Original Research Article

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CLINICAL SAMPLES IN SPECIALIST HOSPITAL, SOKOTO

ABSTRACT

Aim: The study was to determine the susceptibility pattern of *Staphylococcus aureus* isolates against some conventional antibiotics.

Study design: Hospital based cross-sectional study.

Place and duration of study: The study was conducted in Specialist Hospital, Sokoto Metropolis, Sokoto State Nigeria between June 2018 and September 2018.

Methodology: One hundred (100) pathogenic *Staphylococcus aureus* strains were used in this study. Gram's staining, catalase, coagulase and mannitol fermentation tests were used to identify and confirm the isolates. Antibiotic susceptibility test was carried out by disc agar diffusion test.

Results: In the present study 63.0% of the *Staphylococcus aureus* isolates were from male subject, while 37.0% were from female subject. The age group with the highest number of isolates was 11-20years (37%) and the least (9%) was seen in 41-50years. Urine sample had the highest frequency of *Staphylococcus aureus* isolates ~~of~~ 32.0% and high vaginal swab had the lowest 6.0%. The antibiotics tested against *Staphylococcus aureus* isolates were clindamycin(40%), ciprofloxacin(64%), erythromycin(57%), Gentamicin(71%), cefoxitin(34%), Quinupristin/Dalfopristin(46%), tetracycline(58%) and Sulphamethaxazole –Trimethoprim(58%) respectively. Screening for MRSA was carried out by antibiotic sensitivity testing using cefoxitin and a prevalence of 66% was obtained. This study showed that Gentamicin and Ciprofloxacin were the most active antibiotics against *Staphylococcus aureus*. Thus it is believed that these antibiotics should be used in the treatment of *Staphylococcus aureus* infections in this region. **Conclusion**: There is the need for consistent on-going antimicrobial resistance surveillance for important and commonly isolated clinically significant pathogens of staphylococcal species to

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form the basis for developing and implementing measures that can reduce the burden of antimicrobial resistance and prevent a probable impending public health problem.

Keywords: Antibiotics, *Staphylococcus aureus*, MRSA, Clinical samples.

1.0 INTRODUCTION

Staphylococcus aureus is a gram-positive cocci, catalase and coagulase positive bacterium. *Staphylococcus aureus* has emerged as one of the main important human pathogens, and has over the past decades, been a leading cause of hospital and community-acquired infections [1]. The bacterium is well characterized and known to have a diverse arsenal of virulence factors that causes a prominent inflammatory response [2]. This pathogen affects both immune competent and immunocompromised individuals, frequently resulting in high morbidity and with complications, which constitute problem to health care institutions [3]. Variety of factors contribute to the ability of *S. aureus* to cause infection (virulence); enzymes, toxins, adhesion proteins, factors that help the bacteria to evade the innate immune defense, and antibiotic resistance mediate survival of the bacteria and tissue invasion at the site of infection [4].

The emergence of multidrug resistance in Gram-positive bacteria (pneumococci, enterococci and staphylococci) is a particularly important development. Perhaps the pathogen of greatest concern is S. *aureus*, because of its intrinsic virulence, its ability to cause an array of life threatening conditions, and its capacity to adapt to different environmental conditions [5]. *S. aureus* is known to be notorious in the acquisition of resistance to new drugs and continues to defy attempts at medical control. The resistance of *S. aureus* isolates to commonly used antibiotics in Nigeria and other different parts of the world has been widely reported [6]. This increase emergence of resistance strains has being attributed to the indiscriminate use of antibiotics in

both human and veterinary medicine especially in the developing countries. Many strains of *S. aureus* carry a wide variety of multi-drug resistant genes on plasmids, which aid the spread of resistance even among different species [7]. In Nigeria, most symptomatic patients usually indulge in indiscriminate use of antibiotics before consulting the physicians when they could no longer control the symptomatic situations. The physicians on the other hand usually treat the patients with broad-spectrum antibiotics before microbiological investigations [8].

MATERIALS AND METHODS

2.1 Study Design: Hospital based cross-sectional study.

2.2 Bacterial Isolates

A total of 100 isolates of *Staphylococcus aureus* was collected from various clinical specimens (wound swab, nasal swab, ear swab, high vagina swab, pus and urine samples) obtained in medical microbiology laboratory of Specialist Hospital using nutrient agar slants and transported to the medical microbiology laboratory in the school of medical laboratory sciences, Usmanu Danfodiyo University Sokoto, Nigeria.

2.3 Identification of Bacteria: Diagnostic procedures consisted of Gram staining, biochemical test, Catalase, Coagulase and Mannitol fermentation tests.

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2.3.1 Gram Staining Technique

A drop of sterile physiological saline was placed on a clean glass slide. With a sterile wire loop, a colony of the test organisms was emulsified in the drop of saline. The smear was allowed to dry, and then fixed over Bunsen flame briefly. The slide was placed on a staining rack, and then flooded with crystal violent. The stain was allowed to stay for 1 minute, after which it was

washed off with water. The slide was flooded with Lugol's iodine solution, and was allowed to stain for 1minute after which it was washed off with water. The smear was decolorized for 20 seconds with acetone solution, and then washed off with water. The smear was finally counterstained with neutral red solution for 2 minutes and washed off with water. The smear was air dried and viewed under the microscope using 100X objective (oil immersion) and the grams reaction of the organisms was recorded as described by [9].

Comment [u4]: There is no need to explain Gram staining the reference is enough

2.3.2 Biochemical Tests

Isolates found to be gram positive cocci were subjected to biochemical tests like catalase and coagulase using technique described by Chessbrough [9] and also, sub cultured on Mannitol Salt Agar.

Catalase Test

Two drops of 3% hydrogen peroxide solution was placed on a cleaned glass slide. A colony of the test organism was collected using a sterile glass rod and then emulsified into the drop of hydrogen peroxide. Bubbles of gas indicated a catalase positive test, while absence of bubbles indicated a catalase negative test [9].

Coagulase Test

Slide Test to detect bound coagulase; A drop of normal saline was placed on two separate cleaned grease free glass slide. A colony of the organism was picked and emulsified in each of the drops to make a suspension. Using a wire loop a loopful of plasma was added onto one of the suspensions, mixed gently and observed for clumping of the plasma immediately. No plasma was added to the second suspension, it served as the negative control of the test. Clumping of the

plasma indicates the organism is *S. aureus* while no clumping indicates other *Staphylococcus species* [9]

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Mannitol Fermentation Test

Isolates were directly inoculated on Mannitol Salt Agar MSA (Oxoid, England), a selective and differential media of *S. aureus* and incubated at 37°C for 24 hours. Organisms that were able to grow on Mannitol Salt Agar (Oxoid, England) with fermentation of Mannitol and acid production to give yellow colonies were characterized as *S. aureus* [9].

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2.4 Antibiotic Susceptibility Testing

The antimicrobial susceptibility testing for *Staphylococcus aureus* was performed in accordance to Clinical and Laboratory Standard Institute (CLSI) [10]. Standard inoculum was prepared by making a direct saline suspension of isolate colonies by selecting from an 18-hours agar plate (nutrient agar). The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard which resulted in a suspension containing approximately 1 to 2 $x10^8$ colony forming unit (CFU)/ml. It was observed using adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black line. Antimicrobial susceptibility was performed on Mueller-Hinton Agar by the standard Kirby-Bauer disk diffusion method. This was done by dipping a sterile swab stick into the bacterial suspension and carefully swabbing the entire surface of Mueller Hinton agar plates. The antibiotic single discs (Oxoid) were then placed on the surface of the inoculated plates and gently

pressed. The plates were incubated at 37^{O}C for 18–24h. The diameter of zone of inhibition was measured in millimeters and isolates were scored as sensitive, intermediate or resistant by comparing with values recommend in the CLSI M100 inhibition zone standard [10].

2.5 Screening for MRSA

Zones of inhibition ≥22mm with 30µg cefoxitin were recorded as Methicillin Susceptible *Staphylococcus aureus* (MSSA), while zones of inhibition ≤21mm with 30µg cefoxitin was recorded as Methicillin Resistant *Staphylococcus aureus* (MRSA) [10].

Comment [u6]: It is best to sense the strains against oxacillin to certify they are MRSA strains because cefoxitin yet a beta-lactamic antibiotic is commonly used for anaerobic microorganisms. Please check

2.6 Statistical Analysis

The data collected was presented in tables, and analyse using Statistical Package for Social Sciences (SPSS) version 25 and the degree of confidence was set at 95% (P = .05). Comparative resistance rates of *S. aureus* strains from the different clinical specimens ~~was~~ statistically analyzed by Chi square - test.

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3. RESULTS AND DISCUSSION

In this study, a total of 100 *Staphylococcus aureus* isolates were collected from clinical samples of patients attending Specialist Hospital Sokoto from the medical microbiology laboratory. Analysis of the gender specific distribution of patients infected with *Staphylococcus aureus* in Specialist Hospital Sokoto shows that Males had higher infection rate (63.0%) than females (37.0%). (Table 1). However, the age group with the highest frequency of *Staphylococcus aureus* infection was found to be individual aged (11-20) and (1-10) while the least was in the

(21-30) years group. (Table 2). Different clinical specimens from which *Staphylococcus aureus* was isolated were analysed, the highest number of isolates was from urine samples 32(32.0%) followed by wound swab 23(23.0%). The least was from high vaginal swab 6(6.0%). (Table 3).

Sensitivity and resistance pattern of *Staphylococcus aureus* to various antibiotics shows that the highest frequency of sensitivity was observed with Gentamicin (71%) followed by Ciprofloxacin (64%) and Tetracycline (58%). The least was observed with cefoxitin (34%) each. (Table 4). Antibiotic resistance pattern of Methicillin resistant *Staphylococcus aureus* (MRSA) shows that Cefoxitin had resistance 66(100%) while Clindamycin had 44(66.7%) and Quinupristin/Dalfopristin had 38(57.6%) resistance.(Table 5).

The importance of *Staphylococcus aureus* as a persistent nosocomial and community acquired pathogen has become a global health concern. In the present study, it has been observed that male subjects were more infected with *Staphylococcus aureus* (63%) than female subject (37%), which is in agreement with what was reported by Kumurya and Ado [11] at Aminu Kano Teaching Hospital that males had (61.8%) and females (38.2%). This is probably due to the nature of job men engage that females do not, especially farming in the Northern part of the country.

Also, in this study the highest frequency of isolates of *Staphylococcus aureus* (37%) was observed in the age group (11-20) years. This is in contrast to previous study by Nwankwo *et al.* [12] who reported the highest frequency (47.3%) among neonates and infants (0-10) years. This contradiction can be attributed to distribution of specimen collection as more were collected from age group 11-20 than 0-10 during the period of this study.

The prevalence of *S. aureus* isolate was found to be higher from urine samples 32.0% compared to other samples. This is in contrast to previous study by Kumurya and Ado [11] who reported the highest prevalence of 38.1% from blood cultures. This may be attributed to the issue of urine contamination with *S. aureus* from the surface during sample collection.

Staphylococcus aureus develops resistance very quickly and successfully to different antimicrobials over a period of time. The highest frequency of susceptibility in this study occurred with Gentamicin and Ciprofloxacin having (71.0%) and (64.0%) respectively. The least was cefoxitin having (34.0%). A similar study depicted that the most potent of all the antibiotics tested was Rifampicin, with 54% sensitivity [13]. The high level of resistance could be associated with earlier exposure of these drugs to the isolates which may have enhanced development of resistance. There is high level antibiotic abuse in this environment arising from self-medication which is often associated with inadequate dosage and failure to comply to treatment and availability of antibiotics to consumers across the counters with or without prescription [14].

Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a serious public health problem of global concern. Screening for methicillin resistant isolates in this study showed a prevalence rate of 66%. This is in line with a study in Zaria [15] where similar prevalence of 69% was obtained. In other studies elsewhere in Nigeria, a lower prevalence of 25.5% was reported from Kano by Nwankwo *et al.* [12] a higher prevalence of 34.7% was reported a few years [16]. In contrast, the prevalence of MRSA was found to be low in studies conducted in other areas in Nigeria such as Jos [17] 43.0%. This may be associated to the ever increasing prevalence of MRSA; in Nigeria prevalence of MRSA ranging between 37.4% and 72.1% has been reported [18,19].

Table 1. Distribution of *Staphylococcus aureus* Isolates According to gender.

Gender	No. tested	Percentage	X^2	P-value
Male	63	63.0	20.885	0.002
Female	37	37.0		
Total	**100**	**100.0**		

Table 2 Distribution of *Staphylococcus aureus* According to age group

Age group (years)	Frequency	Percentage (%)	X^2	P-value
1-10	28	28	81.317	0.000
11-20	37	37		
21-30	10	10		
31-40	16	16		
41-50	9	9		
Total	**100**	**100**		

Table 3. Distribution of *Staphylococcus aureus* According to Source of Isolates.

Type of specimen	No. tested	percentage %
Nasal	9	9.0
Urine	32	32.0
Wound	23	23.0
Pus	9	9.0
HVS	6	6.0
Semen	9	9.0
Ear	12	12.0
Total	**100**	**100.0**

Table 4. Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolates

Antibiotic	Sensitive (%)	Resistant (%)
Clindamycin	40	60
Quinupristin/Dalfopristin	46	54
Cefoxitin	34	66
Tetracycline	58	42
Sulphamethoxazole/Trimethoprim	58	42
Erythromycin	57	43
Ciprofloxacil	64	36
Gentamicin	71	29

Table 5. Antibiotic Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA).

Antibiotic	Sensitive (%)	Resistant (%)
Cefoxitin	0.0 (0.0)	66 (100.0)
Clindamycin	23(38.7)	44 (66.7)
Quinupristin/Dalfopristin	28 (34.7)	38 (57.6)
Erythromycin	39 (50.3)	27 (40.9)
Tetracycline	34 (36.6)	32 (48.5)
Sulphamethoxazole/Trimethoprim	38 (40.9)	28 (42.4)
Ciprofloxacil	36 (46.3)	30 (45.5)
Gentamicin	39 (59.1)	27 (40.9)

CONCLUSION

In this study, males (63%) where more infected than females (37%) and the highest frequency of *Staphylococcus aureus* isolates was observed in the age group 11-20years. The sample with high

prevalence was urine (32%) and a prevalence of MRSA (66%) was obtained in this study. This study showed that Gentamicin and Ciprofloxacin were the most active antibiotics against *Staphylococcus aureus*.

COMPETING INTERESTS

Authors have declared that no competing interests exist

CONSENT

It is not applicable

ETHICAL APPROVAL

Ethical approval to conduct this study was obtained from the ethics and Research committee of Specialist Hospital, Sokoto in accordance with the university standard.

REFERENCES

1. Yassin NA, Mohammed HH, Ahmad AM. Antibiograming Profiles of *Staphylococcus aureus* Isolated from Various Clinical Specimens in Duhok City Iraq. Adv Trop Med and Pub Health Intern. 2013;3(1):25-31.
2. Jombo GT, Akpan S, Epoke J, Akaa PD, and Eyong KI. Methicillin Resistant *Staphylococcus aureus* and their antibiotic sensitivity pattern in Kano, Nigeria. J of Medic and Med Sci. 2010;1(3):043-046.
3. Onanuga A, and Awhowho GO. Antimicrobial resistance of *Staphylococcus aureus* Strains from Patients with Urinary Tract Infections in Yenagoa, Nigeria. J of Pharm Bioallied Sci. 2012;4(3):226-30.

4. Speziale P, Pietrocola G, Provenzano M, and Visai L. Structural and functional role of *Staphylococcus aureus* surface components recognising adhesives matrix molecule of the host. J of Nat Med Assoc. 2009;4(2):1337-352.
5. Karchmer AW. From Theory to Practice: Resistance in *Staphylococcus aureus* and New Treatments. Clin Microbio Infect. 2006;12(8):15-21.
6. Mincey BA, and Parkulo MA. Antibiotic Prescribing Practices in a Teaching Clinic: Comparison of Resident and Staff Physicians. South Med J. 2001;94(4):365-69.
7. Todar K. Bacterial Resistance to Antibiotics. Textbook of Bacteriology. 4th ed. Edinburg: Churchill Livingstone; 2011
8. Aboderin OA, Abdu A, Odetoyin BW, and Lamikanra AS. Antimicrobial Resistance in Escherichia coli strains from Urinary Tract Infections. J of Nat Med Assoc. 2009;101(12):1268-273.
9. Chessbrough M. District Laboratory practice in tropical countries. 2nd ed. Cambridge: University Press; 2009;62-70;105-114
10. Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Disc Susceptibility Tests. 11th ed. CLSI document M02-A11:2012;323-337.
11. Kumurya AS, and Ado ZG. Detection of clindamycin resistance among Methicillin Resistant *Staphylococcus aureus* Isolates in Kano, Nigerian. Access J of microbio. 2017;1(2):34-40.
12. Nwankwo EOK, Abdulhadi S, Magaji A, and Ihesiulor G. Methicillin Resistant *Staphylococcus aureus* and their antibiotic sensitivity pattern in Kano, Nigeria. Afri J of Clin and Exp Microbio. 2010;11(1):129-36.
13. Onanuga A. Prevalence and Susceptibility Pattern of Methicillin- Resistant *Staphylococcus aureus* Isolates Among Healthy Women in Zaria, Nigeria. Afri Journal of Biotech. 2005;4:1321-324.
14. Odugbemi T. The use and abuse of antibiotics. Nig Med Pract. 1998;1(1):4-8
15. Onanuga A, and Temedie TC. Nasal carriage of multi-drug resistant Staphylococcus aureus in healthy inhabitants of Amassoma in Niger delta region of Nigeria. Afri Health Sci. 2011;11(2):176-81.

16. Kumurya AS, and Ado ZG. Detection of clindamycin resistance among Methicillin Resistant *Staphylococcus aureus* Isolates in Kano, Nigerian. Access J of microbio. 2015;1(2):34-40.
17. Ikeh EI. Methicillin resistant Staphylococcus aureus at Jos University Teaching Hospital (JUTH). Afri J of Clin Exp Microbio. 2003;4(1):52-55
18. Taiwo SS, Bamidele M, Omonigbehin EA, Akinsinde KA, Smith SI, Onile B.A. et al. Molecular epidemiology of methicillin resistant Staphylococcus aureus in Ilorin, Nigeria. West Afri J of Medic. 2005;24(2):100-6.
19. Onanuga A, Oyi AR, Olayinka BO, and Onaolapo JA. Prevalence of Community-Associated Multi-Resistant *Staphylococcus aureus* Among Healthy Women in Abuja, Nigeria. Afri J of Biotech. 2005;4(9):942-45.