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Coexistence of the *blaZ* gene and selected virulence determinants in multidrug-resistant *Staphylococcus aureus*: insights from three Nigerian tertiary hospitals

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Abstract

Background and purpose Infections caused by β-lactamase-producing strains of *Staphylococcus aureus* have become increasingly difficult to treat due to the expression of multiple virulence factors. This has heightened concerns about managing *S. aureus*-related infections. This study was conducted to characterize the *blaZ* gene and selected virulence determinants in β-lactam resistant *S. aureus* from human sources in three Nigerian tertiary hospitals.

Materials and methods Three hundred and sixty samples were collected for the study. *S. aureus* was isolated and characterized following standard microbiological protocols and *nuc* gene amplification. Antibiotic susceptibility and minimum inhibitory concentration tests were performed using the disk diffusion method and E-tests, respectively. Biofilm formation and β-lactamase production were assessed using Congo red agar and nitrocefin kits, while the *blaZ* gene was examined using conventional PCR. Capsular polysaccharide genotyping, accessory gene regulator (*agr*) detection, Panton-valentine leucocidin (PVL), and PVL proteins were performed using PCR and Western blotting.

Results *S. aureus* was recovered from 145 samples, 50 (34.5%) of these isolates exhibited multidrug resistance, with MICs ranging from 0.125 to 1.00 µg/mL, and showed significant resistance to aminoglycosides, fluoroquinolones, and β-lactams. Of these, 31 strains produced β-lactamases, 30 of which carried the *blaZ* gene in combination with *cap8* (80%) or *cap5* (20%). Biofilm formation and PVL gene were observed in 85% of the 20 randomly selected *blaZ*-positive multidrug-resistant (MDR) strains. The *agr2* allele was predominant, found in 70% of the selected MDR strains. No significant difference in the occurrence of the *blaZ* gene was found among the three clinical sources ($p \leq \alpha 0.05$).

Conclusion The co-occurrence of the *blaZ* gene with PVL, capsular polysaccharide genes, and *agr* alleles is associated with biofilm formation, indicating a high risk of β-lactam-resistant *S. aureus* infections. Our findings highlight the need for continuous molecular surveillance to enhance infection management, treatment options, and patient outcomes in the study locality. A limitation of this study is the random selection of MDR isolates, which may affect the comprehensiveness of the analyses.

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Keywords Multidrug-resistant *Staphylococcus aureus*, B-lactamase, Penicillinase, Virulence determinants, Biofilm

Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive opportunistic human pathogen that causes many infections, ranging from minor skin infections to life-threatening infective endocarditis, pleuropulmonary, bloodstream, and device-related infections in community and hospital settings. *Staphylococcus aureus* has a propensity to acquire antibiotic resistance genes (ARGs) and disseminate them via horizontal gene transfer (HGT), making it a pathogen of global public health concern [1]. The emergence of antibiotic-resistant and toxin-producing strains of *S. aureus* is associated with severe infections and tissue damage in their hosts. This has heightened concerns regarding the management of *S. aureus*-related infections. In addition, β-lactamase-producing strains that exhibit targeted affinity for penicillins can persist in the presence of β-lactam antibiotics [2]. Consequently, the production of penicillinase encoded by the *blaZ* gene renders β-lactam antibiotics ineffective during treatment. Recently, the co-occurrence of ARGs and virulence factors (VFs) in *S. aureus* has been reported [3, 4]. The regulation of *blaZ* involves two other genes, namely *blaR1* and *blaI*. When exposed to β-lactam antibiotics, *blaR1*, a sensor transducer, undergoes autocatalytic cleavage, leading to cleavage of the *blaI* repressor. This process enables the transcription of the *blaZ* gene [5, 6].

Virulence factors in *S. aureus* contribute to its pathogenicity. These factors include various proteins and molecules on the surface of the bacteria, as well as secreted proteins and toxins that can damage or kill host cells [7]. Panton-valentine leukocidin (PVL) is a toxin produced by some strains of *S. aureus*, which aids in the immune evasion of the bacterium and enhances host tissue damage. It is a non-hemolytic toxin that mediates the destruction of various white blood cells including polymorphonuclear cells, monocytes, and macrophages. PVL encodes two separate exoproteins, lukS-PV, and lukF-PV [8, 9]. Furthermore, *S. aureus* is covered in a layer of capsular polysaccharides (protective surface structures), which play a crucial role in the development of staphylococcal infections (pathogenesis). There are several distinct types of capsular polysaccharides and most strains produce either type 5 (*Cap5*) or type 8 (*Cap8*) polysaccharides. These polysaccharide layers serve as the primary exopolysaccharide defense mechanism that shields the pathogen from being engulfed by phagocytes and is associated with increased virulence [8, 10].

In contrast, the accessory gene regulator (*agr*) locus influences the expression of many virulence genes, including exotoxins and capsular polysaccharides during *S. aureus*-related infections [11]. This regulation is thought to connect the expression of the bacterial VFs to the stage of infection when they are necessary for obtaining nutrients and evading the host's immune responses [12]. The *agr* locus has been grouped into four major categories, namely *agr* I to IV, based on their specific genetic makeup [13]. A previous study suggested that interference with virulence gene expression may be caused by different *agr* gene groups [14]. In addition, the *agr* gene has been associated with the control and activation of the *agr*-quorum sensing system [8, 15].

The formation of biofilms in clinical strains of *S. aureus* protects them from the action of antibiotics and the host's immune response by impeding the penetration of antimicrobial agents and obstructing the drug's ability to reach and inhibit the bacteria dwelling within the biofilm. Consequently, this bacterium can persist in the host and cause chronic infections [16, 17]. In many instances, the conventional antibiotic treatment approach is ineffective and often inadequate for biofilm-related infections and requires prolonged and higher doses of antimicrobials that can cause significant systemic toxicity [18, 19]. However, detailed studies on the co-occurrence of the *blaZ* gene and virulence determinants in clinical isolates of β-lactamase-producing *S. aureus* in Nigeria are scarce. To address this gap, this study investigated the carriage of the *blaZ* gene and selected virulence determinants in β-lactam-resistant *S. aureus* strains of clinical origin isolated from three Nigerian tertiary hospitals.

Materials and methods

Sample collection and *S. aureus* Isolation

A total of 360 clinical samples (wounds, high-vaginal, and pyodermal infection swabs) were collected from surgical wards, female wards, and general medical wards in three tertiary hospitals (University of Ilorin Teaching Hospital, Ilorin, University College Hospital, Ibadan, and National Orthopedic Hospital, Igbobi, Lagos) between June and December 2021. The samples were transported in ice-packed boxes to the microbiology laboratory of the University of Ilorin, and the Nigerian Institute of Medical Research (NIMR), Lagos, and streaked on mannitol salt agar (MSA) and blood agar plates (L: S Biotech, San Diego, USA). After incubation at 37 °C for 48 h, presumptive *S. aureus* isolates were subjected to Gram-staining and biochemical tests (catalase, coagulase, DNase, and oxidase). The Vitek-2 system (Biomerieux, USA) was used for species validation. Each bacterial isolate was suspended in 3 milliliters of 0.45% physiological saline to achieve a density of 0.5 to 0.60 McFarland, measured using the DensiCHEK Plus instrument (Biomerieux, USA). These suspensions were then used for species

validation with the Vitek 2 Compact system (Biomerieux, USA), following the manufacturer's instructions. Gram-positive (GP) cards were filled with the prepared bacterial suspensions, sealed, and placed in the incubation chamber of the Vitek 2 reader, where they were incubated at 35.5 °C for 8–18 h. After the incubation period, bacterial isolates were identified based on their metabolic activities, enzymatic functions, and biochemical tests, as previously described [20].

Antimicrobial susceptibility testing

The antibiotic susceptibility patterns of the isolates were determined using the Kirby-Bauer disk diffusion method with standard antibiotic disks (Oxoid, UK). The antibiotics tested included: penicillin G (10 µg), oxacillin (1 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), pefloxacin (5 µg), gentamicin (10 µg), erythromycin (15 µg), amikacin (30 µg), amoxicillin plus clavulanic acid (20 µg+10 µg), ampicillin (25 µg), and doxycycline (30 µg). Bacterial inoculum was prepared in sterile Muller Hinton broth (MHB), and adjusted to optical density 0.5 (OD_{600}), and a 0.1 mL aliquot was plated on sterile Muller Hinton agar (MHA) plates. Subsequently, the antibiotic disks were aseptically placed on the MHA plates and incubated at 37 °C for 24 h. The zones of inhibition were interpreted using the CLSI 2020 guidelines [21]. Minimum inhibitory concentrations (MICs) were determined using E-test strips (penicillin G, oxacillin, ceftriaxone, and cefuroxime), and multidrug-resistant (MDR) strains were selected based on their resistance to three or more classes of antibiotics.

Assays for β-lactamase production

Forty randomly selected MDR *S. aureus* strains resistant to at least one penicillin or cephalosporin were screened for β-lactamase production using nitrocefin test kits (Becton, Dickinson and Company, Sparks, MD, USA). A drop of sterile distilled water was dispensed onto the reaction area of the nitrocefin kit using a sterile Pasteur pipette. Discrete colonies of the organism to be tested were then smeared onto the moistened reaction area using a sterile disposable inoculating loop. The reaction was observed for color change over 10 to 30 min; a shift from yellow to pink color indicated the production of β-lactamases [22]. ATCC49230 was used as the negative control (β-lactamase negative strain).

Detection of *blaZ* and virulence genes in *S. aureus*

The genomic DNA of the isolates was extracted using the DNA Universal Kit (Zymo Research, Irvine, CA, USA). The quantity of the extracted DNA was then assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). The *nuc* gene was amplified using specific primers as previously described [23]. The positive control was a methicillin-susceptible *Staphylococcus aureus* (MSSA), and the negative control was *Staphylococcus epidermidis* ATCC 12,228. Next, the capsular polysaccharides (*cap5* and *cap8*), *blaZ*, and PVL genes of thirty selected MDR strains were examined using uniplex PCR as previously described, respectively [24–26]. The *agr* gene of twenty randomly selected β-lactamase-positive and biofilm-forming, *blaZ*-positive *S. aureus* isolates were examined using multiplex PCR as described elsewhere [27]. The primers used for the study, designed and manufactured by Wizbiosolutions, South Korea, are listed in Table 1 below.

Quantification of PVL proteins using western blotting

The expression of PVL-associated protein in the PVL-positive *S. aureus* strains was examined using overnight cultures of *S. aureus* prepared and incubated in a shaker at 37°C and 160 rpm in 10 mL brain heart infusion broth and centrifuged in a 50 mL tube at 5700 rpm at 4°C for 15 min. The supernatant was transferred into a 15 mL tube and 1 mL trichloroacetic acid was added. This was precipitated for 30 min on ice and then centrifuged at 5700 rpm at 4°C for 20 min. The supernatant was discarded and the pellet was resuspended in 2 mL acetone, transferred into a 2 mL Eppendorf tube, and centrifuged at 13 000 rpm at 4°C for 15 min. The filtrate was discarded and the pellet was air-dried and resuspended in 200 µL buffered sodium dodecyl sulfate (SDS, urea buffer) shaken for 10 min, and then stored at −4°C.

After the extraction of the PVL proteins, the gel was placed on a nitrocellulose membrane which was sandwiched between filter papers. The proteins were transferred from the gel onto a nitrocellulose membrane by electroblotting for 30 min at 12 V. The membranes were blocked with 3% bovine serum albumin (BSA) in Tris-buffered saline and Tween 20 (TBS-T) for 1 h and washed once with TBS-T. The membranes were then incubated with rabbit anti-lukS-PV at a dilution of 1:1000:2000 in TBS-T for 1 h and washed 5 times in TBS-T. Afterwards, the membranes was incubated with goat anti-rabbit IgG at a dilution of 1:1000:2000 in TBS-T for 1 h and washed with TBS-T before the addition of the following staining substrates; nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in alkaline phosphatase (AP) buffer. The reaction was stopped in water and the membrane was air-dried and then analyzed. The expression of PVL was determined semiquantitatively in three categories: no PVL production --, low PVL production +, and high PVL production ++.

Table 1 Primers used for the study

Genes	Description	Primers	PCR Protocols	Amplicon size	References
nuc	Extracellular thermostable nuclease	Forward: GCGATTGATGGTGATACGGTT Reverse: AGCCAAGCCTTGACGAACTAAAGC	Initial denaturation: 94 °C for 60s, Annealing:55 °C for 30s, Extension:72 °C for 90s, Final extension: 72 °C for 3.5 min.	279 bp	[23]
blaZ	Penicillinase (β-lactam resistance)	Forward: CAAAGATGATATAGTTGCTTATTC Reverse: TGCTTGACCACTTTTATCAGC	Initial denaturation: 95 °C for 5 min, Denaturation: 95 °C for 30 s (35cycles), Annealing: 50 °C for 30 s, Extension: 72 °C for 30 s, Final extension: 72 °C for 7 min	Variable (Compared with ATCC control)	[24]
luk-PV	Panton-Valentine Leukocidin	Forward: CGCCTCGCTATATGAACTAT Reverse: ATCATTAGGTAAAATGTCTGGAA	Denaturation: 94 °C for 30 s, Annealing: 55 °C for 30 s, Extension: 72 °C for 1 min, Cooling: 12 °C	433 bp	[26]
cap5	Capsular genotype	Forward: TGCTTGACCACTTTTATCAGC Reverse: GAAAGTGAACGATTAGTAGAA	Initial denaturation: 95 °C for 15 min, Denaturation: 94 °C for 30 s, Annealing: 45 °C for 30 s, Extension: 72 °C for 30 s, Final extension: 72 °C for 10 min.	532 bp	[25]
cap8	Capsular genotype	Forward: GTACGAAGCGTTTTGATAGTT Reverse: GTGGGATTTTTGTAGCTTTT		437 bp	[25]
agr1	accessory gene regulators	Forward: ATGCACATGGTGCACATGC Reverse: GTCACAAGTACTATAAGCTGCGAT	Initial denaturation: 94 °C for 4 min, Denaturation: 94 °C for 1 min, Annealing: 50 °C for 2 min, Extension: 74 °C for 3 min, Post-elongation: 74 °C for 3 min.	439 bp	[27]
agr2	accessory gene regulators	Forward: ATGCACATGGTGCACATGC Reverse: TATTACTAATTGAAAAGTGCCATC		572 bp	[27]
agr3	accessory gene regulators	Forward: ATGCACATGGTGCACATGC Reverse: GTAATGTAATAGCTTGTATAATAG		321 bp	[27]
agr4	accessory gene regulators	Forward: ATGCACATGGTGCACATGC Reverse: CGATAATGCCGTAATACCCG		657 bp	[27]

Keys: s-seconds, mins-minutes

Table 2 Distribution of *S. Aureus* in Clinical samples from selected tertiary hospitals

Sampling Locations	Sampling sources					
	Wound swabs		High vaginal swabs		Pyodermal swabs	
	Number of samples taken	Number of S. aureus recovered (%)	Number of samples taken	Number of S. aureus recovered (%)	Number of samples taken	Number of S. aureus recovered (%)
N.O.H.I.L	70	30 (42.86)	-	-	10	2 (20.00)
U.I.T.H	30	12 (40.00)	60	25 (41.67)	40	18 (45.00)
U.C.H.I	40	16 (40.00)	60	19 (31.67)	50	23 (46.00)
Total	**140**	**58** (**41.43**)	**120**	**44** (**36.67**)	**100**	**43** (**43.00**)

Keys N.O.H.I.L- National Orthopedic Hospital, Igbobi, Lagos; U.I.T.H- University of Ilorin Teaching Hospital, Ilorin; U.C.H.I- University College Hospital, Ibadan

Assessment for biofilm production

The formation of biofilm in selected MDR strains was assessed using the Congo red agar (CRA) method as described elsewhere with slight modifications [28].

Data analysis

The statistical significance of the occurrence of the *blaZ* gene among the three sources of clinical samples was determined by analyzing the data via two-way ANOVA using IBM SPSS version 20 (Illinois, USA).

Results

Among the 360 clinical samples obtained from the three selected tertiary hospitals, 145 strains were confirmed to be *S. aureus* (Table 2).

Antibiotic susceptibility testing (AST) revealed that 50 (34.5%) of the 145 *S. aureus* strains recovered exhibited multiple resistance to a minimum of three classes of antibiotics. Antibiogram patterns revealed significant resistance to aminoglycosides, fluoroquinolones, tetracycline, and β-lactams, respectively (Table 3). A total of 12 resistant phenotypes were identified (Table 4). The results of the E-tests carried out on the 50 MDR isolates indicated that 32 (64%) of the isolates were susceptible to the selected antibiotics, with MICs ranging from 0.125 to 1.00 µg/mL.

β-lactamase production and detection of the *blaZ* gene

Of the forty (40) randomly selected MDR strains of *S. aureus* screened for β-lactamase production, 31 (77.5%) were β-lactamase-positive, while nine (22.5%) were β-lactamase-negative. The reference strain ATCC49230 was negative for β-lactamase production according to the nitrocefin kit. The *blaZ* gene was detected in 30 out of 31

Table 3 Antibiograms of the *S. aureus* strains on standard antibiotic disks

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
PEN	68 (46.9)	44 (30.3)	33 (22.8)
OXA	63 (43.4)	59 (40.7)	23 (15.9)
OFX	66 (45.5)	37 (25.5)	42 (29.0)
CIP	72 (49.7)	47 (32.4)	26 (17.9)
CEF	88 (60.7)	39 (26.9)	18 (12.4)
CFT	60 (41.4)	60 (41.4)	25 (17.2)
CZD	73 (50.3)	45 (31.0)	27 (18.7)
PEF	65 (44.9)	55 (37.9)	25 (17.2)
GEN	50 (34.5)	49 (33.8)	46 (31.7)
ERY	79 (54.5)	45 (31.0)	21 (14.5)
AMK	88 (60.7)	37 (25.5)	20 (13.8)
AUG	75 (51.7)	50 (34.5)	20 (13.8)
CTX	93 (64.1)	29 (20.0)	23 (15 0.9)
AMP	48 (33.1)	69 (47.6)	28 (19.3)
DOC	38 (26.2)	77 (53.1)	30 (20.7)

Keys Penicillin G-(PEN); Oxacillin-(OXA); Ofloxacin-(OFX); Ciprofloxacin-(CIP); Cefuroxime-(CEF); Ceftriaxone-(CFT); Ceftazidime-(CZD); Pefloxacin-(PEF); Gentamicin-(GEN); Erythromycin-(ERY); Amikacin-(AMK); Augmentin-(AUG); Cefotaxime-(CTX); Ampicillin-(AMP); Doxycycline-(DOC)

Table 4 Multiple antibiotic-resistant (MAR) patterns among *S. Aureus*

Resistant phenotypes	Number of Isolates
CIP, PEN, OXA, OFX, PEF, AUG	7
CIP, AMK, GEN, OFX, AMP	5
PEN, OXA, CFT, AUG, AMP, ERY	7
PEN, OXA, CZD, DOC	6
GEN, OFX, PEF, CTX	8
PEN, OXA, OFX, GEN, CIP	7
PEN, AMK, CEF, ERY	6
PEN, AMK, CEF, DOC	4
Total Resistance Pattern 12	**50 (34.5%)**

Keys: Penicillin G-(PEN); Oxacillin-(OXA); Ofloxacin-(OFX); Ciprofloxacin-(CIP); Cefuroxime-(CEF); Ceftriaxone-(CFT); Ceftazidime-(CZD); Pefloxacin-(PEF); Gentamicin-(GEN); Erythromycin-(ERY); Amikacin-(AMK); Augmentin-(AUG); Cefotaxime-(CTX); Ampicillin-(AMP); Doxycycline-(DOC)

β-lactamase-positive MDR *S. aureus* strains, representing a significant association (96.8%, $p<0.001$). This strong correlation indicates that the *blaZ* gene is a major contributor to β-lactamase production in the *S.aureus* strains examined.

Detection of selected virulence factors

Twenty randomly selected MDR isolates harboring the *blaZ* gene were subjected to a phenotypic assay for biofilm formation, 17 (85.0%) were biofilm formers, as indicated by the blackening of the CRA surfaces. In comparison, 3 (15.0%) were non-biofilm formers, showing no blackening of the CRA. Furthermore, the capsular polysaccharides genotypes *cap8* (80%; $n=24$) and *cap5* (20%; $n=6$) were detected in all 30 selected *blaZ*-positive strains examined. In addition, of the 20 randomly selected *blaZ*-positive isolates examined for virulence genes, a majority harbored *agr2* (70%; $n=14$), and the PVL gene (85%; $n=17$) was detected. The expression of PVL proteins in ten (10) selected PVL-positive strains showed that most (70%; $n=7$) of the isolates examined exhibited high PVL protein expression.

Discussion

Staphylococcus aureus is an opportunistic pathogen that contributes to severe healthcare-associated and community-acquired infections in developed and developing nations [29]. This bacterium has been linked to treatment failure because it can acquire and harbor various ARGs and form biofilms. Thus, *S. aureus* infections burden the economy considerably due to increasing morbidity and mortality rates and high treatment costs [30–32]. Of the 360 clinical samples obtained for the study, 40.28% tentative *S. aureus* strains were recovered based on phenotypic tests, and the genotypic detection of the *nuc* gene. Detecting the *nuc* gene (a conserved extracellular thermostable nuclease gene) in *S. aureus* allows for accurate and precise identification of this bacterium in clinical specimens [33]. All the recovered strains were coagulase-positive and showed positive DNase activity. The production of coagulase by *S. aureus* has been shown to help form protective structures that shield the bacteria from clearance by phagocytes during infection [34, 35]. The distribution of *S. aureus* in wounds (41.43%), high vaginal (36.67%), and pyodermal swabs (43.00%) in this study is higher compared to previous studies in southwestern Nigeria, which reported lower rates in wounds (22.1%), urogenital tissue (20.7–38.6%), and abscesses (22.6%). This implies that these sites are significant sources for isolating *S. aureus* (Table 2) [36, 37].

Most of the MDR strains recovered in this study exhibited resistance to aminoglycosides, fluoroquinolones, tetracycline, and β-lactams (penicillins and cephalosporins). There have been increasing reports of inappropriate empirical prescription patterns and usage of antibiotics, including newer generations of cephalosporins in Nigerian secondary and tertiary hospitals for pre and post-surgical prophylaxis, with the highest usage in intensive care units (ICUs). These inappropriate empirical prescriptions have significantly driven the development of antibiotic resistance in clinically relevant isolates [38–40]. Notably, the resistance patterns observed in this study mirror the most commonly prescribed antibiotics in the study area, underscoring an urgent need for enhanced antibiotic stewardship.

Furthermore, 77.5% (31/40) of the MDR isolates screened were β-lactamase-positive strains. Notably, the β-lactamase-stable antibiotic tested in this study (amoxicillin plus clavulanic acid) inhibited only some β-lactamase-producing strains (Table 3). This implies an increasing vulnerability of β-lactamase-stable

antibiotics to the degradative activity of the enzyme β-lactamase and can render antibiotics ineffective against β-lactamase-producing strains of *S. aureus*. This confirms that β-lactam antibiotics can trigger the expression of β-lactamase and penicillinase, as previously reported [41, 42]. Grasping these induction mechanisms is crucial for developing effective strategies to address the β-lactam resistance in clinical strains of *S. aureus*.

Additionally, the phenotypic expression of β-lactamase was validated by the detection of the *blaZ* gene, which can be encoded on genomic DNA or plasmid-borne genes [5, 6]. A majority (96.8%; 30/31) of the β-lactamase-positive strains examined in the present study harbored the *blaZ* gene. Okiki et al. (2020) and Ayepola et al. (2015) reported a high prevalence of *blaZ* carriage in *S. aureus*, ranging from 65 to 97.2%, among isolates recovered from high vaginal swabs and soft tissue infections in Southwest Nigeria [36, 37]. A combination of β-lactamase stable antibiotics with a wide spectrum of activity against the enzymatic degradation of β-lactamase may be warranted for treating wound, soft tissue, and skin infections at the study location to improve patient outcomes.

The coexistence of biofilms and ARGs in clinical and subclinical *S. aureus* strains has become increasingly difficult to treat and is commonly achieved through the administration of β-lactam antibiotics [43]. Among the *blaZ*-positive strains examined for biofilm formation in this study, the predominant (85.0%; 17/20) were biofilm-forming strains. The production of biofilms by clinically relevant bacteria is a public health threat because it contributes to the persistence of the pathogen in the presence of antimicrobial agents, making it challenging to effectively treat and manage associated illnesses [44]. Furthermore, the presence of the *agr* locus on the *S. aureus* genome is associated with the activation of the quorum sensing (QS) system. The *agr* quorum-sensing system is believed to be important in various biological processes and plays a crucial role in controlling the formation of biofilm in *S. aureus* [45]. In this study, a single *agr* allele was detected in each of the randomly selected isolates examined, with the majority (70%; 14/20) harboring the *agr2* gene.

Yarwood et al. (2004) explored the impact of *agr* on biofilm development and investigated *agr*-related gene expression in biofilms. Under certain conditions, interference with *agr* expression had no noticeable effect on biofilm formation. However, under certain circumstances, they either prevent or boost biofilm formation [45]. In chronic infections, when the *agr*-QS system is suppressed, *S. aureus* adapts its behavior, forms biofilms, and displays increased resistance to antimicrobial agents [46]. Additionally, the *S. aureus agr*-QS system regulates the production of VFs. These VFs, including toxins and proteases, are crucial for causing disease but may be less important once a chronic infection is established. However, they are also targets for the immune system, so their repression or loss is essential for bacteria to survive and persist in the host. By downregulating their production, bacteria can evade the immune system and prevent further recruitment of immune cells, ultimately allowing them to thrive in the host [47–49]. This is in tandem with the findings of our current study, most of the MDR *S. aureus* strains examined harbored the *agr2* gene, harbored virulence determinants, formed biofilms, and exhibited resistance to multiple classes of antibiotics.

The *cap8* (80%) and *cap5* (20%) genotypes were detected in all the selected *blaZ*-positive strains examined for capsular polysaccharides. However, a specific lineage of *S. aureus* that does not produce capsules has been reported to cause infections in hospitals and communities in the U.S. This is significant because it suggests that other mechanisms may be used to evade the immune system, which is important for designing anti-*S. aureus* vaccines that have previously relied on the components of capsular polysaccharides [50–53]. Eleven types of capsular polysaccharides have been identified in *S. aureus*, among which, type 5 and type 8, encoded by *cap5* and *cap8*, respectively, are found in approximately 80–90% of clinical strains of *S. aureus* [54–56]. This finding is consistent with our findings in the current study.

On the other hand, the presence of PVL has been associated with skin and subcutaneous tissue infections such as furuncles and severe community-acquired necrotizing pneumonia. The PVL gene was detected in 85% of the strains examined in the current study, and of these, PVL proteins were detected in 58.8% of the selected strains that harbored the PVL gene. PVL has been found in both community-acquired and hospital-acquired strains of methicillin-resistant and methicillin-susceptible *S. aureus*. Although it is produced by less than 5% of *S. aureus* strains, it is commonly detected in large numbers among those associated with severe skin infections. The African continent is considered a region where PVL-positive methicillin-susceptible *S. aureus* is widespread [7, 57]. PVL-producing *S. aureus* is more likely to cause fatal infections than strains that do not produce this toxin. When these bacteria invade the skin, they can cause ulcers and hemorrhage, by destroying neutrophils and macrophages. Panton-Valentine Leucocidin-producing strains have been linked to other severe skin conditions, including boils and severe necrotizing skin infections [58]. The production of PVL by *S. aureus* is controlled by the lukS-PV and lukF-PV genes, which are encoded by prophage insertions of the bacteriophage ϕSa2. The specific relationships between PVL genes and other virulence factors are not fully understood. However, some studies have suggested that PVL genes are encoded by prophage insertions containing 160 potential

open reading frames (ORFs), some of which may contribute to virulence, including lukS-PV and lukF-PV expression [59, 60]. The detection of the PVL gene in 85% of *S. aureus* strains examined with high expression of PVL proteins in these strains underscores the potential role of PVL in severe infections. This highlights the importance of monitoring PVL expression when assessing *S. aureus* infection risk and planning treatment strategies.

Conclusion

This study describes the co-occurrence of the *blaZ* gene and several virulence factors in clinical strains of *S. aureus*. These findings underscore the need for continuous molecular surveillance of clinically relevant isolates and could provide better treatment options that can improve patient outcomes. The detection of antibiotic resistance determinants and VFs in clinical strains poses a significant threat to public health, these ARGs and VFs can be disseminated to other pathogenic species via horizontal gene transfer. Due to limited resources in this self-funded study, we randomly selected MDR isolates based on our criteria, allowing us to include a substantial number of MDR isolates in the analyses, this choice is noted as a limitation of the study. Further research on circulating clones, sequence types, and their genetic relatedness is needed to enhance our understanding of the evolution and epidemiology of high-risk clinical strains of *S. aureus* in the study locality.

Supplementary Information

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Supplementary Material 1

Author contributions

AMK and OEA conceptualized and designed the study; AMK performed laboratory analyses, AMK and EDA analyzed and interpreted the data; EDA wrote the draft and final manuscript. All authors have read and approved the final version of the manuscript.

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Data availability

Data included in the article are available in the supplementary materials.

Declarations

Ethics approval and consent to participate

The Ethical Review Committee of the Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria, reviewed and approved the study protocol (NHREC/05/01/2008a). Informed consent was obtained from all patients before sample collection, which was performed according to relevant guidelines and regulations.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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