Original Research Article

COMPARATIVE STUDY OF OTITIS MEDIA AND ITS ANTIBIOTICS SUSCEPTIBILITY PATTERN IN A COHORT OF HIV POSITIVE AND NEGATIVE PATIENTS

ABSTRACT

Aims: This study examined the comparative study of otitis media and its antibiotics susceptibility pattern in a cohort of HIV positive and negative patients.

Study design: A survey and laboratory analytical method was employed in the study. While a survey research design was used to sample a total of 110 patients out of a total population of 561,066 living in the study area. Laboratory analyses of the samples collected from the patients were used to analyze the data collected.

Methodology: A total of 110 ear swabs were collected, 60 from HIV positive subjects and 50 from HIV negatives subjects with otitis media and the samples were analyzed.

Results: From the analysis of the data collected, results showed that 52 (86.7) samples from HIV positive patients and 44 (88%) samples from HIV negative patients yielded bacteria growth The most predominant isolates from middle ear of HIV positive was Staphylococcus aureus, with total occurrence of 22(42.4%), followed by Pseudomonas aeruginosa, Streptococcus pneumonia, Klebsiella pneumoniae and Escherichia coli with occurrence of 12(23%), 7(13.5%), 6(11.5%), 5(9.6%) respectively. For HIV negatives patients, the most predominant was Staphylococcus aureus, followed by Streptococcus pneumonia, Pseudomonas aeruginosa, Klebsiella pneumoniae and Streptococcus pyogenes with occurrence of 16(36.4%), 10(22.7%), 8(18.2%), 6(13.6%), and 4(9.1%) respectively. The result of their antibiotics susceptibility test showed that all the bacteria isolates from both subjects were fully sensitive to fluoroquinolones, while a high level of resistance was seen with the use co-trimoxazole in HIV-positive subjects.

Conclusion: Bacteria isolates from HIV positive patients were highly resistant to co-trimoxazole as compared to isolates from HIV negative patients that were sensitive to co-trimoxazole. It was also noted in the result that the sensitivity pattern of otitis media to antibiotics differs from HIV positive and HIV negative patients, thus requiring different management approaches.

INTRODUCTION

Human Immunodeficiency Virus (HIV) is a virus that attacks cells in the immune system, which serves as the body's natural defense against illnesses. HIV virus is a single stranded RNA virus, which is converted to double-stranded DNA after infection and replication in the host DNA [1]. HIV virus is a devastating pandemic, affecting the lives of millions of people in the world. Globally it is estimated that 38 million people are living with HIV/AIDS. Of these, 36.2 million were adults and 1.8 million were children and 25.5 million of these are in sub-Saharan Africa [2,3]. Nigeria has the fourth-largest HIV epidemic in the world and one of the highest rates of new infection in sub-Saharan Africa (3). According to Nigeria's HIV/AIDS indicator and impact survey (NAIIS), an estimated 1.9 million Nigerians are currently living with HIV/AIDs.

In response to the raging epidemic of HIV/AIDS, the government of Nigeria in partnership with international collaborations established the national antiretroviral therapy (ART) program in 2002 which led to increasing access to HIV care and treatment [4]. Anambra State was among the four states in the Federation with the highest rates of HIV/AIDS cases. About 2.4% of the people living in the state now live with HIV/AIDS virus [3].

People living with HIV have an increased risk of acquiring many diseases. This risk depends on the number of CD4 lymphocytes cells in their blood. CD4 cells are white blood cells that play an important role in the immune system [5]. HIV infects the CD4 cells and other immune cells. The infection causes functional impairment and depletion of CD4 cells resulting in immunosuppression and development of opportunistic infections [6]. If the CD4 lymphocyte cell exceeds 500ml/mm^3, usually the patients have no chronic symptoms of the disease but when the CD4 lymphocytes count of an infected person decline below critical level i.e. 200 cell/mm^3 of which the normal value is 1200 cell/mm^3 , the person becomes prone to the opportunistic infection [5,6, 7,).

Opportunistic infections are illnesses that occur more frequently and are more severe in people living with HIV. This is because they have damaged immune systems. The most common otologic problems reported in HIV-infected patients are otitis media and serous recurrent acute otitis media [6]. Otitis media is also known as a middle ear infection. It is caused as a result of inflammation of the middle ear [8]. The incidence of otitis media is high among those living in the developing world and people living with HIV. Bacterial infections of otitis media in people living with HIV are usually caused by common pathogens that attack a person of normal immune defense. Serious otitis media occur more frequently in HIV positive patients than HIV negative patients, although causative agents are similar as in the general population [9]. A serious otitis media in HIV patients requires immediate examination by ENT to exclude malignant tumor of the middle ear, if left untreated can cause serious hearing loss [8].

The emergence of antimicrobial-resistant organisms is a global problem, not restricted to people with HIV. HIV infection has been reported to be a risk factor for colonization of antibiotic-resistant organisms. There are two main factors that lead to antimicrobial resistance: the volume of antimicrobial used and the spread of resistant organisms. The widespread of co-trimoxazole therapy upsets the patient's commensal flora and results in the selection of resistant strains among people living with HIV [10]. Some research has found out the High prevalence of Antibiotic-Resistant otitis media among people living with HIV in Tanzania [11]. Additional research has suggested that antiviral medication does not result in antibiotics resistance. Therefore it is uncertain how widespread resistance to antibiotics is among people living with HIV. At this time, there is no evidence that HIV-infected patients have a higher incidence of antibiotic resistance of otitis media compared with the general population particularly in the study area. There are few studies looking at antibiotic resistance pattern of pathogens isolated from a middle ear infection in relation to people living with HIV infection in the developing world. To gain some insights into antibiotic resistance of organisms isolated from a middle ear infection in relation to HIV positive patients attending general Hospitals in Onitsha, this study was carried out. The aim of the study was to determine the etiological agents of otitis media in HIV positive and negative patients and the antibiogram of the organisms. It is expected that the findings of the study will help contribute to effective antibiotic therapy of some of the middle ear infections arising in HIV patients in the study area.

2. MATERIALS AND METHODS

2.1 Study Area

The sample where collected at General Hospital Onitsha in Anambra State. The choice of the selection of this area is because of the large number of HIV patients attending the hospital. The hospital is a government Hospital situated in Onitsha, a fast urbanizing city with a mixed population of traders, bankers, civil servants and Artisans. Significant proportions of patients seen at the hospital are uneducated traders. The Virology Departments runs every day, the Ear Nose and Throat (ENT) clinic has a part time specialist who visits twice weekly. The population of Onitsha is 561066 according to the Geo Names geographical database (2012). It is located at 61^o longitude at an elevation/altitude of meters. The neighboring towns

are Obosi, Nkpor, Oba, Ogidi, Umunya and Nkwelle-Ezunaka. It also has the biggest market in the country. The city grew from its settlement around the River Niger. Onitsha's location on River Niger is advantageous as it is accessible by river and secondly the bridge over the Niger also makes the town the only gateway between southeastern and south western Nigeria.

2.2 Data collection and study participant

The study participants were all HIV- positive and HIV negative patients who had complained of ear infection during the study period. A well-structured questionnaire was used to collect patients' data on age and sex. Information on the use of antibiotics for prophylaxis of opportunistic infection which could be associated with current observation on antibiotics resistance was noted and other information relevant to the study.

2.3 Sample Collection

A total of 110 samples of middle ear soaked swabs were collected from 60 HIV positive and 50 HIV negative patients. The specimens were obtained by swabbing the ear with a sterile swab stick, avoiding contact of the stick with the external meatus in order to prevent contamination by the normal floral.

2.4 Sample Analysis

The sample was analyzed and processed at the General hospital within 24 hours of collection. Each swab was inoculated into Blood and MacConkey agar plates and aerobically incubated at 37^{o}C in an incubator for 24h. The culture plates were later examined for evidence of growth. The colony characters were studied; smears were stained by Gram's stain and examined under the 100x objective. Pure cultures were maintained on nutrient agar slants and kept in the refrigerator at 4^{o}C for further studies.

2.5 Characterization and identification of Bacterial Isolates:

The bacteria isolate were characterized based on colonial morphology, cultural characteristics and biochemical test as describes [12,13].

2.6 Antimicrobial Sensitivity Test:

Antibiotic sensitivity was done on Mueller- Hinton agar (Oxoid, England) using Kirby Bauer disk diffusion method [14]. The isolates were subjected to susceptibility testing against 7 widely used antibiotics, namely; co-trimoxazole (25µg), ofloxacin (5µg), cephalexin (30µg), ciprofloxacin (15µg), gentamicin (10 µg), erythromycin (15 µg) and ampicillin (10µg) (Oxoid England). The zones of inhibition produced after 24hrs incubation at 37^{o}C were measured according to Clinical and Laboratories Standards Institute [15].

2.7 Statistical Analysis

Data collected were all subjected to two-way ANOVA analysis using sigma plot version 12 statistical software. Chi-square test was employed to compare the proportion of bacterial isolates and their antimicrobial resistance pattern and also to ascertain the level of significance at 0.05%.

2.8 Ethical Consideration

Following permission to conduct the study from the hospital authorities, all participants were clearly informed of the objectives of the study and were provided with both verbal and written informed consent. They were also guided that all information gathered as well as the laboratory result will be used for academic purpose and the betterment of their health care at large. In order to maintain confidentiality, data were coded and then entered into a computer database for analysis and interpretation. Neither the patients name nor any other personal details were disclosed.

RESULTS

A total of 110 study participants with Otitis media were included in the study. Out of the 110 participants, 60 (54.5%) were HIV positive and 50 (45.5%) were HIV negative. Among these, 62 were males and 48 were females with the age range of 10 to 60 years. The majority of the study participants (58.2%) were in the age group of 21 – 40 years, and 63 (57.3 %) lived in rural areas of whom 57 (51.8%) were traders. Table 1 show the socio-demographic characteristics of study participants among HIV-positive and HIV-negative patients

Table 1: Socio-demographic characteristics of study participants with Otitis media among HIV positive and negative patients attending General Hospital Onitsha.

Characteristics	Frequency	Percent
Patients setting		
HIV positive	60	54.5
HIV negative	50	45.5
Sex		
Male	62	56.4
Female	48	43.6
Age in (years)		
≤20	8	7.3
21- 40	64	58.2
41- 60	38	34.5
Residence		
Urban	47	42.7
Rural	63	57.3
Occupation		
Traders	57	51.8
Civil servants	22	20
Students	21	19.1
Others*	10	9.1
Overall	110	100

**Farmers, Housewife, Drivers, and Jobless*.

The overall result of bacteriological investigation revealed that out of 60 samples collected from the middle ear of HIV positive cases, 52(86.7%) cases yielded growth on culture media while 8 (13.3%) cases yielded no growth on culture media. Whereas, out of 50 samples collected from HIV-negative subjects, 44(88%) cases yielded growth while 6(12%) cases yielded no growth. there is no significant difference among the bacteria load present in positive and negative HIV samples. This, therefore, means that the presence of bacteria load in the middle ear of the samples is not dependent on the level of HIV status. The most predominant isolates from the middle ear of HIV positive subjects were *Staphylococcus aureus* 22 (42.4%), followed by *Pseudomonas aeruginosa* 12(23%). *Streptococcus pneumoniae 7*(13.5%), *Klebsiella pneumoniae* 6(11.5%) and *Escherichia coli* 5 (9.6%). whereas the organisms isolated from HIV negative ear swabs were; *Staphylococcus aureus* 16(36.4%), *Streptococcus pneumoniae* 10(22.7%), *Pseudomonas aeruginosa* 8(18.2%), *Klebsiella pneumoniae* 6(13.6%), and *Streptococcus pyogenes* 4(89.1%). (Table 2)

Table 2. Bacterial isolates from middle ear of HIV-positive and negative patients attending General Hospital Onitsha.

Organisms isolated	HIV positive n (%)	HIV negative n (%)	X±SD

S. aureus	22(42.4)	16(36.4)	18.250±4.08[a]
P. aeruginosa	12(23)	8(18.2)	9.00±2.72 [a]
S. pneumoniae	7(13.5)	10(22.7)	11.500±2.72 [a]
K. pneumoniae	6(11.5)	6 (13.6)	3.750±4.08 [a]
S. pyogenes	-	4(8)	1.750±4.08 [a]
E. coli	5(9.6)	-	6.250±4.08 [a]
Total	52 (100)	44(100)	

P≥ 0.05

Mean with subscript ' a' are not significant . n = number

Table 3 and 4, show the antimicrobial susceptibility patterns of bacterial isolates from HIV positive and HIV negative subjects. A total of 7 widely used antibiotics in the study area were tested against the bacterial species for antibacterial susceptibility pattern. The antimicrobial susceptibility patterns of HIV positive patients and HIV negative patients were presented in table 3 and 4 respectively. The most predominant isolate from both subjects *Staphylococcus aureus* were more resistant to ampicillin and gentamicin while being least resistant to erythromycin in HIV negative subjects. On the other hand *Staphylococcus aureus* isolated from middle ear of HIV positive subjects showed extensively multidrug resistance pattern as it was resistant to all tested antibiotics except ofloxacin and ciprofloxacin.
Pseudomonas aeruginosa from HIV positive subjects were susceptible to ciprofloxacin, ofloxacin and cephalexin while it showed resistance to ampicillin, erythromycin, co-trimoxazole and gentamicin, compared to *P. aeruginosa* from HIV negative subjects that were sensitive to all the tested antibiotics except ampicillin and gentamicin.
Streptococcus pneumoniae and *Klebsiella pneumoniae* from middle ear of HIV negative subjects were more susceptible to ofloxacin, ciprofloxacin and cephalexin compared to *Streptococcus pneumoniae* and *Klebsiella pneumoniae* isolated from the middle ear of HIV positive subjects. Both isolates showed resistance to ampicillin and gentamicin and moderately susceptible to erythromycin and cotrimoxazole.
Escherichia coli was only isolated from the middle ear of HIV positive patients and showed a high level of resistance to cotrimoxazole, erythromycin and ampicillin, however, they were moderately susceptible to other antibiotics. *Strptococcus pyogenes* were only resistant to cephalexin, ampicillin, and gentamicin but susceptible to other antibiotics tested.

Table 3: Antimicrobial susceptibility patterns of bacterial isolates from HIV positive subjects.

Bacterial Isolates	Pattern	number of resistance pathogens to antimicrobial agents (%) OFX	CIP	CL	ER	CT	GM	AP
S, aureus	S	22(100)	20(90.9)	16(72.7)	10(45.5)	6(27.3)	0 (0)	0(0)

(n= 22)	R	0 (0)	2(9.09)	6(27.2)	12 (54.5)	16(72.7)	0(0)	0(0)
P. aeruginosa (n = 12)	S	11(91.7)	9(75	9(75)	4(33.3)	0(0)	4(33.3)	0(0)
	R	1(8.3)	3(25)	3(25)	8(66.7)	12(100)	8(66.7)	0(0)
S. pneumoniae (n =7)	S	7(100)	7(100)	5(71.4)	4(57.2)	3(42.8)	0(0)	2(28.6)
	R	0 (0)	0(0)	2(28.6)	3(42.8)	4(57.2)	7(100)	5(71.4)
K. pneumoniae (n= 6)	S	6(100)	6(100)	5(83.3)	3(50)	3(50)	1(16.7)	1(16.7)
	R	0(0)	0(0)	1(16.7)	3(50)	3(50)	5(83.3)	5(83.3)
E. coli (n= 5)	S	3(60)	3(60)	0(0)	1(20)	0(100)	2(40)	0(100)
	R	2(40)	2(40)	5(100)	4(80)	5(100)	3(60)	5(100)

S= Susceptible, R= Resistance, Ofx =Ofloxacin, CIP= Ciprofloxacin, CL= cephalexin, ER= Erythromycin, CT= Cotrimoxazole, GM= Gentamicin, AP= Ampicillin

Table 4: Antimicrobial susceptibility patterns of bacterial isolates from HIV negative subjects.

Bacterial Isolates	**Pattern**	**number of resistance pathogens to antimicrobial agents (%) OFX**	**CIP**	**CL**	**ER**	**CT**	**GM**	**AP**
S, aureus (n= 16)	S	16(100)	15(93.6)	15(93.5)	12(75)	0(0)	6 (37.5)	2(12.5)
	R	0(0)	1(6.25)	1(6.25)	4 (25)	16(100)	10(62.5)	14(87.5)
P. aeruginosa (n = 8)	S	7(87.5)	8(100)	6(75)	3(37.5)	3(37.5)	2(25)	0(0)
	R	1(12.5)	0(0)	2(25)	5(62.5)	5(62.5)	6(75)	8(100)
S. pneumoniae (n =10)	S	9(90)	9(90)	8(80)	5(50)	6(60)	1(10)	1(10)
	R	1(10)	1(10)	2(20)	5(50)	4(40)	9(90)	9(90)
K. pneumoniae (n= 6)	S	6(100)	6(100)	4(66.7)	3(50)	3(50)	0(100)	2(33.3)
	R	0(0)	0(0)	2(33.3)	3(50)	3(50)	0(0)	4(66.7)
S. pyogenes (n= 4)	S	4(100)	4(100)	4(100)	2(50)	2(50)	1(25)	0(0)
	R	0(0)	4(100)	4(100)	2(50)	2(50)	3(75)	4(100)

S= Susceptible, R= Resistance, Ofx =Ofloxacin, CIP= Ciprofloxacin, CL= cephalexin, ER= Erythromycin, CT= Cotrimoxazole, GM= Gentamicin, AP= Apicillin.

DISCUSSION

Otitis media are particularly common in HIV-infected patients as one of the opportunistic infections experienced in people living with HIV.

The result of the study showed that the majority of the bacterial isolates were identified in the age group 21-40 years (58.2%) which is also in agreement with study conducted in Nigeria [16]. However,

the findings of this study disagreed with the [17] who reported higher frequency in the age group of 1-10 years.
Analysis of the gender incident in the present study revealed that otitis media was found to be more common in males 54.5% than in female 45.5%. A similar finding was reported in study conducted in Tanzania [11] with a male preponderance of 54.4% and [18] with 63.7%, though differing with observed findings done elsewhere with female propensity by [19]. As this study involved a random selection of cases, the prevalence of male patients over female patients may be only an incidental finding.
In the present study, the bacterial isolation rate was 87.3% which was similar to the study conducted in Nigeria [20] with an isolation rate of 84.6%. However, it was higher than studies reported by Hailu, (80.4%) and Argaw-Denoba et al, with an isolation rate of 83.6% [21, 22], but lower than the report from Jimma town [23] with an isolation rate of 100%. The high rate of bacterial isolation in the present study may be due to the differences in the quality of the middle ear swab collected and differences in culture method used.

The most predominant isolate from the middle ear of HIV positive subjects and negative subjects was *S. aureus* 42.4% in HIV positive subjects and 36.4% in HIV negative subjects, followed by *P. aeruginosa* 23% for HIV positive and 18.2% for HIV negative subjects and *S. pneumoniae* with prevalence rate of 22.7% and 13.5% respectively. These findings were Similar to observational study done in Mekelle by [24] and [25] Unlike our findings, Argaw-Denoba *et al*, and Reuben et al, reported *Pseudomonas aeruginosa* as the predominant isolates causing middle ear infection [22, 26].

In our study, *S. aureus*, *P. aeruginosa, S. pneumoniae* and *K. pneumoniae* were seen in the middle ear of both HIV positive and negative subjects. However, various studies have been carried out, establishing the significant of bacteria species like *S. aureus, P. aeruginosa, S. pneumoniae, E. coli, P. mirabilis, K. pneumoniae*, in their connection with otitis media [27, 24]. The organisms causing otitis media in HIV positive subjects were similar to those in HIV-negative subjects but with variations in the number of isolates obtained. There is no significant difference among the bacteria load present in positive and negative HIV samples, this therefore means that the presence of bacteria load in the middle ear of the samples is not dependent on the level of HIV status.
Escherichia coli was only isolated from middle ear of HIV positive subjects. The findings of the present study agree with the work of [28] that isolated similar organism from HIV infected patients with the otorhinolaryngological disorder. In contrast to this report, *E. coli* was reported from ear swab of HIV negative patients from a study conducted in Ethiopia by [23]. Isolation of *E. coli* which is known to be fecal bacteria in the middle ear of people living with HIV, suggests that HIV-positive people are at high risk of infection probably due to suppressed immune system.

An antimicrobial susceptibility test was done for 7 different types of antibiotics commonly used in the study area. The sensitivity result obtained from this study showed resistance to some antimicrobial agents. A high level of resistance was observed to commonly used antibiotic such as ampicillin, gentamicin and cotrimoxazole in HIV-positive subjects, compared to isolates from HIV negative subjects that showed high resistant to only ampicillin and gentamicin. Our findings correlate with report from [29, 23]who reported high rate of resistance to these drugs. All the bacteria isolates from both HIV positive and negative subjects were fully susceptible to fluoroquinolones (Ofloxacin and ciprofloxacin). The high sensitivity of fluoroquinolones has been reported by many authors, [24, 25]. These antimicrobial drugs should be used for the treatment of otitis media in both HIV-positive and HIV-negative patients. However, it was inconsistent with a study conducted in China by Jianghong *et al,* that observed a high prevalence rate of fluoroquinolones- resistant strain [30].

In the study, most of the isolated organisms from HIV-positive subjects were susceptible to co-trimoxazole antibiotic tested. Ofloxacin and ciprofloxacin were found to be the most effective drugs against pathogens causing otitis media in both HIV-positive and HIV-negative patients.

Poor susceptibility result to cotrimoxazole which is used as prophylaxis of opportunistic infections among people living with HIV was observed from isolates in HIV positive subjects compared to isolates from middle ear of HIV negative that are moderately sensitive to these antibiotics. Our findings correlate with report from [10] who reported high resistant rate to cotrimoxazole in HIV-positive patients. However, it was inconsistent with study conducted in Tanzania by [11] that showed high susceptibility rate to cotrimoxazole and gentamicin. The resistance of the isolated organisms to these antibiotics may be due to emerging drug resistance to these classes of antibiotics; drug abuse by the HIV infected population and suppressed immune system in people living with HIV.

CONCLUSION

The findings of this study suggest that opportunistic infection such as otitis media still remain a challenge as one of the OIs among HIV infected population in Nigeria.
Sensitivity pattern of otitis media to antibiotics differs from HIV positive and HIV negative patients, thus requiring different management approaches. These might be as a result of local antimicrobial prescribing practices as well as the prevalence of resistant bacterial strains. Resistance to cotrimoxazole observed in our study is an indication of emerging resistance to these widely used antibiotics as prophylaxis in HIV positive subjects.

RECOMMENDATION

From the result of the current study, it is suggested that; Anambra state government should enforce a control strategy against the spread of HIV/AIDS. HIV-infected patients should be provided with all the necessary treatment and control measures to boost their weakened immune response. Antibiotics sensitivity tests need to be done before the administration of antibiotics. Enforce mass health education campaigns against HIV/AIDS as well as opportunistic infections.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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