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Isolation and Characterization of Staphylococcus Species from Clinical Samples Obtained from some Hospitals in Kano Metropolis, Nigeria

Sadiya M. Ibrahim[1], Isma'il Ahmad[2], Sani M. Yahaya[3], Muhammad Ali[4*]

[1]*Department of Integrated Science, Sa'adatu Rimi College of Education Kumbotso, Kano*

[2]*Department of Microbiology, Kano University of Science and Technology Wudil*

[3]*Department of Biochemistry, Kano University of Science and Technology Wudil*

[4]*Department of Microbiology, Federal University Gusau*

Corresponding Author:** ***Muhammad Ali, *Department of Microbiology, Federal University Gusau,*
E-mail: *alimuhd4real@gmail.com*

Abstract: *The study was aimed to isolate and characterize Staphylococcus species among patients attending some Hospitals in Kano, Northern Nigeria. Three hundred (300) samples from ear swab, high vaginal swab (HVS), wound swab and urine were collected from patients (133 males and 167 females) attending the Hospitals over a period of eight months (October, 2016 to May, 2017). The samples were collected and inoculated onto the surface of freshly prepared Nutrient agar for colony formation and isolation. Each colony was isolated in a pure form by sub culturing for further studies and identification using Gram staining, biochemical characterization and bacteriological method.The result showed that the S. aureus isolates were able to ferment Mannitol, showed Golden yellow coloration onNutrient agar and produce β-haemolysis on blood agar. They also found to be positive for both Catalase DNase and Coagulase test. The coagulase negative Staphylococcus showed negative for both haemolysis and mannitol salt fermentation. Statistical analysis of the distribution of Staphylococcus species showed considerable significant difference at $p<0.05$. It is that Staphylococcus species are one of the most frequent aetiologic agents of various human infections.*

Keywords: *Clinical samples, infections, Kano, prevalence, Staphylococcus species,*

1. Introduction

Staphylococci are group of bacteria frequently isolated as etiologic agents of various infectious diseases with Staphylococcus aureus being the most important human pathogen [1]. S. aureus has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles and cellulitis. Although most Staphylococcal infections are not serious, S. aureus can cause serious infections such as blood stream infections, pneumonia, or bone and joint infections [2]. S. aureus can also cause serious infections such as pneumonia (infection of the lungs) or bacteremia (bloodstream infection), symptoms of these infections include: difficulty breathing, malaise, fever or chills [2]. In addition, two coagulase-negative staphylococcal species, S. epidermidis and S. saprophyticus, are also recognized as important agents of human infections. S. epidermidis is associated with infections of indwelling devices, osteomyelitis, wound infections, peritoneal dialysis catheter-associated peritonitis, and nosocomial bacteremia [3]. S. saprophyticus is recognized primarily as a cause of acute urinary tract infections in young women [4]. Together, these two coagulase-negative species comprise the greater majority of the clinically significant coagulase-negative staphylococci recovered from human specimens [5]. Staphylococcus epidermidis is isolated prevalently from human epithelia and colonizes predominantly the axillae, head, and nares [6]. S. epidermidis belongs to the group of coagulase-negative staphylococci (CoNS), which is discriminated from coagulase-positive Staphylococci, such as S. aureus by its lack of the enzyme coagulase [7].

Indeed, this pathogen is part of the human epithelia micro flora and for this reason has a

benign relationship with the host, but S. epidermidis has emerged as a pathogen causing different infections. Particularly, S. epidermidis represents the most frequent causative agent involved with infections involving any kind of medical devices, such as peripheral or central intravenous catheters [8]. Specifically, catheter-related infections are associated with increased mortality and contribute to an increased length of hospital stay and higher healthcare costs, which are problematic in limited-resource settings [9].

Staphylococcus saprophyticus is uniquely associated with un- complicated urinary tract infection (UTI) in humans. It has special urotropic and ecologic features that are distinctly different from other staphylococci and from Escherichia coli. This article will consider the epidemiology, ecology, pathogenesis, and clinical features of infections caused by this microorganism. Much more needs to be learned about the epidemiology and natural history of UTI caused by S. saprophyticus as well as the role of S. saprophyticus in human and animal health and disease.

A series of research questions are offered to address these issues. Coagulase-negative staphylococci were considered to be urinary contaminants prior to the 1960s. In 1962, Torres Pereira [10] reported the isolation of coagulase-negative staphylococci possessing antigen 51 from the urine of women with acute UTI. In subsequent years, additional reports supported this concept [11]. The laboratory identification of S. aureus has traditionally depended on the demonstration of coagulase production by the tube coagulase test [12].

Susceptibility to novobiocin is a factor widely used in clinical laboratories for the presumptive identification of S. saprophyticus [13]. The study was aimed to isolate and characterize Staphylococcus species among patients attending some Hospitals in Kano, Northern Nigeria.

2. Materials and Methods

2.1. Study Area

The research was conducted in Kano central area which lies between Latitude 11.90 North and Longitude 8.50 East in North western Nigeria, Kano state occupies 20,131 square kilometers and is bounded to the north by Katsina State, to the east and south by Jigawa State and to the west by Kaduna state. The area is densely populated comprising of 9,383,682 people [14]

2.2. Ethical Clearance

Ethical approval was obtained from Kano State Hospital Management Board based on the consent of Murtala Muhammad Specialist Hospital, Muhammad Abdullahi Wase Specialist Hospital and Aminu Kano Teaching Hospital ethical committees.

2.3. Sample Size

A total of 300 samples were collected, a standard epidemiological formula (Fisher's formula) was used to calculate the sample size. The prevalence and antimicrobial susceptibility of MRSA and CoNS isolated from healthy students in Ota, Nigeria as reported by Joshua and Ronke [15] was 78%. This was scaled to 300 at 95% confidence interval, and the sample size was calculated using a formula by Fishers.

$N = Z2pq/d2$

Where:

N=sample size

Z= Standard normal deviate at 95% confidence interval.

P= Proportion of target population

q= 1-p

d= degree of freedom.

$Z=1.962$, $p=0.78$, $q=1-0.0.78=0.22$ $d=0.052$

Thus;

$N=0.65921856/0.0025 =263.7$

Therefore, a total of 263.7 with 14% (36.8) of this subject will be added to the research for attrition, making a total of approximately 300 sampleswas involved in the study.

2.4. Sample Collection

Three hundred (300) samples from ear swab (n=75), high vaginal swab (HVS) (n=75), wound swab (n=75) and urine (n=75) were collected from patients attending three different hospitals within Kano State metropolis (Murtala Muhammad Specialist Hospital, Muhammad Abdullahi Wase Specialist Hospital and Aminu Kano Teaching Hospital) using sterile swab sticks and bottles over a period of eight months (October, 2016 to May, 2017).

2.5. Isolation of Staphylococcal isolates

The swab and urine samples collected inoculated onto the surface of freshly prepared Nutrient agar (Biomark). The plates were

incubated at 37ºC for 24 h for colony formation. Each colony was isolated in a pure form by sub culturing for further studies and identification. Discrete colonies of each isolate were kept in peptone water. The bacterial strains were then stored at 4oC for further experiments [16].

2.6. Biochemical Tests

2.6.1. Catalase Test

3ml of 3% hydrogen peroxide solution was poured into a clean test tube using a sterile syringe; a portion of the test organism was removed and immersed in the hydrogen peroxide solution. Presence of immediate bubble indicate positive test [17].

2.6.2. Coagulase Test

Drop of distilled water was placed on each end of a glass slide; the test organisms then emulsified in each of the drops to make a thick suspension, loop fulof plasma was added to one of the suspension and mix gently. Clumping of the organism within 10 seconds indicate positive test. Tube coagulase test was also done in which 3ml of plasma was poured into a test tube, it was then inoculated with the isolated colony of the test organism and incubated for 1 hour and then observed. Cloud/clump formation indicate positive test [18].

2.6.3. Dnase Test

A colony of the test organism was inoculated onto the DNase agar (with methyl green indicator) which the surface has been dried using a sterile wire loop and incubated at 37°c for 18-24 hours. The DNA was hydrolyzed turning the medium colorless around the test organism in the positive results [18].

2.6.4. Mannitol Fermentation Test

The pure colonies on nutrient agar were picked using a sterile inoculating loop and sub-cultured onto the surface of freshly prepared Mannitol Salt Agar (Oxoid, UK). The plates were incubated at 370 C for 24 hours [18].

2.6.5. Heamolysis Test

The pure colonies on nutrient agar were picked using a sterile inoculating loop and sub-cultured onto the surface of freshly prepared 5% Blood Agar (Oxoid, UK). The plates were incubated at 370 C for 24 hour s[19].

2.7. Statistical Analysis

The data generated were subjected to descriptive statistical analysis using percentages and Chi – square analysis was used in determining the prevalence rates. $p<0.05$ was considered indicative of a statistically significant difference.

3. Results

3.1. Sample Sources

A total of three hundred (300) samples from ear swab (n=75), high vaginal swab (HVS) (n=75), wound swab (n=75) and urine (n=75) were collected from patients (133 males and 167 females) attending the three different hospitals under study.

Table1. *Sample sources and number*

S/N	Sample source	Number
1	Ear swab	75
2	High vaginal swab	75
3	Wound swab	75
4	Urine	75
	Total	**300**

3.2. Characterization of *Staphylococcus* Species

Table 2 described the biochemical reactions and Gram staining of Staphylococcus isolated where microscopic appearance, heamolysis, catalase, coagulase tests and other biochemical reactions was demonstrated.

Table2. *Biochemical characterization of the Staphylococci isolated*

Isolates	GS	CAT	COA	DNase	HEA	MF	NST	Suspected organism
IS_1	+	+	+	+	+	+	+	*Staphylococcus aureus*
IS_2	+	+	-	-	-	-	+	*Staphylococcus epidermidis*
IS_3	+	+	-	-	-	-	-	*Staphylococcus saprophyticus*

Key: + = positive, - = negative, GS= Gram staining, CAT= Catalase, COA= Coagulase, HEA= Heamolysis, MF= Mannitol Fermentation, NST=Novobiocin Sensitivity Test

3.3. Prevalence of *Staphylococcus* Species

Table3 described the distribution of Staphylococcus aureus and other Coagulase Negative Staphylococcus in the clinical samples for the three hospitals, S. aureus was isolated most from wound swab (51 isolates) followed by ear swab (46 isolates) and then H.V.S and urine with 28 isolates each.

CoNS was isolated most from urine sample (11 isolates) followed by H.V.S (9 isolates) then ear swab (8 isolates) and wound swab (2 isolates).

Table3. *Prevalence of Staphylococcus aureus and other Non- Staphylococcus aureus in the Clinical Samples from the Three Hospitals*

Sample source	*S. aureus* (%)	Non- *S. aureus* (%)	Total (%)	X^2
Ear swab	46 (30.07)	08 (26.66)	54 (29.51)	11.9245*
Wound swab	51 (33.33)	02 (06.67)	53 (28.96)	
HVS	28 (18.30)	09 (30.00)	37 (20.22)	
Urine	28 (18.30)	11(36.67)	39 (21.31)	
Total	**153 (100)**	**30 (100)**	**183 (100)**	

Key: * *The table value is .007646, and the result is significant at $p<0.05$.*

Plate1. *S. saprophyticus on blood agar*

Plate2. *S. aureus growth on blood agar*

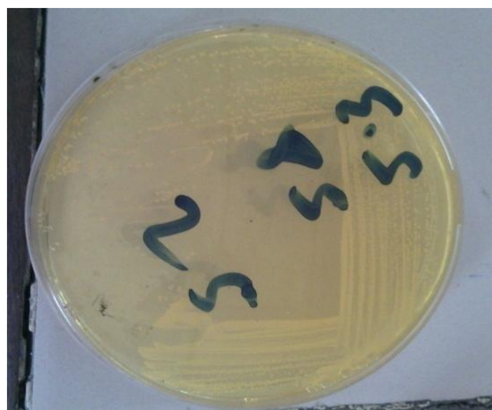

Plate3. *S. aureus on Mannitol Salt Agar*

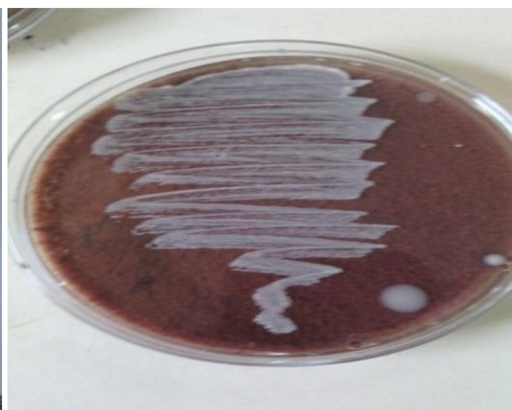

Plate4. *S. epidermidis on blood agar*

4. Discussion

Staphylococcus is innocuous in most environment but with remarkable adaptability and versatility which has equipped it as a commensal and pathogen. S. aureus is one of the most infectious agents with high prevalence in various communities and healthcare institutions [20]. The study aimed at isolation, identification and determination of antimicrobial susceptibility pattern of some Staphylococcus species from clinical samples obtained from some Hospitals in Kano Metropolis. A total of 183 Staphylococcus species from ear swab, H.V.S, urine and wound swab samples was isolated out of 300 samples collected from three Hospitals for the research.

Identification of S. aureus in the present study was based on Gram staining, cultural characteristics and biochemical characterization. All the S. aureus were able to ferment Mannitol

producing yellow colony, they also showed β-haemolysis on blood agar medium enriched with 5% sheep blood. Gram staining of the isolates exhibited a cluster of Gram positive cocci. The isolates were positive for catalase, coagulase and DNase test. In catalase test, hydrogen peroxide was broken down into water and oxygen by enzyme catalase. The production of oxygen was indicated by bubble formation [21]. The positive result of coagulase test was confirmed by the formation of curd like clotting compared to negative control [13]. Earlier findings by Jahanet al.[21]; Amengialueet al. [22]; Yabayaet al. [23]; Aliet al. [24] identified and characterized Staphylococcus on the basis of cultural characteristics, Gram staining and Biochemical characterization. The coagulase negative Staphylococcus showed negative for both haemolysis and mannitol salt fermentation. S. saprophyticus was differentiated from S. epididymis due to resistivity to Novobiocin. The presence of Staphylococcus in the samples from the subjects in the present study demonstrated that the isolates are one of the causative organism associated with infectious diseases. The Staphylococcus produced enterotoxin while multiplying in different parts of the body. S. aureus is known to produce six serologically different types of enterotoxins (A, B, C1, C2, D and E) that differ in toxicity [25]. The infections are caused by invasion of viable bacteria which then grow and establish themselves in the host and subsequently produce a toxin in the host.

The higher incidence Staphylococcus in of sample could be attributed to poor personal hygiene and exposure of the wounds, which might have made it more prone to contamination and infection. Furthermore, most people in this area tend to treat their wounds on their own or employ services of ill-trained quacks before seeking medical attention which could account for the level of colonization by S. aureus and other Staphylococcus species in wounds. The non-coagulase Staphylococci identified amongst these samples might have been contaminants or opportunistic pathogens [26]

5. Conclusion

Based on the finding of the study, Staphylococcus species are one of the etiological agents of various infectious diseases such as urinary tract infection, wound infection, ear infection and pelvic inflammatory diseases. Isolates were confirmed as Staphylococcus species by Cultural characteristic, Gram staining and Biochemical tests. The S. aureus were able to ferment Mannitol, showed Golden yellow coloration onNutrient agar and produce β-haemolysis on blood agar. They also found to be positive for both Catalase and Coagulase test. The coagulase negative Staphylococcus showed negative for both haemolysis and mannitol salt fermentation. It is recommended that individual should practice good hygiene exercise to avoid the spread of infection with Staphylococci

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