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IN-VITRO EFFICACY OF ANTIMICROBIAL AGENTS USED IN THE TREATMENT OF BACTERIAL EYE INFECTIONS IN IBADAN, NIGERIA

[1]D. Olusoga Ogbolu, [1]O. A. Terry Alli, [1]Ephraim I. E, [2]F. A. Olabiyi, [3]O. A. Daini
[1]Department of Biomedical Sciences, College of Health Sciences, (Osogbo Campus), Ladoke Akintola University of Technology, Ogbomoso.
[2]Department of Chemical Pathology, University College Hospital, Ibadan
[3]Department of Biochemistry, College of Health Sciences, Olabisi Onabanjo University, Ago-Iwoye, Remo Campus, Ikenne, Nigeria.

Running Title: *In-vitro* efficacy of antimicrobial agents in Ibadan, Nigeria

Correspondence: Ogbolu D. Olusoga (olusogadave@yahoo.com)

Abstract
Failure to cure eye infections, and reduced potency in topical antimicrobials had been observed in South Western Nigeria, this study sought to evaluate *in vitro*, the efficacy of antimicrobial agents in the treatment of ocular infections. A total of 46 ocular bacterial isolates were recovered from the diagnostic laboratory of the University College Hospital, Ibadan, from conjunctival swabs of patients having underlying eye diseases (Cataracts, glaucoma and esotrapia), and from patients presenting with other symptoms of eye infections. The pathogens incriminated were *Staphylococcus aureus* (73.5%), Coagulase negative *Staphylococci* (13.3%), *Klebsiella* species (10.3%), and *Pseudomonas aeruginosa* (2.0%). Disc diffusion tests (Bauer-Kirby method) were carried out using ciprofloxacin, gentamicin, chloramphenicol, erythromycin, augmentin, cefuroxime and levofloxacin. Broth dilution techniques were thereafter performed using gentamicin, chloramphenicol and ciprofloxacin. The microlide- erythromycin was 63.0% efficacious, augmentin and cefuroxime showed 71.1% and 76% efficacy. Minimum inhibitory concentrations (MIC) of commonly used topical antibiotics however showed different levels of resistance. Resistance to the aminoglycosides was marked, yielding 53.4% with MIC50= 8, MIC90 > 256, Resistance to chloramphenicol was even more marked 69.6% with MIC50= 16, MIC90= 64, the fluoroquinolones showed high efficacy- levofloxacin and ciprofloxacin showed 93.4% and 82.6% susceptibility respectively with MIC50 < 0.5, though slightly demonstrable resistance was observed (MIC90= 8). The study thus recommends the discontinuation of empirical therapy by physicians in order to stem the tide of resistance; it justifies the inclusion of the fluoroquinolones in susceptibility testing of ocular bacterial isolates, and its first line of choice if cure is warranted.

Introduction

Ocular bacterial flora includes *Corynebacterium xeroisis, Moraxella catarrhalis,* and *Staphylococcus epidermidis* (1). The coagulase negative *staphylococci,* a subject of debate in the 1980s, regarding its role in pathogenicity, are remarkable for its opportunism. It has thus been incriminated in chronic blepharitis (2), corneal ulcers, and endophthalmitis after traumatic eye surgery (3). Globally, *S. aureus* is the leading cause of conjunctivitis (3). The incidence of methicillin resistant *Staphylococcus aureus* (MRSA) in ocular infections is on the rising side (4). In Onitsha, Nigeria, *S. aureus* is the leading cause of conjunctivitis and keratitis (5). *Pseudomonas aeruginosa* is also leading causes of corneal ulcers especially among contact lens wearers (6). *Neisseria gonorrheae* and *Chlamydia trachomatis* cause severe conjunctivitis in the newborn - ophthalmia neonatorum (7). *Haemophilus species, Streptococcus pneumoniae* (8) and non fermenting *coliforms* have been implicated in ocular infections (6).

The aminoglycosides gentamicin and tobramycin are well established as first-line therapy for external ocular infections, and possess a broad spectrum of activity against Gram positive and Gram negative organisms (9,10). However, resistance to these antibiotics has been reported. For example, resistance to topical aminoglycoside therapy may be encountered in as many as 8% to 10% of ulcerative keratitis cases caused by *Pseudomonas aeruginosa* (11). Resistance appears to be even greater in ocular infections caused by Gram positive organisms (12). The *in-vitro* studies of antibacterial susceptibility tests by various authors have shown increasing resistance of commonly used antibacterials; gentamicin (21%) (4,13), chloramphenicol, though potent against MRSA strains, has just been demonstrated to show significant reduction in its bacteriostatic action in Europe (14.1%), (14). However, ciprofloxacin, which is still comparatively the most efficacious, has also shown reduced potency; resistance at 35% was shown in Pittsburg, USA (15), ciprofloxacin has also shown reduced potency against ocular MRSA isolates in the United States (16).

These differing levels of resistance impel an evaluation of these drugs in Nigeria in order to ascertain their efficacy and have a documented level of susceptibility to these agents. The study was therefore aimed at evaluating *in-vitro* susceptibility patterns of ocular clinical isolates to commonly used antibiotics, with emphasis on gentamicin, chloramphenicol and ciprofloxacin due to the availability of their topical applications (eye drops).

Materials and Methods

Bacterial strains

A total of 46 bacterial isolates were isolated by standard procedures (17) from 136 eye swabs and scrapings sent to the diagnostic laboratory of Medical Microbiology and Parasitology department, University College Hospital, Ibadan from January to October 2009.

Disc susceptibility testing

Varying concentrations of antibiotics discs; gentamicin (10 µg), ciprofloxacin (10 µg), methicillin (5 µg), chloramphenicol (10 µg), erythromycin (5 µg), ampicillin (10 µg), cloxacillin (5µg), cefuroxime (30 µg), augmentin (10 µg), and levofloxacin (10 µg) were used. Inhibition zone diameters around the discs were measured to the nearest millimetre using a calibrated transparent ruler. The susceptible inhibition zone diameter break point used throughout the study for each antibiotic to the various organisms was based on CLSI recommendation (18). The diameters of the zone of inhibition were recorded. Growth within the zone of inhibition was recorded as resistant (18). Sensitivity patterns for *Staphylococcus*, *Pseudomonas* and *Klebsiella* were compared with the standard *S. aureus* ATCC 29213; *P. aeruginosa* NCTC 10662 and *E. coli* NCTC 10418, respectively.

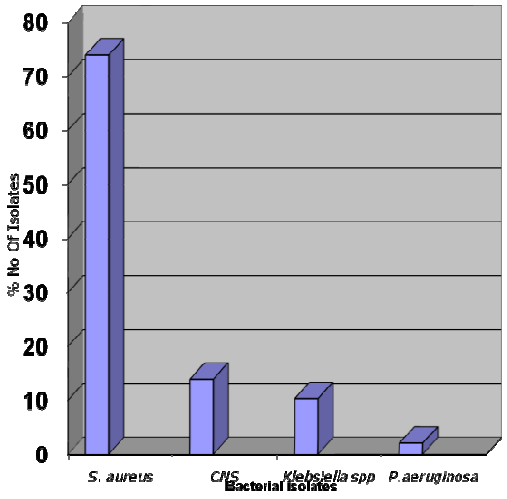
Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of 3 antibiotics; gentamicin, chloramphenicol and ciprofloxacin for all the bacterial strains was determined as described by Goldstein and Acar (19). The antibiotics were supplied in powdery formulations, namely; gentamicin, chloramphenicol and ciprofloxacin by SIGMA-ALDRICH, U.K. Serial doubling dilutions of these antibiotics were made, ranging from 0.0625 to 512 µg/ml. A drop (0.02 ml) of standard inoculum (0.5 Macfarland) of organisms was introduced and these were then incubated at 37˚C for 18 hours. MIC was interpreted as the least concentration or highest dilution with no observable turbidity. Controls were set up namely; sterility control: Mueller Hinton broth only, viability control: Mueller Hinton broth and test organism, positive control: Mueller Hinton broth with antibiotics and the control organisms. They were incubated at 37ºC overnight.

Result

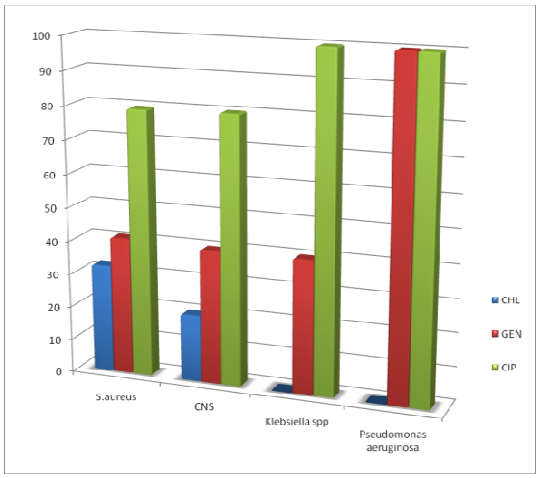
Bacteria isolates recovered were *Staphylococcus aureus* 34 (73.9%), coagulase negative *staphylococci* (CNS) 6 (13%), *Klebsiella* species 5 (10.8%) and *Pseudomonas aeruginosa* 1 (2.2%). The distribution of the various isolates with underlying eye conditions, and ocular infections are shown in Figure 1.

Figure 1: Percentage (%) distribution of conjunctival bacterial isolates.



All the strains examined showed resistance to one or more of the eight antibiotics used for this study. The results depicted a high level resistance. The fluoroquinolones showed slightly lower level of resistance than the rest of the antibiotics including the third generation cephalosporins. More isolates were sensitive to levofloxacin (93.4%) and ciprofloxacin (76%) than all the antibiotics tested (Table 1). Similarly, susceptibility of the strains to methicillin was 10.4%; 30.4% for chloramphenicol while gentamicin had 41.3% (Figure 2). It is noteworthy that chloramphenicol had no activity against *Klebsiella* species and *Pseudomonas aeruginosa* in this study.

Figure 2: Percentage susceptibility to chloramphenicol, gentamicin and ciprofloxacin (disk diffusion test).



The MIC results also showed that the level of resistance to many antibiotics was high. MIC50 and MIC90 of ciprofloxacin were lower than the rest of the antibiotics. MIC_{90} of gentamicin to the strains was very high with value >256 µg/ml (Table 2).

Discussion

The study showed *S. aureus* as the most frequently incriminated conjunctival pathogen, this is consistent with previous studies in Nigeria (20), and outside the country (21). Methicillin-resistant *S. aureus* (MRSA) and Methicillin resistant coagulase negative *staphylococci* (MRCNS) are intraocular pathogens. They all however have a common source; the anterior nares (22), which via the nasolacrimal duct, may reach the conjunctiva or infect deeper ocular structures; endophthalmitis may ensue (23). The isolation of MRSA from patients having cataracts and glaucoma (92.3%) is therefore alarming. The definitions of these conditions do not presuppose a microbial cause. Their incrimination may thus imply the likelihood of subsequent infections that further destroy ocular tissues, and increased susceptibility of these patients to ocular infections. Ultimately, if it be ascertained that these pathogens are simply microflora, it should be alarming from a general epidemiological perspective of resistance, as well as the serious dangers posed these patients if a surgery would be carried out to correct these conditions.

Table 1: Susceptibility patterns of bacterial pathogens using disc diffusion

Organism	MET			CHL			GEN			CIP			ERY			AUG			LEV			CXM		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
S. aureus (34)	3	0	31	11	0	23	14	2	18	26	2	6	24	0	10	27	0	7	31	0	3	30	0	4
CNS (5)	2	0	3	1	0	4	2	0	3	4	0	1	3	0	2	3	0	2	5	0	0	3	0	2
Klebsiella spp (5)	0	0	5	0	0	5	2	0	3	4	0	1	2	0	3	2	0	3	5	0	0	2	0	3
P. aeruginosa (1)	0	0	1	0	0	1	1	0	0	1	0	0	0	0	1	1	0	0	1	0	0	0	0	1

MET- methicillin, CHL- chloramphenicol, GEN- gentamicin, CIP- ciprofloxacin, ERY-erythromycin, AUG- augmentin, LEV- levofloxacin, CXM- cefuroxime, S- susceptible, I- intermediate, R- resistant.

Table 2: Minimum inhibitory concentrations of common antimicrobials to the pathogens

Organism	Antimicrobial Agent	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)
MRSA (30)	Chloramphenicol	24	64	0.25 – 256
	Gentamicin	8	> 256	0.25 – 256
	Ciprofloxacin	0.25	8	0.25 – 256
MSSA (3)	Chloramphenicol	16	32	0.25 – 256
	Gentamicin	4	> 256	0.25 – 256
	Ciprofloxacin	0.25	8	0.25 – 256
Klebsiella spp (5)	Chloramphenicol	8	16	0.25 – 256
	Gentamicin	64	> 256	0.25 – 256
	Ciprofloxacin	2	8	0.25 – 256
P. aeruginosa (1)	Chloramphenicol	-	64	0.25 – 256
	Gentamicin	-	64	0.25 – 256
	Ciprofloxacin	-	4	0.25 – 256

The coagulase negative staphylococci generate fewer controversies on its pathogenicity these days. They have been incriminated in chronic blepharits (2), Keratitis (24), and endophthalmitis (22). In Nigeria, the pathogenicity of the CNS has been established and is incriminated in various disease conditions; Ogbolu *et al.* thus advocate processing when isolated from repeated cultures (25). Their incrimination and inclusion in this study is thus not surprising, and their resistance patterns justify the discourse, which further show that resistance mechanisms are equally been evoked by these organisms, thus increasing their endemic status and enhancing their pathogenicity, especially when they reach deeper structures.

Most ocular isolates showed resistance using disc diffusion techniques to gentamicin (54.6%) but more marked levels of resistance was demonstrated (MIC_{50} = 32, MIC_{90} >256 µg/ml) by broth dilution technique. Resistance to Gram negative rods and *Staphylococcus aureus* including MRSA strains was demonstrated. Third generation fluoroquinolone - levofloxacin was most efficacious using the disc diffusion test technique (93.4%). The efficacy of this drug buttresses the reliability of the fluoroquinolones against conjunctival bacterial pathogens, especially the MRSA. Ciprofloxacin also had demonstrable clinical efficacy using both techniques. Susceptibility of MRSA strains was 83% using disc diffusion test, confirmed by broth dilution techniques (MIC_{50} < 0.5). Resistance was however also demonstrated, with MIC_{90} = 8.0 µg/ml. These results are in sharp variance with Kotlus *et al*'s observation in the United States, where 94% resistance to ciprofloxacin was observed, with MIC_{50} = 8.0 µg/ml, in the study, gentamicin was most efficacious (16). The efficacy of ciprofloxacin has also been demonstrated in Nigeria, this conforms to the study of Idu *et al.* where it was found to be most potent among other topical fluoroquinolones (20).

Abuse of fluoroquinolones in the U.S may have led to the potent drug, whereas it's comparatively new status in Nigeria still ensures its potency.

On definitive prophylaxis pre-ocular surgery, this study may be quick to recommend the use of ciprofloxacin in topical formulations in Nigeria. However, discouragement of empirical therapy for the treatment of ocular infections is essential considering the increasing levels of resistance to commonly used antibiotics as has been shown in this study. The study affirms the high levels of the superbug MRSA among these species in eye infections in Nigeria, having varying resistance patterns. It also quantitatively validates reports of significantly reduced potency to commonly used topical antimicrobials - gentamicin and chloramphenicol. It high rate of resistance to this 1 however shows the fluoroquinolones as unparalleled in the treatment of ocular infections. We therefore advocate for discontinuation of empirical therapy by physicians, and advise isolation of the causative bacterial agents, and subsequent susceptibility testing which should include the fluoroquinolones. The inability to isolate typically fastidious ocular pathogens like *Streptococcus pneumoniae, Haemophilus influenzae,* and *Haemophilus aegypticus,* should impel a clamour for the use of transport swabs in routine diagnosis of eye infections. Periodic re-evaluation of antimicrobial agents is essential in order to guide therapy, as well as to track and monitor resistance by organisms in this sensitive organ of vision.

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