ANTIBIOTIC SUSCEPTIBILITY OF *STAPHYLOCOCCUS AUREUS* FROM PATIENTS ATTENDING DALHATU ARAF SPECIALIST HOSPITAL, LAFIA, NIGERIA.

[1]NKENE, I.H., [1]*NGWAI, Y.B., [2]UNGOKORE, H., [3]ATAI, E., [4]ABDULLAHI, A.M., [1]ABAH, E.J., [1]ABDULLAHI, U., [5]ZAKOU, M.A., [1]NGADDA, N.J.

[1]Department of Biological Sciences, Nasarawa State University, Keffi, Nasarawa State, Nigeria
[2]Department of Pharmaceutics and Pharmeceutical Microbiology, Usman Danfodio University, Sokoto.
[3]Department of Microbiology, Ahmadu Bello University, Zaria.
[4]Department of Medicine, Ahmadu Bello University Teaching Hospital.
[5]Department of Laboratory, Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State.

***Corresponding Author e-mail; ngwaiyb@yahoo.com**.

ABSTRACT

Staphylococcus aureus is a major commensal and pathogen of humans. Study on the antibiotic susceptibility of S. aureus from different clinical samples of patients attending Dalhatu Araf Specialist Hospital Lafia, Nigeria was carried out. A total of 100 urine samples, 30 ear swabs, 40 blood samples, 60 wound swabs, 30 High Vaginal Swabs (HVS) and 25 Endocervical swab samples were collected from February to April, 2014. Staphylococcus aureus was isolated using standard method and antibiotic susceptibility test was carried out in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The frequency of S. aureus irrespective of the source of the sample decreased in order: High vaginal swab (36.7%) > Endocervical swab (28.0%) > urine (14.0%) > Blood (7.5%) > Ear swab (3.3%) > Wound swab (3.3%). The S. aureus isolates were highly susceptible to gentamicin (94.7%), ofloxacin (68.4%), ciprofloxacin (81.6%) and streptomycin (52.6%) nitrofuratoin (50.0%) and less susceptible to pefloxacin (47.4%), augmentin (34.2%), ampicillin (23.7%), cotrimoxazole and ceftriaxone (5.3%) respectively. Thirty six (94.7%) of isolates showed multiple antibiotic resistance (MAR); MAR indices of 0.2 and above. The high susceptibility of the isolates to ciprofloxacin and gentamicin is evidence that these antibiotics may be useful for the treatment of S. aureus infections in this locality.

Keyword: Antibiotics; Susceptibility; Staphylococcus aureus; Multiple Antibiotic Resistance

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is by far the most important and virulent pathogen among the staphylococci and can cause infections such as urinary tract infections (UTIs), skin and soft tissue infections, bacteremia or sepsis, pneumonia, endocarditis, osteomyelitis and toxic shock syndrome. The infections caused by this pathogen have been on increase globally with serious implication for public health (Stanley *et al.,* 2013). The ability to cause disease is via two mechanisms, namely; toxin production and proliferation of the organism, which causes tissue destruction (Egbuobi *et al.*, 2014). Most infections remain localized at entry portals and are usually self-limiting and non-life threatening. Much less frequently, more serious infections may occur when the organism is able to invade deeper into the body (osteomyelitis, septicemia, pneumonia etc). These deeper infections may be extremely serious and even fatal because infections of *S. aureus* occur at a higher rate than that of many bacteria (Egbuobi *et al.,* 2014). The costs incurred for hospitalization and treatment can be tremendous.

The pathogenicity of *S. aureus* has long been established and the infections caused by this multi-drug resistant pathogen in humans are often difficult to treat (Lowry, 2003; Onemu *et al.,* 2013). These multi-drug resistant strains of *S. aureus* are characteristically resistant to three or more classes of antibiotics other than beta-lactams (Onemu *et al.,* 2013). The prevalence of antibiotic resistant bacteria is a result of misuse or abuse of antibiotics in human and veterinary medicine. The greater the duration of exposure, the greater the risk of the development of resistance irrespective of the severity of the need for antibiotics. As resistance towards antibiotic becomes more common, a greater need for alternative treatments arises. However, despite a push for new antibiotic therapies, there has been a continued decline in the number of newly approved drugs. Antibiotic resistance therefore poses a significant problem.

Staphylococcus aureus has been isolated from several clinical samples from different parts of Nigeria

(Ehinmidu, 2003; Kolawole *et al.,* 2005; Orji *et al.,* 2012; Nworie *et al.,* 2013; Onemu *et al.,* 2013) and the prevalence varies from 34.7% to 71.2% (Taiwo *et al*., 2004; Onanuga *et al.,* 2005; Onemu *et al.,* 2013). This study was designed to determine the isolation frequency and antibiotic susceptibility of *S. aureus* isolated from different clinical samples of patients attending Dalhatu Araf Specialist Hospital (DASH), Lafia, Nasarawa State, Nigeria.

MATERIALS AND METHODS

Sample Collection

This study was carried out in Dalhatu Araf Specialist Hospital, Lafia, Nigeria. A total of 100 urine samples, 30 ear swabs, 40 blood samples, 60 wound swabs, 30 High Vaginal Swabs (HVS) and 25 Endocervical Swabs (ECS) were collected between February to April, 2014 and were analyzed in the Microbiology Laboratory at Dalhatu Araf Specialist Hospital Lafia, Nasarawa State, Nigeria.

Ethical Consideration

Permission was sought from the Ethical Committee of the Dalhatu Araf Specialist Hospital Lafia before samples were allowed to be collected from in-patients.**Media, Chemicals and Antibiotic Discs**

Media such as chocolate agar, Mannitol salt agar (Oxoid, Co. Ltd, UK), Nutrient agar (Oxoid, Co. Ltd, UK) and Mueller-Hinton agar (Oxoid, Co. Ltd, UK) were prepared in accordance with manufacturer's instructions. Chemicals (all from BDH Chemicals, UK) such as ethanol, hydrogen peroxide (H_2O_2), sulphuric acid (H_2SO_4), barium chloride dihydrate and sodium chloride were used.

Antibiotic discs (Optum Lab. Ltd, Nigeria) containing gentamicin (10µg), ciprofloxacin (10µg), pefloxacin (10µg), ofloxacin (10µg), ampicillin (30µg), augmentin (30µg), nitrofurantoin (30µg), streptomycin (30µg), cotrimoxazole (30µg) and ceftriaxone (30µg) were obtained from the hospital.

Isolation and Identification of *Staphylococcus aureus*

The specimens were inoculated on mannitol salt agar and blood agar plates using the streak technique to obtain discrete colonies. Blood samples were first inoculated into brain heart infusion broth, incubated for 24 h before being transferred to the mannitol salt and blood agar. The transfer was repeated every day for 7 days, before it was considered negative ("no bacterial growth"). The plates were incubated at 37°C for both mannitol salt and blood agar for 24 h. For all the isolates, the colonial morphology was noted; and the Gram reaction was also determined. Biochemical identification of the isolates was done using catalase and coagulase tests.

Antibiotic Susceptibility Testing

The antibiotic susceptibility testing for *S. aureus* was carried out using Kirby's Bauer disc diffusion method as modified by Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2007). Briefly four (4) colonies of the isolates were transferred into 5 ml of sterile normal saline in a tube such that the turbidity of the bacterial suspension is equivalent to 0.5 McFarland Standard. The sterile swab was dipped in the bacterial suspension and streaked on Muller-Hinton agar and each antibiotic disc was aseptically placed with a sterile pair of forceps on the surface of the inoculated Mueller-Hinton agar (MHA) plate. The plate was incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured using metre rule and the result was interpreted in accordance with the susceptibility break point as earlier described (CLSI, 2007).

RESULTS AND DATA ANALYSES

Isolation of *Staphylococcus aureus*

Isolates that were Gram-positive, cocci, catalase-positive and coagulated human plasma were considered as *S. aureus*. The isolation frequency from the different samples of patients is shown in Table 1. The isolation frequency decreased in order: HVS (36.67%) > ECS (28.00%) > urine (14.00%) > Blood (7.50%) > Ear swab (3.33%) > Wound swab (3.30%).

Table 1: Isolation Frequency (%) for *Staphylococcus aureus* with from patients

Sample	No. Examined	No. Positive (%)
Urine	100	14(14.0)
Ear swab	30	1(3.3)
Blood	40	3(7.5)
Wound swab	60	2(3.3)
HVS	30	11(36.7)
ECS	25	7(28.0)
Total	**285**	**38(13.3)**

Antibiotic Susceptibility Testing

The antibiotic susceptibility of the *S. aureus* isolates is as shown in Table 2. The *S. aureus* from different clinical samples were highly susceptible to gentamicin, ofloxacin, ciprofloxacin, streptomycin and nitrofuratoin and less susceptible to pefloxacin, augmentin, ampicillin, cotrimoxazole and ceftriaxone.

Antibiotic Resistance Phenotype

The antibiotic resistant phenotype of *S. aureus* from clinical samples of patients is as shown in Table 3. The commonest phenotypes were Pn-Aug-Nit-Str-Cot-Cro (13.2%); Pn-Aug-Cot-Pfx-Ofl-Cro-Nit-Str (7.9%); Cot-Pfx-Ofl-Cro-Nit (5.3%); Pn-Aug-Cro-Cot (5.3%); Cot-Cro (5.3%) and Pn-Aug-Cot-Pfx-Ofl-Cro (5.3%).

Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance, defined here as resistance to two or more antibiotics, was present in 36 (94.7%) of the isolates tested. The most common MAR index was 0.6 observed in 11(30.6%) of the MAR isolates as shown in Table 4.

DISCUSSION

Infections caused by *S. aureus* have been on the increase globally with serious implications for public health (Stanley *et al.*, 2013). This study has revealed that *S. aureus* can be found in urine, ear swabs, blood, wound swabs, high vaginal swabs and endocervical swabs as earlier described (Bells and Jounldge, 2002; Nworie *et al.*, 2013; Onemu *et al.*, 2013).

The high isolation rate for *S. aureus* observed shows the versatility of this organism amongst other staphylococci which makes it the most endemic pathogen in clinical setting and is not different from other previous studies reported elsewhere (Nworie *et al.*, 2013; Egbuobi *et al.*, 2014).The high isolation frequency of *S. aureus* in high vaginal swabs and endocervical swabs might be attributed to the level of staphylococcal infection in this study area and poor personal hygiene. The low isolation rate for *S. aureus* observed in this study is in contrast with other previous studies (Obiazi *et al.*, 2007; Orji *et al.*, 2012; Nworie *et al.*, 2013). The high isolation frequency of this pathogen in wound could be attributed to poor personal hygiene and exposure of the wounds, which might have made it more prone to contamination and infection. Furthermore, the low isolation rate for *S. aureus* observed in urine (14.0%) samples might be attributed to the level of staphylococcal infection in this study area and poor personal hygiene. The isolation rate for *S. aureus* from blood was also low from this study.

The low susceptibility of *S. aureus* to pefloxacin, streptomycin, nitrofuratoin, augmentin, ampicillin, cotrimoxazole and ceftriaxone was expected; and this might be due to fact that inappropriate practices like misuse and abuse and unskilled practitioners can also lead to emergence of resistance in bacteria. Expired antibiotics, self-medication, counterfeit drugs, inadequate hospital control measures can as well promote the development of antimicrobial resistance in clinical isolates (Chikere *et al.*,2008, Prescott *et al.*, 2008). In developing countries like Nigeria, self-medication is a common practice and this might probably be a major cause of antibiotic resistance in clinical isolates since patients only think of going to the hospitals when they are unable treat themselves (Iheanyi *et al.*, 2009).

The high susceptibility of *S. aureus* to gentamicin, ofloxacin and ciprofloxacin though not surprising is in contrast with observation from a previous study (Stanley *et al.*, 2013). The high susceptibility to fluoroquinolones observed in this study justifies their common use as drug of choice for treatment of staphylococcal infection. The high susceptibility to gentamicin was expected and this might be due to the fact that gentamicin is not costly and is in injectable form. The injectable antibiotics are not easily abused because of pains and discomfort experienced by patients when administered. Furthermore, the fluoroquinolones particularly ciprofloxacin and ofloxacin are very costly and are not easily abused by patients.

An isolate with MAR index greater than 0.2 indicates that it originates from an environment where antibiotics are freely available and misused (Krumpermann, 1983).

In conclusion, the high isolation frequency for *S. aureus* from clinical samples observed in this study might have serious implication for public health. The high susceptibility of these isolates to gentamicin, ciprofloxacin and ofloxacin is evidence that these drugs may be useful for treatment of *S. aureus* infections in this locality. Further studies on the molecular characterization of antibiotic resistant *S. aureus* should be carried out.

Table 2: Antibiotic susceptibility of *Staphylococcus aureus* isolated from patients

S/No	Sample	No of *S. aureus* Isolates	Gen (10µg)	Cpx (10µg)	Pfx (10µg)	Ofl (10µg)	PN (30µg)	Aug (10µg)	Nit (30µg)	Str (30µg)	Cot (30µg)	Cro (30µg)
1	Urine	14	13(92.85)	11(78.57)	8(57.14)	10(71.42)	2(14.28)	5(35.71)	6(42.85)	6(42.85)	0(0)	0(0)
2	Ear swab	1	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)
3	Blood	3	3(100)	3(100)	2(66.66)	2(66.66)	0(0)	0(0)	1(33.33)	1(33.33)	0(0)	0(0)
4	HVS	11	10(90.90)	8(72.72)	4(36.36)	9(81.81)	3(27.27)	4(36.36)	7(63.63)	7(63.63)	2(18.18)	2(18.18)
5	Wound swab	2	2(100)	2(100)	2(100)	2(100)	1(50)	1(50)	1(50)	0(0)	0(0)	0(0)
6	ECS	7	7(100)	6(85.71)	2(28.57)	3(42.85)	3(42.85)	3(42.85)	3(42.85)	5(71.42)	0(0)	0(0)
		38	**36(94.73)**	**31(81.57)**	**18(47.36)**	**26(68.42)**	**9(23.68)**	**13(34.21)**	**19(50)**	**20(52.26)**	**2(5.26)**	**2(5.26)**

Gen = Gentamicin; CPx = Ciprofloxacin; Pfx = Pefloxacin; Ofl = Ofloxacin; PN = Ampicillin; Aug = Augmentin; Nit = Nitrofurantoin; Str = Streptomycin; Cot = Co-trimoxazole; Cro = Ceftriaxone

Table 3: Antibiotic Resistance Phenotypes of *Staphylococcus aureus* from clinical samples of patients

Antibiotic Resistant Phenotypes	**No. (%) isolates**
Cot	1(2.5)
Cot-Cro	2(5.3)
Pn-Cro	1(2.6)
Pn-Aug-Cot	1(2.6)
Cot-Str-Cro	1(2.6)
Cro-Cot-Pn	1(2.6)
Str-Cot-Pfx-Cro	1(2.6)
Pn-Aug-Cro-Cot	2(5.3)
Cro-Pn-Aug-Nit-Cot	1(2.6)
Cro-Ofl-Pfx-Str-Cot	1(2.6)
Cot-Pfx-Ofl-Cro-Nit	2(5.3)
Pn-Cpx-Nit-Cot-Cro	1(2.6)
Pn-Aug-Str-Cot-Cro	1(2.6)
Pn-Aug-Cro-Str-Cot	1(2.6)
Pn-Str-Cro-Cot-Nit	1(2.6)
Pn-Aug-Nit-Str-Cot-Cro	5(13.2)
Pn-Aug-Cot-Cro-Nit-Pfx	1(2.6)
Pn-Aug-Cro-Ofl-Cpx-Pfx	1(2.6)
Pn-Aug-Str-Cot-Cro-Gen	1(2.6)
Pn-Aug-Cot-Pfx-Ofl-Cro	2(5.3)
Nit-Cot-Ofl-Cpx-Pfx-Cro	1(2.6)
Pn-Aug-Cro-Cpx-Pfx-Nit-Cot	1(2.6)
Pn-Aug-Nit-Str-Pfx-Cro-Cot	1(2.6)
Pn-Str-Cro-Cot-Cpx-Pfx-Aug	1(2.6)
Pn-Aug-Ofl-Pfx-Cro-Cot-Str	1(2.6)
Pn-Aug-Cot-Pfx-Ofl-Cro-Nit-Str	3(7.9)
Pn-Aug-Str-Nit-Cot-Cro-Pfx-Nit-Gen	1(2.6)

Gen = Gentamicin; CPx = Ciprofloxacin; Pfx = Pefloxacin; Ofl = Ofloxacin; PN = Ampicillin; Aug = Augmentin; Nit = Nitrofurantoin; Str = Streptomycin; Cot = Co-trimoxazole; Cro = Ceftriaxone

Table 4: Multiple Antibiotic Resistance index of *Staphylococcus aureus* from patients

No. of antibiotics resistant to (a)	No. of antibiotics (b) tested	No. (%) of MAR isolates	MAR Index (a/b)
9	10	1(2.8)	0.9
8	10	3(8.3)	0.8
7	10	4(11.1)	0.7
6	10	11(30.6)	0.6
5	10	8(22.2)	0.5
4	10	3(8.3)	0.4
3	10	3(8.3)	0.3
2	10	3(8.3)	0.2

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