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In vitro Evaluation of antibacterial agents against ocular Bacterial isolates from a Tertiary Hospital, South-West of Nigeria.

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ABSTRACT

Failure to cure eye infections and reduced potency in ocular antibacterial agents had been observed in South Western Nigeria, this study sought to evaluate in vitro, the efficacy of antibacterial agents used in the treatment of eye infections. A total of 135 bacterial isolates were recovered from the diagnostic laboratory of the University College Hospital, Ibadan, from conjunctival swabs of patients having underlying eye diseases (Cataracts and glaucoma), and from patients presenting with other symptoms of eye infections (conjunctivitis, keratitis and dacryocystitis). The pathogens incriminated were Staphylococcus aureus (75.5%), Coagulase negative Staphylococci (11.1%), Klebsiella species (11.1%), and Pseudomonas aeruginosa (2.2). Disc diffusion tests (Kirby-Bauer method) were carried out using ciprofloxacin, gentamicin, chloramphenicol, erythromycin, amoxicillin-clavulanate, cefuroxime and levofloxacin. Broth dilution technique was thereafter demonstrated using gentamicin, chloramphenicol and ciprofloxacin. The macrolide-erythromycin was 54.4% efficacious, amoxicillin-clavulanate and cefuroxime showed 69.9% and 72.8% efficacy. Minimum inhibitory concentrations (MIC) of commonly used antibiotics however showed different levels of resistance. Resistance to the aminoglycosides was marked, yielding 51.6%, with MIC_{50} = 8, MIC_{90} > 256, resistance to chloramphenicol was also marked, yielding 76.9%, with MIC_{50} = 8, MIC_{90} = 64. The fluoroquinolones showed high efficacy; levofloxacin and ciprofloxacin showed 91.1% and 75.5% susceptibility respectively, with MIC_{50} < 0.5, though slightly demonstrable resistance was observed (MIC_{90} = 8). This study recommends 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 bacterial isolates and its first line of choice if cure is warranted.

Keywords: Eye infections, antibacterial agents, MIC (Minimum inhibitory concentration), efficacy, Resistance.

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INTRODUCTION

The eye, the basic organ of vision is in constant exposure to the external environment, which is a reservoir of microorganisms. While the eye is relatively impermeable to microorganisms, if structural damage occurs, sight threatening bacterial and fungal infections easily develops [1]. It is unlikely therefore that any infection would ensue if the pre-corneal tear film and corneal integrity are intact [2]. Tears aided by the blink reflex, constantly bath the eyes and also contain the antibacterial lysozyme, lactoferrin, secretory immunoglobulins and defensins [3], with which it ensures 'near sterility' in this organ.

Mechanical damage may therefore be essential in the aetiology of bacterial infections in the anterior segment of the eye, resulting in conjunctivitis or even bacterial Keratitis. Infection in the posterior segment of the eye could ensue by one of these means; (i) as a consequence of intra-ocular surgery [4], (ii) penetration of the globe [5] (iii) via haematogenous spread of bacteria from distant anatomical site, this could result in endophthalmitis [12]. Endophthalmitis is a devastating eye complication that can occur following an intraocular surgery [17], and been vision threatening, it requires prompt antibiotic therapy [1].

Globally, Staphylococcus aureus is the leading bacterial agent incriminated in ocular infections [6 and 16]. The incidence of methicillin resistant Staphylococcus aureus (MRSA) in ocular infections is on the rising side [7]. In Onitsha-Nigeria, Staphylococcus aureus is the leading cause of conjunctivitis and Keratitis [8]. Staphylococcus aureus is also as the leading cause of conjunctivitis in South Western Nigeria [9, 15].

Aside bacterial agents, eye infections may have fungal or viral aetiologies; irritations in this organ could also be due to allergies [1]. This is considered by most persons as one which can be cured by 'eye drops', without a consideration of its constituents. Thus self-prescription and over-the-counter administration of antibiotics may have contributed to the emergence of resistant bacterial strains.

On a cosmopolitan scale, due to resistance, reduced efficacy has been associated with gentamicin (21%) [7], Chloramphenicol, though potent against MRSA strains, has shown reduced efficacy in Europe (14.1%) [10], However, ciprofloxacin, which is still comparatively the most efficacious, has also shown reduced potency; resistance at 35% was shown in Pittsburgh [11], ciprofloxacin has also shown reduced potency against MRSA isolates in United States (94%, MIC_{50} = 8.0) [12]. Paucity of reports of reduced antibacterial activities of these drugs in topical and systemic formulations has also been noted in South Western Nigeria. All these differing levels of susceptibility impel a re-evaluation of these antibiotics, so as to ascertain their efficacy, and have a documented level of susceptibility to these agents in our locality, where abuse of antibiotics even in topical formulations has been observed.

This study is therefore aimed at evaluating in vitro, the susceptibility pattern of ocular clinical isolates to commonly used antibacterial agents, with emphasis on gentamicin, chloramphenicol and ciprofloxacin in Nigeria.

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MATERIALS AND METHODS

Sources of clinical Specimens:

Clinical specimens' namely conjunctival swabs were routine samples received by the diagnostic laboratory of University College Hospital (UCH) Ibadan, a referral health institution in South Western Nigeria. These specimens were received from Ophthalmology Clinic and Out Patient Departments of the hospital, from patients presenting with varying symptoms of eye infections between July 2009 and June 2010.

Isolation and identification:

Specimens were inoculated onto 5% lysed blood agar almost immediately. Blood and Mac-conkey agar were also inoculated and streaked. These were incubated aerobically at 37˚C, and the lysed blood agar incubated under microaerophillic conditions with 5-10% CO_2 at 37˚C for 18 hours. Smears made from clinical specimens were stained by Grams technique.

The cultural characteristics of the strains recovered were determined together with Gram reaction for species identification. Further identification and biochemical characterisation was done using standard microbiological techniques [13].

Antimicrobial susceptibility testing:

In vitro antibacterial susceptibility tests were performed on each isolate using antibacterial drugs. Plates were prepared with Mueller Hinton's agar for use in the Kirby-Bauer method. Broth cultures were adjusted to turbidity standard, equivalent to McFarland 0.5. This was then used for disc diffusion according to CLSI (Clinical and Laboratory Standard Institute) criteria, using erythromycin (5 µg), gentamicin (10 µg), ciprofloxacin (5 µg), amoxicillin-clavulanate (10 µg), Chloramphenicol (10 µg) levofloxacin (5 µg), cefuroxime (30 µg) (all from Oxoid, United Kingdom). Methicillin-resistance of the strains was determined by the Kirby-Bauer-disk diffusion method using 1 µg oxacillin disk according to the criteria of the Clinical and Laboratory Standards Institute [14].

Quantitative validation of antimicrobial susceptibility was carried out using broth dilution technique. Overnight broth cultures were chosen based on susceptibility pattern in the Bauer-Kirby technique. 512 µg/ml antibiotic concentrations were prepared using the specific diluents (ethanol, distilled water and 10% glacial acetic acid for chloramphenicol, gentamicin and ciprofloxacin respectively); doubly dilutions were now made to cover the range 256 µg/ml-0.5 µg/ml. The standard inoculums of organisms were introduced and these were incubated at 37˚C for 18 hours. MIC was interpreted as the least concentration or highest dilution with observable turbidity.

Controls were set up namely; Sterility control: Mueller Hinton's broth only, Viability control: Mueller Hinton's broth and test organism, Positive control: Antibiotic and Staphylococcus aureus NCTC 6571. The antibiotics were supplied in powdery formulations by SIGMA (United Kingdom).

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RESULTS

During the period under study, 138 bacterial isolates were identified, and the antimicrobial susceptibility tests performed, using commonly used antibiotics, with an emphasis on gentamicin, chloramphenicol and ciprofloxacin. About 55.4% of isolates recovered were from patients having severe cataracts and glaucoma. Other isolates recovered were from patients presenting with other eye infections; conjunctivitis (37.7%), Keratitis (2.2%) and Dacryocystitis (4.4%).

Bacterial isolates recovered were Staphylococcus aureus 102 (75.5%), Coagulase negative Staphylococci 15 (11.0%), Klebsiella species 15 (11.0%), and Pseudomonas aeruginosa 3 (2.2%). The distribution of the various bacterial isolates from patients with underlying eye conditions, and ocular infections are shown in Table 1. A histogram showing the distribution of bacterial isolates is shown in figure 1.

Table 1: Bacterial isolates from ocular tissues

Clinical Condition	*S. aureus*	CNS	*Klebsiella. Species*	*Pseudomonas aeruginosa*	Total
Opthalmic condition					
Cataracts	48 (35.6)	6 (4.4)	9 (6.6)	-	63 (46.6)
Glaucoma	9 (6.6)	3 (2.2)	-	-	12 (8.8)
Eye infection					
Conjunctivitis	36 (26.7)	6 (4.4)	6 (4.4)	3 (2.2)	54 (37.7)
Keratitis	3 (2.2)	-	-	-	3 (2.2)
Dacryocystitis	6 (4.4)	-	-	-	6 (4.4)
Total	102 (75.5)	15 (11.0)	15 (11.1)	3 (2.2)	135

( ) = value on percentage, CNS-Coagulase negative Staphylococci

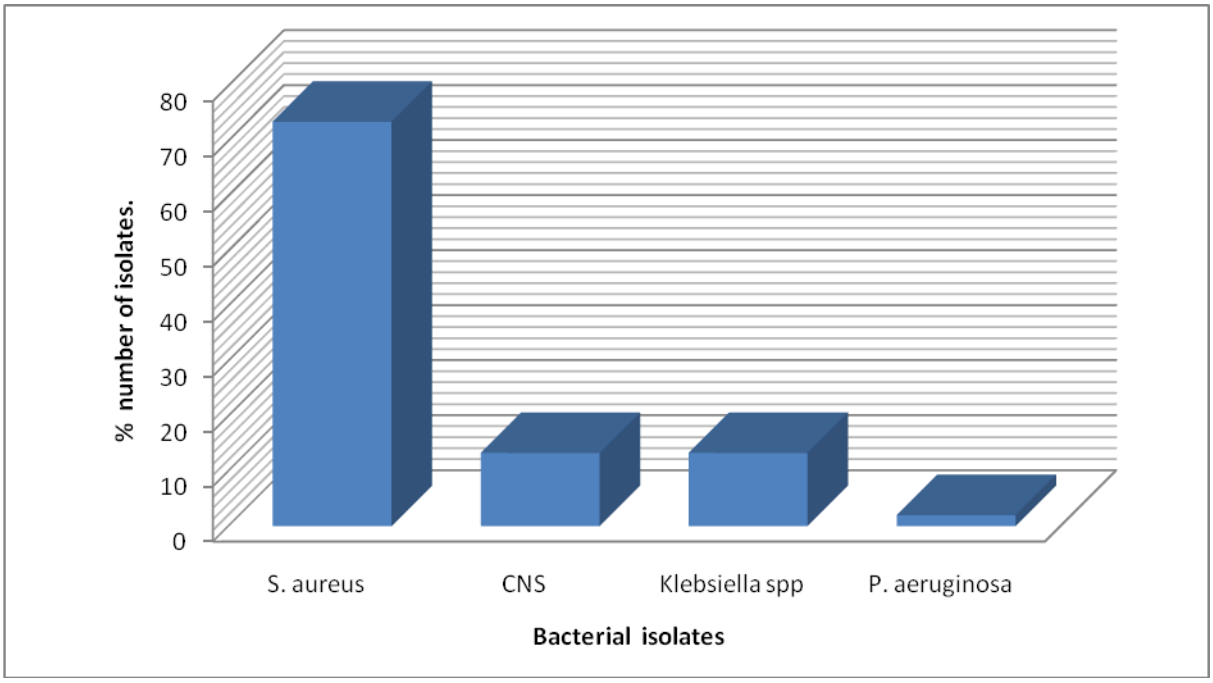

Fig 1

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Table 2 shows the antibacterial profile to commonly used antibiotics using the disk diffusion test (Kirby-Bauer technique). Susceptibility of isolates to levofloxacin was 91.1%, erythromycin was 54.4% efficacious. Amoxicillin-clavulanate showed 69.9% potency and susceptibility of isolates to cefuroxime was 72.8%. Bacterial strains recovered showed susceptibility to oxacillin (12.4%), 23.1% of isolates showed susceptibility to chloramphenicol, 48.4% showed susceptibility to gentamicin and susceptibility to ciprofloxacin was 75.5%.The percentage resistance of bacterial isolates is shown in table 3, summarily, the average resistance to Chloramphenicol and gentamicin was 76.9% and 51.6% respectively.

Table 2: Susceptibility pattern of Bacteria isolated using Disc diffusion.

Bacterial isolates	OX S I R	CHL S I R	GEN S I R	CIP S I R	ERY S I R	AUG S I R	LE S I R	CXM S I R
Staphylococcus aureus (102)	9, 0, 93	33, 0, 69	52, 6, 54	78, 6, 18	72, 0, 30	81, 0, 21	93, 0, 9	90, 0, 12
CNS (15)	6, 0, 9	6, 0, 9	6, 0, 9	12, 0, 3	9, 0, 6	9, 0, 6	14, 0, 1	9, 0, 6
Klebsiella species (15)	0, 0, 15	2, 1, 12	6, 0, 9	12, 0, 3	6, 2, 7	6, 0, 9	13, 0, 2	6, 0, 9
Pseudomonas aeruginosa (3)	0, 0, 3	0, 0, 3	1, 1, 1	2, 0, 1	0, 1, 2	3, 0, 0	3, 0, 0	0, 0, 3

Ox– oxacillin, CHL – Chloramphenicol, GEN – gentamicin, CIP – ciprofloxacin, ERY – erythromycin, AUG – amoxicillin – clavulanate, LE – levofloxacin, CXM – cefuroxime.
Key: S- susceptible, I- intermediate, R- resistant. Value in parentheses = number of isolates, CNS-As in table 1.

Table 3: Percentage Resistance of Bacterial Isolates.

Bacterial isolates	OX	CHL	GEN	CIP	ERY	AUG	LE	CXM
Staphylococcus aureus (102)	91.2	67.6	53.0	17.6	29.4	20.5	8.8	9.0
CNS (15)	60.0	60.0	60.0	20.0	40.0	40.0	6.6	40.0
Klebsiella species (15)	100	80	60	20.0	46.6	60.0	13.3	60.0
Pseudomonas aeruginosa (3)	100	100	33.3	33.3	66.6	0.0	0.0	0.0

Value in parentheses = number of isolates, Ox– oxacillin, CHL – Chloramphenicol, GEN – gentamicin, CIP – ciprofloxacin, ERY – erythromycin, AUG – amoxicillin – clavulanate, LE – levofloxacin, CXM – cefuroxime, CNS-As in table 1.

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Table 4: MIC's of Bacterial Isolates.

Bacterial isolates	MIC_{50}	MIC_{90}	Range (µg/ml)
	Chloramphenicol		
MRSA (93)	16.0	64.0	1.0 – 512.
CNS (15)	4.0	32.0	1.0 – 512.
Klebsiella species(15)	8.0	16.0	1.0 – 512.
Pseudomonas aeruginosa (3)	32.0	64.0	1.0 – 512.
	Gentamicin		
MRSA (93)	8.0	> 256	0.5 – 256.
CNS (15)	2.0	> 256	0.5 – 256.
Klebsiella species (15)	64.0	> 256	0.5 – 256.
Pseudomonas aeruginosa (3)	4.0	64.0	0.5 – 256.
	Ciprofloxacin		
MRSA (93)	< 0.5	8.0	0.5 – 256.
CNS (15)	< 0.5	8.0	0.5 – 256.
Klebsiella species (15)	1.0	8.0	0.5 – 256.
Pseudomonas aeruginosa (3)	1.0	4.0	0.5 – 256.

Value in parentheses = number of isolates, MRSA – Methicillin resistant Staphylococcus aureus, CNS- As in table . 1

Using broth dilution techniques, MIC_{50} and MIC_{90} was determined for the various organisms, and the results are seen in table 4. Ciprofloxacin was most efficacious using this technique, with MIC_{50} < 0.5 µg/ml, MIC_{90} = 8.0 µg/ml. Gentamicin had the poorest demonstrable antibacterial activity, with MIC_{50} > 2.0 µg/ml, MIC_{90} > 256 µg/ml, except for the Coagulase negative Staphylococci, with MIC_{50} = 2.0 µg/ml.

DISCUSSION

This study sought to evaluate in vitro, the efficacy of antimicrobial agents used in the treatment of ocular infections. Drug resistant species were thus incriminated in this sensitive organ of vision, commonly used antibacterial agents-namely gentamicin and Chloramphenicol, were shown to be of low efficacy, this study however places the fluoroquinolones as unparalleled in the treatment of ocular infection.

Staphylococcus aureus was the most frequently incriminated ocular pathogen during this study. This is consistent with previous studies in Nigeria [9, 15], and outside the country [16]. Methicillin resistant Staphylococcus aureus (MRSA) and Methicillin Resistant Coagulase Negative Staphylococci (MRCNS) were also incriminated during this study. MRSA's and MRCNS' are intraocular pathogens. They all however have a common source; the anterior nares [18], which via the nasolacrimal duct, may reach the conjunctiva. The isolation of MRSA's from patients having glaucoma and cataracts is therefore alarming. The aetiopathogenesis of these conditions does not presuppose a microbial cause. Their incrimination may imply an increased susceptibility of these patients to subsequent eye infections. Ultimately, if it were ascertained that these organisms are ocular microflora, the result would still be alarming from an epidemiological perspective of resistance, as well as the dangers posed these patients if a surgery is carried out to correct these conditions, since vision-threatening endophthalmitis is not an uncommon complication accompanying the procedure [25].

The Coagulase negative Staphylococci (CNS) generate fewer controversies on its pathogenicity these days. They have been incriminated in chronic blepharitis [19], Keratitis [20] and endophthalmitis [17]. In Nigeria, the pathogenicity of the CNS has been established and is incriminated in various disease conditions; antimicrobial susceptibility testing has been advocated when isolated from repeated cultures [21]. Their incrimination and inclusion in this study is not surprising, and their resistance pattern justifies the discourse.

Bacterial isolates showed differing levels of resistance to gentamicin in the disc diffusion technique (51.6%). More pronounced levels of resistance were however noticed using broth dilution technique (MIC_{90} > 256 µg/ml). The gram negative rods also showed marked levels of resistance to the drug. While Khosravi et al reports wide coverage of the drug against gram negative rods and MRSA's in Iran [20], this study repudiates any such assertion in South Western Nigeria, and infers a slightly moderate activity.

Resistance to the most frequently used topical antimicrobial in this region-chloramphenicol was demonstrated. About 76.1% of bacterial isolates were resistant in the disc diffusion technique. Resistance was also shown in MRSA's using broth dilution technique to this drug, was once renowned for its bacteriostatic activity against the MRSA's. The gram negative rods also showed demonstrable resistance. Chloramphenicol has been known to induce blood dyscrasias (resulting from bone marrow aplasia) even in topical formulations [22, 24], resulting deaths have been reported [22, 23]. The number of chloramphenicol induced blood dyscrasia cases in Nigeria can best be imagined. Self-medication and over-the-counter administration of antibiotics accompanied with poor documentation of patient history could be contributory factors, since clinicians would hardly ask if patients had previously used the drug. Considering the high MIC's demonstrated in this study, individuals engaged in self- prescription may find themselves increasing the frequency of usage of the drug in topical formulation, having observed poor outcome.

The third generation fluoroquinolone-levofloxacin was most efficacious using disc diffusion technique. The efficacy of this drug buttresses the reliability of the fluoroquinolones against conjunctival pathogens, especially the MRSA's. Ciprofloxacin also had demonstrable clinical efficacy using both techniques. Susceptibility of MRSA strains was marked, confirmed by broth dilution techniques (MIC_{50} < 0.5 µg/ml). Resistance was

however also demonstrated, with MIC_{90} = 8.0 µg/ml. These results are however in sharp variance with Kotlus et al's observation in the United States, where resistance to ciprofloxacin was observed, with MIC_{50} = 8.0 µg/ml; in the study, gentamicin was most efficacious [12]. The efficacy of ciprofloxacin has also been demonstrated in this institution using disc diffusion tests [9], and in South Western Nigeria, where it was most potent among other first and second generation fluoroquinolones tested [15]. Abuse of fluoroquinolones in the United States may have led to induction of resistance mechanisms in this potent drug, whereas it's comparatively new status in Nigeria still ensures its potency.

On definitive prophylaxis before ocular surgery which remains inconclusive [25], this study may be quick to recommend the use of the ciprofloxacin and the third generation fluoroquinolones in topical and systemic formulations in South Western Nigeria. However, discouragement of empirical therapy is essential considering the increasing levels of resistance to commonly used antibiotics as demonstrated in this study.

In conclusion, this study confirms Staphylococcus aureus as the most common organism causing eye infections; it affirms high levels of the superbug MRSA's among these species in eye infections in Nigeria, having varying resistance patterns. It also quantitatively validates reports of reduced potency to commonly used topical antimicrobials-gentamicin and chloramphenicol. It however places the fluoroquinolones as unparalleled in the treatment of eye infections.

We recommend the use of transport swabs in routine diagnosis of eye infections in Nigeria, since typically fastidious ocular pathogens like Streptococcus pneumonia, Haemophilus influenza and Haemophilus aegyptius were not isolated during the study. We also advocate discontinuation of empirical therapy by physicians, and advise isolation of causative bacterial agents, and subsequent susceptibility testing, which should include the fluoroquinolones.. Periodic re-evaluation of antimicrobial agents is essential in order to guide therapy, as well as track and monitor resistance by organisms in this sensitive organ of vision.

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