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Phenotypic Characters of *Staphylococcus Aureus* Isolates from Clinical Samples in Aminu Kano Teaching Hospital, Kano, Nigeria

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Abstract: Bacterial colonies can differ greatly in their morphologies. These differences can help us in identifying different species of bacteria. The clinical isolates of *Staphylococcus Aureus* are subjected to standard biochemical tests, to observe the biochemical characteristics. One hundred and ten (110) pathogenic *Staphylococcus Aureus* strains were used in this study. Characteristics of these strains were determined by biochemical tests such as catalase, coagulase, DNase test, test for beta haemolysin, fermentation of mannitol and lactose. *Staphylococcus Aureus* [American Type Culture Collection (ATCC) 25923] was used as a reference control organism. From this study, males were more infected than females, having 60 (61.8%) and 42 (38.2%) respectively. The age group with the highest number of isolates was (0-10) years while blood culture had the highest frequency of *Staphylococcus Aureus* isolates with a frequency of 42 (38.1%). It was also observed that 100% of *Staphylococcus Aureus* showed positive results in catalase and tube coagulase, about 101 (91.8%) were positive for slide coagulase production test, 102 (92.7%) strains were positive to DNase test, 63 (57.3%) were positive to beta haemolysin while 79 (71.8%) and 106 (96.4%) strains fermented Mannitol and lactose respectively. This study reveals that no single test that can be used for the identification of *Staphylococcus Aureus*, hence the need for sequel testing using proper biochemical tests should be used for the identification of *Staphylococcus Aureus* isolates.

Keywords: *Staphylococcus Aureus,* Biochemical, Characteristics, Fermentation, Identification

1. Introduction

Staphylococcus Aureus is a Gram-positive bacterium belonging to the family *Staphylococcaceae* and is often found as a commensal on the skin, skin glands and mucous membranes particularly in the nose of healthy individuals [20]. *Staphylococcus Aureus* has emerged as one of the most important human pathogens, being over the past several decades, a leading cause of hospital and community acquired infections [14]. It is associated with variety of clinical infections including septicemia, pneumonia, wound sepsis, septic arthritis, osteomyelitis and post-surgical toxic shock syndrome with substantial rates of morbidity and mortality [4, 5, 21]. It can cause infections commonly in newborns, surgical, burns, diabetic patients and persons who are taking drugs suppressing the immunodeficiency diseases [25].

In developing countries, phenotypic tests are routinely used in the diagnosis of staphylococcal infections, in which coagulase tests are usually confirmatory for *Staphylococcus Aureus* [3, 15]. Several studies in Nigeria indicated that *Staphylococcus Aureus* is among the most frequently isolated bacteria in Nigeria [1, 19, 24].

Staphylococcus Aureus is usually isolated on non-specific media (e.g. nutrient agar) and then presumptively identified before definitive overnight characterization [12]. In an attempt to achieve presumptive isolation in a single step, mannitol salt agar (MSA) was developed in 1945 for the

selective isolation of pathogenic staphylococci in the clinical microbiology laboratory. The growth and production of yellow colonies, due to the high salt content of the medium and fermentation of mannitol, is regarded as a presumptive tool in the identification of *Staphylococcus Aureus*. It is also described as a characteristic for the differentiation of coagulase-positive staphylococci from coagulase-negative staphylococci [7].

However, there are reports that some coagulase-negative staphylococci can also produce yellow colonies on mannitol salt agar [11, 22, 26].

Single phenotypic test is inefficient for the identification of *Staphylococcus Aureus*. However a combination of mannitol salt agar and DNAse improves the tube coagulase test [16].

Since *Staphylococcus Aureus* appears to be the major cause of both hospital and community acquired infections, it is important that proper identification and characterization of this organism is carried out. It was because of this that this study was carried out to characterize *Staphylococcus Aureus* isolated from clinical samples in Aminu Kano Teaching Hospital, Kano, Nigeria.

2. Materials and Methods

2.1. Study Area

This study was carried out in Medical Microbiology Laboratory of Aminu Kano Teaching Hospital and was restricted to the *Staphylococcus Aureus* Isolates obtained from various clinical samples processed in the laboratory.

2.2. Sample Size

The prevalence of *Staphylococcus Aureus* in clinical isolate was found to be 7% in a study of conventional and rapid methods for identification of *Staphylococcus Aureus* from clinical specimens at Zaria, Nigeria [18]. Thus for this study the prevalence was used to calculate sample size as follows

$$n=\frac{Z^2Pq}{d^2}$$

Where

n = number of samples

Z = statistic for level of confidence at 95% = 1.96

P = prevalence = 7% (0.07)

D = allowable error of 5%, (0.05)

q=1-p

$$n = \frac{1.96^2 \times 0.07(1-0.07)}{0.05^2} = 100.03 \text{ hence } n \approx 110$$

2.3. Bacterial Isolates

A total of 110 consecutive non-duplicated *Staphylococcus Aureus* isolates were obtained from various clinical samples such as wound swab, blood culture, eye swab, ear swab, throat swab, catheter tips and vaginal swab samples and was identified using standard bacteriological procedures [6]. The quality control and rejection criteria of specimen [10] were followed. *Staphylococcus Aureus* (ATCC 25923) was used as control in every test run.

2.4. Identification of Isolates by Standard Bacteriological Procedures

2.4.1. Gram Stain

Gram staining was carried out on all the bacterial isolates. A drop of sterile normal saline was placed on a well labeled clean grease-free glass slide using a sterile inoculating loop; a colony of an overnight culture of the bacterial isolate was emulsified with the sterile normal saline to make a thin smear. The smear was air dried and then heat fixed. The slide was flooded with crystal violet (primary stain) for 60 s after which the stain was rinsed from the slide with water. The smear was flooded with Lugol's iodine (mordant) to fix the primary stain. The smear was be rinsed with water after 30 s. The slide was then flooded with acetone and rinsed off almost immediately. The counter stain; neutral red was added and left for 60 s before being rinsed off. The stained smear was air dried and then observed under the microscope using X100 oil immersion objective lens of the microscope. A cluster of purple round colonies is indicative of Staphylococci [6].

2.4.2. Catalase Test

Staphylococci produce catalase an important virulence factor which degrades the microbicidal (hydrogen peroxide) H_2O_2 into oxygen (O_2) and water (H_2O). This ability differentiates Staphylococci from Streptococci.

A drop of 3% hydrogen peroxide was placed on a clean grease-free glass slide. About 2 colonies of the bacteria was picked from a culture plate using a sterile wire loop and placed on the hydrogen peroxide; presence of bubbles indicates a positive catalase test [6].

2.4.3. Coagulase Test

Staphylococcus Aureus is distinguished from other Staphylococci by the production of coagulase an enzyme that clots plasma [6].

(i). Bound Coagulase

About one or two drops of blood plasma was placed on a clean grease-free glass slide and 2 colonies of the organism were picked using a sterile wire loop from a culture plate. The colonies will be emulsified in the plasma and observation of a clot indicates a positive coagulase test [6].

(ii). Free Coagulase

Three small test tubes were labeled: 'T' for Test organism (18h broth culture), 'P' for Positive control (18h *Staphylococcus Aureus* broth culture) and 'N' for Negative control (sterile broth). About 0.2ml of serum was added into each tube, 0.8 ml of the test organism broth culture will be added to tube labeled T, P and N each. The tubes was mixed gently, and incubated at 37°C. After 2 hours, the tubes were examined for clotting [6].

2.4.4. Sugar Fermentation Test

(i). Mannitol Salt Agar (MSA)

Using a sterile wire loop a small colony of the test organism was inoculated onto the surface of Mannitol Salt Agar plate and streaked. The culture plate was incubated aerobically at 37°C. The plate was examined for Mannitol fermentation after 24 hours [6].

(ii). MacConkey Agar

Using a sterile wire loop a small colony of the test organism was inoculated onto the surface of MacConkey Agar plate and streaked. The culture plate was incubated aerobically at 37°C. The plate was examined for Lactose fermentation after 24 hours [6].

2.4.5. Deoxyribonucleic Test (DNase Test)

DNase plates was divided into 6 sections by drawing lines on its bottom and Using a sterile wire loop the media was spot inoculated on to a small area in the middle of the marked sections with both the Test and Control organisms. It was incubated at 37°C overnight. After 24 hours, the surface of the plate was flooded with weak HCl acid solution. The excess acid tipped off and then after 5 minutes the colonies was examined [6].

2.4.6. Haemolysin Activity Test

The test organism was inoculated on blood agar by an agar overlay method and incubated at 37°C for 24 hours. After 24 hours, the plates will be observed for the presence of haemolysis [6].

3. Results

Of the One hundred and ten isolates tested, 68 were from male patients (61.8%) and 42 from female patients (38.2%) (Table 1). The age group 0-10 years has the highest percentage of the isolates (44%). This is followed by 61-70, 21-30, 11-20 and 51-60 years with 27.2%, 20%, 5.5% and 1.8% respectively. The least was found in the age group of 31-40 and 41-50 years with both having 0.9% each (Table 2).

The source of isolates with the highest frequency was blood culture (38.1%), followed by wound swab and eye swab with 32.7% and 8.2% respectively. Catheter tip, ear swab and throat swab have 4.6% each. The source with the least number of isolates were high vaginal swab and semen with both having 3.6% each (Table 3).

Of the One hundred and ten isolates tested, 57.3% showed beta hemolysis, 92.7% were DNase positive, 71.8% were Mannitol positive, 96.4% were lactose positive and all the isolates were positive for catalase. While only 91.8% were positive for the slide coagulase production test, 100% were positive for the tube coagulase test (Table 4).

Table 5 depicts the division of the 110 isolates into biotypes based on their ability to ferment Mannitol and lactose. Of the 110 isolates, biotypes I represent the 78 (70.9%) strains that are able to ferment both sugars, biotypes II represent the 1 (0.9%) that only fermented Mannitol but not lactose. Biotypes III represent those 27 (24.5%) that fermented lactose but not Mannitol. Biotypes IV represent those 4 (3.6%) that neither fermented Mannitol nor lactose.

Table 1. *Showing distribution of Staphylococcus Aureus Isolates according to gender (N=110).*

GENDER	FREQUENCY	PERCENTAGE (%)
MALE	68	61.8
FEMALE	42	38.2
Total	110	100

Table 2. *Showing distribution of Staphylococcus Aureus Isolates according to age group.*

AGE GROUP (YEARS)	FREQUENCY	PERCENTAGE (%)
0-10	48	44
11-20	6	5.5
21-30	22	20
31-40	1	0.9
41-50	1	0.9
51-60	2	1.8
61-70	30	27.2
Total	110	100

Table 3. *Showing distribution of Staphylococcus Aureus according to source of isolates.*

SOURCE	FREQUENCY	PERCENTAGE (%)
Blood cultures	42	38.1
Wound swab	36	32.7
Eye swab	9	8.2
Catheter tips	5	4.6
Ear swab	5	4.6
Throat swab	4.6	5
High vaginal swab	4	3.6
Semen	4	3.6
Total	110	100

Table 4. *Showing biochemical and some carbohydrate fermentation characteristics of the Staphylococcus Aureus isolates.*

Tests	No. positive	% positive
Catalase	110	100
Slide Coagulase	101	91.8
Tube Coagulase	110	100
DNase	102	92.7
Beta hemolysin	63	57.3
Mannitol	79	71.8
Lactose	106	96.4

Table 5. *Showing distribution of Staphylococcus Aureus isolates into biotypes based on their ability to ferment mannitol and lactose.*

Biotypes	Mannitol	Lactose	No. of positive (%)
I	+	+	78(70.9)
II	+	-	1(0.9)
III	-	+	27(24.5)
IV	-	-	4(3.6)

4. Discussion

The advance and increase of bacterial strains that are resistant to antibacterial drugs has emerged as a global

problem [23]. In this study, males (61.8%) were more infected than females (38.2%), the reason for this is not clearly understood but this agrees with previous studies conducted at Aminu Kano Teaching Hospital [8].

Also in this study the highest frequency of isolates of *Staphylococcus Aureus* (44%) was observed in the (0-10) year age group in which neonates and infants were included, concurring with previous studies conducted at Aminu Kano Teaching Hospital [8]. It is believed that their immunity is not properly developed at this stage to cope with bacterial infections hence they are vulnerable and easily infected especially when hospitalized. A study in Ilorin reported wound infections of 38% as the highest frequency of *Staphylococcus Aureus* isolates [17]. This is in contrast to the present study where the highest frequency was reported in blood culture (38.1%), followed by wound swab (32%).

In this study, 96.4% of the isolates showed a positive result in lactose fermentation test. Lactose fermenting bacteria usually developed into pink colours on the MacConkey agar plates because it contains crystal violet and bile salt [9]. This result is in slight agreement with previous studies conducted in 8 health institutions in 6 states in Northwestern Nigeria [13] where 100% of the isolates showed positive results in lactose fermentation. It was observed in this study that 71.8% fermented mannitol, 92.7% produced DNase and that 57.3% was positive to Beta hemolysin. This is in contrast to previous studies conducted in Zaria [18].

5. Conclusion

This study reveals that no single test that can be used for the identification of *Staphylococcus Aureus*, hence the need for sequel testing using proper biochemical tests should be used for the identification of *Staphylococcus Aureus* isolates. *Staphylococcus Aureus* appears to be the major cause of both hospital and community acquired infections, therefore it is important that proper identification and characterization of this organism is carried out.

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