Research Article

Prevalence and Drug Susceptibility Pattern of Bacterial Pathogens from Ocular Infection in St. Paul's Hospital Millennium Medical College, Ethiopia

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Abstract

Background: Ocular infection is a major public health problem in developing countries. It is main causes of morbidity and blindness worldwide. The aim of this study was to assess the prevalence of bacterial pathogens among external ocular infection attending St. Paul Hospital Millennium Medical College.

Methodology: A facility based cross sectional study was conducted from April to August 2016. Conjunctival and eyelid margin swabs and corneal scraping were collected. Demographic data were collected using structured questionnaire. All Specimens were processed for microbiological analysis as per standard procedures. The data was analyzed using SPSS version 20. P-value <0.05 was considered as statistically significant.

Results: A total of 215 patients were enrolled in this study. Almost half of the study participants were males 109(50.7%). The mean age of the study participants was 42.34 (Sd.±20.55) and majority were within the age range of 25-44 years 72(33.5%). About 118(54.9%) were found to be culture positive. Staphylococcus aureus 32(27.1%) was the commonest isolate followed by coagulase negative Staphylococci25 (21.2%). Ceftriaxone 38(97.4%), Gentamycin 76(96.2%), Tobramycin 70(88.6%), were effective. Gram positives and gram negatives were showed high resistance against Penicillin 66/88(75%) and Ampicillin 20/27(81.5%) respectively.

Conclusion: The prevalence and drug resistance of bacterial pathogens was higher among external ocular infection.

Keywords: External ocular infections; Conjunctivitis; Blepharitis; Dacryocystitis; Susceptibility

Abbreviations

ATCC: American Type Culture Collection; CLSI: Clinical and Laboratory Standard Institute; CoNS: Coagulase Negative *Staphylococci*; DST: Drug Susceptibility Testing; MDR: Multidrug Resistance; MRCoNS: Methicillin Resistant Coagulase Negative *Staphylococci*; MRSA: Methicillin Resistant *Staphylococcus aureus*; SPSS: Statistical Package for Social Science; WHO: World Health Organization

Background

Ocular infection is a major public health problem in developing countries including Ethiopia. Bacteria, viruses, fungi and parasites can cause these ocular infections [1,2]. Bacteria are the most common microorganisms that cause external ocular infections. This is because the bacterial pathogens inhabit the ocular surface [3,4].

Haemophilus influenza and *Streptococcus pneumoniae* in children and Staphylococcus aureus in adults are the commonest bacteria causing ocular infection. Multidrug resistant bacteria isolates like Methicillin-resistant *S. aureus* (MRSA) are emerging more important pathogen. But, generally gram positive pathogens are responsible for 60% to 80% of acute infections [4-6].

The external ocular infections are responsible for increased incidence of morbidity and blindness world widely. Keratitis is a major cause of vision loss and blindness second to cataract and is the most common in developing countries. Blepharitis can also result in patient discomfort and decrease in vision. Moreover, untreated lacrimal abscess can progress to orbital cellulitis, superior ophthalmic vein thrombosis and these may lead to life threatening infections. Infections of the conjunctiva can also spread to the cornea and cause a perforation [1,7-10]. Bacterial infection is a common cause of conjunctivitis and accounts for up to 50% of all cases of conjunctivitis in adults and 70% to 80% of all cases in children [4,11].

In Ethiopia the prevalence of blindness was about 1.6% and it is estimated that 87.4% of the cases are caused by untreated bacterial, viral and fungal infections. The prevalence of bacterial infections and development of multidrug resistance are becoming increasing in country this makes difficult in treatment of external ocular infections where the diagnosis is without laboratory confirmation [2,9,13].

Multidrug resistance is becoming the very serious problem. The emergence of bacterial resistance towards antimicrobial agents may increases the risk of treatment failure. In our country, the blindly use of antibiotics without physicians prescription may contribute

the increasing of drug resistance [13,14]. The antibiotic susceptibility pattern of bacterial isolates which are implicated to cause ocular infections must be evaluated periodically. Therefore, the aim of this study was to assess the antibiotic susceptibility profile of bacterial pathogen from external ocular infections among patients attending St. Paul Hospital Millennium medical college.

Method and Materials

Study design, period and area

A facility based cross sectional study was conducted from April to August, 2016, at St. Paul's Hospital Millennium Medical College, which is a referral hospital in Addis Ababa under the Ethiopian Federal Ministry of Health (FMOH). It is the second largest public hospital in the nation, built by the Emperor Haile Selassie in 1961 with the help of the German Evangelical Church. The hospital was established to serve the economically under privileged population, providing services free of charge to about 75% of its patients. It is providing medical specialty services to an estimated 110,000 people annually who are referred from all over the country.

Source population

All patients who were attended St. Paul hospital Millennium medical college eye clinic

Study population

All patients attended St. Paul hospital Millennium medical college eye clinic clinically suspected with ocular infections

Eligibility criteria

Inclusion criteria

• Clinically diagnosed patients suspected with external ocular infections.

• Patients who were willing to give their consent were enrolled in this study.

Exclusion criteria

• Patients on topical antibiotics treatment.

Sampling technique

Systematic random sampling technique was used by taking the first participant with lottery method from the first three patients then the other participants were recruited in every 3 individuals and a total 215 ocular sample were collected from April to August 2016.

Data Collection and Laboratory Analysis

Specimen collection and transportation

Upon admission to the hospital, patients were examined physically and with the help of slit lamp microscope for external ocular infections by the ophthalmologist. During examination 2 to 4 conjunctival and eyelid swabs were collected aseptically by using sterile cotton tipped swab pre-moistened with sterile physiological saline by asking the patient to look up, the lower lid was pulled down using thumb with an absorbing tissue paper and the swab was rubbed over the lower conjunctival sac from medial to lateral side and back again. Pus from lachrymal sac was collected using dry sterile cotton tipped swab either by applying pressure over the lachrymal sac and allowing the purulent material to reflux through the lachrymal punctum. Corneal scraping was collected after instilling 2 to 3 drops of local anesthetic (Tetracaine hydrochloride 0.5%) into the conjunctiva and patient was asked to wait for 2 to 3 minutes and corneal surface was cleaned for debris and discharge using dry sterile cotton tipped swab and with the help of slit lamb the edge of the ulcer was scraped using 21gauge needle. All swabs and the scraped material obtained on the needle directly were transferred into amies transport media and Brain Heart Infusion Broth 2ml (BHIB) (Oxoid, Basingstoke, UK) respectively [15,16]. All samples were transported to clinical bacteriology and mycology laboratory of Ethiopian Public Health Institute (EPHI). All ocular samples were collected by the ophthalmologist. Demographic data, clinical data and associated factors of study participant were collected by using pretested structured questionnaire and face to face interview.

Laboratory Processes

Bacterial cultivation and Identification

All swab samples were inoculated onto Blood agar base (Oxoid, Basingstoke, UK) to which 10% sheep blood is incorporated, chocolate agar/heated blood agar (Oxoid, Basingstoke, UK) and MacConkey agar (Oxoid, Basingstoke, UK). The inoculated cultures were incubated at 37°C for 24 hours with in candle jar (5-10% CO_2) except MacConkey agar and if no growth under overnight incubation re-incubated for further 24 hours. Pure isolates of bacterial pathogen were preliminary characterized by colony morphology, gram stain, and catalase and hemolytic reactions on blood agar plates. Identification of bacteria down to species level was done by employing an array of routine biochemical tests such as catalase, coagulase, Optochin test and Bacitracin test for gram positive identification and oxidase test, motility test, indole production test, Urease test, citrate utilization test, lysine decarboxylation test, carbohydrate fermentation, gas production and H_2S production for gram negative bacterial identification and using X and V factors test for Haemophilus species identification [17,18].

Drug susceptibility testing

A modified Kirby-Bauer disc diffusion technique for Drug Susceptibility Test (DST) was performed among all identified bacterial isolates as recommended by Clinical and Laboratory Standard Institute (CLSI), 2015 on Mueller-Hinton agar and Mueller-Hinton agar supplemented with 5% sheep blood for fastidious bacterial isolates (Oxoid Ltd Basingstoke, Hampshire, UK). The bacterial suspension prepared equivalent to the McFarland standard (0.5 CFU) was seeded on Muller-Hinton agar and after few minutes put the paper impregnated antibiotic disks (Oxoid Ltd Basingstoke, Hampshire, UK) then incubate for 18-24hrs at 37°C based on the organisms tested. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a caliper and classified as sensitive, intermediate, and resistant. The following antibiotics which are currently recommended by CLSI version 2015 were tested such as: Amoxicillin-clavulanic acid (20/10µg), Ampicilin (10µg), Amikacin (30µg), Gentamycin (10µg), Erythromycin (15µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Norfloxacine (10µg), Tetracycline (30µg), Trimethoprim-sulphamethoxazole (1.25/23.75µg), Penicillin (10µg), Vancomycin (30µg), Clindamycin (2µg), Cefoxitin (30µg), Oxacillin (30µg), Chloramphenicol (30µg),

Table 1: Demographic and Clinical characteristics of study participants and their association with bacterial positivity at St. Paul's Hospital Millennium Medical College, 2016.

Variable		Frequency (%)	Pos (N,%)	AOR (95%, CI)	P-value
Sex	Male	109(50.7)	59(54.1)	1.064(0.622-1.821)	0.82
	Female	106(49.3)	59(55.6)	1	
Residence	Rural	63(29.3)	29(46.0)	1.656(0.917-2.992)	0.09
	Urban	152(70.7)	89(58.5)	1	
Age in year	<14	19(8.0)	16(84.2)	0.205(0.052-0.806)	0.21
	15-24	24(11.2)	10(43.5)	1.424(0.516-3.928)	0.49
	25-44	72(33.5)	37(50.7)	1.066(0.504-2.253)	0.86
	45-64	56(26.0)	32(57.1)	0.821(0.371-1.817)	0.63
	>65	44(20.7)	23(52.3)	1	
Educational	Illitrate	94(43.7)	50(53.2)	1.100(0.465-2.601)	0.83
Background	Preschool	12(5.6)	10(83.3)	0.250(0.046-1.365)	0.11
	Litetrate	109(50.7)	58(53.2)	1	
Occupation	Labor worker	64(29.7)	31(48.4)	1.310(0.689-2.490)	0.41
	Employee	60(27.9)	38(63.3)	0.689(0.354-1.342)	0.27
	Unemployed	91(42.3)	49(53.8)	1	
Trauma	Yes	34(15.8)	17(50.0)	1.262(0.606-2.629)	0.53
	No	181(84.2)	101(55.8)	1	
Previous	Yes	32(14.9)	18(56.3)	0.937(0.440-1.997)	0.86
Surgery	No	183(85.1)	100(54.6)	1	
Systemic	Yes	21(9.8)	9(42.8)	1.710(0.689-4.246)	0.24
Diseases	No	194(90.2)	109(56.1)	1	
Contact lens	Yes	0(0.0)	0(0.0)	NA	
Wearing	No	215(100)	118(54.9)		
Frequency of	Less frequent	109(50.7)	54(49.5)	3.064(0.895-10.49)	0.07
face washing	Frequent	87(40.5)	49(56.3)	2.099(0.611-7.206)	0.24
	More frequent	19(8.8)	15(78.9)	1	
Hospitalization	Yes	6(2.8)	2(33.3)	2.495(0.447-13.92)	0.29
for long period	No	209(97.2)	116(55.5)	1	

Key: `World Health Organization Age Group, AOR- Adjusted Odds Ratio, CI-Confidence Interval, 1-Reference.

Piperacilin (100μg), Tobramycin (10μg), Ceftazidime (10μg) and Meropenim (10μg). Bacterial isolates which were resistant for two or more classes of antibiotics were considered as Multidrug Resistant (MDR) [18,19].

Quality Control

To maintain the quality of the work from sample collection up to final laboratory identification the standard operating procedure of sample collection and laboratory analysis were followed strictly. All the equipment were checked for their proper functionality. The prepared culture media were checked for sterility by incubating the five percent for overnight and observe for the presence of any growth. Capacity of the prepared media supporting the growth of organisms was checked by inoculating control strains. The known control organisms were used such as *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853). Questionnaires used to collect demographic data and associated factors were pretested prior to data collection and supervision of the data collection

was done regularly on daily basis and in which incompletely filled questionnaires were discarded.

Statistical Analysis

Data were collected, entered, cleaned and analyzed using SPSS version 20 software according to the study objectives. The descriptive summaries were presented with text and tables. Binary logistic regression was used to determine the association between the prevalence of bacterial pathogens and selected demographic characteristics and associated risk factors. P-value less than 0.05 were considered as statistically significant.

Ethical Consideration

Ethical clearance was obtained from Departmental Research and Ethical Review Committee (DRERC) of Medical laboratory Science, School of Allied Health Science, College of Health Science, Addis Ababa University and St. Paul's Hospital Millennium Medical College. The permission from the hospital management office was

Table 2: Frequency of bacterial isolates among different clinical features at St. Paul's Hospital Millennium Medical College, 2016.

Clinical feature	Total cases	Bacterial isolates
Conjunctivitis	87(40.5)	53(60.9)
Blepharitis	48(22.3)	24(50.0)
Blepharoconjunctivitis	27(12.6)	13(43.1)
Dacryocystitis	22(10.2)	15(63.2)
Keratitis	9(4.2)	2(22.2)
Post-truamatic	9(4.2)	5(55.5)
Others*	13(6.0)	6(46.2)
Total	215(100)	118(54.9)

*Eye lid abscess, Malignancy super infection, External hordeolum, Pre-septal cellulitis.

obtained. Written informed consent was also obtained from each study participants. Study participant's confidentialities were strictly maintained during the interview process as well as anonymity was kept during data processing and report writing. Laboratory confirmed cases were treated with effective antibiotics tested. So that patients were benefited from this study.

Results

Demographic characteristics of study participants

A total of 215 patients with external ocular infection were enrolled in this study. Majority of the participants were males 109 (50.7%). The mean age of the study participants was 42.34(Sd.±20.55) and majority of participants age were within the age range of 25-44 years 72(33.5%). Most of the participants lives in urban 152(70.7%), literate 109(50.7%) and house wives 51(23.7%) in occupation. On the other hand, 34(15.8%) and 32(14.9%) of study participants had trauma history and previous eye surgery respectively. Most of study participants had less frequent face washing habit 109(50.7%) and 21(9.8%) had chronic diseases (Table1).

Spectrum of bacterial isolates

Among 215 samples collected 118(54.9%) were found to be bacterial culture positive. The predominant bacterial pathogen isolated were gram positives 88(74.6%). *S. aureus* 32(27.1%) and *K. pneumoniae* 9(7.6%) were the dominant gram positive and gram negative bacterial pathogens respectively (Table 2).

Bacterial isolates and clinical features

In this study, the prevalence of bacteria were higher accounting 15(68.2%) among Dacryocystitis followed by conjunctivitis 53(60.9%), blepharitis 24(50%). However, there was no statistically significant variation of bacterial positivity among clinical features (Table 2). *S. aureus* was the commonest organism in conjunctivitis, blepharitis, and blephero-conjunctivitis. But, in Dacryocystitis CoNS were the dominant isolate. *S. aureus* and *S. pneumoniae* were also isolated from keratitis (Table 3).

Antibiotic susceptibility patterns of bacterial isolates

In this study a total of 118(54.9%) bacterial isolates were recovered. All those isolates were tested for the drug sensitivity test by Kirby-Bauer disc diffusion method. The most effective antibiotics for both gram positive and gram negative bacteria were Ceftriaxone 38(97.4%), Gentamycin 76(96.2%), Tobramycin 70(88.6%), and Ciprofloxacin 75(86.2%). Gram positive cocci were sensitive for Erythromycin 67(76.1%), Clindamycin 75(85.2%). *Streptococci* were sensitive for Vancomycin 24(77.4%) and penicillin 20(64.5%) but 55(94.7%) of *staphylococci* isolates were resistant to penicillin. 7(12.3%) of Cefoxitin resistant *staphylococci* were isolated. Gram negative rods were 100% sensitive for Amikacin, Ceftazidim, and Meropenem, however, they were resistant against Ampicillin 20(81.5%), and Amoxicillin 16(59.3). All *Moraxella* species were sensitive for all antibiotics tested. *S. aureus* was highly sensitive for Tobramycin 32(100%), Gentamycin 31(96.9%), Clindamycin 30(93.8%), and Ciprofloxacin 29 (90.6%) whereas Penicillin 31(96.9%) and Tetracycline 18(56.2%) were

Table 3: Bacterial pathogens distributions in different clinical features at St. Paul's Hospital Millennium Medical College, 2016.

Bacterial isolate	Conjunctivitis	Blepharitis	B/Conjunct-ivitis	Dacryocystis	Keratitis	Post-truamatisc	Others	Total
	N=87	N=48	N=27	N=22	N=9	N=9	N=13	N=215
S. aureus	14(26.4)	4(16.7)	3(23.1)	7(46.7)	1(50)	0(0.0)	3(50.0)	32(27.1)
CoNS	12(22.6)	0(0.0)	2(15.4)	8(53.3)	0(0.0)	2(40)	1(16.7)	25(21.2)
S. pneumoniae	10(18.8)	2(8.3)	1(7.7)	3(20)	1(50)	1(20)	0(0.0)	18(15.3)
S. pyogenes	1(1.9)	1(4.16)	1(7.7)	0(0.0)	0(0.0)	0(0.0)	1(16.7)	4(3.4)
S. viridian	3(5.7)	2(8.3)	2(15.4)	1(6.7)	0(0.0)	1(20)	0(0.0)	9(7.6)
Moraxella spp	2(3.8)	1(4.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(2.5)
H. influenzae	2(3.8)	2(3.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(16.7)	5(4.2)
E. coli	3(5.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(2.5)
K. pneumoniae	4(7.5)	1(4.2)	1(7.7)	2(13.3)	0(0.0)	1(20)	0(0.0)	9(7.6)
P. aeruginosa	0(0.0)	1(4.2)	1(7.7)	1(6.7)	0(0.0)	0(0.0)	0(0.0)	3(2.5)
E. aerogenes	1(1.9)	0(0.0)	1(7.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.7)
P. mirabilis	0(0.0)	1(4.2)	0(0.0)	1(6.7)	0(0.0)	0(0.0)	0(0.0)	2(1.7)
Citrobacter spp	0(0.0)	0(0.0)	1(7.7)	1(6.7)	0(0.0)	0(0.0)	0(0.0)	2(1.7)
Alkaligenes spp	1(1.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.8)
T0tal	53(44.9)	24(20.3)	13(11.0)	15(12.7)	2(1.7)	5(4.2)	6(5.1)	118(100)

Table 4: Antibiotics sensitivity pattern of gram positive bacterial isolates at St. Paul's Hospital Millennium Medical College, 2016.

Bacterial isolates	Pattern	PE	FOX	TE	E	DA	CIP	CN	TOB	SXT	C	VA	CRO
						Antibiotics							
S. aureus	S	3.1	87.5	43.8	81.3	93.8	90.6	96.9	100	81.2	78	ND	ND
	R	96.9	12.5	56.2	18.7	6.2	9.4	3.1	0	18.8	22		
CoNS	S	8	88	36	64	76	72	96	76	76	72	ND	ND
	R	92	12	64	36	24	28	4	24	24	28		
S. pneumoniae	S	66.7	94.4	72.2	83.3	88.9	ND	ND	ND	72.2	78	83.3	ND
	R	33.3	5.6	27.8	16.7	11.1				27.8	22	16.7	
S. pyogenes	S	100	ND	50	75	100	ND	ND	ND	25	100	100	ND
	R	0		50	25	0				75	0	0	
S. viridian	S	44.4	ND	88.9	77.8	66.7	ND	ND	ND	77.8	89	55.6	100
	R	55.6		11.1	22.2	33.3				22.2	11	44.4	0
Total	S	26.1	76.1	52.3	76.1	85.2	82.5	96.5	89.5	75	78	77.4	100
	R	73.9	23.9	47.7	23.9	14.8	17.5	3.5	10.5	25	22	22.6	0

C-Chloramphenicol (30µg), CIP- Ciprofloxacin (5µg), CN- Gentamycin (10µg), CRO- Ceftriaxone (30µg), DA- Clindamycin (2µg), E- Erythromycin (15µg), OX- Oxacilin (30µg), PE- Penicillin (10unit), SXT- Trimethoprim-Sulphamethoxazole (1.25/23.75µg), TE- Tetracycline (30µg), TOB-Tobramycin (10 µg), VA- Vancomycin (30µg), CoNS- Coagulase Negative Staphylococci, N- Number and ND- Not done.

Table 5: Antibiotic susceptibility pattern of gram negative bacterial isolates at St. Paul's Hospital Millennium Medical College, 2016.

Bacteria isolates	Pattern	AMP	AMC	TE	AK	NOR	CIP	CN	TOB	SXT	C	CRO	CAZ	MEM	PEP
							Antibiotics								
E. coli	S	0	66.7	66.7	100	66.7	33.3	66.7	33.3	33.3	0	100	100	100	ND
	R	100	33.3	33.3	0	33.3	66.7	33.3	66.7	66.7	100	0	0	0	
K. pneumoniae	S	0	33.3	44.4	100	100	100	100	88.9	88.9	66.7	100	100	100	ND
	R	100	66.7	55.6	0	0	0	0	11.1	11.1	33.3	0	0	0	
E. aerogenes	S	0	0	100	100	100	100	100	100	100	100	100	100	100	ND
	R	100	100	0	0	0	0	0	0	0	0	0	0	0	
P. aeruginosa	S	0	0	66.7	100	100	100	100	100	33.3	66.7	100	100	100	100
	R	100	100	33.3	0	0	0	0	0	66.7	33.3	0	0	0	0
P. mirabilis	S	0	0	100	100	100	100	100	100	100	100	100	100	100	ND
	R	100	100	0	0	0	0	0	0	0	0	0	0	0	
Citrobacter spp	S	0	0	50	100	100	100	100	100	100	50	100	100	100	ND
	R	100	100	50	0	0	0	0	0	0	50	0	0	0	
Alkaligenes spp	S	0	0	100	100	100	100	100	100	100	100	0	100	100	ND
	R	100	100	0	0	0	0	0	0	0	0	100	0	0	
Moraxella spp	S	ND	ND	100	ND	ND	100	ND	ND	100	100	100	100	100	ND
	R			0			0			0	0	0	0	0	
H. influenza	S	100	100	60	ND	ND	100	ND	ND	40	100	100	ND	ND	ND
	R	0	0	40			0			60	0	0			
Total	S	0	25.9	66.7	100	95.5	93.3	95.5	86.4	73.3	73.3	96.7	100	100	100
	R	100	74.1	33.3	0	4.5	6.7	4.5	13.6	26.7	26.7	3.3	0	0	0

AMP- Ampicillin (10µg), AMC- Amoxicillin-Clavulanic acid (20/10µg), AK- Amikacin (30µg), CAZ- Ceftazidime (10µg), MEM- Meropenim (10µg), NOR- Norfloxacin (10µg), PEP- Piperacilin (100µg), N- Number and ND- Not Done.

showed less susceptibility. *K. pneumoniae* was highly sensitive for Ciprofloxacin 9(100%), Gentamycin 9(100%), Ceftriaxone 9(100%), Amikacin 9(100%), Ceftazidime 9(100%), Meropenim 9(100%), and Tobramycin 8(88.8%) but resistant for Ampicillin 8(88.9%), Amoxicillin 6(66.7%), and Tetracycline 5(55.6%) (Table 4,5).

Multidrug resistance of bacterial isolates

In this study, the overall prevalence of multidrug resistance (bacteria resistant for two or more classes of antibiotics tested) was 84(71.2%). Most of the gram negative enteric bacteria isolated were resistant for at least one antibiotic (Table 6).

Table 6: Multiple antibiotics resistance pattern of bacterial isol ates at St. Paul's Hospital Millennium Medical College, 2016.

Bacterial isolates	R0	R1	R2	R3	R4	R5 and above	Total
			Multidrug resistance				
S. aureus	1(8.3)	8(36.4)	13(32.5)	4(22.2)	3(17.6)	3(33.3)	32(27.1)
CoNS	0	3(13.6)	8(20.0)	2(11.1)	9(52.9)	3(33.3)	25(21.2)
S. pneumoniae	5(41.7)	5(22.7)	4(10.0)	3(16.7)	0	1(11.1)	18(15.3)
S. pyogenes	1(8.3)	1(4.5)	1(2.5)	1(5.6)	0	0	4(3.4)
S. viridian	0	3(13.6)	5(12.5)	0	1(5.9)	0	9(7.6)
Moraxella spp	3(25.0)	0	0	0	0	0	3(2.5)
H. influenzae	2(16.7)	1(4.5)	2(5.0)	0	0	0	5(4.2)
E. coli	0	0	0	0	1(5.9)	2(22.2)	3(2.5)
K. pneumoniae	0	0	4(10.0)	3(16.7)	2(11.8)	0	3(2.5)
P. aeruginosa	0	0	0	2(11.1)	1(5.9)	0	3(2.5)
E. aerogenes	0	1(4.5)	1(2.5)	0	0	0	2(1.7)
P. mirabilis	0	0	2(5.0)	0	0	0	2(1.7)
Citrobacter spp	0	0	0	2(11.1)	0	0	2(1.7)
Alkaligenes spp	0	0	0	1(5.6)	0	0	1(0.8)
T0tal	12(10.2)	22(18.6)	40(33.9)	18(15.3)	17(14.4)	9(7.6)	118(100)

R0-Sensitive for all antibiotics tested, R1-Resistant for 1 antibiotic, R2- Resistant for 2 antibiotics, R3-Resistant for 3 antibiotics, R4-Resistant for 4 antibiotics, R5-Resistant for 5 and above antibiotics.

Bacterial prevalence and associated risk factors

The associated risk factors such as sex, residence, educational background, occupational status, trauma, presence of systemic diseases, previous surgery, hospitalization for long time and age were assessed. The binary logistic regression was done to determine the association. However, there was no statistically significant association between bacterial prevalence and those associated factors (Table 1).

Discussion

Ocular infection is a major public health problem especially in developing countries like Ethiopia. The external ocular infections are responsible for increased incidence of morbidity and blindness worldwide, their morbidity vary from self-limiting light infection to sight threatening infection [1,2].

In this study, out of 215 patients, 54.9% were found to be positive for bacterial pathogen. This finding is in agreement with the previous studies carried out in Gondar, 54.2% [21], in Iran, 52.4% [22], in Hawassa, 50.7% [13], and it is comparable with studies in Addis Ababa (47.4%) [23], in Borumeda (59.4%) [9]. Higher prevalence were reported in Jimma 74.7% [2], in Babylon, 92.1% [5] and in India, 64% [3]. However, our finding showed higher prevalence than study conducted in Bangalore, 34.5% [24]. Possibly reasons for varying rate of isolation of bacterial pathogens may be because of the variations in geographical location, study period, study design as well as socioeconomic status population studied.

In the current study, the most common type of external ocular infection was conjunctivitis (40.5%) followed by blepharitis (22.3%), Dacryocystitis (12.6%). This is the same with the previous works in our country like in Borumeda, Hawassa, Jimma, Addis Ababa [2,9,13,23]. Keratitis cases were lower in number when compared to other studies in Ethiopia and abroad [2,3,13,25,23]. This may be due to the difference in geographical location, climate variation, the period study done. The bacterial culture positivity among different types of external ocular infections in this study was revealed that Dacryocystitis 68.2%, followed by conjunctivitis 60.9%, blepharitis 50%. This is in line with a study in Addis Ababa and other studies elsewhere, study in India showed that Conjunctival swabs yielded 52% bacterial isolates [23,24].

The most commonly isolated bacterial pathogens in this study were gram positive cocci 88(74.6%). In line with the current study, there are reports in other part of Ethiopia, like 93.7% in Borumeda [9], 52% in Jimma [2], 61.5% in Hawassa [13]. And other studies abroad also reported that gram positive cocci as the dominant bacteria in Navodaya, Cambodia, Babylon, Iran, China and Nigeria [1,22,26-29]. In general most studies revealed that gram positive cocci were responsible for causing external ocular infections. This may be because of abnormal multiplication of the normal biota, contaminated fingers or skin [30].

In the present study, *S. aureus* 32(27.1%) was the predominant pathogen followed by CoNS 25(21.2%) *S. pneumoniae* 18(15.3%), *S. viridians* 9(7.6%), *K. pneumoniae* 9(7.6%). Our result is in agreement with other studies in Ethiopia like in Addis Ababa, Gondar, Hawassa, Jimma [2,13,21,23] and in other parts of the world like in Bangalore, Yemen, Babylon and Cambodia also reported *S. aureus* as a predominant organism followed by coagulase negative *Staphylococci* [3,24,26,31]. However, some other studies reported CoNS as the commonest isolate such as in South India [32], in Uganda [19], in Borumeda [9] and studies in Gondar [33,34]. In this study *S. aureus* was the commonest bacterial isolate in conjunctivitis, blepharitis, and blepharo-conjunctivitis. This finding is supported by studies conducted in Jimma [2], Nigeria [35], in India [25]. However, in Dacryocystitis, coagulase negative *Staphylococci* were the predominant bacteria. This is consistent with a study in Gondar [34]. In contrast, studies in

Hawassa [13] and Addis Ababa [23] reported that *S. pneumoniae* was the predominant bacteria in Dacryocystitis. The high coverage of *S. aureus* and CoNS in blepharitis and blepharo-conjunctivitis may be attributed with the bacterial virulence factors such as exo-enzymes, surface slime may contribute for the pathogenesis [13,36].

In this study about 22(18.8%) of enteric gram negative bacteria were isolated. Similar studies reported higher in locally (Gondar) 44.5% [21] and internationally (India) 29(35%) [24]. However, our study showed higher prevalence than studies in Borumeda and Jimma accounted 6.5% and 11.5% respectively [2,9]. Among gram negative bacteria *K. pneumoniae* was the predominant. This is supported by studies conducted in Navodaya [1], and Egypt [37]. But other studies in Jimma, Yemen and Gondar, reported *P. aeruginosa* as the dominant gram negative bacterial isolate [2,31,34]. *E. coli* also reported in other study as a predominant organism [5].

In the current study, the demographic characteristics and associated factors were assessed. However, there was no statistically significant association between bacterial prevalence and those associated factors. This disagrees with other studies conducted in Egypt, India, Jimma, and Gondar in which they showed the statistically significant association between bacterial prevalence rate and age variation specifically bacterial infection was more common in children less than 2 years age [2,25,31,3]. The reason for increased susceptibility to infection in infants and children may be due to that they are at a greater risk after their maternal immunity has disappeared and before their own immunity system had matured [25,33].

Since the treatment of ocular infection is on empirical basis with first line broad spectrum antibiotics and the increasing of drug resistance among pathogens causing external ocular infection, continuously updated data on antimicrobial susceptibility patterns would be beneficial for the trend of empirical therapy. In the current study, Gentamycin (96.2%), Tobramycin (88.6%), Ciprofloxacin (85%) and Chloramphenicol (77.4%) were effective against overall bacterial isolates. This is in line with other previous studies elsewhere in India [3], Iran [25], Uganda [19] and in Navodaya [1]. Studies in Ethiopia like in Hawassa [13], Jimma [2], and Addis Ababa [38] also reported the same pattern with our finding. A study in Gondar also showed that 74.2% of overall bacterial isolates were sensitive for ciprofloxacin, tetracycline, Chloramphenicol, co-trimoxazole [34]. However, another study in Gondar showed that most of bacterial isolates were resistant for Gentamycin (45.2%), penicillin (71%), trimethoprim-sulphamethoxazole (58.1%), and tetracycline (64.6%) [33]. In addition, a study in Borumeda showed lower coverage of tetracycline, norfloxacine, ceftriaxone and ciprofloxacin against gram negative bacteria [9].

On the other hand, gram positive cocci were sensitive for erythromycin (79.5%), Clindamycin (85.2%) and vancomycin (77.4%) while gram negative isolates were 100% sensitive for Ceftazidime, Meropenim and Amikacin.

However, 73.9% of gram positive cocci were resistant for penicillin especially *Staphylococci* (94.7%) and tetracycline (47.7%) and most gram negative isolates were resistant for ampicillin and amoxicillin 81.5% and 59.3% respectively and also 26.7% of gram negative bacteria were resistant for tetracycline and Chloramphenicol. This is similar

with other studies conducted in Navodaya, Iran, Gondar, Jimma and Hawassa [1,2,13,21,22]. This may be due to these antibiotics are more likely utilized to treat infection empirically and patients can purchase easily from pharmacies [2,21].

S. aureus was highly sensitive for Tobramycin 32(100%), Gentamycin 31(96.9%), Clindamycin 30(93.8%), and Ciprofloxacin 29(90.6%) however, highly resistant to Penicillin 31(96.9%) and Tetracycline 18(56.2%). This finding is consistent with previous studies such as in Jimma and Gondar [2,34]. The result of this study also showed that 12.3% of *Staphylococci* were Cefoxitin resistant. Of this 12.5% and 12% of *S. aureus* and CoNS were Cefoxitin resistant respectively. This is higher than study in Cambodia reported 4.3% of *S. aureus* was resistant for Cefoxitin [26]. This is lower as compared with study in Uganda reported relatively higher percentage 31.9% and 27.6% of Methicillin resistant *S. aureus* and CoNS respectively [19]. A review paper in United States reported that from 3% to 64% of ocular staphylococcal infections were due to Methicillin-resistant *S. aureus* [39]. And this condition is becoming more common and the organisms are resistant to many antibiotics [40].

In this study, the prevalence of multidrug resistant was 71.2%. This is comparable with previous studies in Hawassa 69.9% [13], Gondar 77.1% [21]. However, another study in Gondar reported the higher prevalence of MDR (87.1%) [33]. This may be due to the difference in practice of wise drug utilization among the population. Resistance among ocular pathogens is becoming increasing in consonance with the increase of resistance among bacteria associated with systemic infections. The factors contributing to the development of drug resistance among ocular bacterial isolates may include overuse of antibiotics for systemic infection as well as overuse of topical antibiotics in the eye with or without physician prescription, improper dosing regimen, misuse of antibiotics for viral infections, extended duration of therapy and due to lack of microbiology laboratory in most health institutions clinicians' advocated use of empirical first line broad spectrum therapy. Therefore these factors may result in increasing of drug resistance development and it needs continuous assessment and measures to be taken by the concerned body [2,13,41].

Conclusion

The prevalence of bacterial pathogens from external ocular infections was found to be higher. Conjunctivitis cases were the most commonly identified type of external ocular infection. The multiple antimicrobial resistances were also high. Therefore, High prevalence of bacterial drug resistance among external ocular infection high lights the need for nationwide study on bacterial external ocular infections and early identification of causative agents as well as periodic evaluation drug susceptibility pattern of bacterial pathogens associated with external ocular infection. It also needs the due attention from all the concerned bodies.

Author's Contribution

AA, AB, WD, NA, EH, YM has participated in the laboratory work, data analysis and interpretation. AA, AB, NA, MW have participated in the laboratory work, data analysis, and interpretation and write up of the manuscript. All authors read and approved the final manuscript.

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