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Age and Gender- Based Antibiotic Susceptibility Patterns of *Staphylococcus aureus* among HIV Patients in Some Selected Hospitals in Anambra Central, Nigeria

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ABSTRACT

Background: *Emergence of resistant S. aureus strains is of great public health concern especially among the immune-compromised subjects.*

Method*: A total of 236 mid-stream urine samples were obtained from the HAART departments of the selected hospitals in Anambra central. The isolates were identified using cultural and biochemical characterization. Their antibiotic susceptibility patterns were evaluated using disk-diffusion method.*

Results*: Sixty-two (62) Staphylococcus aurues isolates were recovered from the 236 clinical samples. The S. aureus isolates showed 38.7% resistance to Vancomycin, 41.93% resistance to gentamicin, 33.8% resistance to ofloxacin, 48.39% resistance to tetracycline, 64.52% resistance to erythromycin and 100% resistance to Amoxicillin-clavulanic acid and Cefuroxime. Twenty-four isolates were Vancomycin Resistant Staphylococus aureus (VRSA).*

Conclusion: *The S.aureus isolates were multidrug resistant.*

Keywords

Antibiotic resistance, *Staphylococcus aureus*, HIV Positive Patients, Anambra.

Introduction

Staphylococcus aureus is a known source of community- and hospital-acquired infections. It is an important opportunistic bacterial pathogen causing significant morbidity and higher risk of death among the immune-compromised individuals like HIV patients [1,2]. HIV-infected subjects are commonly susceptible to this pathogen and HIV infection has been a known risk factor for methicillin-resistant *S. aureus* (MRSA) carriage and infections [3,4]. Colonizing feature of *S. aureus* is a potential factor for infection as an individual colonized with MRSA strain has a higher risk of subsequent infection [5,6] and infection of MRSA has been reported more in HIV-infected persons [4,7,8]. This might be linked to continued viral replication, increased viral load and the consequent decline in CD4+ T cells among the HIV-infected persons [3,4]. *S.aureus* has been identified as being responsible for morbidity and mortality among HIV patients, produces important virulence factors and readily acquires resistance to different antibiotics [3].

Accumulation of resistance factors by *S. aureus* has rendered the bacterium dominant and immune to many antibiotics and this has sharply increased the burden of MRSA which is often associated with increased morbidity and mortality of the patients [7,9]. The increased incidence of MRSA has led to more frequent use of vancomycin, a reliable drug against MRSA infections. However, irrational use of antibiotics has led to the emergence of vancomycin resistance phenotype among *S. aureus* strains [10,11]. There are increasing reports on emergence of *S. aureus* strains with decreased susceptibility to vancomycin and other glycopeptides [12,13]. Vancomycin-resistant *Staphylococcus aureus* (VRSA)

strains may contain two vancomycin resistance genes, vanA and vanB genes [10]. This study was therefore designed to evaluate antibiotic resistant *Staphylococcus aureus* among HIV Patients in some selected Hospitals in Anambra Central senatorial district, Anambra Nigeria.

Methods

Isolation and Identification

Two hundred and thirty-six (236) mid-stream urine samples were collected from patients in the out-patient heart to heart clinics of the Chukwuemeka Odimegwu Ojukwu University Teaching Hospital, Amaku, Regina-Caeli Specialist Hospital and St. Joseph Hospital, Adazi-Nnukwu all in Anambra central district. Only one sample was collected per patient. Samples were then transported in rigid outer boxes to the Microbiology laboratory at Nnamdi Azikiwe University, School of Pharmacy, Agulu within 1hr of collection. Verbal informed consent was obtained from all patients prior to specimen collection and the study was conducted after obtaining due ethical approval from the ethical committee of the hospital **(COOUTH/CMAC/ETH.C/VOl.1/0053).** *S. aureus* isolates were identified based on standard microbiological techniques. Identification of isolates was by colony morphology, Gram staining, catalase test and coagulase test. Thereafter, isolates that were positive to Gram staining, catalase and coagulase tests were considered as *S. aureus* [1,9,14].

Antibiotic Susceptibility Study

Antibiotic susceptibility testing was done using the Kirby Bauer's Disk diffusion method. The antibiotic discs (Oxoid and Abtek, UK) containing the following antibiotics was used: Vancomycin (30ug), Gentamycin (10ug), Ofloxacin (5ug), Tetracycline (30ug), Amoxicillin-clavulanic acid (30ug), Erythromycin (5ug), Cefuroxime (30ug), Fusidic acid (10ug), Ceftazidime (10ug) and Ceftriaxone (30ug). Isolates were classified as either resistance or intermediate or sensitive according to the CLSI (2016).

Results

The prevalence of *S. aureus* based on age of the participants is shown in Table 1. The prevalence of *S. aureus* was highest among the age groups 22-32 years and 33-43 years with prevalence of 7.2 % and 9.74% respectively. This was followed closely by 6.36% prevalence in the age group of 44-54 years. The age group 0-10 and 11 – 21 years had a prevalence rate of 0.85% respectively and in the age group ≥ 55 years had a prevalence rate of 1.27%.

Table 1: Prevalence of *S. aureus* based on age.

Age (Years)	Number Tested	Number Negative	Number positive	Prevalence (%)
0 – 10	18	16	2	0.85
11 – 21	29	27	2	0.85
22 – 32	47	30	17	7.20
33 – 43	63	40	23	9.74
44 – 54	56	41	15	6.36
≥55	23	20	3	1.27
TOTAL	**236**	**171**	**62**	**26.27**

The prevalence of *S. aureus* based on gender of the participants involved is indicated in Table 2. Analysis of the prevalence showed that females had a significantly higher prevalence of *S. aureus* compared to male counterparts. Table 3 shows the susceptibility /resistance percentage of *S. aureus* to selected antibiotics. The isolated organism, *S. aureus* showed 38.7% resistance to Vancomycin, 41.93% resistance to Gentamicin, 33.8% resistance to ofloxacin, 48.39% resistance to Tetracycline, 64.52% resistance to erythromycin and 100% resistance to Amoxicillin-clavulanic and Cefuroxime. The multiple antibiotic resistance index (MARI) in the study was 0.6. This is ≥ 0.2 thus indicating that *S. aureus* in this study was from a high risk source of contamination where several antibiotics have been used. Table 4 shows the distribution of resistance based on age. The prevalence of Vancomycin resistance was highest with the age group 33-43 years (20.97%).

Table 2: Prevalence of *S. aureus* based on Gender.

Gender	Number Tested	Number Negative	Number positive	Prevalence (%)
Male	95	76	19	8.05
Female	141	98	43	18.22
TOTAL	**236**	**171**	**62**	**26.27**

Table 3: Susceptibility of *S. aureus* to various antibiotics.

Antibiotics (%)	Susceptible	Intermediate (%)	Resistant (%)	MRI
Vancomycin (30ug)	21 (33.87)	17(27.42)	24 (38.71)	0.60
Gentamicin (10ug)	35 (56.45)	1 (1.61)	26 (41.93)	
Ofloxacin (5ug)	38 (61.29)	3 (4.83)	21 (33.87)	
Tetracycline (30ug)	19 (27.41)	13 (20.97)	30 (48.39)	
Amoxicillin – Clavulanic acid (10ug)	-	-	62 (100)	
Erythromycin (15ug)	10 (16.13)	12 (19.35)	40 (64.52)	
Cefuroxime (30ug)	-	-	62 (100)	

*CLSI standards for zone diameter interpretive criteria (nearest whole mm).

The MRI was calculated using = a/b×c.

Table 4: Distribution of Resistance Based On Age.

Antibiotics	Age	Quantity	Prevalence of resistance	Correlation coefficient	p-value
Vancomycin	0-10	1	1.61	0.322	0.534
	11-21	1	1.61		
	22-32	2	3.23		
	33-43	13	20.97		
	44-54	5	8.06		
	> 55	2	3.23		
Gentamicin	0-10	0.0	0.0	0.122	0.817
	11-21	0.0	0.0		
	22-32	12	19.35		
	33-43	9	14.52		
	44-54	5	8.06		
	> 55	0	0.0		
Ofloxacin	0-10	2	3.23	-0.018	0.973
	11-21	1	1.61		
	22-32	9	14.52		
	33-43	5	8.06		
	44-54	2	3.23		
	> 55	2	3.23		

Tetracycline	0-10	2	3.23	0.146	0.783
	11-21	2	3.23		
	22-32	6	9.68		
	33-43	15	24.19		
	44-54	2	3.23		
	> 55	3	4.84		
Erythromycin	0-10	2	3.23	0.149	0.389
	11-21	2	3.23		
	22-32	7	11.29		
	33-43	22	35.48		
	44-54	6	9.68		
	> 55	1	1.61		
Amoxicillin –Clavulanic acid	0-10	2	3.23	0.292	0.287
	11-21	2	3.23		
	22-32	17	27.42		
	33-43	23	37.09		
	44-54	15	24.19		
	> 55	3	4.84		
Cefuroxime	0-10	2	3.23	0.292	0.287
	11-21	2	3.23		
	22-32	17	27.42		
	33-43	23	37.09		
	44-54	15	24.19		
	> 55	3	4.84		

Table 5: Distribution of antibiotic resistant *S. aureus* based on Gender.

Antibiotics	Gender	Prevalence of res.	Correlation coeff.	p-value
Vancomycin	Male	5 (8.06)	1.000**	0.00001
	Female	19 (30.65)		
Gentamycin	Male	16 (25.81)		
	Female	10 (16.13)		
Ofloxacin	Male	7 (11.29)		
	Female	14 (22.58)		
Tetracycline	Male	22 (35.48)		
	Female	9 (14.52)		
Erythromycin	Male	16 (25.81)		
	Female	24 (38.71)		
Amoxicillin – Clavulanic acid	Male	19 (30.65)		
	Female	43 (69.35)		
Cefuroxime	Male	19 (30.65)		
	Female	43 (69.35)		

** Correlation is significant to the 0.01 level.

The distribution of antibiotic resistant *S. aureus* based on gender of participants is shown in table 5. In females, Vancomycin resistance was 30.65% while in males, the prevalence was 8.06%. Gentamicin resistance in males was 25.81% and 16.13% in females. Erythromycin resistance in male participants was 25.81% and 38.71% in females whereas the prevalence of Tetracycline resistance in males was 35.48% and 14.52% in females. However, there was a 100% prevalence of Amoxicillin –Clavulanic acid and Cefuroxime in both male and female participants. Figure 1 shows the distribution of Vancomycin resistant *S. aureus* and Vancomycin susceptible and intermediate *S. aureus* among the recovered staphylococci isolates.

Discussion

Human immunodeficiency virus (HIV) is a major health problem in Nigeria. HIV positive patients are much more prone to many opportunistic infections as their immune status are compromised [15]. There has been an increase in the rate of infection and diseases caused by MRSA throughout the world. The situation is even more alarming among patients with reduced immunity [16]. Understanding the antibiotic resistance among *S. aureus* strains infecting HIV positive patients is important for the design of treatment and control strategies. Out of the 236 samples derived from the various HAART Clinics, 62 *S. aureus* were isolated .The finding from this study was higher than 9.3% prevalence reported by Olivo *et al*., [17] in Brazil and 5.4% in Nigeria by Moses *et al*., [18]. This variation could be linked to the regional difference in infection control and antibiotic use policies [19]. Twenty –one out of the 62 *S.aureus* isolates (33.87%) were Vancomycin susceptible *S. aureus* (VSSA), 17 (27.42%) were Vancomycin intermediate *S. aureus* (VISA) and 24 (38.71%) of them were VRSA (Figure 1). In our study, the prevalence of VRSA was higher among the females (30.65%) than in the males (8.06%). A similar higher rate of resistance among female subjects had been reported in Southern Iran by Farhad et al., [19].In contrast, higher rate of *S. aureus* colonization was reported among the male in Central Nepal by Neupane *et al.,* [4].

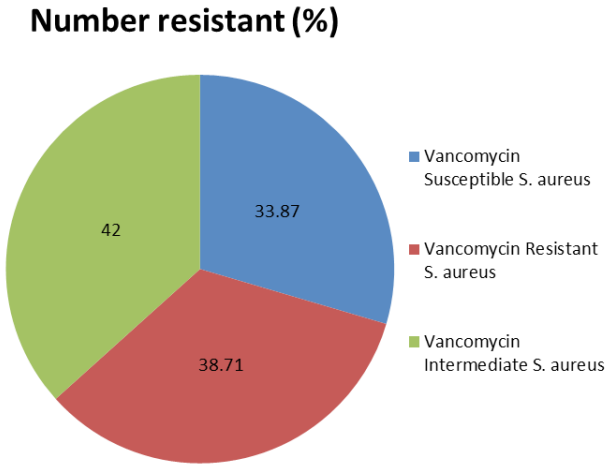

Figure 1: Distribution of Vancomycin resistant *S. aureus* and Vancomycin susceptible and intermediate *S. aureus* among the recovered staphylococci isolates.

In line with our findings, Neupane et al. [4] equally reported a higher rate of *S. aureus* colonization among patients of age group 31–40 years among HIV Patient. Similarly, Reinato et al. [21] reported 36.1% of *S. aureus* colonization rate among the HIV patients of age group 30–39 years. Some of the risk factors that have been linked with the emergence of *S.aureus* infections among HIV infected subjects include living conditions, previous hospitalization, use of fluoroquinolones and third generation cephalosporins, other secondary co-infections and low level of CD4 cells [4].

In this study, all the isolates showed resistance to a minimum of 7 antibiotics including vancomycin, a finding that is comparable to what was reported by Adegoke and Okoh [21]. Vancomycin

resistance is acquired by mutation and thickening of cell wall due to accumulation of excess amounts of peptidoglycan [22]. The increased thickness and poor cross-linked nature of cell wall layer of a VISA strain increases the amounts of D-Ala-D-Ala (building blocks of cell wall as a binding site for vancomycin). These impede the penetration of vancomycin towards pentapeptide targets resulting in diminished effects of vancomycin due to competition [13,23]. Resistance to vancomycin occurs due to the function of the *van* gene complex. The product of the *vanA* gene is a ligase that alters the dipeptide residue from D-Ala-D-Ala to D-alanyl-D-lactate (D-Ala-D-Lac), a dipeptide with substantially reduced affinity for the antibiotic [12,13].

Conclusions

Our study reports a high recovery of *S.aureus* from clinical samples of HIV positive patients in Anambra Central. The *S.aureus* isolates were multidrug resistant. However, vancomycin in clinical settings should be used cautiously to curtail the appearance and spread of new resistant strains.

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