Antibiotic Susceptibility Pattern and Bacterial Spectrum Among Patients with External Eye Infections at Menelik II Referral Hospital in Addis Ababa, Ethiopia

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Background: Eye infections can cause loss or impairment of visual function and can lead to severe impairment. Bacteria are the most common pathogens that affect the structure of the eye. As a result, quick identification of the causative agents and testing of their medication susceptibility are essential for effective treatment of eye infections. This study was intended for determining the extent of bacterial isolates from external eye infections (EEIs) and their susceptibility to antibiotics.

Methods: A facility-based cross-sectional study was conducted among patients attending Menelik II Referral Hospital. The study comprised patients who had EEIs verified. EEI samples were collected using sterile methods. Bacterial isolates were identified using gram stain, colony morphology, and biochemical tests. The Kirby-Bauer disk diffusion technique was used to conduct a drug susceptibility test.

Results: Totally, 323 participants were recruited for this study and 184 bacterial isolates were obtained from 175 (54.5%) participants. The main clinical diagnosis was blepharitis 122 (37.8%), followed by conjunctivitis 73 (22.6%) and keratitis 57 (17.6%). The gram-positive isolates were 171 (92.9%). CoNS with a frequency of 76 (41.3%) was the most common bacterial isolates, followed by *S. aureus* 67 (36.4%), *Viridans streptococcus* 16 (8.7%), and *Klebsiella* species 6 (3.3%). Gram-positive isolates were sensitive to tobramycin, gentamicin, chloramphenicol, vancomycin, and ceftriaxone. In contrast, 94.0% of these gram-positive isolates showed resistance to penicillin. Multidrug resistance (MDR) was observed in both gram-positive and negative bacteria at rates of 123 (72%) and 12 (92.1%), respectively. The overall MDR rate among the isolates was 135 (73.4%).

Conclusion: In this study, blepharitis was the major EEI, followed by conjunctivitis. The predominant bacterial species isolated from EEIs were CoNS, followed by *S. aureus*. More than half of the isolates were drug-resistant, with a large number being multidrug-resistant, highlighting the necessity for continued and coordinated surveillance to hunt for infections that are known to be resistant.

Keywords: external eye infection, bacterial isolate, antibiotic susceptibility status, Ethiopia

Background

Eye infections are common in most developing countries.¹ Based on the site of infection, it can be divided into two main groups: external infections (EEI) and intraocular infections. Both forms of infection can cause visual impairment and are a major health problem.² Both are common causes of eye disease worldwide, including Ethiopia.^{3,4} The most common EEIs, such as conjunctivitis, blepharitis, keratitis, Dacryocystitis, orbital and periorbital cellulitis, are recognized forms of eye infections. Keratitis is an inflammation of the cornea that threatens vision.^{3,5}

Various variables affect the development of bacterial EEI, including unsanitary conditions, socioeconomic conditions, living conditions, contact lens use, previous eye trauma, chronic epithelial defects, and immune status.⁶ In addition, ocular surface and eyelid epithelium are relatively impervious to microorganisms but factors that facilitate infections are

trauma, surgery, or systemic disease.⁵ EEI can cause many signs and symptoms. The causative organism can result from a systemic infection carried by the external environment or blood.⁷ The normal bacterial community have a tremendous impact on the health of the external eyes. Their mechanisms and hosts regulate the normal microbial community of the eyelids and conjunctiva, and alterations in this normal bacterial community may contribute to ocular infections.⁸ Many opportunistic microorganisms are increasingly encountering ocular infections as a result of the extensive use of local and systemic immunosuppressants.^{9,10}

The most essential pathogens in bacterial conjunctivitis are *S. aureus* and *S. pneumoniae*. *Pseudomonas aeruginosa* and *Proteus mirabilis* have also been isolated less frequently from bacterial conjunctivitis samples.^{11–13} In many cases, a single infection with Gram-negative bacilli may predominate. Several different sorts of microorganisms may also be implicated in the pathogenesis of chronic lacrimal pouchitis (Dacryocystitis). Usually, the majority of the infected individuals harbor more than one microorganism.¹⁴ *P. aeruginosa* and *S. aureus*, on the other hand, are two of the most prevalent bacteria that cause microbial keratitis.⁹

Generally, infection of the eye can lead to loss or impairment of visual function causing major Disability. Hence, there is a need for immediate treatment for the serious bacterial eye infection that threatens visions of the eye.^{15,16} On the other hand, bacterial resistance has been emerging worldwide, concerning the irrational use of antimicrobial agents.^{17,18} Because bacterial aetiology, susceptibility, and resistance patterns differ depending on geographic location,¹⁹ locally generated up-to-date information is essential for appropriate antimicrobial therapy and management of eye infection.⁹ Although Menelik II Referral Hospital is a referral hospital designated for all ophthalmology related complicated diseases in Ethiopia, enough up-to-date published data are not found from this hospital on this topic. Therefore, this study aimed to determine antimicrobial susceptibility to bacterial eye infections, risk factors, and bacterial isolates in eye infections at Menelik II Referral Hospital.

Methods and Materials

Study Design and Settings

A health facility-based prospective cross-sectional study was implemented among patients suspected of external eye infections from January to April 2019 from Menelik II Referral Hospital, Department of Ophthalmology, and Addis Ababa, Ethiopia. Three hundred and twenty-three patients with suspected eye infections were enrolled in the study.

Sample Size and Sampling Technique

A single proportion formula was used to determine the sample size by considering 30% of the prevalence rate, which was documented previously.²⁰ By taking the margin error (d) of 0.05 and 95% confidence interval, the calculation uses the following formula.

$$\text{Sample size, where: } n = \frac{(Z_{\alpha/2})^2 \cdot P(1-P)}{d^2}$$

Where:

n= the required sample size

p= proportion

d= Expected margin of error =0.05

Z $\alpha/2$ = 95% confidence interval (C.I.) =1.96

p=prevalence of the previous study found from literature review=30%.

$$\text{Hence, } n = \frac{(1.96)^2 \times 0.3 \times 0.7}{(0.05)^2}$$

Therefore, 323 study participants were recruited. Sampling was done conveniently every other day from EEI confirmed patients who fulfilled the sampling criteria.

Inclusion and Exclusion Criteria

All ages and sex groups with the presence of EEI and who had inclined to offer their consent had been enrolled in the study. However, patients who had taken antibiotics for the last two weeks and those unwilling to give consent were excluded from the study.

Study Participants and Socio-Demographic Data

Patients who visited the ophthalmology department, were clinically diagnosed with external eye infections, and met the eligibility criteria during the study period were included in the study. Subjects were recruited prospectively based on thorough clinical examination by clinicians/ophthalmologists using a slit lamp biomicroscope to rule out inflammation. Only patients willing to give their consent were enrolled in the study. Structured questionnaires and physical examinations were used to collect demographic data (age, sex, monthly income, educational level, occupation, and address) and ophthalmic clinical data (history of repeated infections, length of stay in the hospital, use of contact lenses, surgery, previous antibacterial therapy, systemic diseases, and use of traditional medicine).

Specimen Collection, Transportation and Processing

Samples were collected prospectively from study participants following the standard practice of the hospital. A qualified ophthalmologist and ophthalmic nurse collected corneal scraping after instilling 2 to 3 drops of local anaesthetic (Tetra Caine hydrochloride 0.5%) into the conjunctiva and waiting for 2 to 3 minutes.¹⁶ Conjunctival specimens were collected using a sterile cotton-tipped swab by passing the swab gently over conjunctiva 2-times.^{3,11}

Dacryocystitis and canaliculitis pus were collected by applying pressure to the lacrimal sac using a dry sterile cotton swab. The primary swab was inoculated into Amies's transport medium and transported to the Microbiology Laboratory. Next, inoculate Brain Heart Infusion Broth (BHIB) for concentration and enrichment, subculture in 5% sheep blood agar medium, MacConkey agar medium, chocolate agar medium, Mannitol salt agar medium, and subculture at 37 ° C and inoculated for 24–48 hours. Aerobic air conditions were maintained in MacConkey agar and Mannitol salt agar, and chocolate agar and 5% sheep blood agar were cultured in a 5–10% CO₂ atmosphere.²² Finally, Gram staining, subculture and biochemical tests were done for species identifications. The isolates were then maintained in sterile BHIB mixed with glycerol for storage for further work.

Antimicrobial Susceptibility Testing

A modified Kirby-Bauer disk diffusion technique was done for drug susceptibility test (DST) on all identified bacterial isolates as recommended by Laboratory Standard Institutes^{21,23} on plain Mueller-Hinton agar non-fastidious isolates and Mueller-Hinton agar supplemented with 5% defibrinated sheep blood for fastidious bacterial isolates. The bacterial suspension equivalent to the McFarland standard (0.5 CFU) was seeded on Muller-Hinton agar followed by putting antibiotic-impregnated disks on the agar suspension after a few minutes of delay (Oxoid Ltd Basingstoke, Hampshire, UK), and then incubating for 18–24 hours at 37°C. The inhibition zone of disk diameter was measured using a calliper and categorized as resistance, intermediate and, sensitive. According to the CLSI guideline 2017, the following antibiotics were tested against gram-positive bacterial isolates. Penicillin (10µg), Erythromycin (15µg), Cotrimoxazole (1.25/23.75µg), Cefoxitin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Tobramycin (10µg), Chloramphenicol (30g), Tetracycline (30µg), Ampicillin (10µg), Ceftriaxone (30µg) and Vancomycin (30µg). Similarly, the following antibiotics were tested against gram-negative isolates, Amikacin (30g), Ampicillin (10µg), Gentamicin (10µg), Cotrimoxazole (1.25/23.75µg), Ciprofloxacin (5µg), Cefoxitin (30µg), Ceftazidime (10µg), Ceftriaxone (30µg), Meropenem (10 µg), Tobramycin (10µg) and Chloramphenicol (30g) Bacterial isolates which are resistant for at least one antibiotic from three or more classes of antibiotics were considered as multidrug-resistant (MDR).²⁴

Quality Control

To maintain the quality of expected outcome, Standard Operating procedures (SOP) were strictly followed during sample collection, identification and susceptibility testing. Subsequently, Visual inspections were performed for the absences of cracks in media or plates, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination. The sterility of culture media was then confirmed by overnight incubation of 5% of prepared media and observation of no growth from each batch. Finally, the prepared media and biochemical tests' abilities to support the growth of organisms were checked throughout the study by inoculating standard reference strains such as (ATCC® 29213) for *S. aureus*, (ATCC® 25922),

for *E. coli*, (ATCC® 49226) for *N. gonorrhoeae*, (ATCC® 700603) for *K. pneumonia* and (ATCC® 27853) for *P. aeruginosa* obtained from Ethiopian public health institute, Addis Ababa, Ethiopia.^{11,21,25}

Data Analyses

Before data was collected, a standardized questionnaire was translated into the local language (Amharic). To produce high-quality and accurate data, all quality control checks were performed before, during, and after data collection. Then, every day, all of the data was entered into a Microsoft-Excel spreadsheet 2016 (Microsoft Cop., USA). Finally, the data were imported and analyzed with Statistical Product and Service Solutions (SPSS) version 25.0. (IBM, USA). Descriptive statistics were calculated, and data were presented in the form of figures and tables. Binary logistic regression was also used to show the association of different independent variables with the dependent variable (bacterial isolates). Moreover, a multivariate logistic analysis was computed to identify factors that independently influenced the occurrence of dependent variables (bacterial isolates) by determining the adjusted odds ratio. In all analyses, P-values less than 0.05 were considered significant.

Ethical Considerations

The study was evaluated and approved by the Research Ethics Review Committee of the Department of Microbiology, Immunology and Parasitology (DRERC) (Ref. No. /07/2019), and the Institutional Review Board (CHS-IRB) (Ref. No. IRB/012/2019) of Addis Ababa University. A support letter was secured from Addis Ababa Health Bureau; and College of Health Science, Addis Ababa University. Moreover, before commencing the study, written informed consent and/or assents were obtained from each participant or guardian. Subject confidentiality and any special data security requirements were maintained and assured.

Physicians were notified of the findings of laboratory testing that had a direct impact on the study participants' health, and the patients received their results when they returned for appointments or retreatments. Overall, the study protocol complies based on the Declaration of Helsinki.

Results

Socio-Demographic Characteristics

Three hundred and twenty-three patients with external eye infections were enrolled. The majority of the participants were males 186 (57.6%) and the mean age was 40.9 years. About 143 (44.3%) of the patients were over 45 years of age, and most of them 206 (63.8%) were urban residents. The majority of 208/323 (64.4%) study patients' educational status were shown primary school level and below. Patients aged 40–60, 31–45 years also showed higher culture-positive results 81 (25.1%), and 69 (21.4%), respectively. Regarding educational status, high culture positivity rates were observed, among elementary school and illiterates with the rate of 109 (34%) and 81 (25.1%), respectively.

In regards to participants' clinical data from 323 study participants, 59 (18.3%) patients had a previous history of eye surgery and only 25 (7.7%) individuals had the systemic disease. The dominant clinical feature was Blepharitis accounting for 122/323 (37.8%), followed by conjunctivitis 73 (22.6%) and Dacryocystitis 39 (12.1%). According to their geographical location, 147 (45.5%) were from Addis Ababa and 27.2% of them were from Oromia, respectively (Table 1).

The Magnitude of Bacterial Isolates and Clinical Features

The ocular specimens of 323 ophthalmic patients were cultured in this investigation, and 175 (54.2%) of them were culture positive. A total of 184 bacteria isolates were obtained and the majority of patients 166/175 (94.9%) had a single bacterial isolate while only 9/175 (5.1%) demonstrated mixed bacterial isolates. Among the isolates, 171 (92.9%) were gram-positive and 13 (7.1%) were gram-negative bacteria. Coagulase-negative *Staphylococci* (*CoNS*) were the predominant isolate 76/184 (41.3%) followed by *S. aureus* 67/184 (36.4%) and *Streptococcus Viridian* 16/184 (8.6%).

Among culture-positive conjunctivitis, Blepharitis, Blepharo-conjunctivitis, and keratitis gram-positive bacteria were constituted 40/41 (98%), 67/71 (94%), 10/10 (100%) and 31/33 (93.9%), respectively. On the other hand, higher

Table 1 Sociodemographic Characteristics of Study Participants

Variables		Frequency	%
Gender	Female	137	42.4
	Male	186	57.6
Residence	Rural	117	36.2
	Urban	206	63.8
Geographical location	Addis Ababa	147	45.5
	Oromia	88	27.2
	Amhara	55	17
	SNNPR	21	6.5
	Harari	4	1.2
	Others	8	2.4
Occupation	Preschool Children	23	7.1
	Student	32	9.9
	Farmer	90	27.9
	House Wife	33	10.2
	Labourer	43	13.3
	Merchant	23	7.1
	Employed	75	23.2
	Unemployed	4	1.2
History of any eye trauma	Yes	51	15.8
	No	272	84.2
Eyeglass uses	Yes	71	22
	No	252	78
Total		323	100

proportions of gram-negative bacterial isolates were obtained from Dacryocystitis 6/23 (26%), Blepharitis 4/71 (5.6%) and keratitis 2/33 (6.1%). Among patients positive for bacterial isolates and clinically categorized as blepharitis (n = 71), *CoNS* were the most common isolates occurring appearing in 36/71 (50.7%) isolates followed by *Staphylococcus aureus* 23/71 (32.4%) isolates. On the other hand, the proportion of Gram-negative bacterial isolates was 13/184 (7.1%); especially, *Klebsiella species* 6/184 (3.3%) were among the common gram-negative isolates (Table 2).

Susceptibility to Antibiotics of Gram-Positive Bacterial Isolates

Only 10/171 (2.9%) of the Gram-positive bacterial isolates examined in this study showed no antimicrobial resistance to any of the drugs tested; an overwhelming majority of the isolates were resistant to one or more antibiotics. Among the 171 gram-positive bacterial isolates, relatively high susceptibility was observed to Tobramycin (94.4%), Vancomycin (92.9%). Among the *S. aureus* isolates, 65/67 (97.0%) and 58/67 (86.5%) were susceptible to Tobramycin and Gentamicin, respectively. On the other hand, significant amounts of gram-positive isolates had lower sensitivity (below 50%) to penicillin (6.0%), ampicillin (20.0%), erythromycin (28.9%), and Cotrimoxazole (36%). Higher

Table 2 The Distribution of Bacterial Isolates in External Eye Infection at Menelik II Referral Hospital, Ethiopia, 2019

Isolated Bacterial Species in Each Case	Categories of External Eye Infection n (%)						Total
	Conjunctivitis 73(22.6)	Dacryocystitis 39(12.1)	Blepharitis 122(37.8)	Keratitis 57 (17.6)	BC 18(5.6)	Other EOI 14(4.3)	323 (100)
Gram-positive isolates							
<i>Staphylococcus aureus</i>	18(43.9)	7(30.4)	23(32.4)	12(37)	5(50)	2(33.3)	67(36.4)
<i>CoNS</i>	14(34.1)	7(30.4)	36(50.7)	13(39)	3(30)	3(50)	76(41.3)
<i>β-Hemolytic Streptococci</i>	0	0	0	1(3)	0	0	1(0.5)
<i>Streptococcus Viridans</i>	4(9.8)	2(8.7)	5(7)	3(9)	2(20)	0	16(8.6)
<i>S. pneumoniae</i>	3(7.3)	0	2(2.8)	1(3)	0	0	6(3.3)
<i>Enterococcus Species</i>	1(2.4)	1(4.3)	1(1.4)	1(3)	0	1(16.7)	5(2.7)
Total isolates	40(97.6)	17(73.9)	67(94.3)	31(94)	10(100)	6(100)	171(93)
Gram-negative isolates							
<i>Escherichia coli</i>	0	1(4.3)	0	0	0	0	1(0.5)
<i>Klebsiella Species</i>	1(2.4)	3(13.1)	2(2.8)	0	0	0	6(3.3)
<i>Acinetobacter Species</i>	0	1(4.3)	0	1(3)	0	0	2(1.1)
<i>Proteus Species</i>	0	1(4.3)	1(1.4)	0	0	0	2(1.1)
<i>P. aeruginosa</i>	0	0	0	1(3)	0	0	1(1.1)
<i>Providencia stuartii</i>	0	0	1(1.4)	0	0	0	1(1.1)
Total isolates	1(2.4)	6(26)	4(5.6)	2(6)	0	0	13(7.1)
Total bacterial species	41(22.3)	23(12.5)	71(38.6)	33(17.9)	10(5.43)	6(3.3)	184(100)

proportions of the *S. aureus* isolates had lower sensitivity to penicillin 2/67 (3.0%) and Erythromycin 21/67 (31.3%). The prevalence of *MRSA*, as determined based on the resistance pattern of Cefoxitin, was 23/67 (34.3%).

Tobramycin was the only antibiotic to which *CoNS* isolates were highly sensitive, accounting for 70/76 (92.1%); and for the rest three drugs *CoNS* isolates were sensitive to Gentamicin 50/76 (67%), Chloramphenicol 45/76 (59.2%), and to Cefoxitin 41/76 (53.9%). All *Enterococcus* spp. and *Streptococcus pneumoniae* isolates 6/6 (100%) were sensitive to Vancomycin (Table 3).

Gram-Negative Bacterial Isolates' Antibiotic Susceptibility

As of entire bacterial isolates, only 13 of them were gram-negative. All of these isolates 13/13 (100%) were showed susceptibility to Tobramycin. In addition, the large majority of these isolates 11/13 (84.6%) were susceptible to Gentamicin. While 10/13 (76.9%) gram-negative bacterial isolates were equally susceptible towards Amikacin and ciprofloxacin. However, the vast majority of them were shown to have a low susceptibility rate towards Ampicillin 1/10 (10%) and Cotrimoxazole 3/13 (23.1%). As far as the susceptibility of each specific isolate is concerned, *Klebsiella* spp. was completely 6/6 (100%) susceptible to Meropenem and Tobramycin but demonstrated reduced susceptibility to Amikacin and Gentamicin with the rate of 5/6 (83.3%) for both antibiotics (Table 4).

Multidrug Resistance Patterns of Bacterial Isolates

In this study, only 5(2.7%) of isolates were sensitive for all classes of tested antibiotics; 18 (9.8%) were resistant to one class of antimicrobials. The overall prevalence of multidrug resistance (bacteria resistant to at least one antibiotic from three or more classes) was 135/184 (73.4%).

Table 3 Antibiotic Susceptibility Pattern of Gram-Positive Bacteria Isolated from External Eye Infections at Menelik II Referral Hospital, Ethiopia, 2019

Bacterial Isolates	Resistance Pattern	Antibiotic Susceptibility Status N (%)											
		PEN	ERY	GEN	COT	CPR	CXT	TOB	CHL	TET	AMP	CFT	VAN
<i>S. aureus</i>	R	65(97)	37(55.2)	3(4.5)	31(46.3)	11(16.4)	23(34.3)	2(3.0)	11(16.4)	–	–	–	–
	I	–	9(13.4)	6(9)	1(1.5)	8(11.9)	–	0	3(4.5)	–	–	–	–
	S	2(3.0)	21(31.3)	58(86.5)	35(52.2)	48(71.6)	44(65.7)	65(97.0)	53(79.1)	–	–	–	–
CoNS	R	75 (99)	57(75)	18(23.7)	56(73.7)	39(51.3)	35(46.1)	4(5.3)	30(39.5)	–	–	–	–
	I	–	6(7.9)	7(9.2)	4(5.3)	6(7.9)	–	2(2.6)	1(1.3)	–	–	–	–
	S	1(1.3)	13(17.1)	50(67)	16(21)	31(40.8)	41(53.9)	70(92.1)	45(59.2)	–	–	–	–
<i>S. pneumoniae</i>	R	1(16.7)	1(16.7)	–	2(33.3)	–	–	–	2(33.3)	2(33.3)	–	–	0
	I	–	0	–	1(16.7)	–	–	–	0	0	–	–	0
	S	5(83.3)	5(83)	–	3(50)	–	–	–	4(66.7)	4(66.7)	–	–	6(100)
<i>B hemolytic Streptococci</i>	R	0	0	–	1(100)	–	–	–	1(100)	1(100)	–	–	0
	S	1(100)	1(100)	–	0	–	–	–	0	0	–	–	1(100)
<i>Streptococcus viridans</i>	R	–	7(43.8)	–	–	–	–	–	2(12.5)	–	–	2(12.5)	2(12.5)
	I	–	1(6.2)	–	–	–	–	–	0	–	–	2(12.5)	0
	S	–	8(50)	–	–	–	–	–	14(87.5)	–	–	12(75)	14 (87.5)
<i>Enterococcus Species</i>	R	–	–	–	–	2(40)	–	–	–	–	4(80)	–	0
	I	–	–	–	–	0	–	–	–	–	–	–	0
	S	–	–	–	–	3(60)	–	–	–	–	1(20)	–	5(100)
Total tested isolates		150	166	143	150	148	143	143	166	7	5	16	28
Resistance isolates n (%)		141(94)	118(71.1)	34(23.8)	96(64)	66(44.6)	58(40.6)	8(5.6)	50(30.1)	3(42.9)	4(80)	4(25)	2(7.1)

Abbreviations: PEN, Penicillin; ERY, Erythromycin; GEN, Gentamicin; COT, cotrimoxazole; CPR, Ciprofloxacin; CXT, Cefoxitin; TOB, Tobramycin; CHL, Chloramphenicol; TET, Tetracycline; AMP, Ampicillin; CFT, Ceftriaxone; VAN, Vancomycin.

Table 4 Antibiotic Susceptibility Pattern of Gram-Negative Bacteria Isolated from External Eye Infections at Menelik II Referral Hospital, Ethiopia, 2019

Bacterial Isolates	Level of Pattern	Antibiotic Susceptibility Status N (%)									
		AMK	AMP	GEN	COT	CPR	CAZ	CFT	MER	TOB	CHL
<i>Acinetobacter</i>	R	1(50)	–	1(50)	2(100)	0	2(100)	–	1(50)	0	–
	S	1(50)	–	1(50)	0	2(100)	0	–	1(50)	2(100)	–
<i>E. coli</i>	R	0	1(100)	0	1(100)	0	1(100)	0	1(100)	0	0
	S	1(100)	0	1(100)	0	1(100)	0	1(100)	–	1(100)	1(100)
<i>Klebsiella</i>	R	1(16.7)	6(100)	1(16.7)	3(50)	0	3(50)	1(16.7)	0	0	2(33.0)
	I	0	0	0	0	2(33.3)	0	2(33.3)	0	0	1(16.7)
<i>Proteus</i> Species	S	5(83.3)	0	5(83.3)	3(50)	4(66.7)	3(50)	3(50)	6(100)	6(100)	3(50)
	R	0	2(100)	0	2(100)	0	0	1(50)	2(100)	0	1(50)
<i>P. aeruginosa</i>	S	2(100)	0	2(100)	0	2(100)	2(100)	1(50)	0	2(100)	1(50)
	R	0	–	0	1(100)	0	1(100)	–	0	0	–
<i>P. stuartii</i>	S	1(100)	–	1(100)	0	1(100)	0	–	1(100)	1(100)	–
	R	1(100)	0	0	1(100)	1(100)	0	0	1(100)	0	0
	S	0	1(100)	1(100)	0	0	1(100)	1(100)	0	1(100)	1(100)
Total tested isolates		13	10	13	13	13	13	10	13	13	10
Resistance isolates n (%)		3(23.1)	9(90)	2(15.4)	10(86.9)	3(23.1)	7(53.8)	4(40)	5(38.5)	0	4(40)

Abbreviations: AMK, Amikacin; AMP, Ampicillin; Gen, Gentamicin; COT, cotrimoxazole; CPR, Ciprofloxacin; CAZ, ceftazidime; CFT, Ceftriaxone; MER, Meropenem; TOB, Tobramycin; CHL, Chloramphenicol; S, sensitive; R, resistant; I, Intermediate.

Among the gram-positive isolates, 123/171 (72%) had multidrug resistance. Similarly, most of the gram-negative bacterial isolates 12/13 (92.3%) demonstrated multidrug resistance. The leading multi-drug resistant gram-positive isolates were *CoNS* with the rate of 70/76 (92%) followed by *S. aureus* isolates with the rate of 45/67 (67.2%). The prevalence of MRSA, as determined based on the resistance pattern of Cefoxitin, was 23/67 (34.3%). However, the lowest 1/5 (20%) multidrug resistance observed among the gram-positive isolates were from the *Enterococcus species*. In this study, there were no XDR and PDR isolates detected (Table 5).

Associated Risk Factors with the Magnitude of Bacterial Isolates

Whereas age, occupation, household income, use of eyeglass, residence, educational status, frequency of face washing, systemic disease, and history of eye trauma were used as possible risk factors for Eye infection in this study. Multivariable logistic regression analysis showed that only three of them were significantly associated with frequency of bacterial isolation from EEI: namely, educational status (including illiterate ($P = 0.005$), elementary school ($P = 0.002$) and pre-school children ($P = 0.001$)) from the demographic data; and previous history of eye surgery ($P = 0.045$) and systemic disease ($P = 0.006$) from the clinical data (Table 6).

Discussions

The overall prevalence of bacterial External eye infection in this study was 175/323 (54.2%). Similar findings were observed in a prior study done in Ethiopia, from Addis Ababa (54.9%)²⁶ and Gondar (60.8%);²⁸ from India, (58.8%) and 54.6%⁸ were reported. However, a relatively lower prevalence was reported in Hawassa, Ethiopia (48.8%)²⁹ and South India (46.3%).³⁰

On the other hand, a relatively higher prevalence of bacterial isolate of external Eye infection was reported in Ethiopia, from Jimma (74.7%)⁴ and other countries such as Saudi Arabia (78.7%).¹⁰ The varying degree of isolation from one place to another might be due to differences in the distribution of bacterial aetiology, the health of the cornea, geographic location and climate, and also tends to vary somewhat over time.⁴ Moreover, most of our study participants were urban settlers so infection prevention practice in that different settings might be lower compared to the study participants in rural areas.

In the present study, statistically significant associations were observed between external Eye infection and history of systemic disease ($P = 0.006$), history of previous eye surgery ($P = 0.045$) and elementary school level ($P=0.005$), being preschool children ($P= 0.002$) and being illiterate ($p= 0.001$). However, there were no significant associations between external Eye bacterial infection and the remaining independent variables. Comparable findings were reported from different parts of the world. For example, the reported results from Australia,³¹ Taiwan³² and India³³ showed that systemic disease and previous eye surgery were the most common predisposing risk factors for bacterial isolates of EEI.

In this study, the predominant type of external eye infection was Blepharitis 122 (37.8%), followed by Conjunctivitis 73 (22.6%). This is different from reports by other studies in Ethiopia such as Hawassa,²⁹ Borumeda¹¹ and Gondar,³ where conjunctivitis was reported to be the predominant clinical future followed by Blepharitis. The reason for this discrepancy could be attributed to the nature of most conjunctivitis cases, which are acute and thus require medication at the nearest medical centre. In the case of our study site, most of the patients being in a chronic presentation are usually handled at the referral hospital, the reason for which Blepharitis might remain predominant EEI at our site.

The leading bacterial isolates of EEI were gram-positive cocci. This is supported by several previous reports from Ethiopia, Jimma,⁴ Borumeda,¹¹ Gondar,³ southern Ethiopia³⁴, and countries like Nigeria,¹⁵ Japan³⁵, Rwanda³⁶ and India.³⁷ *CoNS* has been in the past overlooked as a cause of severe infections since it is considered normal flora. *CoNS* has developed as a prominent source of nosocomial bloodstream infections in recent years, owing to an increased usage of intravascular devices and an increase in the number of hospitalized immunocompromised patients.^{38,39} Thus, it is alarming that *CoNS* was the most predominant pathogen in this study with an overall prevalence of 76/184 (41.3%). This dominance is in concordant with previous study reports from Ethiopia, Gondar accounting for 27.42%,²⁸ and in Iran the leading bacterial isolates *CoNS* 90%.^{39,40} Additionally, *CoNS* was predominant isolates in EEI in Uganda 65.9%, and Rwanda 51.4% were reported.^{36,41}

Table 5 Multidrug Resistance Patterns of Bacterial Isolates from an External Eye Infection at Menelik II Referral Hospital, Ethiopia, 2019

Bacterial Isolates	MDR Status of Bacterial Isolates n (%)							
	R ₀	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇
Gram-positive isolates								
<i>S. aureus</i>	1 (1.5)	8 (12)	13 (19)	22 (32.8)	9 (13.4)	12 (17.9)	2 (3.0)	0
<i>CoNS</i>	0	0	6 (8.0)	8 (10.5)	20 (26.3)	25 (33.0)	15 (19.7)	2 (2.6)
<i>S. pneumoniae</i>	3 (49.9)	0	1 (17)	1 (16.7)	1 (16.7)	0	0	
<i>B. hemolytic Streptococci</i>	0	0	0	1 (100)	0	0	0	0
<i>Streptococcus viridans</i>	1 (6.3)	6 (38)	4 (25)	3 (18.7)	2 (12.5)	0	0	0
<i>Enterococcus Species</i>	0	4 (80)	1 (20)	0	0	0	0	0
Total n (%)	5 (2.9)	18 (11)	25 (15)	35 (20.5)	32 (18.1)	37 (21.6)	17 (9.9)	2 (1.2)
Gram-negative isolates								
<i>Escherichia coli</i>	0	0	0	0	1 (100)	0	0	0
<i>Klebsiella Species</i>	0	0	0	2 (33.3)	3 (50)	1 (16.7)	0	0
<i>Proteus Species</i>	0	0	0	0	0	2 (100)	0	0
<i>P. aeruginosa</i>	0	0	1 (100)	0	0	0	0	0
<i>Acinetobacter Species</i>	0	0	0	1 (50)	1 (50)	0	0	0
<i>Providencia stuartii</i>	0	0	0	0	1 (100)	0	0	0
Total n (%)	0	0	1 (7.7)	3 (23.1)	6 (46.1)	3 (23.1)	0	0
Over all n (%)	5 (2.7)	18 (9.8)	26 (14.1)	38 (20.6)	38 (20.6)	40 (21.7)	17 (9.2)	2 (1.1)

Abbreviations: R₀, sensitive to all antimicrobials; R₁, resistant to 1 antimicrobial; R₂, resistant to 2 antimicrobials; R₃, resistant to 3 antimicrobials; R₄, resistant to 4 antimicrobials; R₅, resistant to 6 antimicrobials; R₇, resistant to 7 antimicrobials.

Table 6 Multivariate Logistic Regression Analysis of Factors Associated Among Patients with External Eye Infection at Menelik II Referral Hospital, Ethiopia, 2019

Variables	Categories	Bacterial Isolates			COR (95% CI)	AOR (95% CI)	P-value
		Yes (%)	No (%)	N (%)			
Age group	≤15	28(62.2)	17(37.8)	45(13.9)	1.0(references)	1.0	
	16–30	34(51.5)	32(48.5)	66(20.4)			
	31–45	30(43.5)	39(56.5)	69(21.4)	2.538(1.25–5.16)	1.18(0.51–2.74)	0.7
	46–60	42(51.9)	39(48.1)	81(25.1)			
	>60	41(66.1)	21(33.9)	62(19.2)			
Educational status	Illiterate	55(67.9)	26(32.1)	81(25.1)	0.15(0.06–0.37)	0.17(0.05–0.59)	0.005
	Preschool children	15(83.3)	3(16.7)	18(5.6)	0.06(0.01–0.27)	0.014(0.0–0.20)	0.001
	Elementary school	67(61.5)	42(38.5)	109(34)	0.19(0.08–0.47)	0.190(0.07–0.55)	0.002
	Secondary School	20(34.5)	38(65.5)	58(18.0)	0.02(0.07–0.50)	0.02(0.07–0.57)	
	College and Above	18(31.6)	39(68.4)	57(17.6)	1.0(references)	1.0	
House hold monthly income	≤ 600 Birr	13(76.5)	4(23.5)	17(5.3)	0.20(0.06–0.70)		
	600–1500 Birr	89(66.9)	44(33.1)	133(41.2)	0.32(0.17–0.63)		
	1500–4500 Birr	52(43.3)	68(56.7)	120(37)			
	>4500 Birr	21(39.6)	32(60.4)	53(16.4)	1.0(references)	1.0	
Previous eye surgery	Yes	44(74.6)	15(25.4)	59(18.3)	0.357(0.18–0.63)	0.471(0.23–0.98)	0.045
	No	131(49.6)	133(50.4)	264(81.7)	1.0(references)	1.0	
Systemic disease	Yes	20(80.0)	5(20.0)	25(7.7)	0.271(0.1–0.74)	0.21(0.07–0.64)	0.006
	No	155(52.0)	143(48.0)	298(92.3)	1.0(references)	1.0	
Face washing frequency	Sometimes	26(72.2)	10(27.8)	36(11.1)	0.32(0.13–0.82)	0.22(0.10–0.71)	
	One Times	65(54.6)	54(45.4)	119(36.8)			
	Two times	64(51.6)	60(48.4)	124(38.4)			
	More than 2 times	20(45.5)	24(54.5)	44(13.6)	1.0(references)	1.0	

The data of the present study showed that the predominant bacteria from blepharitis cases were *CoNS* followed by *S. aureus* accounting for 36/71 (50.7%) and 23/71 (32.4%), respectively. This is in agreement with the previous study done in Ethiopia, Hawassa, from blepharitis cases (*CoNS* 35.6% and *S. aureus* 32%).²⁹ More bacterial isolates were found in the eyelid sample compared to the conjunctival sample due to bacterial colonization and repeated penetration from adjacent skin to the edge of the eyelid.^{5,7,8} In the Dacryocystitis case, *S. aureus* and *CoNS* account equally for 30.4% (7/23). The result is supported by several reports from Ethiopia, Gondar *CoNS* 29.0% and *S. aureus* 29.4%;¹⁴ *CoNS* 25.0% and *S. aureus* 17.1%.¹

In regards to sensitivity, significant amounts of gram-positive isolates had lower sensitivity to penicillin (6.0%) and ampicillin (20.0%). Among those, *S. aureus* isolates, 65/67 (97.0%) and 58/67 (86.5%) were susceptible to Tobramycin and Gentamicin, respectively. In the current study, Tobramycin was the only antibiotic to which *CoNS* isolates were highly sensitive accounting for 70/76 (92.1%). However, higher proportions of *CoNS* isolates indicated to be lower sensitivity to many of the commonly used antibiotics; Penicillin 1/76 (1.3%), Erythromycin 13/76 (17.1%) and Ciprofloxacin 31/76 (40.8%). This is comparable with similar studies done in Rwanda in regards to Erythromycin (29%) and Ciprofloxacin (41.2%) were reported to be lower.³⁶ Improper selection of antibiotics, improper dosages, and inadequate adherence to treatment can all play an important role in increasing drug tolerance.^{17,18,29}

Among the *S. aureus* isolates, 65/67 (97.0%) and 58/67 (86.5%) were susceptible to tobramycin and gentamicin, respectively. However, higher proportions of the *S. aureus* isolates were lower sensitivity to penicillin 2/67 (3.0%) and erythromycin 21/67 (31.3%). In agreement with another study report from Ethiopia, Gondar had 3.1% sensitivity to penicillin; but in other ways, higher sensitivity to Erythromycin 71.9% was reported.³ The lower sensitivity to Erythromycin in our study might be a result of time and geographic variation as well as study populations. Furthermore, the prevalence of *MRSA* infection, as determined based on the resistance pattern of Cefoxitin in this study was 23/67 (34.3%). Although the gold standard for identifying *MRSA* is the detection of the *mecA* gene,⁴² the study result depended only on the detection of Cefoxitin since genotypic tests are expensive. Formerly, a study in Taiwan using molecular characterization, and Antibigram of *MRSA* demonstrated that patients with bacterial conjunctivitis simultaneously had *S. aureus* isolates, 34/59 (57.6%) of them were *MRSA* positive.⁴³ On the other hand, based on Cefoxitin resistance status a report from Brazil 56/566 (9.9%) *S. aureus* isolates were resistant to methicillin and concluded that *MRSA* is yet an infrequent cause of EEI in Brazil.⁴⁴ This shows a significant variation in the prevalence of *MRSA* eye infections geographically and at different time points.³ This may reflect real differences in the prevalence of *MRSA*, the standards of microbiology tests, or even epidemiological changes over time.

The drug susceptibility status of gram-negative bacterial isolates to Tobramycin and Gentamicin were 13/13 (100%) and 11/13 (84.6%), respectively. Additionally, 10/13 (76.9%) of gram-negative bacterial isolates were susceptible to Amikacin and ciprofloxacin. This is in agreement with the previous studies from Pakistani.³⁸

Prevalence of multidrug resistance (MDR) is bacterial isolate at least having one or more commonly prescribed antimicrobials resistant from three or more classes of antibiotics.²⁷ MDR was observed in 135/184 (73.4%) of the bacterial isolates. The overall MDR pattern of the gram-negative bacterial isolates was 12/13 (92.3%). This is in agreement with the previous study reports with a higher prevalence of MDR documented from Gondar.³⁰ However, in Hawassa relatively low prevalence of multidrug resistance 100/143 (69.9%) was reported.³¹

Among gram-positive isolates, 123/171 (72%) had multidrug resistance. This indicates that most of the isolates had shown higher resistance to commonly used antibiotics. This is because of lack of access to microbiology laboratory in most health institutions, clinicians' use broad-spectrum or empirical therapy; that concerns' the risk of multidrug resistance expansion.^{4,11} The higher prevalence of MDR in our study might be due to the irrational use of antimicrobial agents and empirical therapy, which can result in the emergence of bacterial strains that show multidrug resistance.²⁷ Since, Menelik II Hospital is a national referral centre, where most of the patients coming for medical attention are those who have already started and failed medication to common topical antimicrobial therapy.

Limitation of the Study

The limitation of our study was that those bacteria which are not easily cultured by routine laboratory diagnosis but that cause Eye infections such as *Chlamydia trachomatis*, *Corynebacterium* species and anaerobic bacteria were not

investigated. Additionally, genotyping analysis was not performed, due to resource constraints. Fungal agents did not address in this study due to resource constraints and the availability of the facility.

Conclusions

The main clinical future of the external eye was blepharitis, followed by conjunctivitis in patients with the external eye. Low education, systemic disease, and history of eye surgery were significantly associated with the presence of bacterial isolates. Overall, CoNS, *Staphylococcus aureus*, *Viridans streptococcus*, and *Klebsiella species* were the predominant isolates. The majority of Gram-positive and all Gram-negative isolates showed some degree of multidrug resistance.

The overall antibiotic susceptibility status of the bacterial isolate indicates that chloramphenicol, following tobramycin and gentamicin, should be considered as the optimal drug for empirical treatment. Microbiological isolation and antibiotic susceptibility testing should be performed as a routine diagnosis of eye infections to reduce the increased incidence of multidrug-resistant strains. In general, the increased pattern of drug resistance seen in this and other studies indicates the need for continued bacterial monitoring and further research.

Abbreviations

AOR, Adjusted Odds Ratio; ATCC, American Type Culture Collection; BHIB, Brain Heart Infusion Broth; CI, Confidence Interval; CoNS, Coagulase Negative Staphylococcus; COR, Crude Odds Ratio; EEI, External Eye Infection; MDR, Multi-Drug Resistance; MRSA, Methicillin-Resistant *Staphylococcus aureus*.

Data Sharing Statement

The first author (Asmamaw Nitsuh) can provide data to support the findings of this paper upon request. The full length of the thesis is found on the Addis Ababa University research Repository website: <http://etd.aau.edu.et/bitstream/handle/123456789/21296/Asmamaw%20Nitsuh.pdf?isAllowed=y&sequence=1>

Consent to Participation and Ethics Approval

The AAU College of Health Sciences, Department of Microbiology, Immunology, and Parasitology, and the Research Ethics Review Committee gave their approval. Addis Ababa Regional Health Bureau Research & Emergency Management, as well as Menelik II Referral Hospital, provided official collaboration letters. Before data was collected, the study participants/guardians gave their written informed consent and assent.

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Disclosure

The authors report no conflicts of interest in this work.

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