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PHENOTYPIC VIRULENCE SIGNATURES AMONGST SKIN-BORNE STAPHYLOCOCCI ISOLATES CULTURED FROM APPARENTLY HEALTHY UNDERGRADUATE STUDENTS IN A TERTIARY INSTITUTION LOCATED IN EKPOMA, EDO STATE

IGBINOSA, N.,[1] WEMAMBU, E. C.[2] AND *OBAYAGBONA, O. N.[3]
[1]Department of Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[2]Department of Microbiology, University of Benin, Edo State, Nigeria
[3]Department of Environmental Management and Toxicology, University of Benin, Edo State, Nigeria
[*]Corresponding author: omoregbe.obayagbona@uniben.edu

ABSTRACT
Staphylococcus species are normal commensals of the human skin but can act as opportunistic pathogens due to their possession of virulence factors and antibiotic resistance traits. This study was aimed at determining the virulence signatures and antibiotic susceptibility patterns of Staphylococci species isolated from the skin of apparently healthy students of Ambrose Alli University, Ekpoma. Thirty (30) skin swab samples were collected from the forearms of undergraduate students and analyzed using standard microbiological and biochemical techniques. A total of twelve (12) Staphylococcus isolates were recovered, comprising Staphylococcus aureus (58.3%) and Staphylococcus epidermidis (41.7%). Findings from this study revealed that the majority of the S. aureus cultures were β-hemolytic (85.7%) and all were coagulase positive (100%), confirming its high virulence potential, while all the identified S. epidermidis cultures were coagulase negative and non-hemolytic but demonstrated biofilm-forming ability (80%). Antibiotic susceptibility results for Staphylococcus aureus revealed penicillin and oxacillin resistance were 72% and 100% respectively and 40% each for Staphylococcus epidermidis in all isolates. S. aureus isolates showed higher oxacillin resistance, suggestive of methicillin-resistant S. aureus (MRSA). Both species were highly sensitive (100%) to vancomycin, suggesting this drug remain most effective options for the treatment of staphylococcal infections in the community. These findings indicate that Staphylococcus species associated with the skin can harbor virulent and drug-resistant traits that may predispose individuals to infections. Hence, strict personal hygiene and continuous monitoring of antibiotic resistance among skin microbiota are recommended to mitigate the risk of staphylococcal infections.

KEYWORDS: *Biofilm production, Coagulase-negative staphylococci, Hemolysis, Skin borne, Staphylococci*

INTRODUCTION

The human skin is a multifaceted and highly dynamic organ that functions as the body's primary protective barrier against external environmental stressors, including harmful pathogenic microorganisms. It naturally hosts a rich and varied array of microbial communities that form the normal skin microbiota. These communities include bacteria, fungi, viruses, and mites, among which bacteria belonging to the genus Staphylococcus represent some of the most abundant and clinically significant groups. These prokaryotic organisms typically exist as commensals on human skin and mucosal surfaces, yet they possess the ability to function as opportunistic pathogens when conditions permit (Murray *et al*., 2023).

The genus *Staphylococcus* encompasses more than 40 recognized species. The most frequently isolated from human skin are *S. aureus*, *S. epidermidis* and *S. saprophyticus*. *S. aureus* stands out for its capacity to trigger a wide range of infections, extending from relatively mild superficial skin problems such as boils and abscesses to life-threatening systemic conditions including bacteremia, pneumonia, osteomyelitis, and endocarditis (Zaghen *et al*., 2023). By contrast, coagulase-negative staphylococci (CoNS) - with *S. epidermidis* as a prominent example - are frequently associated with hospital-acquired (nosocomial) infections, particularly those linked to indwelling medical devices and implants (Bekoe *et al.,* 2022).

While staphylococci commonly reside as harmless commensals on the skin, several triggers—including disruption of the skin barrier, immunosuppression, competition from other microbes, and the expression of virulence factors—can drive their shift from benign colonizers to pathogenic organisms. Among the most important virulence factors are hemolysins (such as alpha-toxin), Panton–Valentine leukocidin (PVL), protein A, phenol-soluble modulins (PSMs), adhesins such as ClfB and SasG, and the ability to form biofilms (Mills *et al*., 2024).

In recent years, the rise of antibiotic-resistant strains, notably methicillin-resistant S. aureus (MRSA), has significantly complicated clinical diagnosis and treatment. These resistant bacteria are no longer restricted to hospital settings; they have spread extensively into community environments, including among healthy individuals (Al-Saleh *et al.,* 2022). Community-associated MRSA (CA-MRSA) strains, which frequently carry PVL genes and exhibit strong biofilm-forming capacity, have become increasingly prevalent even in otherwise healthy hosts (Kao and Fritz, 2025). The asymptomatic carriage of these virulent and resistant strains raises serious public health concerns, given their potential for transmission and their ability to cause infections when favorable conditions arise (Brooks *et al*., 2023).

With reference to this concern, this study aimed at evaluating the virulence signatures and antibiotic susceptibility patterns of staphylococci species cultured from the skin surfaces of apparently healthy undergraduate students of Ambrose Alli University, Ekpoma.

MATERIALS AND METHODS

Study Area

This study was carried out among apparently healthy undergraduate final year students of Microbiology department, Faculty of Life Sciences,

Ambrose Alli University, Ekpoma, Edo State. The final 400 level class of Microbiology for that academic session had an estimated number of 118 students while the faculty is comprised of five departments.

All microbiological analyses were conducted at the Microbiology Laboratory, Department of Microbiology, Faculty of Life Sciences, Ambrose Alli University, Ekpoma.

Sample Collection

Skin swab samples were collected from the forearm region of 30 apparently healthy undergraduate students at Ambrose Alli University, Ekpoma. A total number of 30 students were enrolled for the study because not enough students in the class were willing to give consent to sample collections and over 50% of the class refused to participate in the study. Prior to sampling, each participant for the study provided oral informed consent. The sampling site was first cleaned with sterile saline to remove surface contaminants. Using sterile cotton swab sticks moistened with sterile physiological saline, the swabs were rolled firmly over the skin surface in a rotating motion to ensure maximum contact. Each swab was immediately transferred into a sterile universal container and labeled appropriately with the sample ID, date, and time of collection. All samples were transported in a cooler box to Microbiology laboratory within one hour of collection and processed immediately to maintain sample integrity and viability of the organisms.

Isolation of Culturable Staphylococci

The skin swab samples collected from the forearms of apparently healthy students were streaked directly onto Mannitol Salt Agar (MSA) plates as described by Cappuccino and Welsh (2020). Plating was performed under aseptic conditions and the plates were incubated aerobically at 37°C for 24 hours.

Identification and Characterization of Staphylococcal isolates

After 24 hours incubation, visible colonies were observed and sub-cultured onto freshly prepared nutrient agar plates to obtain pure cultures. Characterization of the staphylococci isolates was based on morphological observations on the agar plates, Gram staining techniques and biochemical characteristics of the isolates. Morphological characteristics such as size, elevation, margin, consistency, pigmentation and suspected *Staphylococcus* isolates, were subjected to Gram staining and some biochemical tests including catalase and coagulase production tests.

Detection of Selected Virulence Markers

According to methods used by Brooks *et al*. (2023), virulence factors were evaluated using specific phenotypic methods and hemolysis was observed on blood agar after 24 hours at 37°C. Observed clear zones represented beta-hemolysis; greenish partial zones, alpha-hemolysis; and absence of change, gamma-hemolysis. Biofilm formation was assessed using the Congo red agar method as described by Kao and Fritz, (2025). Congo red agar was prepared by adding 0.08 g of Congo red dye and 3.6 g of sucrose to 100 mL of standard Nutrient agar, which was then autoclaved and poured into sterile Petri dishes. Each isolate was streaked onto the prepared plates and incubated at 37 °C for 24–48 hours. Black, dry, crystalline colonies indicated biofilm production, while red or pink colonies was indicative of non-biofilm producers.

Antibiotic Susceptibility Profiling of the Staphylococcal isolates

The antibiotic susceptibility profile of the isolates was determined using the modified Kirby-Bauer disk diffusion method utilizing Mueller Hinton Agar as described by Vandepitte *et al*. (2003) and this procedure was performed under aseptic conditions. Isolates were emulsified in 5 ml of sterile peptone broth and adjusted to 0.5 McFarland standard. The inoculum was swabbed uniformly on Mueller Hinton agar (MH) plates. Commercially available antibiotic discs; Penicillin (10 µg), Oxacillin (1 µg), Erythromycin (15 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Tetracycline (30 µg) and Vancomycin (30 µg) were aseptically placed. The inoculated MH agar plates were incubated at 37°C for 18–24 hours. Resultant zones of inhibition were measured with the aid of a ruler, and interpretations were made in accordance to CLSI (2023) guidelines.

RESULTS

Thirty (30) skin swab samples were collected from the forearms of consenting apparently healthy undergraduate students in this study. A total of twelve (12) *Staphylococcus* strains were recovered and identified as *Staphylococcus aureus* and *Staphylococcus epidermidis*. Table 1 shows the cultural, morphological, and biochemical characteristics of the *Staphylococcus* isolates obtained from the skin of apparently healthy students. *S. aureus* was identified by its large, golden-yellow colonies, positive to catalase and coagulase reactions, and mannitol fermentation on mannitol salt agar while *S. epidermidis* produced small, white colonies, which were catalase positive, coagulase negative, and did not ferment mannitol. Both isolates were Gram-positive cocci arranged in clusters.

The frequency and percentage occurrence of the isolated *Staphylococcus* species are presented in Table 2. *S. aureus* had the highest occurrence (58.3%), followed by *S. epidermidis* (41.7%).

Table 3 shows the hemolytic characteristics of the isolates cultured on blood agar. *S. aureus* exhibited strong β-hemolysis, while *S. epidermidis* showed no hemolysis (γ-a), indicating it is a free commensal and non-pathogenic in nature.

The results of the coagulase test are presented in Table 4. Only *S. aureus* produced the enzyme coagulase, confirming its distinction as the main coagulase-positive species. *S. epidermidis* was coagulase negative, which supports its classification as a coagulase-negative *staphylococcus* (CoNS). The results of the biofilm production test are shown in Table 5. *S. epidermidis* exhibited stronger biofilm-forming ability (80%) compared to *S. aureus* (42.9%), indicating that biofilm formation is a common persistence mechanism among coagulase-negative staphylococci.

The antibiotic susceptibility profiles of *S. aureus* and *S. epidermidis* strains is presented in Table 6 and 7 respectively. The results revealed that all the staphylococcal strains (100%) were sensitive to vancomycin while all the *S. aureus* strains (100%) were resistant to oxacillin respectively.

Table 1: Morphological and Biochemical Characteristics of the Skin borne Staphylococcal Isolates

Morphology (NA)	Shape	Gram Reaction	MANN	CAT	COA	Tentatively identified
Golden Yellow, smooth colonies	Cocci in clusters	+	+	+	+	*Staphylococcus aureus*
Small, whitish Colonies	Cocci in clusters	+	-	+	-	*Staphylococcus Epidermidis*

KEY: NA= Nutrient agar, MANN = Mannitol Fermentation, Test CAT = Catalase Test, COA = Coagulase Test, + = Positive - =Negative

Table 2: Frequency and Percentage Occurrence of Staphylococcal Isolates from Skin Swabs

Bacterial Isolates	Frequency	Percentage (%)
Staphylococcus aureus	7	58.8
Staphylococcus epidermidis	5	41.7
Total	12	100

Table 3: Hemolytic Pattern of Staphylococcal Isolates

Isolate	No. of strains tested	β-Hemolysis n (%)	γ-Hemolysis n (%)
Staphylococcus aureus	7	6(85.7%)	1(14.3%)
Staphylococcus epidermidis	5	0(0%)	5(100%)
Total	12	6(50%)	6(50%)

Table 4: Coagulase Activity among Staphylococcal Isolates

Isolate	No. of strains tested	Coagulase Positive n(%)	Coagulase Negative n(%)
S. aureus	7	7(100%)	0(0%)
S. epidermidis	5	0(0%)	5(100%)
Total	12	7(58.3%)	5(41.7%)

Table 5: Biofilm Formation Ability of Staphylococcal Isolates

Isolate	No. of strains tested	Strong/Moderate Biofilm n(%)	Weak/None n(%)
S. aureus	7	3(42.9%)	4(57.1%)
S. epidermidis	5	4(80%)	1(20%)
Total	12	7(58.3%)	5(41.7%)

Table 6: Antibiotic Susceptibility Pattern of the *S. aureus* strains

Number of isolates (N=7)	VAN (30µg)	PEN (10µg)	OXA (1 µg)	CPX (30µg)	TET (30µg)	CN (10µg)	ERY (15µg)
S	7	2	0	6	4	4	6
I	0	0	0	0	0	0	0
R	0	5	7	1	3	3	1
% of resistant strains	0	72	100	14	43	43	14

KEY: PEN= Penicillin, OXA= Oxacillin, ERY= Erythromycin, CPX= Ciprofloxacin, CN= Gentamicin, TET= Tetracycline, VAN= Vancomycin, R= Resistant, I= Intermediate, S= Sensitive

Table 7: Antibiotic Susceptibility Pattern of the *S. epidermidis* strains

Number of isolates (N=5)	VAN (30µg)	PEN (10µg)	OXA (1 µg)	CPX (30µg)	TET (30µg)	CN (10µg)	ERY (15µg)
S	5	3	3	4	2	4	3
I	0	0	0	0	1	0	0
R	0	2	2	1	2	1	2
% of resistant strains	0	40	40	20	40	20	40

KEY: PEN= Penicillin, OXA= Oxacillin, ERY= Erythromycin, CPX= Ciprofloxacin, CN= Gentamicin, TET= Tetracycline, VAN= Vancomycin, R= Resistant, I= Intermediate, S= Sensitive.

DISCUSSION

This study demonstrated that the forearm skin of apparently healthy undergraduate students harboured two *Staphylococcus* species: *S. aureus* and *S. epidermidis*. *S. aureus* was the predominant isolate, comprising 58.3% of the total, which aligns closely with the results of Becker *et al.* (2014). Those researchers observed that *S. aureus* frequently colonizes moist, intermittently exposed body sites such as the axilla, nares, and forearm. Although it forms part of the normal microbiota in many healthy individuals, *S. aureus* functions as an opportunistic pathogen that can trigger diverse infections - including furuncles, abscesses, wound infections, and bacteremia - whenever host defenses are compromised (Alsolami *et al.,* 2025).

The detection of *S. epidermidis* at 41.7% frequency as a common commensal organism is in line with its extensively documented position as one of the most abundant and stable members of the normal skin flora (Ejyir *et al*., 2022). This species readily colonizes the uppermost layers of the skin and mucous membranes, where it supports host protection by competing with pathogenic bacteria for space and nutrients. Nevertheless, although it is typically non-pathogenic under ordinary physiological conditions, *S. epidermidis* can act as an opportunistic pathogen, particularly in immunocompromised hosts or when introduced into normally sterile body sites *via* indwelling medical devices such as catheters or prosthetic implants (Madigan *et al*., 2021).

The virulence traits identified in this study demonstrated clear distinctions between the two *Staphylococcus* species. *S. aureus* stood out as the sole coagulase-positive isolate and displayed β-hemolysis on blood agar plates, pointing to its substantial pathogenic capacity. Coagulase production is to known to serve as a key virulence factor, enabling the bacterium to form clots that can shield it from engulfment by host immune cells (Tsai *et al*., 2024). The observed β-hemolytic activity in *S. aureus* aligns with the expression of α-hemolysin, a powerful cytotoxin responsible for rupturing red blood cells and promoting tissue damage (Di Bella *et al.,* 2025).

In comparison, *S. epidermidis* was coagulase-negative and non-hemolytic, aligning with its profile as a low-virulence commensal prokaryote. These contrasting features underscore the divergent survival approaches within the *Staphylococcus* genus: *S. aureus* deploys robust and aggressive virulence tools, whereas *S. epidermidis* is known to be primarily reliant on long-term persistence as well as strong surface attachment to establish colonization.

The ability of both species to form biofilms is particularly noteworthy. A greater proportion of *S. epidermidis* strains exhibited stronger biofilm production than *S. aureus* strains. This observation is consistent with the work of Lister and Horswill (2024), who identified biofilm formation as a hallmark characteristic of coagulase-negative staphylococci (CoNS).

Biofilms are known to consist of organized communities of bacteria embedded within a self-produced extracellular matrix. These structures adhere firmly to various surfaces, such as skin and indwelling medical devices. By shielding bacteria from host immune defenses and the effects of antibiotics, biofilms significantly improve bacterial survival and persistence. Consequently, biofilm formation represents a key virulence mechanism. This is especially relevant for *S. epidermidis*, which relies on it to establish prolonged colonization on the skin and to facilitate hospital-acquired infections (Severn and Horswill, 2023).

Antibiotic susceptibility testing showed that *S. aureus* strains displayed elevated levels of resistance to penicillin (72%) and oxacillin (100%), while *S. epidermidis* strains exhibited 40% resistance to each of these antibiotics. The elevated oxacillin resistance observed in *S. aureus* would suggest the presence of methicillin-resistant *S. aureus* (MRSA) strains, a trend that aligns with a previous community-based study (Al-Saleh *et al*., 2022). Notably, both species demonstrated complete sensitivity (100%) to vancomycin, indicating that this antibiotic has remained one of the most reliable treatment options for staphylococcal infections in community settings.

These results indicated that the skin of apparently healthy individuals can serve as a reservoir for both commensal and potentially pathogenic *Staphylococcus* species. The simultaneous presence of virulent (*S. aureus*) and strong biofilm-forming (*S. epidermidis*) strains underscores the importance of maintaining good personal hygiene and implementing ongoing surveillance of antibiotic resistance in skin-colonizing bacteria.

CONCLUSION

This study investigated the virulence factors and antibiogram profiles of *Staphylococcus* species recovered from the skin of apparently healthy undergraduate students. The findings verified the occurrence of both *S. aureus* and *S. epidermidis* - representing coagulase-positive and coagulase-negative staphylococci, respectively - in every skin swab examined. These bacterial strains displayed diverse virulence attributes, such as hemolysin production, coagulase activity, and biofilm formation, along with resistance to several commonly prescribed antibiotics.

The results indicate that although the skin microbiota generally serves a beneficial protective function, certain resident staphylococcal strains possess pathogenic potential and can trigger opportunistic infections under suitable conditions. Therefore, ongoing surveillance, proper personal hygiene, and prudent antibiotic usage remain essential for curbing the dissemination of virulent or drug-resistant *Staphylococcus* strains in the community.

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