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Research Article

Prevalence, Characterization and Determination of Antibiotics Sensitivity Profile of Clinical Isolates of *Staphylococcus aureus*

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Abstract: The study was aimed to determine the prevalence, characterize and evaluate antibiotic sensitivity profile of clinical isolates of *Staphylococcus aureus* 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 collected 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. Identification of the isolates was conducted using Gram staining, biochemical test and microbiological analysis of the isolates. The result showed that the isolates were positive for Gram staining, catalase, coagulase, DNase and Mannitol fermentation test. The isolates showed β-haemolysis on blood agar plates. The prevalence of *S. aureus* for the three hospitals treated was 153 positive samples out of 300 total samples which accounted for 51%. The highest incidence of *S. aureus* was seen in wound swab with 51 isolates. The antibiotic susceptibility pattern of the isolates shows that *S. aureus* was susceptible to Ciprofloxacin 105 (68.63%), Gentamicin 102 (66.67%), Levofloxacin 95 (62.08%) and Amikacin 90 (58.82%) while resistant to Cefuroxime 153 (100%), Ceftazidime 150 (98.04%), Augmentin 120 (78.43%), Cloxacillin 111 (72.55%) and Cefoxitin 110 (71.89%). Statistical analysis of the result showed significant difference in the prevalence of *Staphylococcus aureus* among the samples examined at $p<0.05$. *S. aureus* is one of the etiologic agents of infectious diseases.

Keywords: Antibiotics, characterization, Kano, prevalence, *Staphylococcus aureus*.

INTRODUCTION

Microbial resistance to antibiotics is one of the most serious health threats threatening human well-being today. Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic (Anonymous, 2012). The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria (Goossens *et al.*, 2005). Infections from resistant bacteria are now too common and some pathogens have even become resistant to multiple types or classes of antibiotics (antimicrobials used to treat bacterial infections).

Staphylococcus 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 (Minnesota, 2010). *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 (Minnesota, 2010). The bacterium appears as grape like cluster when viewed through a microscope, and has large round, golden yellow colonies, often with hemolysis, when grown on blood agar plate (Ryan and

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Ray, 2004). *Staphylococcus aureus* is catalase – positive meaning it can produce the enzyme catalase so is able to convert hydrogen peroxide (H_2O_2) to water and oxygen which make the catalase test useful to distinguish Staphylococci from Enterococci and Streptococci. A small percentage of *Staphylococcus aureus* can be differentiated from most other Staphylococci by the coagulase test. *Staphylococcus aureus* is coagulase positive (meaning it can produce the enzymes coagulase) that causes the clot formation, whereas most other *Staphylococci* sp are coagulase – negative.

Staphylococcus aureus are highly successful colonizers of humans and animals. They reside mainly on the skin, particularly in moist areas such as the anterior nares (nose), axilla and groin. Between one-third and three-quarters of individuals carry these organisms at any one time. Staphylococcal infections occurs worldwide and newly emerging hyper virulent or multi-resistant strains spread rapidly over wide geographical areas. The bacteria survive in the air, on objects or in dust for days; therefore they can contaminate environments (such as hospitals) and continue to be transmitted over long periods of time. Some individuals may shed the organism more heavily than others. Staphylococcal infections are acquired from either self (endogenous) or external (exogenous) sources (Irving *et al.*,, 2006).

Staphylococcus aureus is a facultative anaerobe that grows at an optimum temperature of 37ºC and an optimum pH of 7.5. *S. aureus* produces white colonies that tend to turn a buff-golden color with time, which is the basis of the species epithet *aureus* (golden). Most, but not all, strains show a rim of clear β-hemolysis surrounding the colony (Ryan and Ray, 2004).On nutrient agar, following aerobic incubation for 24 hours at 37ºC, colonies are 1 – 3mm in diameter, have a smooth glistening surface, an entire edge and an opaque pigmented appearance. In most strains, pigmentation is golden with orange, yellow and cream varieties. On MacConkey agar, colonies are small to medium in size and pink or pink-orange in colours (Mackie and McCartney, 1989). The objective of the study is to determine the prevalence, characterize and evaluate the antibiotic sensitivity pattern of clinical isolates *S. aureus* obtained from some tertiary hospitals in Kano, Nigeria.

MATERIALS AND METHODS

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.

Study Area

The research was conducted in Kano central area which lies between Latitude 11.9^0 North and Longitude 8.5^0 East in North western Nigeria, Kano state occupies 20,131 square kilometers and is bounded to the North west by Katsina State, North east by Jigawa State to the north east and south by Bauchi and Kaduna respectively. The area is densely populated comprising of 9,383,682 (NPC, 2006).

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 (2015) was 78% this was scaled to 300 at 95% confidence interval, and the sample size was calculated using a formula by Fishers.

$N= Z^2 pq/d^2$

Where:

N=sample size

Z= Standard normal deviate at 95% confidence interval.

P= Proportion of target population

q= 1-p

d= degree of freedom.

$Z=1.96^2$, p=0.78, q=1-0.0.78=0.22 $d=0.05^2$

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 samples was involved in the study.

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 (133 males and 167 females) 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).

Table 1: 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**

Isolation and Identification of *Staphylococcal* aureus

The ear swab, high vaginal swab (HVS), wound swab and urine samples collected inoculated onto the surface of freshly prepared Nutrient agar (Biomark). The plates were incubated at 37ºC for 24 hours 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 4^{o}C for further experiments (APHA, 1992). The isolated organisms were subjected to Gram staining, Mannitol fermentation and Biochemical tests (Catalase, coagulase, DNase, haemolysis test) as described by Zaved *et al.*(2008) and Cheesbrough (2010) for identification.

Antibiotics Susceptibility Testing

The bacteria isolates were subjected to antibiotic susceptibility testing using agar diffusion method as described by Bauer *et al.*, (1966). Mueller Hinton agar (Oxoid, UK) plates were inoculated with overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were then aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were then incubated at 37°C for 24 hours. Sensitivity pattern of the isolates to levofloxacin 5µg, amikacin 30µg, cefepime 30µg, ceftazidime 10µg, imipenem 10µg, gentamicin 10µg, nitrofurantoin 300µg, ceftriaxone 30µg, ciprofloxacin 5µg, cefoxitin 30µg, augmentin 30µg, ofloxacin 30µg, cloxacillin 5µg, erythromycin 5µg and cefuroxime 30µg. The result of each antibiotic was reported as susceptible, intermediate or resistant using the standard given by the National Committee on Clinical and Laboratory Standard as described by Nwoire *et al.*(2013).

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.

RESULTS

Identification of the Isolates

The identification of *Staphylococcus aureus* from the samples obtained from the Hospitals is presented in the Table below 2. The *S. aureus* was identified using Gram staining, biochemical test and Mannitol fermentation test. Result showed that the isolates were positive for Gram staining, catalase, coagulase, DNase and Mannitol fermentation test. The isolates showed β-haemolysis on blood agar plates.

Table 2: Morphological and Biochemical Identification for *S. aureus*

S/N	Test	Inference
1	Catalase	+
2	Coagulase	+
3	DNase	+
4	Mannitol test	Golden yellow colony
5	Blood agar growth	β-haemolysis
6	Nutrient agar growth	Yellow colony

Prevalence of *Staphylococcus aureus*

The prevalence of *Staphylococcus aureus* in the samples obtained from the Hospitals is presented in Table 3 below. The result shows that higher number of isolates was obtained from wound swab 51 (33.33%), followed by ear swab 46 (30.07%) while least number was obtained from HVS and urine with 28 isolates each which accounted for 18.3%. Statistical analysis of the result showed that it is significant at $p<0.05$.

Table 3: Prevalence of *Staphylococcus aureus* in the Samples obtained from the Hospitals

S/N	Sample source	No. of samples	*S. aureus* + (n)	Prevalence (%)	X^2
1	Ear swab	75	46	30.07	11.9245*
2	Wound swab	75	51	33.33	
3	HVS	75	28	18.30	
4	Urine	75	28	18.30	
	Total	**300**	**153**	**100**	

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

Antibiotics Sensitivity Test

The average zones of inhibition and standard deviation of the isolates against standard antibiotics used is presented in Tables 4. The isolates were classified into either resistant, susceptible or intermediate depending on the figure obtained as according to NCLSI: ≥ 17= S, 11-16= I and ≤10= R.

Table 4: Mean Diameter of Zone of Inhibition and standard deviation for the isolates against some antibiotics

Antibiotics	Concentration	*S. aureus*	Inference
LEV	5µg	18.89 ±2.84	Sensitive
AK	30µg	18.77±1.46	Sensitive
FEP	30µg	8.21 ±4.07	Resistance
CAZ	10µg	07.72 ±1.39	Resistance
IMI	10µg	12.39 ±3.07	Intermediate
CN	10µg	17.38 ±3.45	Sensitive
F	30µg	16.36 ±2.46	Intermediate
CRO	30µg	15.96 ±2.11	Intermediate
CIP	5µg	18.36 ±2.30	Sensitive
FOX	30µg	18.69 ±1.61	Resistance
ERY	5µg	13.84±2.65	Intermediate
CXC	5µg	08.65±1.80	Resistance
CRX	30µg	08.44±1.38	Resistance
AUG	30µg	08.29±1.90	Resistance

Key**:** CAZ-Ceftazidime, FEP-Cefepime, FOX-Cefoxitin IMI-Imipenem, CRO-Ceftriaxone, CIP-Ciprofloxacin, F-Nitrofurantoin, AK-Amikacin, CN-Gentamicin, LEV-Levofloxacin, AUG-Augmentin, CXC-Cloxacillin, CRX-Cefuroxime and ERY-Erythromycin.

Number and Percentage of Susceptible, Intermediate and Resistant Isolates

Table 5 represents the number and percentage of *Staphylococcus* aureus susceptible, intermediate and resistant to selected antibiotics. The isolates were highly susceptible to Ciprofloxacin 105 (68.63%), Gentamicin 102 (66.67%), Levofloxacin 95 (62.08%) and Amikacin 90 (58.82%) while resistant to Cefuroxime 153 (100%), Ceftazidime 150 (98.04%), Augmentin 120 (78.43%), Cloxacillin 111 (72.55%) and Cefoxitin 110 (71.89%).

Table 5: Number and Percentage of *Staphylococcus* aureus Susceptible, Intermediate and Resistant to selected antibiotics (n=153).

Antibiotics	Susceptible	Intermediate	Resistant
Levofloxacin	95 (62.09%)	43(28.10%)	15 (9.80%)
Amikacin	90 (58.82%)	23(15.03%)	40 (26.14%)
Cefepime	39 (25.49%)	21(13.73%)	93 (60.78%)
Ceftazidime	3 (1.96%)	0(0.00%)	150 (98.04%)
Imipenem	48 (31.37%)	20(13.07%)	85 (55.56%)
Gentamicin	102 (66.67%)	28(18.30%)	23 (15.03%)
Nitrofurantoin	70 (45.75%)	33(21.57%)	50 (32.68%)
Ceftriaxone	35 (22.88%)	20(13.07%)	100 (65.36%)
Ciprofloxacin	105 (68.63%)	18(11.76%)	30 (19.61%)
Cefoxitin	40 (26.14%)	3(1.96%)	110 (71.89%)
Erythromycin	40 (26.14%)	14(9.15%)	99 (64.71%)
Cloxacillin	40 (26.14%)	2(1.31%)	111 (72.55%)
Cefuroxime	0 (0.00%)	0(0.00%)	153 (100%)
Augmentin	18 (11.76%)	15(9.80%)	120 (78.43%)

DISCUSSION

Identification of *Staphylococcus aureus* in this study was based on cultural characteristics, Gram staining and biochemical characterization. All the 34 isolates 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 (Jahan *et al.*,2005). The positive result of coagulase test was confirmed by the formation of curd like clotting compared to negative control (Amengialue *et al.*, 2015). Earlier finding by Ali *et al.* (2019) identified and characterized *S. aureus* on the basis of cultural characteristics, Gram staining and Biochemical characterization.

The prevalence of *S. aureus* for the three hospitals treated was 153 positive samples out of 300 total samples which accounted for 51%. The highest incidence of *S. aureus* was seen in wound swab 51 (33.33%) followed by ear swab 46 (30.07%), then urine and H.V.S with 28 (18.30%) each. This result was in conformity with the findings of Gambo *et al.*, (2018) who found high percentage of *S. aureus* from wound samples. The higher incidence in wound swab 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* in wounds. It is well known that other *Staphylococci* though normal commensals are opportunistic pathogen of man (Baba *et al.*, 2002).

The antibiotic susceptibility pattern of the isolates shows that *S. aureus* was susceptible to Ciprofloxacin 105 (68.63%), Gentamicin 102 (66.67%), Levofloxacin 95 (62.08%) and Amikacin 90 (58.82%) while resistant to Cefuroxime 153 (100%), Ceftazidime 150 (98.04%), Augmentin 120 (78.43%), Cloxacillin 111 (72.55%) and Cefoxitin 110 (71.89%). Several studies were conducted on antibiotic susceptibility pattern of *S. aureus*. The finding of this study justifies the one of Ali *et al.* (2017) on evaluation of antimicrobial susceptibility pattern of *Staphylococcus* species from clinical samples obtained from some hospitals in Kano metropolis, Nigeria who found the isolates were susceptible to ciprofloxacin and gentamicin while resistant to Augmentin and cloxacillin

Plate 1: S. aureus growth on blood agar

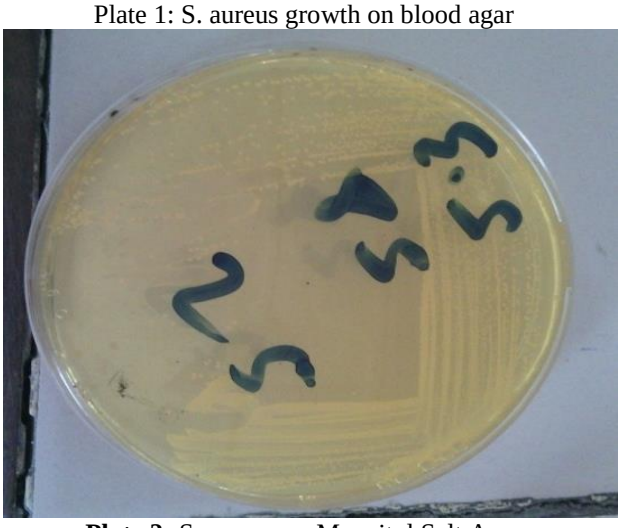

Plate 2: S. aureus on Mannitol Salt Agar

CONCLUSION

Based on the findings of the present study, the prevalence of *S. aureus* for the three hospitals treated was 153 positive samples out of 300 total samples which accounted for 51%. The highest incidence of *S. aureus* was seen in wound swab with 51 isolates followed by ear swab 46 isolates, then urine and H.V.S with 28 isolates each. The antibiotic susceptibility pattern of the isolates shows that *S. aureus* was susceptible to Ciprofloxacin, Gentamicin, Levofloxacin and Amikacin while resistant to Cefuroxime, Ceftazidime, Augmentin, Cloxacillin and Cefoxitin. The multiple antimicrobial resistant indexes shows increase in the rates of resistance in *S. aureus* thus making antimicrobial susceptibility surveillance and testing more crucial in selecting empiric regimen.

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