Original Research Article

Assessment of Bacterial profile of ocular infections among subjects undergoing ivermectin therapy in onchocerciasis endemic area in Nigeria

ABSTRACT

Bacteria are the major contributor of ocular infections worldwide. This can damage the structures of the eye with possible blindness and visual impairments, If left untreated. This study was undertaken to determine bacterial agents of conjunctivitis among individuals from onchocerciasis endemic area in Anambra State, Nigeria. This was across-sectional study involving ninety-two (92) randomly recruited test subjects undergoing ivermectin therapy aged between 11-80 years with signs and symptoms of onchocerciasis infection and bacterial conjunctivitis at Isu-Anaocha community in Anambra state between October and November, 2016. Ninety-two (92) apparently healthy subjects without signs and symptoms of onchocerciasis and bacterial conjunctivitis with normal vision, within the age of 11-80 years from the same community were used as control subjects, making a total of 184 participants. Visual acuity test was conducted on the participants using Snellen's alphabet chart and illiterate E chart. Phenotypic and antibiotic susceptibility tests of these isolates were carried out according to Kirby - Bauer disc diffusion method guideline. In the test group, 22 out of 92 conjunctival samples yielded bacterial growth, giving percentage prevalence of 23.9%, with age group 41-50 years having the highest frequency 7(31.8%) among individuals co-infected with onchocerciasis infection whereas 72 out of 92 conjunctival samples in the control group yielded bacterial growth giving percentage prevalence of 78.3%, with age group 41-50 years having the highest frequency 35(37.2%) among individuals that had no signs and symptoms of onchocerciasis infection . The predominant bacteria isolated was *Staphylococcus aureus* 8(36%) in the test group and 20 (28%) in the control group, giving a total of 28 (30%). Other bacterial isolates were *Streptococcus pneumoniae* 17 (18%), *Staph. epidermidis* 15 (16%), *Escherichia coli* 13(14%), *Pseudomonas aeruginosa* 12(13%) and *Salmonella spp.* 9(9%).

Antibiotic susceptibility testing showed that Gentamicin and fluoroquinolones are most active against Gram positive bacteria in conjunctivitis while Augmentin was found to be the most active against Gram negative organisms, therefore, recommended as first line drug. Visual acuity (VA) test done on the test group showed that 52 (57%) persons had normal vision, 20 (22%) had mild visual impairment, 15 (16%) had moderate visual impairment, 5(5%) had severe visual impairment but none was blind. All forms of visual impairment are not wholly attributable to bacterial conjunctivitis as some could be due to ageing and uncorrected refractive errors.
Key words: Bacterial conjunctivitis, onchocerciasis, visual impairment.

INTRODUCTION

Bacterial conjunctivitis is the inflammation of the conjunctiva causing redness, discomfort or discharge from the affected eye which is brought about by bacteria [1]. Normal conjunctival bacterial flora include *Staphylococcus epidermidis,* non hemolytic streptococci, *Neisseriae, Moraxella* species, *Micrococci* and aerobic diphtheroids, *Staphylococcus aureus* and *Haemophilus* species [2]. Bacterial conjunctivitis are caused by *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae, Niesseria gonorrhoeae, Niesseria meningitides, Moraxella lacunata Gram negative enteric flora*, and *Treponema pallidum* [3].

Chlamydial conjunctivitis is known to be caused by *Chlamydia trachomatis* (causes trachoma and inclusion conjunctivitis of the newborn) [3]. Bacteria are generally associated with many types of ocular infections such as conjunctivitis, keratitis, endophthalmitis, blepharitis, orbital cellulitis and dacryocystitis manifestations [4]. Conjunctivitis, inflammation of the mucosa of conjunctiva, is the most frequent ocular case with noticeable economic and social burdens [5]. Blepharitis which is an inflammation of the eyelid can cause loss of eye lash [6]. The infection may not remain localized and is known to spread to other anatomical sites of the eye [7]. Keratitis, the most serious eye infection is the leading cause of corneal blindness. Moreover, the disease can also progress to endophthalmitis if not diagnosed early [8].Exogenous endophthalmitis is an infective complication of primary cataract, intraocular surgery and ocular trauma due to the introduction of infectious pathogens like bacteria whereas the endogenous one is commonly due to systemic dissemination of the pathogens. Both keratitis and endophthalmitis are potentially devastating ocular infections if not diagnosed early [9].Dacryocystitis is an inflammation of the nasolacrimal duct. During chronicity the disease is associated with infection, inflammation of the conjunctiva, accumulation of fluid and chronic tearing. This can be potentially dangerous to ocular tissues such as the cornea; leading to post surgery endophthalmitis [10].

Onchocerciasis is also not a significant cause of blindness according to work carried out by [11], despite the fact that the sample included clusters from areas where onchocerciasis has been known to be endemic. Previous studies report that onchocerciasis is an important cause of blindness in endemic areas of Africa, including Nigeria [12; 13] and ocular manifestations of onchocerciasis have been recorded in the savannah as well as in the rain forest areas. Despite its size, there has been no earlier national estimate of the prevalence and causes of blindness and visual impairment in Nigeria. Data from surveys of special groups or in focal areas cannot be extrapolated to the entire country, because of its cultural, economic, ethnic and geographical diversity [11]. However, Visual acuity should be done on regular basis on this endemic regions, thus providing a mechanism for monitoring change in prevalence of disease and visual impairment over time.

MATERIALS AND METHODS

Study design

This was a cross-sectional study involving ninety-two (92) randomly recruited subjects undergoing ivermectin therapy aged between 11-80 years with signs and symptoms of onchocerciasis infections and conjunctivitis at Isu-Anaocha community in Anambra state between October and November, 2016.

Study Area

Isu-Anaocha is a town in Awka-North Local Government Area of Anambra State, Nigeria. It is a rural community and a riverine area. The major occupation of the inhabitants is farming. It is a choice area for the study because of the presence of fast flowing river/ stream like Mgboji, Mpili and Oyi which may possibly attract the black fly, vector for onchocerciasis (river blindness).The anti-parasitic drug, ivermectin(mectizan) is routinely distributed to the inhabitant of this area because of endemicity of onchocerciasis.

Inclusion criteria

Participants that were included in the study were residents of Isu-Anaocha undergoing ivermectin therapy with signs and symptoms of onchocerciasis such as skin nodules, dermatitis or previous history of the disease. Participants of 11-80 years of age with mucopurulent eye discharge, red eye, itchy eyes without ny use of antibiotic eye drop within the past two weeks of sample collection.

Exclusion criteria

Subjects with history of eyes surgery and those within ages 0-10 years (infants and little children.Subjects with acute loss of vision and eye trauma(mechanical or chemical).Paticipants wearing contact lenses and who administer eye drop(systemic or local antibiotics) or herbal eye drops within the previous two weeks priormto sample collection.

Controls

Ninety-two, (92) apparently healthy individuals between the ages of 11-80 years,undergoing ivermectin therapy without signs and symptoms of Onchocerciasis or bacterial conjunctivitis within the Isu-Anaocha community were used as control subjects.

Examinations and Assessment of the participants

The physical examination included investigation of the degree of redness (peripheral,whole conjunctiva or whole conjunctiva and pericorneal, the kind of discharge(watery, mucuous, purulent), and bilateral involvement. For the control participants, after declaring to participant in the study, visual acuity test, interviewer-administered questionnaire and physical examination was completed. The physical examination included careful investigation of body for absence of signs of onchocerciasis like skin nodule, dermatitis and then of the eye/conjunctiva for absence of signs of bacterial conjunctivitis as eye discharge, redness.

These examinations and sampling were carried out early in the morning. The participants were instructed not to take their baths so that the eye discharge will not be washed off (if present).For each participant, one eye was designated the "study eye". In the case of two diseased eyes(bilateral involvement), the one with worse signs or symptoms was the study eye, in the case of two equally affected eyes, the first affected eye was the study eye. In the case of one affected (unilateral involvement), the affected eyes was the study eye [14].

Visual Acuity Screening

Distance visual acuity was employed in assessing the participants. The participants stood at a measured distance of six from the chart and were asked to read the Snellen chart [for the lettered participants] and the illiterate E chart [for the non-lettered participants] shown to them by the examiner [15]. Both eyes were assessed separately.

World Health Organization categories of vision loss were used to define blindness and severe visual impairment, allowing international comparisons to be made [16; 17].

.Isolation and identification of conjunctival bacterial pathogens

Ninety-two conjunctival swabs were transported to Medical Microbiology Laboratory for analysis and inoculated on Blood agar, MacConkey agar and Chocolate media and incubated at 37°C for 24hrs. Discrete colonies of bacterial isolates recovered were subjected to Gram-staining and various biochemical tests for identification.

Preparation and standardization of inocula

Standard bacterial suspensions were prepared as follows: Colonies of each strain of bacterial isolate were emulsified in 3ml volume of saline to the turbidity of 0.5 MacFarland standards.

Antiobiotic susceptibility testing

Sterile swabs were then dipped into these standard bacterial suspensions and plated on Mueller-Hinton agar sensitivity plates. The plates were covered for five minutes to dry. Various antibiotic sensitivity discs (six per plate) were placed on each plate and incubated at 37°C for 24hours. The bacterial pathogens were tested against the following antibiotics: gentamycin, erythromycin, tetracycline, ampicillin, chloramphenicol, cloxacillin, streptomycin, Pefloxacin, penicillin and ciprofloxacin. These were the antibiotics that were readily available in the environment under study. After 24hrs incubation, the plates were examined for zones of inhibition around each of the antibiotic disc.These were measured and compared with interpretive chart to determine the sensitive and the resistant strains.Demographic information regarding age, Occupation and sex of patients were also obtained and recorded

Table 1: Zone of inhibition diameter	Interpretive criteria
< 14 - 25	Resistant
26 - 32	Intermediate
33 - ∞	Sensitive (Susceptible)

Statistical Analysis

Result Data were presented as Mean, Standard deviation and percentage for categorical data. Comparative analyses between two variables were done using Z-test for continuous variables and Chi-square for categorical variable. Level of significant was also considered at $P < 0.05$.

RESULTS

The findings from the visual acuity (VA) of the participants tested showed that 52(57%) had normal vision while 20(22%) had mild visual impairment. Other categories observed included moderate visual impairment 15(16%) and severe visual impairment 5(5%) (Table 2). Prevalence of bacterial conjunctivitis among the test subjects is 22/92(23.9%)[Table 3]. Table 4 shows that age group 41-50 years has the highest frequency of bacterial isolates from their conjunctiva in both the test and control group with 7(31.8%) and 28(38.9%) respectively. There is a significant difference in the development of bacterial conjunctivitis in individuals co-infected with onchocerciasis infection between the age and occupation status of the participants as seen in Table 5.

Table 2: Distribution of Visual Acuity (VA) of Participants

Grades of VA	Code	Test (n=92)	Control (n=92)	Total (n=184)
Normal vision	4	52(57.0%)	92(100.0%)	144(78.0%)
Mild visual impairment	3	20(22.0)	0(0.0%)	20(11.0%)
Moderate visual impairment	2	15(16.0%)	0(0.0%)	15(8.0%)
Severe visual impairment	1	5(5.0%)	0(0.0%)	5(3.0%)
Blindness	0	0(0.0%)	0(0.0%)	0(0.0%)
		92(100.0%)	92(100.0%)	184(100.0%)

Distance visual acuity test [18]

Table 3: **Comparison between Bacterial profile of conjunctivitis con-infected with onchocerciasis and without onchocerciasis among subjects.**

Isolates	Test (n=22)	Control (n=72)	Total (n=94)
Staphylococcus aureus	8(36.0%)	20(28.0%)	28(30.0%)
Staphylococcus epidermidis	5(23.0%)	10(14.0%)	15(16.0%)
Streptococcus pneumonia	2(9.0%)	15(21.0%)	17(18.0%)
Pseudomonas aeruginosa	3(14.0%)	9(12.0%)	12(13.0%)
Escherichia coli	2(9.0%)	11(15.0%)	13(14.0%)
Salmonella spp.	2(9.0%)	7(10.0%)	9(9.0%)
TOTAL	22 (100.0%)	72(100.0%)	94(100.0%)

Table 4:Distribution of of sociodemographic characteristic of the participants

Age range(yr)	Test	Control	Total
11-20	1(4.5%)	3(4.2%)	4(4.3%)
21-30	2(9.1%)	8(11.1%)	10(10.6%)
31-40	4(18.2%)	12(316.7%)	16(17.0%)
41-50	7(31.8%)	28(38.9%)	35(37.2%)
51-60	5(22.7%)	21(29.2%)	26(27.7%)
61-70	2(9.1%)	0(0.0%)	2(2.1%)
71-80	1(4.5%)	0(0.0%)	1(1.1%)
Total	22(100.0%)	72(100.0%)	94(100.0%)
Occupation	Test	Control	Total
Farmer	42(46.0%)	15(16.0%)	57(31.0%)
Trader	20(22.0%)	22(24.0%)	42(23.0%)
Artisan	11(12.0%)	32(35.0%)	43(23.0%)
Civil servant	9(10.0%)	10(11.0%)	19(10.0%)
Student	5(5.0%)	5(5.0%)	10(6.0%)
Unemployed	3(3.0%)	5(5.0%)	8(4.0%)

Table 5: Relationship between socio-demographic characteristics and development of bacterial conjunctivitis in individuals con-infected with onchocerciasis

	Age	Occupation	
X^2	10.69	17.99	4.5536
P-value	* 0.01	* 0.005	* 0.05

* Indication of significant at P = 0.05

Table 6 shows the antibiogram profile of Gram Positive bacterial isolates from conjunctival swabs. Gentamicin was found to be the most active agent, with 25(89.3%) isolates of *Staph. aureus* sensitive to it, while only 3(10.7%) isolates of *Staph. aureus* were resistant to gentamicin. 15(88.2%) of the 17(100%) isolates of *Streptococcus pneumoniae* were sensitive to ciprofloxacin, and so ciprofloxacin is the most active agent against this organism; followed by pefloxacin 14(82.4%) and gentamicin 13(76.5%). Penicillin was shown to be less active drug against the Gram positive bacterial isolates with only 2(7.1%) of *S. aureus,* 3(20.0%) of *S.epidermidis* and 4(23.5%) of *Streptococcus pneumoniae* isolates sensitive to it. 14(93.3%) of the*Staph. epidermidis* isolates were sensitive to ciprofloxacin which seemed to be the most active agent against this organism, followed by pefloxacin 13(86.7%) and gentamicin 12(80.0%). Fluoroquinolones(ciprofloxacin and pefloxacin) were shown to be the most active antibacterial drugs against Gram positive bacterial isolates, followed by gentamicin, and so should be recommended first. Table 7 shows the antibiogram profile of Gram negative bacterial isolates from conjunctival swabs. Augmentin was shown to be the most active agent against *E. coli*, with 9(69.2%) out of the 28(100%) *E.coli* isolates sensitive to Augmentin. Augmentin was also shown to be the most active agent against *Pseudomonas aeruginosa* , with all 12(100%) of the isolates sensitive to it, while none was resistant to augmentin. Augmentin and cotrimoxazole were shown to be the most active agent against Salmonella species, with all 9(100%) and 8(88.9%) of the isolates sensitive to augmentin and cotrimoxazole respectively , while none was resistant to both.

Table 6: Antibiogram profile of Gram positive bacterial isolates from conjunctival swab

Gram positive bacterial isolates

Antibiotics (µg) tested	*Staphylococcus aureus (n=28)*		*Staphylococcus epidermidis (n=15)*		*Streptococcus pneumoniae (n=17)*	
	Sensitive	*Resistant*	*Sensitive*	*Resistant*	*Sensitive*	*Resistant*
Gentamicin (10)	**25 (89.3%)**	**3(10.7%)**	**12(80.0%)**	**3(20.0%)**	**13(76.5%)**	**4(23.5%)**
Erythromycin (5)	**16(57.1%)**	**12(42.9%)**	**9(60.0%)**	**6(40.0%)**	**10(58.8%)**	**7(41.2%)**
Tetracycline (10)	**9(32.1%)**	**19(67.9%)**	**12(80.0%)**	**3(20.0%)**	**13(76.5%)**	**4(23.5%)**
Ampicillin (2)	**6(21.4%)**	**22(78.6%)**	**10(66.7%)**	**5(33.3%)**	**11(64.7%)**	**6(35.3%)**
Pefloxacin (5)	**17(60.7%)**	**11(39.3%)**	**13(86.7%)**	**2(13.3%)**	**14(82.4%)**	**3(17.6%)**
Penicillin (1)	**2(7.1%)**	**26(92.9%)**	**3(20.0%)**	**12(80.0%)**	**4(23.5%)**	**13(76.5%)**
Streptomycin (10)	**14(50.0%)**	**14(50.0%)**	**11(73.3%)**	**4(26.7%)**	**12(70.6%)**	**5(29.4%)**
Cloxacillin (5)	**10(35.7%)**	**18(64.3%)**	**7(46.7%)**	**8(53.3%)**	**8(47.1%)**	**9(52.9%)**
Ciprofloxacin (5)	**20(71.4%)**	**8(28.6%)**	**14(93.3%)**	**1(6.7%)**	**15(88.2%)**	**2(11.8%)**
Chloramphenicol (10)	**13(46.4%)**	**15(53.6%)**	**5(33.3%)**	**10(66.7%)**	**6(35.3%)**	**11(64.7%)**

Table 7: Antibiogram profile of Gram negative bacterial isolates from conjunctival swabs

Gram negative bacterial isolates

Antibiotics (µg) tested	*Escherichia coli (n=13)*		*Pseudomonas aeruginosa (n=12)*		*Salmonella species (n=9)*	
	Sensitive	*Resistant*	*Sensitive*	*Resistant*	*Sensitive*	*Resistant*
Cotrimoxazole (25)	**7(53.8%)**	**6(46.2%)**	**10(83.3%)**	**2(16.7)**	**8(88.9%)**	**1(11.1%)**
Cloxacillin (5)	**8(61.5%)**	**5(38.5%)**	**9(75.0%)**	**3(25.0%)**	**7(77.8%)**	**2(22.2%)**
Erythromycin (5)	**2(15.4%)**	**11(84.6%)**	**7(58.3%)**	**5(41.7%)**	**5(55.6%)**	**4(44.4%)**
Gentamicin (10)	**4(30.8%)**	**9(69.2%)**	**5(41.7%)**	**7(58.3%)**	**4(44.4%)**	**5(55.6%)**
Augmentin (30)	**9(69.2%)**	**4(30.8%)**	**12(100.0%)**	**0(0.0%)**	**9(100.0%)**	**0(0.0%)**
Streptomycin (10)	**3(23.1%)**	**10(76.9%)**	**7(58.3%)**	**5(41.7%)**	**5(55.6%)**	**4(44.4%)**
Tetracycline (10)	**3(23.1%)**	**10(76.9%)**	**8(66.7%)**	**4(33.3%)**	**6(66.7%)**	**3(33.3%)**
Chloramphenicol (10)	**5(38.5%)**	**8(61.5%)**	**10(83.3%)**	**2(16.7%)**	**8(88.9%)**	**1(11.1%)**

DISCUSSIONS

The findings from the visual acuity (VA) of the participants tested showed that 52(57%) had normal vision while 20(22%) had mild visual impairment. Other categories observed included moderate visual impairment 15(16%) and severe visual impairment 5(5%). Low vision of some of the tested subjects can be attributed to ageing and uncorrected refractive errors. Tested participants and control group had various bacterial isolates from their conjunctiva. This suggested that presence of bacterial isolates in the conjunctiva does not affect vision. This agrees with the findings of [2] which stated that pink eye(conjunctivitis) does not cause any changes in vision and that the pupil should be normally reactive and visual acuity normal. Persons in the control group had normal vision. The visual acuity findings showed that 40/92(43.4%) of the tested group had visual impairment while 52/92(57%) had normal vision. Prevalence of bacterial isolates from conjunctiva revealed that *Staphylococcus* species constituted more than half of the bacterial yields found in the test group 13/22(59%) while control group had 30/72(42%). There are similar bacterial isolates from the two groups. *Staphylococcus* species in the tested group has significant difference with those from control group. The overall bacterial yields did not show any significant difference between the test and control groups. Age group 41-50 years has the highest frequency of bacterial isolates from their conjunctiva with test group having 7/22(31.8%) and then 28/72(38.9%) in the control group.

Prevalence of bacterial conjunctivitis in association with occupation of the studied subjects revealed that the test group were predominantly farmers 42/92(46%) while artisans 32/92(35%) were higher in the control group. As the bacterial yields were similar for the test and control groups, it was likely that the cause of visual impairment was due to some other reasons or causes. The demographic distribution of the participants showed that 63% were aged 41 years and above in the test group while 27% was found in the control group). It is known that presbyopia and other refractive errors, cataract and glaucoma are nearly always restricted to those 45 years of age and above [1]. This could have contributed significantly to the visual impairment of the test subjects). Further, onchocerciasis has as its major ocular complications cataract from anterior uveitis, chorioretinitis, and optic atrophy. These are the dominant causes of blindness and visual impairment in river blindness patients in the rain forest zones [18]. The agrarian occupation of majority of the test participants 42/92(45.7%) could have also exposed them more to vegetative traumatic eye diseases which impair vision.

Staphylococcus aureus has the highest occurrence with 8(36%) and 20(28%) in the test and control groups respectively. This is in line with the findings of[19] that reported 43.1% prevalence for *Staph. aureus* out of 72(69.2%) bacteriologically positive samples of 104 conjunctival swabs analysed at UITH, Ilorin, Nigeria. This equally agrees with the findings of [20] that reported 72(41.4%) of 174 bacterial isolates to be *Staph. aureus* in a study on conjunctivitis among children in University College Hospital(UCH)Ibadan, South-West of Nigeria.

However, this does not agree with the findings of [14] that reported highest occurrence of *Streptococcus pneumoniae* 27(47%), followed by *Stapylococcus aureus* 13(23%) out of 57 bacterial isolates in a study on predicting bacterial cause in infectious conjunctivitis in Amsterdam. This difference could be attributed to seasonal and environmental differences Isolation of *Staphylococcus aureus* and *Streptococcus pneumoniae* from the conjunctiva of the test group could be suggestive of an acute bacterial conjunctivitis[21]. Isolation of *Pseudomonas aeruginosa* from the conjunctiva of both the test and control could be due to contamination from the use of traditional eye medications(TEMs) or from vegetation, probably during farming [21] or due to growth of *P. aeruginosa* in eye drops and ointments [16] which are commonly used by these subjects. Contamination of the eye due to use of TEMs by the subjects can lead to ocular complications like corneal ulcers [22].

However, intense eye itch experienced by some of these subjects could be said to be due to *Wolbachia pipientis*, an endosymbiont of *Onchocerca volvulus* -the nematode responsible for onchocerciasis. When the worms die, their *Wolbachia* symbionts are released, triggering a host immune system response that can cause severe itching, and can destroy optical tissue in the eye[18].For this reason, it is necessary to include an antibiotic treatment in addition to antiparasitic drug(Ivermectin) in the management of these subjects to take care of the bacterial endosymbiont.

Antimicrobial susceptibility testing showed that fluoroquinolones (ciprofloxacin and pefloxacin) and gentamicin are most active against Gram positive agents of bacterial conjunctivitis, while augmentin was found to be the most active against Gram negative organisms, and are therefore recommended as first line drugs. This is in line with the findings of [19] and [20].

Conclusions

Visual impairment is not only attributed to infectious diseases (onchocerciasis, bacterial conjunctivitis or trachoma) but some could be due to aging or uncorrected refractive errors. There is need for proper laboratory diagnosis in cases of bacterial conjunctivis and onchocerciasis infection instead of empirical treatment. It is also recommended that antibiotic treatment using doxycycline should be used in addition to ivermectin as an adjunct therapy for the treatment of onchocerciasis. However, Visual acuity test and other eye care services should be provided to inhabitants of onchocerciasis endemic regions so as to enable early detection and prevention of ocular morbidity .

Disclaimer regarding Consent and Ethical Approval:

As per university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

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Author's contributions

Okeke-Nwolisa, B.C-Sample collection, processing and data analyses. Enweani, I.B –Conceived and supervised the Research work. Oshim,I.O - participated in literature review, manuscript writing and editing. Uzozie,C.C. was involved in training of the researcher on the collection of conjunctival swab samples. Urama, E.U, Odeyemi, O and Olise, A.N - were also involved in editing and reviewing of the manuscript.

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