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METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* COLONIZATION AMONG HIV PATIENTS ATTENDING A TERTIARY HOSPITAL IN KADUNA, NIGERIA

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ABSTRACT

Introduction: Methicillin resistant *S. aureus* is known to have significant negative impact on both immunocompetent and immunocompromised individuals (HIV patients).
Aim: This study evaluated the antibiotics susceptibility profile of MRSA *staphylococcus aureus* isolated from HIV patients attending Barau Dikko Teaching Hospital Kaduna.
Methods: Using sterile wet swabs, within the period of 3 months (January – March, 2020), a total of 180 samples were collected from the skin, ear and nasal cavity of 60 HIV patients, who gave their consent to be part of the survey and *S. aureus* was isolated. Isolation, bacteria characterization and antimicrobial susceptibility tests were carryout using the methods described by Chesbrough and Kirby-Bauer respectively.
Results: Bacteria isolation rate was 76.7% (138/180) in the samples collected, with 56.5% (78) been Gram positive. The incidence of *S. aureus* and MRSA were 12.8% and 8.6% respectively. From the sample sources, the distribution of *S. aureus* was more in nostrils followed by skin and ear swab. Resistance was observed against imipenem (86.9%), vancomycin (65.3%), tetracycline (65.3%) and erythromycin (60.9%) while susceptible to ciprofloxacin (60.9%), streptomycin (43.5%) and gentamycin (52.2%). High percentage (91.3%) of the *S. aureus* were multidrug resistant and had MARI > 0.2.
Conclusion: This study established that high resistant *S. aureus* with possible MRSA gene could be responsible for frequent staphylococcus associated infection and resistant to treatment among HIV patients in Kaduna, Nigeria.
Keywords: *Staphylococcus aureus*, HIV patients, antibiotics resistance, MRSA, Kaduna

INTRODUCTION

Human immunodeficiency virus (HIV)-infected individuals are often at risk of various opportunistic bacterial infections including *S. aureus* (1). This *S. aureus* is the leading cause of community and nosocomial infections, and it's been implicated in bacteremia, joint and bone infection, surgical wound infections, endocarditis, osteomyelitis, meningitis and pneumonia (2). They are also observed to acquire drug resistance to various classes of antibiotics including all beta lactams including methicillin (3, 4). Among the deadliest *S.*

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aureus strain with drug resistant profile is the methicillin resistant *S. aureus* (MRSA). MRSA is known with constantly evolving epidemic strains and different genetic diversities, which has made treatment options difficult and to include additional care such as infection source control, regular consultations and echocardiography due to their compromised immune system. These accruing factors have significantly influenced the rate of mortality and morbidity associated with MRSA infections. MRSA have been isolated from different sources both in human and animal, especially environment were antibiotics have been abused. Although the prevalent rate of MRSA is on the increase, recent studies showed that it poses high clinical threat among HIV patients. According to Crum-Cianflone *et al.*, (5) study in 2007, which assessed the retrospective data of HIV patients from 1993 to 2005, showed that there was 18-fold increase in incidence of community associated methicillin resistant Staphylococcus aureus among HIV patients in USA with 90% of the infections resulting to soft tissue/skin infection (SSI). HIV patient with this SSI also had evidence of scrotal abscess or buttocks injury, and 21% them do have recurrent infection. Further assessment of their study showed that common risk factors associated with HIV patient infected with MRSA are high viral load, low CD4 count, syphilis infection and frequent use of beta-lactams. Also, in a study conducted in southern India by Swathirajan *et al.*, (6) revealed that there was a progressive increase in MRSA incidence geometrically from 51.8% in 2012 to 86% in 2017 among HIV patients with significant morbidity and mortality. Another study conducted by Shinohara *et al.*, (7) using whole genomic sequencing of 28 isolates from 2016 – 2019 reported an apparent emergence of new clone ΨUSA300 among community associated MRSA (CA-MRSA) HIV patients in HIV/AIDS referral hospital Tokyo, Japan. The isolated new USA300 strain as at 1990s to early 2000 were only isolated among people living with HIV harboring MRSA in America. In this environment USA300 clone have never been reported and was extremely rear in Japan; indicating possible new introduction as a result of migration, horizontal gene transfers and other possible factors. This strain is known to cause altered soft tissue/skin infection (SSTI) due to the presences of staphylococcal cassette chromosome mec (SCCmec) type IV, sequence type (ST) 8 possessing arginine catabolic mobile element (ACME) and Panton-Valentine leucocidin (PVL) in their genome (1). The reports of Jung *et al.*, (8) and Huang *et al.*, (9) further revealed that the incidence of MRSA among HIV patients is on the increase in Korea and Taiwan respectively. Also, among people living with community acquired pneumonia, in both hospital and community settings, high incidence of CA-MRSA clones has been reported to be endemic and spreading rapidly in China (10). In northern Taiwan, Hsu *et al.*, (11) report nasal colonization of MRSA that are susceptible to trimethoprim-sulfamethoxazole and fluoroquinolones

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among HIV patients. Their study further identified risk factors among those infected with hepatitis C virus, cancer, smoking and those with frequent antibiotics use history within 1 year. In Ghana, West Africa and Ethiopia, East Africa, the studies of Boison *et al.,* (3) and Muhaba *et al.,* (12) also reported high nasal colonization of multidrug resistant MRSA among HIV patients with high occurrence among patients whom have been hospitalized. In Kano and Sokoto, Northwest Nigeria, nasal colonization of MRSA has been reported in both Children infected with HIV and non-HIV infected patients respectively (13, 14). Study by Garba (15) had documented that Kaduna state had the second highest HIV prevalence in Nigeria while the report of Joshua *et al.,* (16) further showed that in Zaria, Kaduna State, Nigeria; 50% of the *S. aureus* isolated harbor MRSA gene but sadly no study has reported the incidence of nasal colonization of MRSA among HIV patients in Kaduna. It's therefore relevant to sure period surveillance of MRSA among HIV patients to manage rising morbidity and mortality associated with the infection as few reports are available on nasal and skin colonization of MRSA among HIV patients in Nigeria including Kaduna, this led to its assessment among HIV patients attending a tertiary hospital in Kaduna, Nigeria.

MATERIALS AND METHODS

Ethical Approval

A letter of ethical approval and informed consent were obtained from Barau Dikko Teaching Hospital Kaduna and the patients respectively. The confidentiality, privacy and autonomy of the patients were preserved throughout the study period.

Study Area, Sample Collection and Preparation

The study was carried out at the Special Treatment and Care (STC) Unit of Barau Dikko Teaching Hospital (BDTH), located at Lafiya road in Kaduna Metropolis. The Hospital was officially established on the 30th March, 2015. Using sterile swab stick moistened with sterile normal saline 180 swab samples from the nostril, ear and skin of 60 HIV patients were randomly collected and transferred on an ice pack to the Pharmaceutical Microbiology Laboratory, Kaduna State University for further analysis. The swab sticks were cut into already prepared and sterilized 5mL peptone water at room temperature, and incubated at 37^{o}C for 24 hours. Growth from the overnight cultures was streaked on Mannitol salt agar and incubated again at 37^{o}C for 24 hours.

Isolation and Identification of *Staphylococcus aureus*

Colonies that grew on Mannitol salt agar plates with golden yellow and smooth edge or surface were Gram stained in accordance with standard Gram staining procedure, and microscopic examination carried out. Biochemical tests such as catalase, coagulase, indole, oxidase and sugar fermentation (lactose, fructose and glucose) tests. Gram staining and biochemical tests were carried out as described by Chesbrough (17).

Gram Staining

A smear of the isolate was made on a clean glass slide and heat fixed. The smear was

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then stained with crystal violet for 30 seconds, fixed with lugols iodine for 30 seconds and decolorized with 95% ethanol for 30 seconds, after which it was counterstained with dilute carbol fuchsin solution for 1 minute. On examination microscopically, the isolates that produced violet cocci (Gram positive) predominantly cocci in clusters were selected for further biochemical identification.

Biochemical Test

Catalase Test

This test shows the ability of the organisms to produce the enzyme catalase. A drop of 30% hydrogen peroxide (H_2O_2) was placed on a glass slide and portions of an overnight culture of the bacteria were picked using a sterile wire loop and make a smear. A positive test was indicated by bubbling and fruiting, which was absent in a negative test.

Oxidase Test

S. aureus produces cytochrome oxidase enzymes during oxidation of the substrate "tetramethyl-p-phenylenediamine dichloride" to indophenols. a dark purple colored end product shows positive reaction. A piece of filter paper was placed in a clean petri-dish and 2-3 drops of freshly prepared oxidase reagent (1% tetra methyl-p-Phenylenediamine dihydrochloride) was added. With the help of inoculation loop, small portion of the isolate was placed on the filter paper and a smear was made. It was then observed for immediate colour change to blue purple within 10 minutes.

Indole Test

Indole is one of the metabolic degradation products of amino acid (tryptophan). Bacteria that possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan to produce indole, pyruvic acid and ammonia. Tryptophan broth was inoculated with the isolated colony and incubated at 37 ^{0}C for 72 hours. 0.5 ml Kovac's reagent was then added to the broth culture and colour change to pink-red was observed. *S. aureus* is indole negative as such no colour change is expected.

Citrate Test

The citrate test shows the ability of a bacteria to utilize citrate in a sodium citrate medium as the only source of carbon and energy and nitrogen from ammonium salts. The utilization of citrate results in the release of ammonia, leading to a colour change in the medium from green to blue. Tubes of Simon's citrate agar were inoculated with the test organism and incubated at 35^{0}C for 48 hours. *S. aureus* utilizes citrate (positive) and this causes a change in the medium from green to royal blue.

Coagulase Test

The coagulase test was used to confirm the presence or absence of coagulase positive or negative Staphylococci isolate. Two drops of physiological saline were placed about 2cm apart on the slide that had been divided into two with a grease pencil. One colony each of the isolate was carefully emulsified on each drop of saline. A loop full of human plasma was added to the bacteria suspension on the side and rocked gently for 60 seconds. Viable clumping of cells showed the presence of coagulase enzyme indicating the presence of coagulase positive Staphylococci.

Antibiotic Susceptibility Testing (AST)

Antibiotic susceptibility testing was carried out on each purified *S. aureus* isolates using CLSI modified disc diffusion method as described by Chesbrough (17). Standardized (0.5 McFarland) inoculums of each isolate were aseptically streaked on a fairly dried surface of

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sterile Mueller Hinton agar plate to evenly cover the surface of the agar, excess was drained off and the surface of the agar was allowed to be absorbed within the agar with the petri dish lid in place for 10 minutes. Single antibiotic discs were aseptically distributed evenly on the inoculated plate with each disc slightly pressed down to ensure its contact with the Mueller Hinton agar. Each plate contains maximum of five different antibiotics. Within 15 minutes of applying the disc, the plate was inverted and incubated aerobically at 30 degrees Celsius for 18 h. After the 18 h incubation, the diameters of the zone of inhibition for each of the isolates were measured underside of the plate to the nearest millimeter (mm). The same procedures were carried out for the other isolates. After incubation the zone of inhibition of each antibiotic was measured and the results were interpreted as susceptible, intermediate and resistant based on the Clinical Laboratory Standards Institute (18) recommendation shown in Table 1 below

Table:1 CLSI Interpretative Chart Diameter Zone of Inhibition for *Staphylococcus aureus* Isolates

S/N	Antibiotic	Zone of Inhibition (mm)		
		Susceptible	Intermediate	Resistant
1	Erythromycin (15 µg)	≥23	14-22	≤13
2	Gentamicin (30 µg)	≥15	13-14	≤12
3	Chloramphenicol (10 µg)	≥18	13-17	≤12
4	Vancomycin (30 µg)	≥15	-	-
5	Augmentin (30 µg)	≥18	14-17	≤13
6	Imipenem (10 µg)	≥16	14-15	≤13
7	Tetracycline (30 µg)	≥19	15-18	≤14
8	Ciprofloxacin (5 µg)	≥21	16-20	≤21
9	Streptomycin (10 µg)	≥15	12-14	≤11
10	Cefoxitin (30 µg)	≥22	-	≤21
11	Oxacillin (1 µg)	≥13	11-12	≤10

Determination of Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the organism was resistant to by the total number of antibiotics tested. This was carried out as described by Christopher *et al*, (19).

Determination of Methicillin Resistant *Staphylococcus aureus*

Staphylococcus aureus suspension equivalent to 0.5 McFarland standard were prepared for all isolates and tested with Cefoxitin (30µg) and Oxacillin (1µg) disc, using Muller Hinton agar. All plates were incubated at 35^0C for 24 hours. Zone of inhibitions were measured and interpreted as guideline recommended by CLSI, (2019). For cefoxitin disc, zone diameter ≤21mm was reported as MRSA and ≥22 mm as MSSA. For oxacillin disc, zone diameter < 10 mm was reported as MRSA and >13 mm as MSSA (Table 1).

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RESULTS

Sample Collection and Bacteria Identification

A total number of 180 samples were collected from 60 HIV patients' skin, ear and nasal cavity of in Barau Dikko Teaching Hospital, Kaduna. There was high occurrence of HIV in female (58.3%) compared to male (41.7%) (Table 2). There was also 76.7% (138/180) bacteria isolation rate of which 43.5% (60) were Gram negative while 56.5% (78) were Gram positive. Skin had the highest bacteria isolation followed by nostril then ear (Table 3).

Table 2: Gender and Samples Source Distribution of HIV Patients in the Hospital

Samples	Gender		Total
	Male %	Female %	
Nose	25	35	60
Ear	25	35	60
Skin	25	35	60
Total	**75(41.7)**	**105(58.3)**	**180**

Table 3: Isolation and Characterization of Bacteria Isolates from HIV Patients in BDTH, Kaduna

Staining	Nose	Skin	Ear	Total	%
Gram +	22	20	18	78	43.5
Gram -	26	28	24	60	56.5
Total	**46**	**48**	**42**	**138**	**100**

Distribution of *Staphylococcus aureus* among Gram Positive Bacteria Isolated

On mannitol salt agar, 60.3% (47/78) of the Gram-positive isolates grew with only 33.3% (26/78) showing golden yellow colonies with smooth surface (Table 4). Further biochemical analysis identified 23 out of the 26 isolates to be *S. aureus* (Table 5).

Table 4: Evaluation on *Staphylococcus spp.* on Mannitol Sugar Agar

CMA	Nose	Ear	Skin	Total (%)
Golden Yellow	10	7	9	26 (33.3)
Red	7	8	6	21 (26.9)
No Growth	6	6	4	31 (39.8)
Total	**23**	**21**	**19**	**78 (100)**

Key: CMA= colour on mannitol

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Table 5: Biochemical Test for Identification of *Staphylococcus aureus*

S/n	Isolate	TSI			Ind.	Cit.	Cat.	Oxi.	Coag.	Organism
		H_2S	Gas	Sugar						
1	F1	-	-	+	-	+	+	-	+	*S. aureus*
2	F2	-	-	+	-	+	+	-	+	*S. aureus*
3	F3	+	+	+	+	+	+	-	-	*S. epidermidis*
4	F6	+	+	+	+	+	+	-	-	*S. epidermidis*
5	F8	-	-	+	-	+	+	-	+	*S. aureus*
6	F10	+	+	+	+	-	+	-	-	*S. epidermidis*
7	F11	-	-	+	-	+	+	-	+	*S. aureus*
8	F12	-	-	+	-	+	+	-	+	*S. aureus*
9	F14	-	-	+	-	+	+	-	+	*S. aureus*
10	F15	-	-	-	-	+	+	-	+	*S. aureus*
11	F16	+	+	-	+	+	+	+	-	*S. epidermidis*
12	F18	+	+	+	-	-	-	-	-	*S. epidermidis*
13	F20	-	-	+	-	+	+	-	+	*S. aureus*
14	F21	+	+	-	-	+	+	+	-	*S. epidermidis*
15	F22	-	-	+	-	+	+	-	+	*S. aureus*
16	F23	-	-	+	-	+	+	-	+	*S. aureus*
17	F25	+	-	+	+	-	+	-	-	*S. epidermidis*
18	F27	-	+	+	+	+	+	+	-	*S. epidermidis*
19	F29	+		-	+	-	+	-	-	*S. epidermidis*
20	F31	+	-	+	-	+	-	+	+	*S. epidermidis*
21	F32	-	-	+	-	+	+	-	+	*S. aureus*
22	F34	-	-	+	-	+	+	-	+	*S. aureus*
23	F35	-	-	+	-	+	+	-	+	*S. aureus*
24	M36	-	+	+	-	+	+	+	-	*S. epidermidis*
25	M37	-	-	+	-	+	+	-	+	*S. aureus*
26	M38	-	-	+	-	+	+	-	+	*S. aureus*
27	M39	-	-	-	-	+	+	-	+	*S. aureus*
28	M40	+	-	+	+	+	+	-	-	*S. epidermidis*
29	M41	-	-	+	-	+	+	-	+	*S. aureus*
30	M43	+	+	+	-	+	+	+	-	*S. epidermidis*
31	M44	-	-	+	-	+	+	-	+	*S. aureus*
32	M47	-	-	+	-	+	+	-	+	*S. aureus*
33	M48	+	-	+	+	+	+	+	-	*S. spp.*
34	M49	+	+	+	-	+	+	+	-	*S. epidermidis*
35	M50	-	-	+	-	+	+	-	+	*S. aureus*
36	M51	+	+	+	-	+	+	+	-	*S. epidermidis*
37	M52	-	-	+	-	+	+	-	+	*S. aureus*

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38	M53	+	-	+	+	+	+	+	-	*S. epidermidis*
39	M54	+	-	+	+	-	-	+	-	*S. spp.*
40	M55	+	-	+	-	+	+	-	-	*S. epidermidis*
41	M56	+	+	+	+	+	+	-	+	*S. aureus*
42	M58	-	-	+	-	+	+	-	+	*S. aureus*
43	M59	-	-	+	+	+	-	-	+	*S. spp.*
44	M60	+	-	-	-	+	+	+	+	*S. epidermidis*
45	F36	-	-	+	+	+	-	-	+	*S. spp.*
46	F38	+	-	-	-	+	+	+	+	*S. epidermidis*
47	F43	+	-	-	-	+	+	+	+	*S. spp.*

Keys: F= Female M= Male + = Positive - = Negative

Percentage of *Staphylococcus aureus* isolates

The incidence of *S. aureus* was 12.8% (23/180) while among the Gram-positive bacteria isolated, 29.5% (23/78) were *S. aureus* (Table 6). Assessment of the distribution of *S. aureus* base on sample source showed that nasal swabs had more *S. aureus* followed by skin and ear (Figure 1).

Table 6: Percentage occurrence of *Staphylococcus aureus* Isolates among HIV Patients in Barau Dikko Teaching Hospital

Sample	% (Number)
S. aureus isolates	29.5 (23)
Other Staphylococci isolates	35 (21)
No Growth	43.6 (34)
Total	**100 (78)**

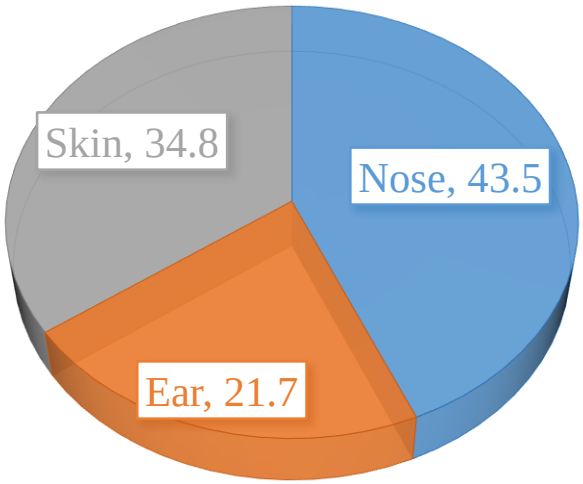

Figure 1: Percentage Distribution of *Staphylococcus aureus* from the Sample Source

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ANTIBIOTIC SUSCEPTIBILITY TEST (AST)

The antibiotics susceptibility profile of the isolates is presented in Table 7 below. The result revealed differences among *S. aureus* isolates in their susceptibility patterns to antibiotics. The highest rate of resistance was observed against Imipenem (86.9%), Cefoxitin (65.3%), Vancomycin (65.3%) and Tetracycline (65.3%) followed by Erythromycin (60.9%). The isolates were more susceptible to Ciprofloxacin (34.8%), Streptomycin (43.5%), Gentamycin (43.5%) and Chloramphenicol (47.8%). High percentage (91.3%) of the *S. aureus* isolated were multidrug resistant while 65.2% were resistant to more than 4 class of antibiotics tested. Also, 30% of the isolates had VA, AUG, IMI, FOX pattern of resistant profile (Table 8). Table 9 showed that 91.3% of the isolates had MARI > 0.2.

Table: 7: Percentage Antibiotics Susceptibility Profile of *Staphylococcus aureus* Isolated from HIV Patients in Barau Dikko Teaching Hospital, Kaduna

S/N	Antibiotics	Resistant	Intermediate	Sensitive
1	VA	15 (65.3)	0 (0)	8 (34.8)
2	AUG	18 (78.3)	2 (8.7)	3 (13.0)
3	IMI	20 (86.9)	1 (4.3)	2 (8.7)
4	FOX	15 (65.3)	11 (47.8)	8 (34.8)
5	C	11 (47.8)	11 (47.8)	1 (4.3)
6	CN	10 (43.5)	1 (4.3)	12 (52.2)
7	TE	15 (65.3)	1 (4.3)	7 (30.4)
8	CP	8 (34.8)	1 (4.3)	14 (60.9)
9	S	10 (43.5)	4 (17.4)	4 (17.4)
10	E	14 (60.9)	4 (17.4)	5 (21.7)

Key: Erythromycin (15µg)-E, Gentamicin (30µg) -CN, Chloramphenicol (10µg)-C, Vancomycin 30 µg –VA, Augumentin (30 µg)-AUG, Imipenem (10 µg) –IMI, Tetracycline (30 µg) - TE, Ciprofloxacin (5 µg) - CIP, Streptomycin (10 µg) –S, Cefoxitine (30 µg) –FOX.

Table 8: Multidrug Resistance Pattern of *Staphylococcus aureus* Isolated from HIV Patients in Barau Dikko Teaching Hospital, Kaduna

S/N	Isolates Code	Antibiotic Resistance Pattern	NART	NCART	CR
1	FI	VA,AUG,IMI,FOX,C,CN,TE,E	8	8	MDR
2	F2	AUG,IMI,CM,S	4	3	MDR
3	F8	IMI,FOX,CN	3	3	MDR
4	F11	VA,AUG,IMI,FOX,CN,TE,CIP,S,E	9	8	MDR
5	F12	VA,AUG,IMI,FOX,C,TE,CIP,S,E	9	9	MDR
6	F14	VA,AUG,IMI,FOX,C,CN,TE,S,E	9	8	MDR
7	F15	AUG,IMI,FOX	3	3	MDR
8	F20	VA,AUG,IMI,TE,S,E	6	6	MDR
9	F22	IMI,FOX,C	3	3	MDR

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10	F23	AUG,FOX	2	2	NMDR
11	F32	VA,AUG,IMI,TE,CIP,S,E	7	7	MDR
12	F34	VA,AUG,IMI,FOX,C,CIP	6	6	MDR
13	F35	IMI,FOX,E	3	3	MDR
14	F37	VA,AUG,FOX,CN,E,CIP,S	7	6	MDR
15	F38	VA,IMI,TE,CIP,E	5	5	MDR
16	F39	VA,AUG,FOX,TE	4	4	MDR
17	M41	VA,AUG,IMI,FOX,C,TE	6	6	MDR
18	M44	VA,AUG,IMI,C,CN,TE,E	7	7	MDR
19	M47	AUG,IMI,FOX,C,TE,E	6	6	MDR
20	M50	VA,AUG,IMI,C,CM,TE,CIP,S,E	9	8	MDR
21	M53	VA,AUG,IMI,FOX,C,CN,TE,CIP,S,E	10	9	MDR
22	M55	IMI,E	2	2	NMDR
23	M58	VA,AUG,IMI,C,CN,TE,S,E	8	7	MDR

Key: NART = Number of Antibiotics Resistance, NCART = Number of Class of Antibiotics Resistant to, CR = Classification Resistance, MDR = Multi Drug Resistance, NMDR = Non Multi Drug Resistance, Erythromycin (15µg) -E, Gentamicin (30 µg) -CN, Chloramphenicol (10 µg) -C, Vancomycin (30µg) –VA, Augmentin (30µg) -AUG, Imipenem (10µg) –IMI, Tetracycline (30 µg) -TE, Ciprofloxacin (5 µg) -CIP, Streptomycin (10µg) –S, Cefoxitin (30µg) –FOX.

Table 9: Multiple Antibiotics Resistant Indices (MARI) of *Staphylococcus aureus* Isolate

MARI	Number of Organism	Percentage
0.1	0	0
0.2	2	8.7
0.3	4	17.4
0.4	2	8.7
0.5	1	4.3
0.6	4	17.4
0.7	3	13.1
0.8	2	8.7
0.9	4	17.4
1	1	4.3
Total	23	100

91.3%

Key: MARI = Multiple antibiotics resistant index

Higher percenatge [65% (15/23)] of the *S. aureus* were methicillin resistant *S. aureu* (MRSA) (Figure 2). This implies that the incidence of MRSA in this study is 8.3%.

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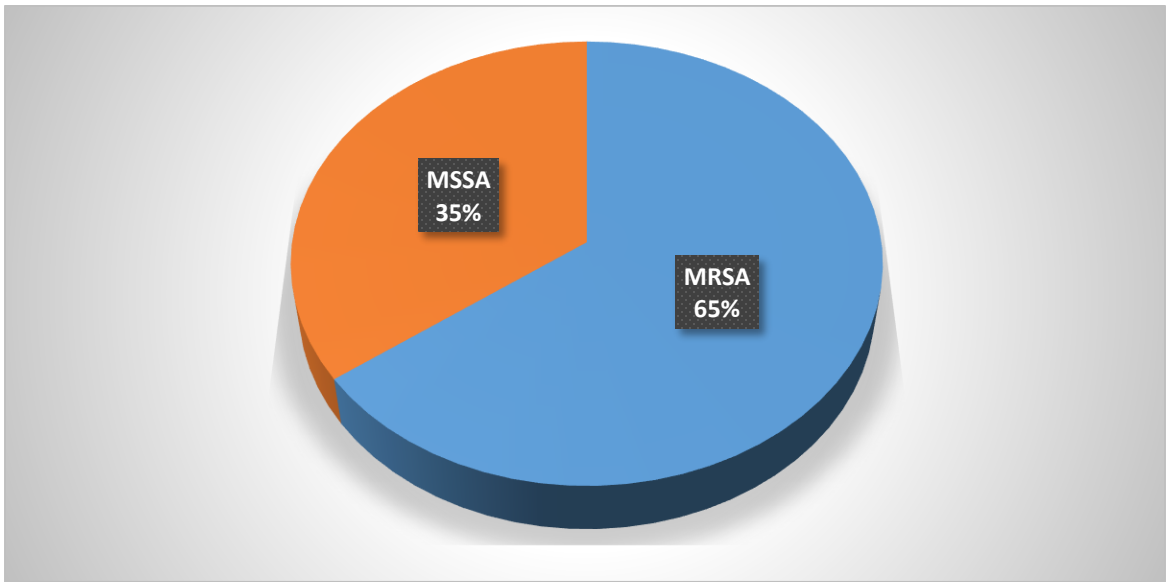

Figure 2: Percentage Occurrence of MRSA Among *Staphylococcus aureus* Isolated from HIV Patient in BDTH.

DISCUSSION

At the end of 2022 survey by WHO, the global incidence rate of HIV infection was estimated around 39million with 66.7% (25.6 million) in Africa. Mortality rate was estimated around 1.62% (630,000) while 1.3 million people were newly infected (20). This shows that HIV infection is still on the raise (3.33%) and infections associated with *S. aureus* such as sepsis, pneumonia, deep tissue infections will frequently be reported in more than 50% patients especially among advanced HIV-1 infected patients. This is evidence in their autopsy studies (21). According to Boison *et al.,* (3), high nasal colonization of MRSA (8.2%) and *S. aureus* (44.7%) multiple antibiotics resistant profile have been reported among HIV infected patients in Ghana. From Onovo *et al.,* (22) study in Nigeria, 2021 statistics showed that the prevalent of HIV in Nigeria was 1.3% with an increase to 2.1% in 2022. The study conducted by Abdullahi *et al.,* (23) revealed that the prevalence of *S. aureus* colonization of nasal cavity (29.6%) of people living with HIV/AIDS were far higher than those isolated from their UTI (6.8%). They further observed the prevalence of MRSA to be 13% among the nasal samples with MSSA-ST15-t084 and MRSA-ST8-t064 been the most common genetic lineages after molecular typing from 3 studies. Knowing the aforementioned, there is need for periodic surveillance of pathogenic infections associated with HIV patients to ensure strict implementation of guidelines to prevent new infections. This will reduce morbidity and mortality rate among HIV patients (24).

Although epidemiological progression of HIV wasn't the primary aim of this study, sex distribution of HIV in this study agrees with USAID (25) report that high percentage of HIV patients were girls and women. USAID (25) further reported that 2.5% of the women

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were commercial sex workers. This dilemma might be linked to the premise stated by Ikuteyijo *et al.,* (26) that greater probability exists for women to use sex as means of livelihood to generate support for their dependents. There was 76.7% bacteria isolation rate among HIV patients evaluated in Kaduna, of which 43.5% (60) were Gram negative while 56.5% (78) were Gram positive. Nasal cavity had the highest bacteria isolation rate followed by the skin then ear. This finding is in line with the study of Kinman *et al.,* (27), who reported that *S. aureus* infection among HIV patients has the propensity to proliferate in both skin and nasal cavity diseases such as lesions, sores and rashes.

Further evaluation showed that only 33.3% (26/78) were *Staphylococcus spp.* with 29.5% been *S. aureus*. This finding agrees with the report of the European Centre for Disease Prevention and Control on microorganisms isolated in HAIs (all HAI types) in acute care hospitals in EU/EEA, ECDC-PPS 2011-2012, which showed possibility of isolating 32.71% *S. aureus* out of the total Gram-positive bacteria isolated (28). Reason to this might be associated with Ogawa *et al.,* (29) report that Gram positive bacteria enhances the susceptibility of HIV patients to infections and HIV replication in monocyte-derived Langerhans cells. According to Adetokunbo *et al.,* (30), *Staphylococcus aureus* isolated from people living with HIV/AIDS leverage on the deficiency of β-cell and dysfunctional macrophage to weaken patients' immune system, exhibit high virulence and pathogenicity.

Antibiotics susceptibility profile revealed high resistance to commonly prescribed drugs against *S. aureus* especially to Imipenem (86.9%), Cefoxitin (65.3%), Vancomycin (65.3%), Tetracycline (65.3%) and Erythromycin (60.9%). High percentage (91.3%) of the *S. aureus* isolated were multidrug resistant while 65.2% were resistant to more than 4 classes of antibiotics tested. Also, 30% of the isolates were sequentially resistant to Vancomycin, Augmentin, Imipenem and Cefoxitin, making the most common pattern of resistant profile. These reports on resistant profile agrees with the study of Muhaba *et al.,* (31), who observed that *S. aureus* isolated from HIV patients were 96.5% multidrug resistant and showed significant resistance to cotrimoxazole (86.2%), erythromycin (84.5%) and tetracycline (53.4%). Their study further showed high rate of *S. aureus* and MRSA nasal colonization. Further analysis showed that 91.3% of the isolates had MARI > 0.2. This implies that the isolates have been pre-exposed to the antibiotics tested and might encode resistance gene to these antibiotics. Although, the causative factors are divers such as low CD4+ T lymphocyte counts (<200 cells/µl), previous hospitalization and antimicrobials (32), high resistance to commonly prescribed antibiotics especially cotrimoxazole and penicillin's by *S. aureus* isolated from HIV patients are on the increase and have bee reported by various studies in recent time (33, 34). Most studies attributes resistance profile of *S. aureus* to encoding MRSA genes and also acknowledged that they limit treatment regimens and challenge available clinical management options for infection control (34). This could inadvertently reduce time cure of HIV patients and also influence increase in secondary infections, loss of time

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hour at work, morbidity and mortality. Irrespective of the high resistant reports from periodic surveillance, susceptible to Ciprofloxacin, Streptomycin, Gentamycin and Chloramphenicol have also been documented. Kengne *et al.,* (33) reported high susceptibility rate to Chloramphenicol and gentamicin.

High percentage (65%) of the *S. aureus* isolates obtained from HIV patients were MRSA. This implies that *S. aureus* isolation rate in the total sample was 12.8% while MRSA was 8.3%. This result concurs with the findings of Abdullahi *et al.,* (2022), who observed that the prevalence of nasal (29.6%) colonization of *S. aureus* is higher than other body sites including blood bacteremia and UTI. But also have high accruing profile of MRSA (13.4%) among the *S. aureus* evaluated. The study conducted by Saud *et al.,* (35) showed 65.3% *S. aureus* isolation among non-HIV patients with 47.4% MRSA and 73.9% multidrug-resistant. These findings signify polarity and wide spread of MRSA among *S. aureus* susceptible candidates. This according to FerreiraI *et al.,* (32), could be attributed to previous hospitalization, ability to produce biofilm and antimicrobials resistant; indicating that possible colonization or infection by MRSA might be related to vast antimicrobial usage HIV and non-HIV patients in both community and hospital patients (35, 36).

CONCLUSION AND RECOMMENDATION

This study evaluated the incidence of *S. aureus* among people living with HIV and found that Gram positive were the most frequently isolated bacteria species from their nasal, ear and skin. The incidence of *S. aureus* and MRSA were 12.8% and 8.3% respectively. High percentage (65.2%) were resistant to more than 4 classes of antibiotics tested, but significantly susceptible to Ciprofloxacin, Streptomycin, Gentamycin and Chloramphenicol. This study recommends periodic surveillance on *S. aureus* among people living with HIV to be able to understand the dynamics and resistant profile within the study area. This will control the development of resistance to most effective drugs for the treatment of *S. aureus* associated infection.

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