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ORIGINAL ARTICLE

BACTERIAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS FROM EXTERNAL OCULAR INFECTIONS AND ASSOCIATED RISK FACTORS AT THE ALL AFRICA LEPROSY REHABILITATION AND TRAINING CENTER (ALERT), ADDIS ABABA, ETHIOPIA.

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ABSTRACT

Background: *Bacteria are major causative agents that frequently cause infections of the eyes and loss of vision. Resistance of bacteria isolated from ocular infections to antimicrobial agents is a global concern.*

Objective: *The aim of this study was to determine the prevalence of different bacterial isolates and their antimicrobial susceptibility pattern in patients with external ocular infections and to determine any associated risk factors.*

Methods: *A cross sectional study was conducted from May to August 2015 in ALERT outpatient and inpatient department of Eye Clinic. A total of 288 samples were collected which were processed for bacterial culture according to standard procedures. Presumptive isolates were further identified by a series of biochemical tests. The antimicrobial susceptibility patterns of the isolates were determined by the disk diffusion method. The data were entered and analyzed using SPSS software version 20.*

Results: *A total of 288 patients were enrolled. The overall prevalence of bacterial pathogens among external ocular samples was 171/288 (59.4%). Gram-positive bacteria were the most common isolates accounting for 70.2% (120/171). Staphylococcus aureus was present in 36.8% of the cases (63/171). Most (91.6%) of the bacteria isolated showed high resistance to Penicillin (120/131) and Tetracycline (70.4%; 119/169). Gentamicin was the most effective antibiotic against gram-positive and gram-negative bacteria (94%, 161/171). The overall prevalence of multiple drug resistance was 159/171 (93%): gram-positive 117/120 (97.5%) and gram-negative 42/51 (82%). Most variables did not have a statistically significant association with presence of ocular infection; only repeated infections were observed to have significant association.*

Conclusion: *The prevalence of bacterial pathogens among external ocular samples was high. Most of the isolates were drug resistant to commonly used antibiotics. Gentamicin and Ciprofloxacin were the most effective antimicrobial agents for both gram-positive and gram-negative bacteria.*

Keywords: *external ocular infections, antimicrobial agents, susceptibility pattern.*

INTRODUCTION

Eye infections are one of the most common diseases and bacteria are the most common causative agents, followed by fungi and viruses (1). Although ocular infection is considered to be a minor infection, it can be vision threatening, leading to loss of vision (1, 2). The external ocular surface acquires microbial flora at birth and some of the commensal flora may become resident in the conjunctiva and eyelids with a potential to become pathogenic. Moreover, some microorganisms derived from the environment can also transiently colonize the eye and, given the opportunity, can invade the ocular tissues. They can cause ocular disease due to virulence and host's reduced resistance in the presence of risk factors such as personal hygiene, living conditions, socio-economic status, decreased immune status, trauma, surgery, contact lens use and systemic diseases (2-5). The parts of the eye that are frequently infected are the conjunctiva, eyelid and cornea.

Clinically, external eye infections present as conjunctivitis, keratitis, blepharitis, canaliculitis, dacryocystitis, external hordeolum and cellulitis (6).

Bacteria, such as *Staphylococus aureus, Streptococus pneumoniae, Pseudomonas* and even *Neisseria meningitidis*, have been reported to have greater virulence (7), whereas *Neisseria gonorrhoeae* rarely causes acute conjunctivitis. Transmission to the eye could be by contact with infected urine or genital secretions. The incidence rates of gonococcal conjunctivitis increase during spring and summer. This is a potentially devastating ocular infection because it can cause severe ulcerative keratitis (8).

The eyelid and conjunctiva have normal microbial flora controlled by its own mechanism and by the host. Modification of this normal flora contributes to ocular infections such as blepharitis, conjunctivitis, dacryocystitis, canaliculitis, orbital cellulitis, and endophthalmitis (4, 12).

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The most common ocular infection seen by primary care physicians worldwide is bacterial conjunctivitis, which is largely present as an acute infection, 78% to 80% of cases being bacterial in origin (9-11). The majority of publications related to acute conjunctivitis reported that *Staphylococcus aureus, Streptococcus pneumoniae*, and *Haemophilus influenzae* are the most common pathogens. Coagulase negative staphylococcus (CoNS) and *S. aureus* are most frequently isolated in chronic conjunctivitis, with a tendency for an increased antibiotic resistance in recent years (3, 10, 13, 14). Gram-positive pathogens are responsible for 60% to 80% of acute infections (15,16).

The development of bacterial resistance to specific antibiotics is an important consideration for clinicians treating ocular infections. Bacterial resistance has been emerging globally, likely due to the widespread and inappropriate dosing of broad-spectrum antibiotics for systemic infections, in addition to intensified inadequate compliance to full treatment duration on the patient's side (10,17). External ocular infections are usually treated on an empirical basis with topical broad spectrum antibacterial drugs because clinicians do not send patients to laboratories since microbiological and susceptibility tests are time consuming and expensive (18, 19). Bacteria cause eye disease because of their virulence and host's immune status which can be impacted by various factors such as socio-economic status, individual hygiene, lifestyle, nutrition, genetic makeup, physiology, contact lens use and age (4). Factors which influence the etiology and pathogenesis of bacterial keratitis vary. Most cases of bacterial keratitis are associated with ocular trauma or ocular surface diseases. However, the widespread use of contact lenses has dramatically increased the incidence of contact lens related keratitis. Besides, the pattern of risk factors predisposing to bacterial keratitis varies with geographical region (20).

PATIENTS AND METHODS

A cross sectional study was conducted from May to August 2015 in ALERT outpatient and inpatient departments of Eye Clinic.

Data collection procedures

Socio demographic and clinical characteristics: External ocular examination using a slit lamp biomicroscope to rule out any focus of infection or inflammation was done thoroughly in all patients by an ophthalmologist. Subsequently, socio demographic data were collected using a structured questionnaire.

Standard operating procedures to be followed throughout the study were prepared by the principal investigator. Physical examinations and sample collection were done by ophthalmologists or experienced Ophthalmic Nurses, and clinical findings and demographic data were recorded on structured questionnaires by the study physician. Specimens were collected only from those patients presenting with external ocular infections.

Laboratory diagnosis

Collection, handling and transportation of specimens: An ophthalmologist or experienced nurse took the swabs from the eyelid and conjunctiva using sterile cotton swabs moistened with sterile physiological saline. The swab was rolled on the internal portion of the eye lid margin from medial to lateral side and back again. Pus was collected from lacrimal sac and blepharitis using dry sterile cotton swabs either by applying pressure over the lacrimal sac to allow the purulent material to reflux through the lacrimal punctum or by irrigating the lacrimal drainage system. While collecting the sample from the refluxing material, we ensured that the lid margins and conjunctiva were not touched. Two swabs were collected from each participant, labeled and inoculated into culture media.

Direct microscopy

Gram stain: Gram staining was done from the swab for identification of gram-positive and gram-negative bacteria and for the presence of polymorph nuclear cells.

Culture

The remaining swab was inoculated on 5% sheep blood agar, MacConkey agar, chocolate agar and mannitol salt agar and incubated at 37°C for 24 -48 hours. After inoculation of the swab onto the culture media, the swab was re-inoculated in sterile brain heart infusion broth. Samples inoculated on MacConkey and mannitol salt agar were incubated under aerobic atmospheric conditions, whereas samples on chocolate agar and 5% sheep blood agar were incubated at 5-10% CO_2 atmospheric conditions. All agar plates were initially examined for growth after 24 hours of incubation and plates with no growth were incubated further for 48 hours.

After getting pure colonies, further identification was conducted using standard microbiological techniques, which included gram stain, colony morphology and biochemical tests. Presumptive gram-negative bacteria were identified using Kligler iron agar, citrate utilization test, lysine decarboxylase test, urease test, motility test, indole test, oxidase test, tributyrin, X and V factors.

Analytical Profile Indexes (API 20 E and API NH) from bioMerieux, Inc. were used for further identification of some bacteria that were difficult to characterize using conventional methods. Gram-positive bacteria were identified using hemolytic activity on sheep blood agar, catalase, coagulase test, bile solubility and optochin disk test (2, 21).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out on each identified bacterium using the disc diffusion method on Muller Hinton agar (MHA) (Oxoid Ltd Basingstoke, Hampshire, UK) based on the CLSI 2014 guideline (22). Briefly, 3-5 colonies of the test organism were transferred into a tube containing sterile physiological saline and mixed gently until the suspension became turbid. The suspension was adjusted to 0.5 McFarland standard. The suspension was uniformly swabbed onto Muller Hinton agar for non-fastidious organisms. For *Neisseriae* spp., MHA with defibrinated sterile 0.5% sheep blood was used and Haemophilus Test was used for *H. influenzae*. The antimicrobial impregnated disks were placed on the agar plate's surface using a disc dispenser and plates were incubated at 37°C for 18-24 hours. The zone of inhibition around the disc was measured to the nearest millimeter using a graduated caliper. Based on the size of the inhibition zone, isolates were classified as sensitive, intermediate, or resistant following the CLSI 2014 standards.

Disks (Oxoid Ltd, Basingstoke, and Hampshire, UK) impregnated with thirteen different antibiotics were used. The antibiotics for disc diffusion testing had the following concentrations: Ampicillin (AMP) 10μg, Amoxicillin-Clavulinic acid (AMC) 20μg, Ceftazidime (CAZ) 30μg, Ceftriaxone (CRO) 30μg, Ciprofloxacin (CIP) 5μg, Trimethoprim sulphamethoxazole (SXT) 25μg, Erythromycin (E) 15μg, Gentamicin (CN) 10μg, Cefoxitin (FOX) 30μg, Tetracycline (TE) 30μg, Chloramphenicol (30μg) Penicillin (P) 10U and Clindamycin (DA) 2ug.

Quality control

All specimens were collected according to prepared SOPs for ophthalmic specimen collection. Culture media were tested for sterility and performance as Quality Control (QC); sterility of culture media was checked by incubating overnight at 35-37 ºC without specimen inoculation. Any physical change such as cracks, excess moisture, color, hemolysis, dehydration, and contamination was assessed and expiry date was also checked. Standard strains *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), *H. influenzae* (ATCC 49247), *N. meningitidis* sero group-A (ATCC 13077), *S. pneumoniae* (ATCC 49619) and *N. gonorrheae* (ATCC 49226) were used as QC throughout the study for culture and antimicrobial susceptibility tests.

Data Analysis

Data analysis and cleaning were done using SPSS version 20.0 software. Chi square (X^2) test was used to determine presence of association between categorical data (risk factors and culture results). Multivariate logistic regression was also used to explain the dependent variable based on the independent variable. Differences were deemed significant at p-values < 0.05 at 95% confidence interval limits.

Ethical Consideration

Ethical approval was obtained from the Research and Ethical Review Committee of the Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University and AHRI/ALERT Ethical Review Committee. Data and samples were collected after informed consent was obtained from each volunteer and guardian.

RESULTS

Socio-demographic characteristics and clinical features of study subjects

A total of 288 patients were enrolled in this study. Study subjects were primarily male 153/288 (53.1%). The mean age of the study participants was 25.1 (SD± 19.65) years. About 30% of participants were 18-39 years of age (89/288). The majority of the study participants were from urban settings (214/288; 74.3%). Based on educational status of participants, the majority had completed elementary school (95/288; 33.0%). Only 4.5% (13/288) of participants reported using traditional medicine to treat an external ocular infection before seeking healthcare at ALERT. Almost 9% (25/288) of participants reported having systemic diseases such as high blood pressure, diabetes mellitus and rheumatoid arthritis. About 3.5%(10/288) of study participants had undergone surgery before a sample was taken for the purpose of this study (Table 1).

In this study, 59% (170/288) of patients reported having suffered from conjunctivitis. The second most frequent cause of ocular disease was blepharitis (20.8%, 60/288) followed by blepharo conjunctivitis (13.9%, 40/288). The dominant ocular infection among both male and female patients was conjunctivitis (Table 2).

Table 1: Socio demographic characteristics of study participant presenting with external ocular infections.

Variables	Total Percentage
Age in years	
0-2	44(15.3)
3-11	48(16.7)
12-17	34(11.8)
18-39	89(30.9)
>=40	73(25.3)
Residence	
Rural	74(25.7)
Urban	214(74.3)
Sex	
Male	153(53.1)
Female	135(46.9)
Occupation	
Student	47(16.3)
Farmer	6(2.1)
Business man	73(25.3)
Civil servant	27(9.4)
Housewives	35(12.1)
Underage	82(28.5)
No job	18(6.25)
Educational Status	
Illiterate	32(11.1)
Elementary	95(33.0)
High school	48(16.7)
Under age	79(27.4)
College and above	34(11.8)

Table 2: Prevalence: of bacterial pathogens across the different clinical features of external ocular infections.

Bacterial isolates	**Conjunctivitis (N=170)**	**Blepharitis (N=60)**	**Blepharoconjunctivitis (N=40)**	**Dacryocystitis (N=12)**	**Post-trauma (N=6)**	**Total (N=288)**
Gram positive cocci						
S. aureus	29(30.9%)	6 (17.1%)	18(64.3%)	8(72.7%)	2(66.7%)	63 (36.8%)
CoNS	30(31.9%)	16 (45.7%)	6(21.4%)	2(18.2%)	0(0.0%)	54 (31.6%)
S. pneumoniae	3(3.2%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	3(1.8%)
Gram Negatives cocci						
Moraxella spp.	8(8.5%)	3(8.6%)	0(0.0%)	0(0.0%)	0(0.0%)	11(6.4%)
N. gonorrhoeae	0(0.0%)	0(0.0%)	1(3.6%)	0(0.0%)	0(0.0%)	1(0.6%)
N. meningitides	2(2.1%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	2(1.2%)
Gram Negatives Rods						
Pseudomonas spp.	0(0.0%)	0(0.0%)	1(3.6%)	0(0.0%)	0(0.0%)	1(0.6%)
H. influenzae	2(2.1%)	1(2.9%)	2(7.1%)	0(0.0%)	0(0.0%)	5(2.9%)
E. coli	12(12.8%)	4(12.4%)	0(0.0%)	1(9.1%)	1(33.3%)	18 (10.5%)
K. pneumoniae	6(6.4%)	3(8.6%)	0(0.0%)	0(0.0%)	0(0.0%)	9(5.3%)
p. mirabilis	1(1.1%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(0.6%)
Citrobacter spp.	1(1.1%)	2(66.7%)	0(0.0%)	0(0.0%)	0(0.0%)	3(1.8%)
Total	94	35	28	11	3	171

Prevalence of bacterial pathogens

Bacteria were isolated from 59.4% (171/288) of the study subjects. Among the isolates, 70.2% (120/171) and 29.8% (51/171) were gram-positive and gram-negative bacteria, respectively. *S. aureus* was the predominant pathogen accounting for 36.8% (63/171) followed by CoNS (54/171; 31.6%). Overall, bacteria were isolated mainly among participants in the age group 18-39 years (89/288; 30.9%) and in male patients (91/288; 31.6%). Among patients with clinically confirmed conjunctivitis (n=170) and blepharitis (n=60), CoNS were the most common isolates (29/171; 30.9%) and (6/171; 17.1%), respectively. A total of 3.2% (3/228) of patients with confirmed conjunctivitis had *S. pneumoniae*.

Antimicrobial susceptibility patterns of bacterial isolates from gram-positive bacteria

The antimicrobial susceptibility patterns of bacterial isolates from patients with external ocular infections showed that a significant number were resistant to one or more antimicrobials. Most isolated bacteria were sensitive to Gentamicin, Clindamycin, Ceftriaxone and Ciprofloxacin whereas 94.7% and 75.4% of *S. aureus* strains were resistant to Penicillin and Tetracycline, respectively. *S. aureus* isolates however were 90.5% sensitive to Ciprofloxacin, 92.0% to Clindamycin and 98.2% to Gentamicin. *CoNS* also showed more or less similar susceptibility patterns to *S. aureus*. Of the isolated *S. pneumoniae* strains, 100% were sensitive to Erythromycin, 100% to Penicillin, 66.7% to Chloramphenicol and 66.7% to Tetracycline while 33.3% were resistant to Trimethoprim-sulphamethoxazole (Table 3).

Antimicrobial susceptibility patterns of bacterial isolates from gram-negative bacteria

From gram-negative bacterial isolates, 96.1% were found to be susceptible to Ciprofloxacin, 58.3% to Amoxicillin-Clavulinic acid, 82.3% to Ceftazidime, 92.2% to Ceftriaxone, 90.1% to Gentamicin and 78% to Chloramphenicol. However, 58.3%, 47% and 38% of isolates showed resistance to Ampicillin, Tetracycline and Trimethoprim-sulphamethoxazole respectively. All isolated gram-negative bacteria, except for *E. coli* and *Moraxella* spp., were highly sensitive to Ceftriaxone (100%) and Ciprofloxacin (100%).

N. gonorrhoeae was 100% sensitive to all antibiotics tested, and *H. influenzae* was 100% sensitive to Chloramphenicol and Ceftazidime (Table 4).

In this study, occupation, residence, education level, surgery, using traditional eye medicine, history of chronic illness, use of contact lenses, presence of any underlying disease, the occurrence of systemic disease and repeated infections were used as possible risk and predisposing factors for ocular infections. However, bivariate logistic regression analysis showed that only repeated infection had a significant association (p=0.012) (Table 5).

DISCUSSION

External ocular infections are a common public health problem in Ethiopia (2). In the current study, the overall prevalence of bacterial external ocular infections was 59.4% (171/288) which is similar to findings from previous studies conducted in northwest Ethiopia that reported an overall prevalence of 60.8%, 54.2% and 59.4%, respectively (4,17,23). However, the prevalence observed in our study was lower compared to studies conducted in India (88%) Nigeria (74.9%) and Southwestern Ethiopia (74.7%) (1,14 and 24). The present finding was higher than reports from Bangalore, Gondar and Addis Ababa, with prevalence of 34.5%, 47.4% and 54.2%, respectively (2,17 and 18). The differences in prevalence between the sites and countries could have occurred due to different distributions of bacterial pathogens with geographic variation, study period, differences in the populations studied and preventive practices.

In this study, gram-positive cocci were the most common isolates (70.2%) which is congruent with several other studies conducted in Ethiopia (1, 4, 18, 23 and 25) and Nigeria (24). In the current study, the predominant bacterial isolates were *S. aureus* 63/171 (36.8%) followed by CoNS 54/171(31.6%), which is similar to previous studies conducted in Ethiopia, (1, 17, 18 and 25), in Nigeria (22) and in India (12). Over the past 15 years, there has been an increase in the reporting of ocular infections caused by CoNS which sugests that these bacteria play an important role in the occurrence of clinical staphylococcal blepharitis and mixed seborrhoeic/staphylococcal blepharitis (26). In several other studies, the predominant isolates were CoNS. The increased prevalence of gram-positive cocci may be due to contamination of the eye caused by the transmission of bacteria from the normal skin flora by touching eyes with hands, cataract extraction, lens implantation, and use of contact lens (4, 15).

The overall prevalence of gram-negative bacteria among patients suffering from external ocular infections in our study was 29.8% (51/171).

Table 3: Antimicrobial susceptibility pattern of gram-positive bacteria isolated from external ocular infections.

Organisms isolated (n=171)		Antibiotics tested CIP	E	DA	SXT	CN	C	P	TE
S. aureus	S I R	57(90.5 %) 4(6.3 %) 2(3.2%)	55 (87.3%) 2(3.2%) 6(9.5%)	58 (92.1%) 4(6.3%) 1(1.6%)	44 (69.8%) 11 (17.5%) 8 (12.7%)	62 (98.2%) 0(0.0%) 1(1.8%)	46 (73.0%) 12 (19.0%) 5(8.0%)	3(5.3%) 0(0.0%) 60 (94.7%)	3(5.3%) 11 (19.3%) 49 (75.4%)
CoNS	S I R	40(74%) 5(9.3 %) 9 (16.7%)	48 (88.9%) 1(1.9%) 5(9.2%)	52 (96.2%) 1(1.9%) 1(1.9%)	32 (59.3%) 7(13%) 15 (27.8%)	87 (98.2%) 3(5.6%) 4(7.4%)	42 (77.8%) 8 (14.8%) 4(7.4%)	0(0.0%) 1(1.9%) 52 (98.1%)	3(5.6%) 5(9.2%) 46 (85.2%)
S. pneumoniae	S I R	ND ND ND	3 (100%) 0(0.0%) 0(0.0%)	ND ND ND	1 (33.3%) 0 (0.0%) 2 (66.7%)	ND ND ND	2 (66.7%) 0(0.0%) 1 (33.3%)	3 (100%) 0(0.0%) 0(0.0%)	2 (66.7%) 0(0.0%) 1 (33.3%)

Table 5: Bivariate analysis of possible risk factors and their association with the prevalence of bacterial infection of the eye among external ocular infections.

Variable	Number positive (%)	(CI 95%)	P-value	(CI 95%)	P-value
Age in years					
0-2	26(39.1)	0.625(0.285-1.362)	0.236	1.604(0.320-8.042)	0.566
3-11	29(60.4)	0.608(0.307-1.144)	0.284	1.082(0.273-4.283)	0.910
12-17	16(47.1)	0.83(0.166-0.887)	0.025	0.424(0.149-1.206)	0.108
18-39	49(55.1)	0.528(0.275-1.014)	0.055	0.603(0.291-1.249)	0.173
≥40	51(69.9)	1		1	
Residence					
Rural	49(66.2)	1.478(0.851-2.568)	0.166	1.309(0.699-2.452)	0.401
Urban	122(57.0)	1		1	
Repeated infection					
Yes	75(68.8%)	0.524(0.318-0.865)	0.011	0.492(0.282-0.856)	0.012
No	96(53.6%)	1		1	
Educational Status					
Illiterate	44(68.8)	1		1	
Elementary	64(67.2)	1.700(.850-3.398)	0.133	2.069(0.465-9.213)	0.340
High school	19(39.6)	1.582 (.796-3.146)	0.191	3.198(0.824-12.415)	0.093
Under age	44(56.4)	0.506(0.244-1.052)	0.068	0.858(0.188-3.907)	0.843
College and above	21(61.8)	1.248(0.548-2.845)	0.598	2.324(0.477-11.325)	0.297
Systemic Disease					
Blood pressure	14(4.9)	0.697(0.238-2.044)	0.510		
Diabetes	4(1.4)	2.090(0.215-20.4)	0.526		
Rheumatoid and Arthritis	7(2.4)	0.83(0.496-35.2)	0.188		
Other's	263(91.3)	1			
Surgery					
Yes	10(3.5)	2.822 (0.588-13.54)	0.205		
No	278(96.5)	1			
Hospitalized					
Yes	22(7.6)	0.404 (0.145-1.129)	0.30		
No	266(92.4)	1			

Bacterial isolated (n=171)		Antibiotics tested % CIP	CRO	AMP	AMC	SXT	CN	C	CAZ	TE
E. coli	S I R	17 (94.4%) 0(0.0%) 1(5.6%)	15 (83.3%) 2(11.1%) 1(5.6%)	2(11.1%) 6(33.3%) 10 (55.6%)	9(50.0%) 6(33.3%) 3(16.7%)	5(27.8%) 4(22.2%) 9(50.0%)	17 (94.4%) 1(5.6%) 0(0.0%)	13 (72.2%) 3 (16.7%) 2 (11.1%)	15 (83.3%) 3(16.7%) 0(0.0%)	2(11%) 3 (16.7%) 13 (72.2%)
K. pneumoniae	S I R	9(100%) 0(0.0%) 0(0.0%)	9(100%) 0(0.0%) 0(0.0%)	0(0.0%) 3(33.3%) 6(66.7%)	5(55.6%) 1(11.1%) 3(33.3%)	5(55.6%) 0(0.0%) 4(44.4%)	9(100%) 0(0.0%) 0(0.0%)	7(77.8%) 2(22.2%) 0(0.0%)	8(88.9%) 0(0.0%) 1(11.1%)	3 (33.3%) 1 (11.1%) 5 (55.6%)
Pseudomonas spp.	S I R	1(100%) 0(0.0%) 0(0.0%)	1(100%) 0(0.0%) 0(0.0%)	0(0.0%) 0(0.0%) 1(100%)	1(100%) 0(0.0%) 0(0.0%)	0(0.0%) 0(0.0%) 1(100%)	0(0.0%) 1(100%) 0(0.0%)	1(100%) 0(0.0%) 0(0.0%)	1(100%) 0(0.0%) 0(0.0%)	0(0.0%) 0(0.0%) 1(100%)
Citrobacter spp	S I R	3(100%) 0(0.0%) 0(0.0%)	3(100%) 0(0.0%) 0(0.0%)	0(0.0%) 0(0.0%) 3(100%)	2(66.7%) 0(0.0%) 1(33.3%)	2(66.7%) 0(0.0%) 1(33.3%)	2(66.7%) 0(0.0%) 1(33.3%)	1(33.3%) 1(33.3%) 1(33.3%)	2(66.7%) 0(0.0%) 1(33.3%)	3(100%) 0(0.0%) 0(0.0%)
P. mirabilis	S I R	1(100%) 0(0.0%) 0(0.0%)	1(100%) 0(0.0%) 0(0.0%)	0(0.0%) 0(0.0%) 1(100%)	0(0.0%) 0(0.0%) 1(100%)	1(100%) 0(0.0%) 0(0.0%)	1(100%) 0(0.0%) 0(0.0%)	1(100%) 0(0.0%) 0(0.0%)	1(100%) 0(0.0%) 0(0.0%)	1(100%) 0(0.0%) 0(0.0%)
H. influenzae	S I R	5(100%) 0(0.0%) 0(0.0%)	5(100%) 0(0.0%) 0(0.0%)	1(20.0%) 2(40.0%) 2(40.0%)	2(40.0%) 3(60.0%) 0(0.0%)	3(60.0%) 1(20.0%) 1(20.0%)	ND ND ND	5(100%) 0(0.0%) 0(0.0%)	5(100%) 0(0.0%) 0(0.0%)	3 (60.0%) 0(0.0%) 2 (40.0%)
N. gonorrhoeae	S I	1(100%) 0(0.0%) 0(0.0%)	1(100%) 0(0.0%) 0(0.0%)	ND ND ND	ND ND ND	ND ND ND	ND ND ND	ND ND ND	1(100%) 0(0.0%) 0(0.0%)	1(100%) 0(0.0%) 0(0.0%)

Table 4: Antimicrobial susceptibility Pattern of gram-negative bacteria isolated from external ocular infections.

Bacterial isolated (n=171)	CIP	CRO	AMP	AMC	SXT	CN	C	CAZ	TE	FOX	P
E .coli S	17 (94.4%)	15 (83.3%)	2 (11.1%)	9 (50.0%)	5 (27.8%)	17 (94.4%)	13 (72.2%)	15 (83.3%)	2(11%)	ND	ND
I	0(0.0%	2 (11.1%)	6 (33.3%)	6 (33.3%)	4 (22.2%)	1 (5.6%)	3(16.7%)	3 (16.7%)	3(16.7%)	ND	ND
R	1(5.6%	1(5.6%)	10 (55.6%)	3 (16.7%)	9 (50.0%)	0 (0.0%)	2(11.1%)	0(0.0%)	13 (72.2%)	ND	ND
K. pneumoniae S	9 (100%)	9(100%)	0(0.0%)	5 (55.6%)	5 (55.6%)	9 (100%)	7(77.8%)	8 (88.9%)	3(33.3%)	ND	ND
I	0(0.0%)	0(0.0%)	3 (33.3%)	1 (11.1%)	0(0.0%)	0 (0.0%)	2(22.2%)	0(0.0%)	1(11.1%)	ND	ND
R	0(0.0%)	0(0.0%)	6 (66.7%)	3 (33.3%)	4 (44.4%)	0 (0.0%)	0(0.0%)	1 (11.1%)	5(55.6%)	ND	ND
Pseudomonas spp. S	1 (100%)	1(100%)	0(0.0%)	1(100%)	0(0.0%)	0 (0.0%)	1(100%)	1(100%)	0(0.0%)	ND	ND
I	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1 (100%)	0(0.0%)	0(0.0%)	0(0.0%)	ND	ND
R	0(0.0%)	0(0.0%)	1(100%)	0(0.0%)	1(100%)	0 (0.0%)	0(0.0%)	0(0.0%)	1(100%)	ND	ND
Citrobacter spp S	3 (100%)	3(100%)	0(0.0%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	1(33.3%)	2 (66.7%)	3(100%)	ND	ND
I	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0 (0.0%)	1(33.3%)	0(0.0%)	0(0.0%)	ND	ND
R	0(0.0%)	0(0.0%)	3(100%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1(33.3%)	1 (33.3%)	0(0.0%)	ND	ND
P. mirabilis S	1 (100%)	1(100%)	0(0.0%)	0(0.0%)	1(100%)	1 (100%)	1(100%)	1(100%)	1(100%)	ND	ND
I	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0 (0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	ND	ND
R	0(0.0%)	0(0.0%)	1(100%)	1(100%)	0(0.0%)	0 (0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	ND	ND
H. influenzae S	5 (100%)	5(100%)	1 (20.0%)	2 (40.0%)	3 (60.0%)	ND	5(100%)	5(100%)	3(60.0%)	ND	ND
I	0(0.0%)	0(0.0%)	2 (40.0%)	3 (60.0%)	1 (20.0%)	ND	0(0.0%)	0(0.0%)	0(0.0%)	ND	ND
R	0(0.0%)	0(0.0%)	2 (40.0%)	0(0.0%)	1 (20.0%)	ND	0(0.0%)	0(0.0%)	2(40.0%)	ND	ND
N. gonorrhoeae S	1 (100%)	1(100%)	ND	ND	ND	ND	ND	1(100%)	1(100%)	1 (100%)	1 (100%)
I	0(0.0%)	0(0.0%)	ND	ND	ND	ND	ND	0(0.0%)	0(0.0%)	0 (0.0%)	0 (0.0%)
R	0(0.0%)	0(0.0%)	ND	ND	ND	ND	ND	0(0.0%)	0(0.0%)	0 (0.0%)	0 (0.0%)
N. meningitides S	2 (100%)	2(100%)	ND	ND	2(100%)	ND	1(50.0%)	ND	ND	ND	ND
I	0(0.0%)	0(0.0%)	ND	ND	0(0.0%)	ND	1(50.0%)	ND	ND	ND	ND
R	0(0.0%)	(0.0%)	ND	ND	0(0.0%)	ND	0(0.0%)	ND	ND	ND	ND
Moraxella spp. S	10 (90.9%)	10 (90.9%)	1(9.0%)	9 (82.0%)	5 (45.5%)	10 (90.9%)	10(90.9%)	9 (82.0%)	6 (54.50%)	ND	3 (27.3%)
I	0(0.0%)	0(0.0%)	5 (45.5%)	1(9.0%)	3 (27.25%)	0 (0.0%)	1(9.1%)	2 (18.0%)	3 (27.25%)	ND	0 (0.0%)
R	1(9.1%)	1(9.1%)	5 (45.5%)	1(9.0%)	3 (27.25%)	1 (9.1%)	0(0.0%)	0(0.0%)	2 (18.25%)	ND	8 (72.7%)

♣ND-not done

The low prevalence of gram-negative enteric bacteria in our study could be explained by improved personal hygiene as the most important mode of transmission for enteric pathogens is fecal-oral contamination of the eye. Moreover, there are reports which documented that the main cause for gram-negative bacteria causing ocular infection is wearing contact lenses (27). In the present study, none of the patients/participants reported wearing contact lenses.

Other gram-negative bacteria isolated in this study include *H. influenzae, P. mirabilis*, *Moraxella* spp. and *N. gonorrheae*. Studies done by Okesola et al. (24) also reported isolation of similar gram-negative bacteria in Nigeria. *N. meningitidis* was isolated in this study similar to the work of Ubani *et al*. in Nigeria (28) and Nigatu *et al*. in Ethiopia (18), The current study showed that external ocular infections were predominantly seen among male patients 91/171(53.2%). The prevalence of conjunctivitis was also found to be higher among male patients, which might be due to their outdoor activities. A higher number of cases of blepharitis were observed among female patients. Our findings can be supported by similar reports from Gondar and Nigeria; however, lower cases of dacryocystitis and trauma in both male and female have been reported in Ethiopia (23) and Nigeria (24). Organisms causing conjunctivitis may be components of the normal eye lid flora (e.g. *S. aureus*) or nasopharyngeal flora (e.g. *S. aureus* and *H. influenzae*), or may be introduced by hand or acquired through aerosol transmission (25).

The degree of inflammation, eye discharge, and symptoms of ocular infections vary. In most cases, up to 80% of the ocular infections become bilateral, justifying bilateral treatment even if the infections are present unilaterally (28). In the current study, conjunctivitis was the dominant type of eye infection 170/288(59%) followed by blepharitis 60/288 (20.8%). Conjunctivitis was found in both male 98/288(34%) and female (72/288 (25%) patients. A similar study, done in Gondar by Shiferaw *et al*. revealed 43.1% of patients suffering from conjunctivitis and 29.4% from blepharitis (23). In this study, CoNS was the most common isolate for conjunctivitis 30/94 (31.9%) and blepharitis 16/35 (45.7%). This is consistent with similar studies conducted in Jimma, Ethiopia (1), India (12) and Nigeria (28). However, *S. aureus* was found to be the predominant isolate in cases of blepharoconjunctivitis 18/28 (64.3%) and dacryocystitis 8/11 (72.7%). This finding was supported by similar studies conducted in Ethiopia (23).

The two Ethiopian studies that were performed in Jimma and Gondar by Tesfaye et al. and (1) Aweke et al. (19) had shown similar results. The high rate of CoNS and *S. aureus* among blepharitis cases may be attributed to a particular virulence factor, an exoenzyme, which produces a surface slime. Furthermore, the enzyme, presumably the Ica proteins make a polysaccharide that promotes biofilm formation.

The rate of bacterial isolation in this study was higher among the age group greater than 40 years (29.8%), similar to a study from Gondar (1) which might be due to dry eye and waning immunity in older age groups. Immediate prescription of antibiotics without susceptibility testing for severe ocular infections is routine in ophthalmic practice and may be responsible for the increase in drug resistance. In this study, most bacterial isolates showed high resistance to Penicillin (91.6%), Ampicillin (58.3%) and Tetracycline (70.6%). The increased resistance to Penicillin and Tetracycline may be attributed to earlier exposure of the isolates to these drugs (allocated as first line drugs). Moreover, these drugs are very common and patients can access them easily at a low price and often over the counter at pharmacies without a prescription. Similar findings have been reported by Tesfaye *et al*. (1), Anagaw et al. (17) and Uganda by Mshangila *et al*. (15). However, we found high susceptibility to Ceftriaxone (90.2%), Chloramphenicol (75.3%), Ceftazidime (84.3%), Erythromycin (88.4%), Clindamycin (93.4%) and Ciprofloxacin (86.9%).

In this study, the presence of a high percentage of single and multiple antibiotic resistance patterns were common. The reason for the observed single and multiple resistances might be the empirical prescription of broad spectrum antimicrobial agents to treat bacterial infections without a definite diagnosis. This was supported by a study done by Tesfaye and his colleague in Jimma, Ethiopia (1). Multiple drug resistance (MDR) against two or more of the commonly prescribed antimicrobials was seen among 97.5% of gram-positive isolates (Table 6).

The MDR rate was highest for *S. aureus* (51.7%), followed by CoNS (44.2%), and least for *S. pneumoniae* (1.7%). MDR was observed in 82% of gram-negative isolates. Overall 159/171 (93%) of the bacterial isolates were resistant to multiple antibiotics in our study. This is in agreement with studies conducted by Muluye *et al*. (4), Barnabas *et al*. (15), Anagaw *et al*. (17) and Aweke *et al.* (19). Similarly, a high prevalence of MDR (87.1%) was previously reported in Gondar, Ethiopia (4).

Table 6: Multiple antibiotic resistance pattern of bacterial isolate from external ocular infection.

Bacterial isolate	Antibiotic resistance patterns					Total
	R1	R2	R3	R4	R5 and more	
S. aureus	1	14	29	11	8	63
CoNS	1	9	21	10	13	54
S.pneumoniae	1	1	0	1	0	3
H. influenzae	2	2	0	1	0	5
P. aeruginosa	0	0	0	0	1	1
E. coli	2	3	7	3	3	18
K. pneumoniae	0	4	2	2	1	9
N. gonorrhoeae	1	0	0	0	0	1
N. meningitides	1	0	0	0	1	2
Moraxella spp.	3	5	2	1	0	11
Citrobacter spp.	0	0	1	1	1	3
P. mirabilis	0	0	1	0	0	1
Total	12	38	63	30	28	171

R1: resistance to one drug, **R2**: resistance to two drugs, **R3**: resistance to three drugs, **R4**: resistance to four drugs, **≥R5**: resistance to five and above drugs

The combined use of antibiotics could provide a broader coverage against infection prior to susceptibility testing (29).

In our study, the prevalence of bacteria between male and female, age groups, occupation type, residence, education level, history of surgery, previous antibiotic use, using traditional eye medicine, history of chronic illness, wearing contact lens and the occurrence of systemic disease were not statistically significant. The reason might be due to the small sample size and the fact that most of our study participants were from urban areas; urban populations are more likely to have access to better sanitation because they have better access to information than patients from rural areas. Similar findings were reported from previous studies conducted in India (12), Gondar, Ethiopia, (17) and Dessie, Ethiopia (19). However, our study found a significant association only in patients with a history of repeated infection (p-value = 0.012). Other studies conducted in Gondar and India had shown statistically significant association in the age group greater than 2 years (4, 12). The reason for increased susceptibility to infection in infants may be that they are at a greater risk after their maternal immunity has disappeared and before their own immunity system had matured (28).

Other factors may include usage of suboptimal quality or substandard antimicrobial drugs, increased use of a particular antimicrobial agent, poor sanitation and cross-contamination from humans or animals. Other contributing factors may include improper dosage regimens during administration which includes difficulty of administration of drops of antibiotics for day time use for adult populations and children (23). In this study post-trauma (2.1%) as a predisposing factor for the external ocular infection was not found to have significant association; however, a history of trauma was the major predisposing factor in Mexico City 25% (30). This result is consistent with multiple microbial keratitis studies involving children where ocular trauma has been associated with ocular infections in up to two thirds of the cases (30).

Conclusion and recommendation

The current study revealed that there is a high burden of bacterial infections associated with external ocular diseases. Moreover, there is an increasing tendency of antimicrobial resistance to the various antibiotics used to treat ocular diseases in the study settings. Hence clinicians should be vigilant about any changes in the drug resistance profile and make optimal use of the effective drugs to treat external ocular infections.

In addition, continuous surveillance of the bacterial profile and their drug resistance patterns is crucial for prudent antibiotic use and reducing the emergence of drug resistance.

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