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Original Article

Susceptibility of conjunctival bacterial pathogens to fluoroquinolones: A comparative study of ciprofloxacin, norfloxacin and ofloxacin

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Abstract

In order to determine the most common bacteria implicated in conjunctivitis, and the effectiveness of the antibiotic Fluoroquinolone for its treatment, a total of 50 subjects (100) eyes, between the ages of 1-30 years with mean age of 16.94 ± 8.06 years with infected eyes, were examined at the Lagos State University Teaching Hospital, Nigeria (LASUTH). Conjunctival swabs were collected and cultured in the laboratory to isolate the pathogens responsible for the infection. Sensitivity and antibiotic susceptibility tests were carried out with discs impregnated with 0.3% concentration of ophthalmic topical solutions of chibroxin (Norfloxacin), ciloxan (Ciprofloxacin), and ocuflox (Ofloxacin), to ascertain the most sensitive of the three drugs. The results showed that the implicated bacteria in order of decreasing frequency were *Staphylococcus aureus* (34%), *followed by Streptococcus pneumoniae* (22%), *Pseudomonas aeruginosa* (14%), *Klebsiella pneumoniae* (12%), *Hemophilus influenzae* (9%), *Escherichia coli* (9%). All the isolated organisms were highly sensitive to the three drugs. However, a one way analysis of variance (ANOVA) showed a significant difference in the sensitivity of the three drugs ($p < 0.05$). ANOVA Post Hoc located Ciprofloxacin as the source of the significance. In conclusion therefore, Ciprofloxacin is the most sensitive of the three drugs and, hence should be the first choice of the fluoroquinolones for the treatment of bacterial conjunctivitis.

Introduction

Bacteria are the most common microorganisms that cause conjunctivitis (inflammation of the conjunctiva). This is so because the bacteria pathogens inhabit the ocular surface (i.e. mucous membrane of the conjunctiva) though the lysosomes and antibodies in tear film along with the blinking mechanism keep their population in check. The most common bacteria microorganisms implicated in bacterial conjunctivitis include *Staphylococcus aureus, Neisserria gonorrhoeae, Streptococcus pneumoniae, Haemophilus influenzae, Moraxella lacunata, Corynebacterium diphtheriae.*(1)

There are two types of bacterial conjunctivitis; acute and chronic. The acute stage is classically recognized by vascular engorgement and mucopurulent discharge with associated symptoms of foreign body sensation, irritation, and sticking together of the lids. The chronic stage is more innocuous in onset, it runs a protracted course and is often associated with the involvement of the eyelids, lacrimal system or low-grade inflammatory reaction. Although majority of bacterial conjunctivitis are self limiting, without need for medical intervention, studies have demonstrated that antibiotic therapy hastens the eradication of bacteria, prevents the dissemination of the infection to other structures, decreases the risk of systemic disease, reducing person to person spread and shortens the symptomatic period allowing the patient to return more quickly to his or her normal activities.(1-3)

Most times primary eye care providers start the treatment of external ocular infection before the causative micro-organisms have been identified, or submitted to antibiotic susceptibility tests. Consequently, broad-spectrum antibiotics are routinely used in the treatment of bacterial conjunctivitis. Of the many antibiotics in use is the group of *fluoroquinolones*- particularly Ciprofloxacin (ciloxan), Ofloxacin (ocuflox) and Norfloxacin (chibroxin). Flouroquinolones are synthetic quinolone derivatives with fluorine atom in the 6th position. The addition of the fluorine atom improves its potency, enhances the antimicrobial activity and alters the pharmacokinetic properties with tremendous therapeutic advantage over nalidixic acid. Their mechanism of action is unique among available antibiotics suggesting that cross resistance to fluoroquinolones can be minimized.(4) The broad spectrum of activity of the fluoroquinolones permits their use in a variety of infections including those affecting the respiratory tract, urinary tract, skin, soft tissues and eyes. The earliest fluoroquinolones were predominantly against gram negative agents especially *Enterobacteriaceae*. The newer fluoroquinolones also are active against gram negative organisms but offer a broader spectrum of activity, including coverage of gram positive and atypical bacteria. They have gained popularity in ocular therapy due to their efficacy in the treatment of bacterial corneal ulcers. These drugs are available in topical and oral forms. The current topical formulation are in 0.3% concentrations. Each has distinct clinical characteristics, differences in solubility and precipitate formation.(5)

Studies directly comparing the efficacy of the fluoroquinolones are sparse. However, studies conducted by some researchers (5-8), have shown that antibiotic of the fluoroquinolone group were more effective in the treatment of ocular infections than some other broad spectrum antibiotics eg. gentamycin, chloramphenicol, tobramycin, erythromycin and tetracycline. The study by Jauch (8) has also shown that *Staphylococcus aureus* is a common pathogen in bacterial infection. The aim of this study therefore was to identify the common bacterial pathogens that cause conjunctivitis and the most sensitive of the three topical fluoroquinolones under study for its treatment.

METHODOLOGY

Study population

The study population comprised of 50 subjects (100 eyes), between the age range of 1-30 years who were diagnosed to have bacterial conjunctivitis at the Lagos State University Teaching Hospital, Nigeria (LASUTH).

Procedure

Conjunctival swabs were collected from both eyes of each subject using sterile cotton tipped applicator wiped twice across the lower conjunctival cul-de sac from the temporal to the nasal region. The swabs were labelled and taken to the laboratory within two hours of collection. In the laboratory, it was placed in a medium and inoculated.

Preparation of medium

Both blood and chocolate agar were used. The powdered medium was mixed with water and steamed to dissolve the agar. The whole mixture was then sterilised in an autoclave at 121° C and subsequently allowed to cool to about 45° C, a temperature at which the agar remained molten. Plates were then prepared by pouring some 15 – 20 ml of the molten agar medium into sterile Petri dishes which were left undisturbed until the agar set.
Blood agar was made by mixing molten agar at about 45°C- 50°C with 5 -10% volume of blood before pouring into the plates.
Chocolate agar was made by heating blood agar to 70°C - 80°C until it became chocolate brown in colour.

Inoculation of medium

The solid medium was inoculated using the streaking method which involved the progressive thinning out of the inoculum in such a way that the separated cells were left in at least some areas of the plate incubated at 37°C for 24 hours.

Antibiotic susceptibility test of organisms isolated using the disc diffusion method

Cultures of the identified organisms on the basis of all the various tests carried out were streaked evenly on the Petri dishes with sterile cotton wool swabs. (Table 1)

Discs impregnated with two drops (5 mcg) each of ophthalmic topical solutions of Ciprofloxacin, Norfloxacin and Ofloxacin were placed on them with the aid of a flamed forceps, incubation was done for 24 hours at 34° C.

Zone of inhibition of growth ranging from 5 mm and above around the specific antibiotic disc indicated sensitivity to that particular antibiotic, while a total absence of such a zone of inhibition indicated complete resistance.

Statistical Analysis

A one way analysis of variance (ANOVA) was used to analyse the sensitivity of the three drugs. ANOVA post Hoc using Bonferroni was used to determine the source of significance.

RESULTS

Table 1 shows the characterization and identification of the bacterial isolates, while Table 2 shows the percentage occurrence of the isolated bacteria. *S. aureus* had the highest occurrence of 34% , while *H. influenza* and *E.coli* had the least occurrence of 9% each. Table 3 and Figs. 1-3 show the zones of inhibition of each drug against the bacterial isolates. Ciprofloxacin had the highest zones of inhibition on all the bacterial isolates followed by Ofloxacin and then Norfloxacin. In order to use a parametric test, the data obtained were transformed into their natural logarithms. The one factor ANOVA showed that the difference in zones of inhibition was statistically significant ($F_{2,15}$= 15.05, $p < 0.05$). Post Hoc using Bonferroni located Ciprofloxacin as the source of the significant declared in one factor ANOVA.

Table 1: Characterisation and identification of bacterial isolates
+ Positive Test; - Negative Test

Morphology	Gram stain	Catalase	Coagulase	Oxidase	Probable identity of isolates
Rods in short chains	-	+	-	-	*E. coli*
Cocci in pairs	-	-	-	+	*N. gonorrhoeae*
Rods in long chains and clusters	-	+	-	+	*P. aureuginosa*
Short rods in pairs	-	+	-	-	*K. pneumoniae*
Cocci in clusters	+	+	+	-	*S. aureus*
Cocci in chains	+	-	-	-	*S. pneumoniae*

Table 2. Percentage occurrence of isolated microorganism

	Micro Organism Isolated	Frequency	% Occurrence
1.	*Staphylococcus aureus*	34	34%
2.	*Streptococcus pneumoniae*	22	22%
3.	*Pseudomonas aeruginosa*	14	14%
4.	*Klebsiella pneumoniae*	12	12%
5.	*Haemophilus influenzae*	9	9%
6.	*Escherichia coli*	9	9%

Table 3. Zone of inhibition of each drug against isolated micro organism

	Micro Organism	Ciprofloxacin	Norfloxacin	Ofloxacin
1.	*Staphylococcus aureus*	17mm	11mm	14mm
2.	*Streptococcus pneumoniae*	16mm	12mm	14mm
3.	*Pseudomonas aeruginosa*	17mm	10mm	13mm
4.	*Klebsiella pneumoniae*	16mm	10mm	13mm
5.	*Hemophilus infuenzae*	18mm	12mm	15mm
6.	*Escherichia coli*	14mm	10mm	9mm

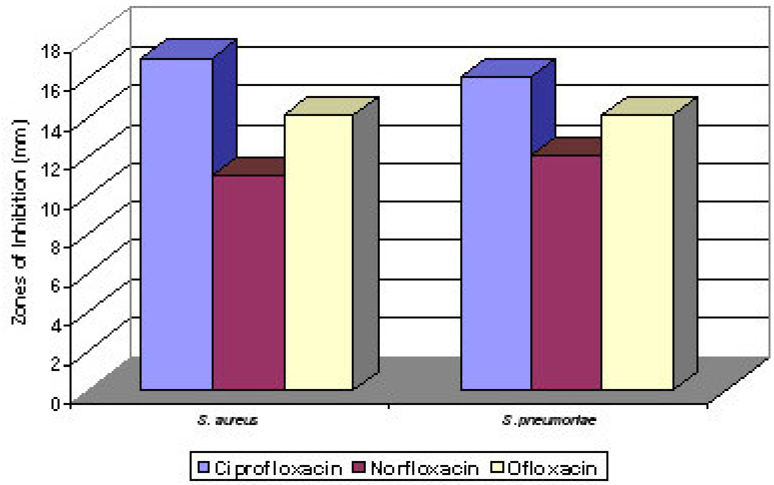

Fig 1: Histogram of zones of inhibition of *S. aureus* and *S. pneumoniae*

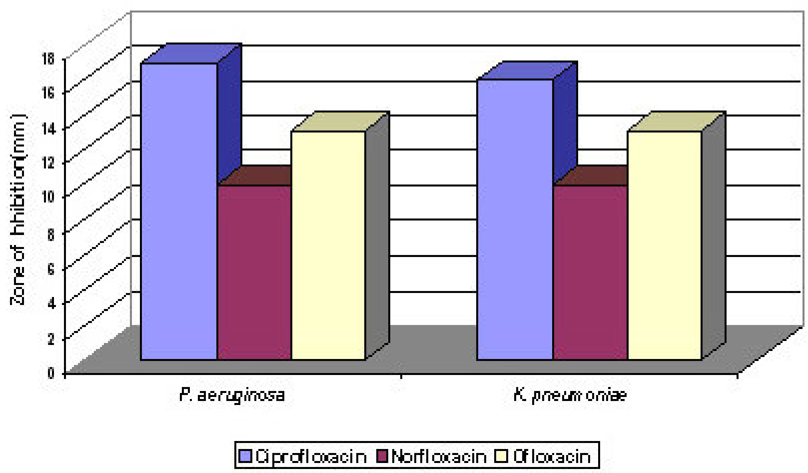

Fig 2: Histogram of zones of inhibition of *P. aeruginosa* and *K. pneumoniae*

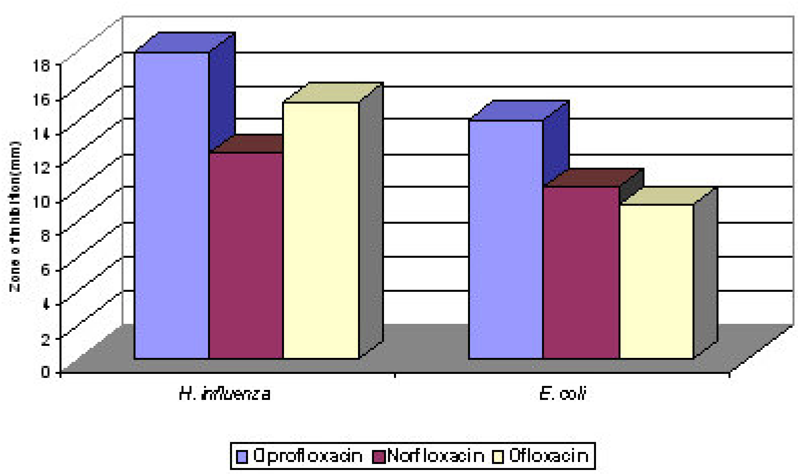

Fig 3: Histogram of zones of inhibition of *H. influenzae* and *E. coli*

Discussion

Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae and *Escherichia coli* were the bacteria pathogens isolated in order of decreasing frequency from the swabs taken to the laboratory. The cause of bacterial conjunctivitis is the alteration in the normal flora, which can occur by external contamination by spread from adjacent sites or via a blood – borne pathway. The primary defense against infection is the epithelial layer covering the conjunctiva. Disruption of this barrier can lead to infection.

Staphylococcus aureus having the highest prevalence amongst the organisms implicated is consistent with the claim that it is the most frequent cause of bacterial conjunctivitis worldwide.(9-11) The gram-positive bacteria isolated were *Staphylococcus aureus* and *Streptococcus pneumoniae* while the gram-negative ones were *Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae* and *Escherichia coli.*

In children, *Haemophilus*, *Streptococcus* and *S. aureus* are the common pathogens isolated. Streptococcal infections are self limiting and may occur in epidemics. They are more frequent in temperate climates and winter months and are associated with sub-conjunctival haemorrhages.(9) Conjunctivitis due to *Haemophilus influenzae* are often epidemic but may be endemic in warmer climates. They are also associated with subconjunctival haemorrhages.(1-3)

Ciprofloxacin had the highest sensitivity over the other two antibiotics as all the organisms isolated showed highest susceptibility to it followed by Ofloxacin and then Norfloxacin. This is consistent with the study carried out by Brower et al (5), which showed the high efficacy of Ciprofloxacin in the treatment of bacterial keratitis. Ciprofloxacin exhibited it's highest sensitivity on *Haemophilus influenzae* with a zone of inhibition of 18 mm. while it's least sensitivity was seen on *Escherichia coli* with 14 mm zone of inhibition. The highest susceptibility to Norfloxacin was exhibited by *S. pneumoniae* and *H. influenzae* with 12 mm zone of inhibition each. The highest susceptibility to Ofloxacin was exhibited by *H. influenzae* with a zone of inhibition of 15 mm.

Conclusion

The study has shown that *Staphylococcus aureus* is the most causative micro-organism implicated in bacteria conjunctivitis. All six isolated bacteria were susceptible to flouroquinolone with Ciprofloxacin as the most sensitive drug, followed by Ofloxacin, and the least was Norfloxacin. Thus Ciprofloxacin is the most effective of the three drugs and it is therefore recommended as the best choice of topical fluoroquinolone antibiotic for the treatment of bacterial conjunctivitis.

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