15 min, cooled on ice, and centrifuged; the supernatant containing DNA was stored at −20°C. DNA quantity and quality were assessed by NanoDrop spectrophotometry.

Conventional PCR was performed on an ABI 9700 thermal cycler using primers specific for fnbA, cna, and hla (Inqaba, South Africa) in a final volume of 30 µL containing 2× Dream Taq Master Mix, 0.4 µM primers, and 50 ng template DNA. Cycling conditions comprised initial denaturation at 94°C for 5 min; 35 cycles of denaturation (94°C, 40 s), annealing (appropriate temperature, 45 s), and extension (68°C, 45 s); and final extension at 68°C for 5 min. Products were resolved on 1.5% agarose gels (ethidium bromide or safer dye) and visualized under blue light or UV illumination. Expected amplicon sizes were approximately 128 bp (fnbA), 560 bp (cna), and 219–427 bp (hla, depending on primer pair). Selected amplicons were sequenced (BigDye Terminator kit, 3510 ABI sequencer, Inqaba Biotechnological, Pretoria).

Statistical Analysis

Data were analyzed with SPSS version 26. Frequencies and percentages were calculated.

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Associations between gene presence and age, gender, or specimen type were assessed by chi-square test of independence; $p < 0.05$ was considered significant.

III. RESULTS

- Table 1 is the demographic distribution of specimens showed that of 106 participants, females predominated across specimen types and age groups. Urine specimens accounted for the largest proportion (males 15.1%, females 50.0%), followed by HVS (females 23.6%). The 31–40-year age group contributed the highest number of specimens (n = 37).

Table 1. Demographic Distribution of Specimens by Gender and Age

Age (years)	NE	URINE		HVS		ECS		Wound Swab		Sputum		Skin Swab	
		M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)
11-20	14	0(0.0)	11(78.6)	0(0.0)	1(7.1)	0(0.0)	2(14.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
21-30	26	2(7.7)	14(53.8)	0(0.0)	10(38.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
31-40	37	4(10.8)	17(46.0)	0(0.0)	8(21.6)	0(0.0)	0(0.0)	0(0.0)	4(10.8)	0(0.0)	2(5.4)	1(2.7)	1(2.7)
41-50	12	2(16.7)	3(25.0)	0(0.0)	5(41.6)	0(0.0)	0(0.0)	0(0.0)	2(16.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
51-60	09	5(55.6)	3(33.3)	0(0.0)	1(11.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
61-70	08	3(37.5)	5(62.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	106	16(15.1	53(50.0)	0(0.0)	25(23.6)	0(0.0)	2(1.8)	0(0.0)	6(5.7)	0(0.0)	2(1.8)	1(1.0)	1(1.0)

Key:
M- Male
F- Female
NE- Number examined
HVS- High vaginal swab
ECS- Endocervical swab

- Table 2 is the distribution of S. aureus by age and gender showed that twenty-six isolates of S. aureus were recovered (24.5%). Females accounted for 18 (69.2%) and males for 8 (30.8%). The highest recovery (12 isolates, 46.2%) occurred in the 31–40-year age group.

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Table 2: Distribution of Staphylococcus aureus by age and gender

AGE (Year)	Male (%)	Female (%)	Total (%)
11 -20	00	02(7.7)	02(7.7)
21 – 30	02(7.7)	04(15.4)	06(23.1)
31 – 40	02(7.7)	10(38.5)	12(46.2)
41 – 50	01(3.8)	01(3.8)	02(7.7)
51 – 60	03(11.5)	00(0.0)	03(11.5)
61 – 70	00(00)	01(3.8)	01(3.8)
Total	08(30.8)	18(69.2)	26(100)

- Table 3-5 are detection of virulence genes showed that eighteen isolates were screened. fnbA was detected in 13 (72.2%), hla in 5 (27.8%), and cna in none (0%). Gene-positive isolates clustered in the 21–30, 31–40, and 51–60-year groups. No statistically significant association was observed with age ($\chi^2 \approx 0.079$, $p > 0.05$), gender ($\chi^2 = 0.004$, $p = 0.952$), or specimen type ($\chi^2 = 0.873$, $p = 0.832$) (Tables 3–5). Urine specimens contributed the majority of gene-positive isolates (hla 4/13, fnbA 9/13). Single wound and skin isolates each carried fnbA.

Table 3: Distribution of Some Virulent genes by Age

AGE (Years)	*HLA* (%)	*FNBA* (%)	*CNA* (%)	TOTAL (%)
11 – 20	00(0.00)	00(0.00)	00(0.00)	00(0.00)
21 – 30	02(28.60)	05(71.40)	00(0.00)	07(100)
31 – 40	02(25.00)	06(75.00)	00(0.00)	08(100)
41 – 50	00(0.00)	00(0.00)	00(0.00)	00(0.00)
51 – 60	01(33.30)	02(66.70)	00(0.00)	03(100)
61 – 70	00(0.00)	00(0.00)	00(0.00)	00(0.00)
TOTAL	05(27.80)	13(72.20)	00(0.00)	18(100)

Table 4: Distribution of some Virulent genes by Gender

GENDER	HLA(%)	FNBA(%)	CNA(%)	TOTAL(%)
MALE	02(40.0)	05(38.50)	00(0.00)	07(38.9)
FEMALE	03(60.0)	08(61.50)	00(0.00)	11(61.1)
TOTAL	05(100)	13(100)	00(0.00)	18(100)

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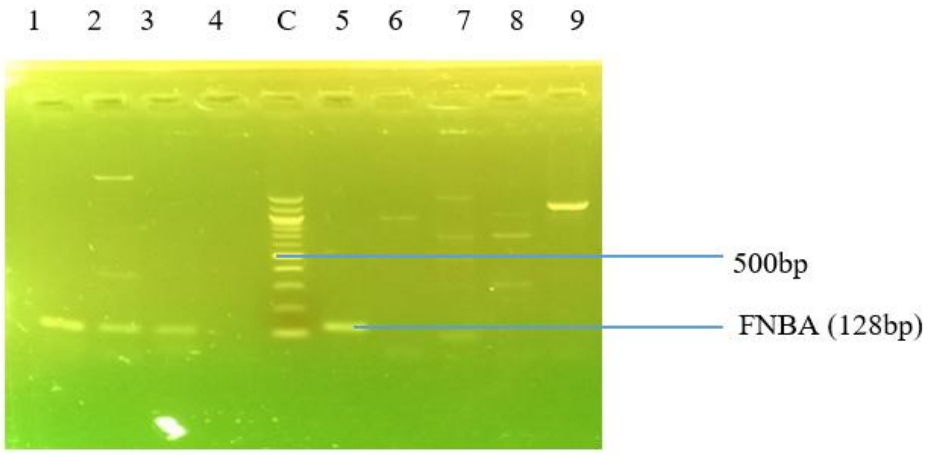
Table 5: Distribution of some Virulent (HLA, FNBA and CNA) Genes by Samples

SAMPLES	NE	HLA (%)	FNBA (%)	CAN (%)	TOTAL (%)
Urine	13	04(30.8)	09(69.2)	00(0.0)	13(100)
HVS	03	01(33.3)	02(66.7)	00(0.0)	03(100)
ECS	00	00(0.0)	00(0.0)	00(0.0)	00(0.0)
Wound	01	00(0.0)	01(100)	00(0.0)	01(100)
Sputum	00	00(0.0)	00(0.0)	00(0.0)	00(0.0)
Skin	01	00(0.0)	01(100)	00(0.0)	01(100)
TOTAL	18	5(27.8)	13(72.2)	00(0.0)	18(100)



Agarose gel electrophoresis showing the amplified genes. Lanes 1-3,5 and 7 are the positive FnbA gene bands at 128bp while lane C represents the 100bp-1500bp molecular ladder.

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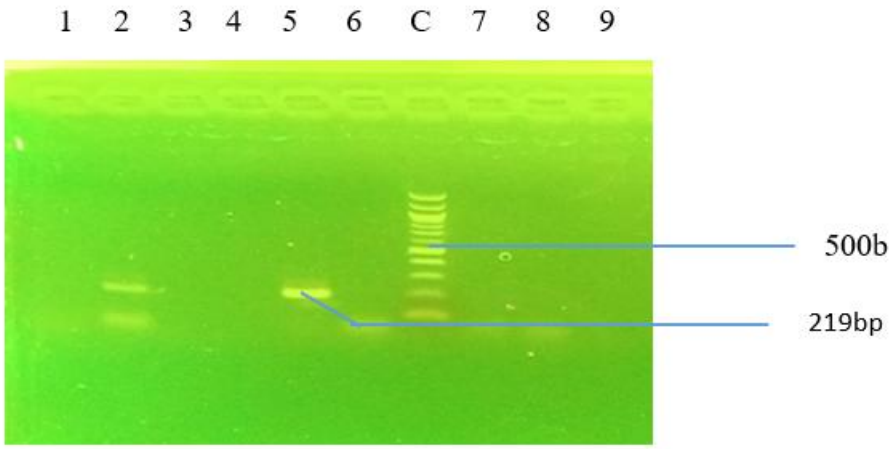
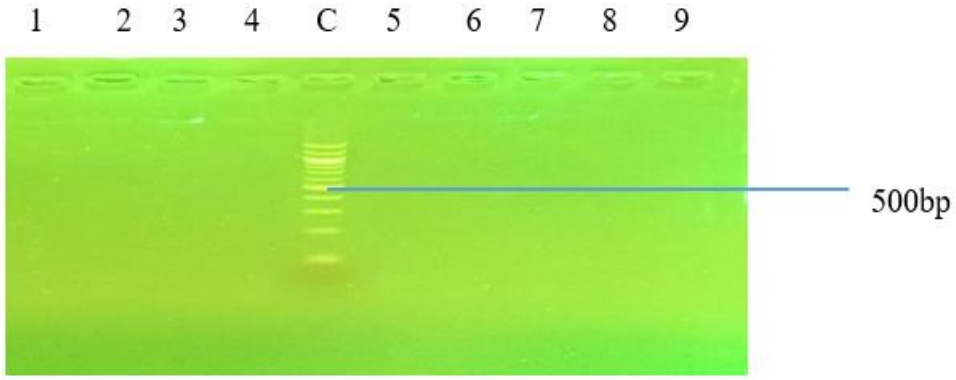


Agarose gel electrophoresis showing the amplified genes. Lanes 2 and 5 are the positive hla gene bands at 219bp while lane C represents the 100bp-1500bp molecular ladder.



Agarose gel electrophoresis of CNA gene of some selected bacterial isolates. Lanes 1- 9 represent the isolates tested for CNA but no gene band was seen after amplification process while lane C represents the 100bp-1500bp molecular ladder.

IV. DISCUSSION

The isolation rate of 24.5% and the marked female predominance (69.2%), with peak recovery in the 31–40-year age group, align with southern Nigerian epidemiological patterns driven by anatomical, hormonal, and healthcare-seeking factors (Egbule et al., 2024; Ogunleye et al., 2025; Bilyaminu et al., 2025; Igbinosa et al., 2023; Otokunefor et al., 2022).

The high prevalence of fnbA (72.2%) indicates that circulating strains possess strong fibronectin-mediated adhesion capacity, facilitating colonization and persistence, particularly in urinary and genital tracts. Moderate hla carriage (27.8%) confers the potential for pore-forming toxin-mediated tissue damage in a subset of isolates. Complete absence of cna is consistent with the predominantly mucosal/urinary specimen mix; collagen-binding protein is more characteristic of bone and joint infections (Peter et al., 2026; Bhattacharya et al., 2026). Lack of statistically significant associations with age, gender, or specimen type is expected once isolates are recovered and mirrors findings from other southern Nigerian molecular series (Ogunleye et al., 2025; Otokunefor et al., 2022; Alli et al., 2015). Comparative data show that hla frequencies vary widely by niche (30–98%), while fnbA is frequently high (50–80%) in clinical soft-tissue and urinary isolates (Okafor et al., 2025; Igbinosa et al., 2023; Peter et al., 2026). Limitations include the modest

number of isolates subjected to molecular analysis (n = 18), single-centre design, restricted gene panel, and absence of phenotypic expression assays. Larger multi-centre studies incorporating WGS and clinical outcome data are warranted.

V. CONCLUSION

fnbA gene were predominates among S. aureus isolates at M.U.T.H, which indicates strong adhesion potential, while hla is moderately distributed and cna is absent. These findings constitute the first molecular virulence-gene profile from the institution and the south-south geopolitical zone, providing essential baseline data for risk classification, antimicrobial stewardship, and infection-control strategies. Future multi-centre studies employing whole-genome sequencing, phenotypic virulence assays, and clinical outcome linkage will further refine understanding of local S. aureus pathotypes.

CONFLICT OF INTEREST

I declare that there is no conflict of interest associated with this study as the research was conducted independently, and no personal, financial or institutional interest influenced the study design, data collection, analysis and interpretation of the results as well as preparation of this manuscript.

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