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Microbial profile and associated factors of external ocular bacterial and fungal infections in Arba Minch General Hospital: a cross-sectional study

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External ocular infections are of serious global concern, which cause significant visual morbidity and even blindness, particularly in low-income and resource-limited countries. Herewith, we are reporting the profile of bacteria and fungi causing external ocular infections and the associated factors in Arba Minch, southern Ethiopia. An institution-based cross-sectional study was conducted among 259 suspected individuals with external ocular infections from 01 January to 10 June 2020 in Arba Minch General Hospital. The demographic data were obtained using a structured questionnaire, while corneal scrapings and conjunctival swabs were collected for culture. Samples were inoculated onto MacConkey, blood, mannitol salt and Sabouraud dextrose agar plates. Bacteria were characterised using conventional microbiological techniques. Fungal isolates were identified by culture and morphology by means of microscopy. Antibiotic susceptibility tests for bacteria were performed via the Kirby-Bauer disk diffusion technique. A logistic regression analysis determined the association between dependent and independent variables; P values ≤ 0.05 were considered statistically significant. The most common clinical manifestations in culture-proven cases were conjunctivitis (38.6%, *n* = 100) and blepharoconjunctivitis (25.9%, *n* = 67). The overall prevalence of external ocular bacterial and fungal infections was 73.2 (*n* = 115) and 26.8% (*n* = 42), respectively. Isolates of *Staphylococcus aureus* (24.8%, *n* = 39) and *Pseudomonas aeruginosa* (13.4%, *n* = 21) were the predominant Gram-positive and Gram-negative bacteria, respectively. The prevalence of multidrug-resistant bacteria was 71.3% (*n* = 82). The World Health Organisation-prioritised bacteria such as methicillin-resistant *S. aureus* (61.5%, *n* = 24), extended-spectrum beta-lactamase (22.6%, *n* = 12), and carbapenemase-producing Enterobacteriaceae (15.1%, *n* = 8) were also detected. The prominent fungi recovered from patients with keratitis and conjunctivitis were Aspergillus spp. and *Candida albicans*, respectively (9.5%, *n* = 15 each). Age (26–49 years) and family income were statistically associated with ocular infections (*P* ≤ 0.05). The results provide insights into the characteristics of major ocular bacterial and fungal pathogens circulating in the ophthalmic patients of Arba Minch. Conjunctivitis is the main ocular manifestation observed, with a predominance of *S. aureus*. More than two-thirds of the ocular bacteria were MDR, and the highest prevalence corresponds to *S. aureus*. The aggravation of multi-drug resistance, including the WHO-prioritised ones, warrants periodic evaluations.

Keywords Blepharitis, Conjunctivitis, External ocular infections, Keratitis, Drug resistance

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The World Health Organization (WHO) reported that the estimated global burden of visual impairment is 2.2 billion, and almost half of these cases, including blindness, could have been prevented if treated promptly[1]. In the African continent, around 26.3 million have some form of visual impairment; out of these, 5.9 million are blind[2]. It is reported that 15.3% of the world's blind population lives on this continent[2]; up to 5% of the entire global blindness is due to ocular trauma and associated infections[3].

External ocular infections comprising blepharitis, conjunctivitis, keratitis, dacryocystitis, pre-septal orbital cellulitis and associated complications are significant challenges that impose high morbidity, resulting in retarded economic growth[4]. Global estimate of keratitis, a cause of unilateral blindness ranges from 1.5 to 2 million cases per year[4]. The diagnosis of ocular infections is challenging due to diverse presentations and lack of prompt interventions culminating in vision impairment[4]; they can result from monomicrobial or polymicrobial invasions. Nevertheless, many etiological agents are responsible for ocular infections, among which bacteria are the major pathogens contributing to more than 50% of the cases[5]. The foremost causative bacteria are *Staphylococcus aureus*, coagulase-negative *Staphylococci*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*[4]. Ocular fungal infections are on the rise, especially in those who are immuno-suppressed and on long-term steroid therapy or in patients suffering from penetrating eye trauma, the causative species being *Aspergillus*, *Fusarium*, and *Candida*[4]. The spectra and prevalence of ocular infections within a community or geographic zone depend on many risk factors related to patients, microbes and the environment, such as virulence and drug-resistance of pathogens, the level of personal hygiene, socioeconomic status, nutrition, genetics, physiology, age, accessibility to healthcare facilities, exposure to pollutants, occupational risks, and co-morbidities[5]. Assessing these factors is essential in understanding their impacts on ocular health, which eventually may aid in developing effective interventions involving early recognition, prevention, and treatment.

Ocular infections are often treated empirically with broad-spectrum antibiotics in the form of eye drops or ointments and intra-ocular formulations via topical application or sub-tenon ways[6]. During the last few decades, ocular bacterial pathogens also started acquiring resistance to different antibiotics belonging to the family of penicillin, fluoroquinolone, and macrolides due to various reasons[7,8], and their antibiograms can diverge according to geographical, social, and biological status[8]. Literature surveys indicate that many programs on ocular antimicrobial surveillance are available and or in the pipeline in developed countries[8–12]. Unfortunately, in some developing countries, antimicrobial surveillance schemes are only in the inception stage. Moreover, there is a stark shortage of prompt diagnostic tools and culture facilities, which can lead to misdiagnosis and incorrect treatment, ultimately contributing to the aggravation of drug-resistance. Therefore, knowledge about the current trends in microbiological profiles and their antibiograms is much needed.

The Ethiopian population, in general, is at a greater risk of contracting ocular infections due to the subtropical climate, financial constraints, eye trauma, domestic air pollution, poor hygiene, scarcity of ophthalmologists, healthcare facilities, and lack of awareness[12–18]. Bacterial ocular infections are the leading causes of visual morbidity in the country, and the overall incidence correspond to 48–74%, with significant regional fluctuations in prevalence and associated factors[12–18]. However, most of the studies so far done did not include fungal pathogens. Ocular infections are one of the most frequently encountered medical conditions and are common among patients attending primary care facilities in our study area. External ocular infections, in general, are treated empirically due to the lack of culture facilities. Understanding the etiological profile, risk factors, diagnosis, and treatments are important since they provide valuable insights into differential diagnoses, aiding improvements in healthcare and, hence, this study.

Materials and methods

Study setting, design, and period

The study was conducted in Arba Minch General Hospital (AMGH), which has an ophthalmology department from 01 January to 10 June 2020. The department offers outpatient and inpatient services and treats more than 500 recorded cases with different ophthalmological manifestations per month.

Study population and eligibility criteria

This study is a single-centre based cross-sectional type, performed among all patients suspected of external ocular infections attending the Ophthalmology Department of the Hospital. The inclusion criteria were clinically diagnosed outpatients (both sexes) suspected of any external ocular infections (such as blepharitis, conjunctivitis, keratitis, and blepharoconjunctivitis), those who had an onset of infection within a period of 24 to 48 h prior to sample collection and were willing to give informed consents or assents. The exclusion criteria include patients with internal eye infections and severe ocular trauma, history of antibiotic usage (topical/systemic) during the two weeks before the commencement of the study, patients who underwent recent ocular surgery, those with incomplete medical records, and those who were seriously ill or were unable to respond well.

Sample size determination and patient recruitment

A single population proportion formula was applied to determine the sample size. A prevalence of 48.8% of external ocular infection was adopted from a previous study conducted elsewhere in Ethiopia[12]. On applying a confidence interval of 95% ($z = 1.96$) and a 6% marginal error ($d = 0.06$), the calculated sample size became 235; 10% non-response rate (24 subjects) was applied, and thus the final sample size was 259. A systematic random sampling method was implemented to recruit the study participants. By considering the previous year's record of suspected cases of external ocular infections for five months, which was 530, we have calculated the K value as 2.04 (530/259). The first participant was chosen by a lottery method, and the rest were recruited every 2 intervals.

Outcome variables

The primary outcome of interest in this study is the prevalence of culture-proven external ocular infections. A positive culture was defined as one having a confluent growth of ocular pathogens on the culture media. The secondary outcomes of this study were the identification of factors associated with culture-confirmed cases of ocular infections among patients, elucidation of bacteriological and fungal profiles, and the determination of antibiotic-susceptibility patterns of bacteria.

Covariates

Sociodemographic factors (age, sex, marital status, place of residence, income, educational and occupational status), clinical variables (type of ocular infections, trauma, history of co-morbidity, history of hospitalisation and surgery, usage of antibiotics and corticosteroids) and behavioural factor (face washing habits) were the selected covariates.

Data collection

Upon admission to the study, patients were examined physically, with the help of a slit lamp bio-microscope to detect the presence of external ocular infections, by an ophthalmologist. Based on the clinical evidence, demographic data and clinical and behavioural factors of patients were collected through a face-to-face interview using a structured questionnaire. Their medical records were assessed while collecting the data, and the initially assigned card numbers served as specific identification codes. The collected data were kept on a shelf with a lock and key system by data collectors until takeover by the principal investigator during supervision; electronic data were also stored in password-protected computers or files.

Ocular specimens

Clinical specimens were aseptically collected from sites of infections (eyelids, conjunctiva, and cornea) from each study participant by an ophthalmologist following standard protocols[19,20]. Sampling was done before the initiation of any ocular antimicrobial or steroidal eye drops. After detailed examinations, conjunctival cul-de-sac and eyelid margin specimens were obtained by means of sterile cotton-tipped swabs pre-moistened with physiological saline. Corneal scraping was performed after instilling two to three drops of a local anaesthetic (0.5% tetracaine hydrochloride) into the conjunctiva, and the edge of the ulcer was scraped using a 21-gauge needle. All swabs and scraped specimens were transferred to brain heart infusion broth (Oxoid, Basingstoke, UK) and then transported to the Microbiology and Parasitology Laboratory.

Culture and identification of ocular pathogens

All the specimens collected were separately plated out on various isolation media, viz., Sabouraud dextrose agar (SDA), blood agar, mannitol salt agar, and MacConkey agar (Oxoid, Basingstoke, Hampshire, UK). In the case of corneal scraping, the specimens were inoculated in the form of a 'C' shaped streak. Bacteriological culture media were incubated aerobically at 37 °C for 24 to 48 h, while the SDA plates were kept at 25 °C for seven days and discarded at the end of the fourth week if found growthless[19–21].

Pure cultures of the respective ocular bacterial isolates were subsequently subjected to species identification and confirmation; macroscopic and microscopic analyses were performed, and the biochemical characteristics were evaluated via standard laboratory methods[19–21]. Briefly mentioning, Gram-positive bacteria were identified using catalase and coagulase tests. Isolates of the members of the Enterobacteriaceae family were identified biochemically through a series of tests: catalase, indole, citrate, urease, H_2S production, methyl red, Voges–Proskauer, and triple-sugar iron. Non–lactose fermenting Gram-negative bacteria were identified by indole, triple-sugar iron, urease, oxidase, and catalase tests. The confluent growth of bacteria on any culture media is a laboratory diagnostic criterion for culture-positive bacterial ocular infections, which can be further confirmed by staining and biochemical tests[21].

Ocular fungal pathogens were morphologically identified by means of the rate of growth, the general topography of the colony (flat, heaped, folded regularly or irregularly), colony texture (moist, glabrous, powdery, granular, velvety, cottony), and pigmentation on the surface and reverse side. All the filamentous septated and non-septated fungi were microscopically identified by lactophenol cotton blue staining; yeast-like colonies were confirmed by Gram staining followed by germ tube test. The laboratory diagnostic criteria to detect fungal infections were the presence of confluent growths at the site of inoculation on SDA, which is re-confirmed by direct microscopic examinations[21].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, UK) employing the Kirby-Bauer disc diffusion method as per the methodology set by the Clinical and Laboratory Standards Institute (CLSI)[22]. Antibiotic discs such as penicillin (10 µg), ampicillin (10 µg), piperacillin (100 µg), amoxicillin-clavulanic acid (20/10µg), cefoxitin (30 µg), cefepime (30 µg), ceftriaxone (30 µg), meropenem (10 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), erythromycin (15 µg), chloramphenicol (30 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), clindamycin (30 µg), and amikacin (30 µg) were utilized. Finally, the isolates were categorised as susceptible, intermediate, or resistant according to the CLSI interpretive criteria. Isolates with intermediate resistance were classified as fully resistant for the purpose of statistical analysis. Multi-drug resistance in this study corresponds to the resistance to at least three or more groups of antibiotics tested[23]. The presence of methicillin-resistant *S. aureus* (MRSA), extended-spectrum beta-lactamase producers (ESBL), and carbapenem-resistant Enterobacterales (CRE) was detected as per the standard procedures (CLSI, 2019).

Quality controls

The questionnaire was pretested to check its clarity and functioning among 5% of the participants before the commencement of the actual work; one-day training was given to data collectors, and the data were checked daily for its completeness, accuracy, clarity, and consistency by the principal investigator; the standard operating procedures were strictly followed. The expiry date of media and reagents, as well as the quality control parameters were checked according to CLSI guidelines. All culture media were prepared as per the manufacturers' instructions, and their sterility was tested by incubating 5% of each batch at 35–37 °C overnight (for the evaluation of any possible contamination). After that, all culture plates and antibiotic disks were stored at the recommended refrigeration temperature (2–8 °C). The quality of media and antibiotic disks were standardised according to the reference strains: *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), and *K. pneumoniae* (ATCC 700603) (for ESBL), *S. aureus* (ATCC 25923), and *S. aureus* (ATCC 29213) (for MRSA). All the reference strains were procured from Ethiopian Public Health Institute.

Statistical analysis

Data were analysed using IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA); the dependent variable was culture-proven ocular infection. Demographic and clinical factors (frequency and percentage) were summarised using descriptive statistics. The prevalence of culture-positive ocular infections was described in terms of proportion with a 95% confidence interval (CI). The susceptibility of ocular bacteria to various antibiotics is presented in terms of percentages. The multi-collinearity among variables was also inspected (variance inflation factor and tolerance test). The effects of covariates on the evaluated outcomes were analysed through multiple logistic regressions. Initially, the data were subjected to a series of bivariable analyses, and those with a cut-off point of P-values ≤ 0.25 were processed further by multivariable analysis. The fitness of the model was checked using the Hosmer-Lemeshow goodness fit test. Adjusted odds ratio (AOR) and 95% CI were applied to determine the strength of association. The significance level was set at P-values ≤ 0.05 in multivariable analysis.

Ethical considerations

The research protocol was approved by the Institutional Review Board, College of Medicine and Health Science, Arba Minch University (Ref. IRB/150/12, Dated 19-12-2019). This study was conducted in line with the tenets of the Declaration of Helsinki and its later amendments. After a thorough briefing of all the study-related procedures and associated risk factors, informed written consents were obtained from the adult participants aged > 18 years, and in the case of younger subjects (< 18), age-appropriate informed consents (assents) were taken from the respective legal guardian through the survey tool, before sample collection.

Results

Sociodemographic and clinical characteristics

Altogether, 259 patients were systematically selected and included, with a 100% response rate; less than a third of them, i.e., 30.1% ($n=78$), were in the age group greater than 50; a wafer-thin majority of them were unmarried, 51.7% ($n=134$), illiterate, 50.2% ($n=130$), and unemployed, 57.9% ($n=150$); detailed sociodemographic characteristics of participants are shown in Table 1.

More than a third of the participants had symptomatic conjunctivitis (38.6% $n=100$), whereas approximately a fourth of them had blepharon-conjunctivitis (25.9%, $n=67$) and keratitis (24.7%, $n=64$), and a tenth of them

Variables	Categories	Frequency (*n*)	Percentage (%)
Sex	Male	173	66.8
	Female	86	32.2
Age (in years)	≤ 25	111	42.8
	26–49	70	27
	≥ 50	78	30.1
Marital status	Married	125	48.3
	Unmarried	134	51.7
Residence	Urban	127	49.0
	Rural	132	51.0
Educational level	Illiterate	130	50.2
	Primary school	85	32.8
	Secondary school and above	44	17.0
Occupational status	Farmer	85	32.8
	Employee	24	9.3
	Unemployed	150	57.9
Family monthly income (Ethiopian Birr)	< 2000 (< 35 USD)	192	74.1
	≥ 2000 (> 35 USD)	67	25.9

Table 1. Sociodemographic characteristics of study participants.

had blepharitis (10.8%, $n=28$). A history of consumption of antibiotics was recorded in the case of 46.3% ($n=120$), and 31.7% ($n=82$) of them experienced eye trauma. Face washing was not practised so often by a sizeable number of participants (42.4% $n=110$), and 66% ($n=171$) had a recent history of some sort of eye infection (Table 2).

Spectra of ocular pathogens

During the study period, 259 patients were clinically diagnosed with external ocular manifestations and were investigated for bacterial and fungal infections. The overall prevalence of culture-confirmed cases of external ocular infections was 60.6% ($n=157$) (95% CI: 54.4, 66.6). There is a preponderance in the number of cases caused by bacteria, i.e., 73.2% ($n=115$), and the rest were fungal infections (26.8%, $n=42$). All the culture-proven cases observed are monomicrobial in nature and are mostly Gram-positive isolates (39.49%, $n=62$). The most common bacterial causative agents were *S. aureus* (24.8%, $n=39$), followed by CoNs (14.6%, $n=23$), *P. aeruginosa* (13.3%, $n=21$), and *E. coli* (9.5%, $n=15$). Bacteria were the major causative agents of conjunctivitis (77%, $n=47$), blepharoconjunctivitis (93.9%, $n=31$), keratitis (63.6%, $n=28$), and blepharitis (47.4%, $n=9$). Four cases of blepharoconjunctivitis were exclusively caused by *N. gonorrhoea* (Table 3).

The predominant fungal etiological species were *Aspergillus* spp. and *Candida albicans*, to an equal extent (9.5%, $n=15$ each), followed by *Fusarium* spp. (5.1%, $n=8$) and Rhizopus spp. (2.5%, $n=4$). Septate, non-septate, and yeast-like fungi were detected in keratitis (36.4%, $n=16$) and also in the case of conjunctivitis (23%, $n=14$) (Table 3).

Antimicrobial susceptibility patterns of ocular bacteria

The Gram-positive bacteria showed some variations among themselves in susceptibility profiles (Table 4). Invariably, all the isolates were susceptible (i.e., 100%) to clindamycin, whereas the entire isolates were fully resistant (i.e., 100%) to penicillin; ciprofloxacin was the second most effective drug (93%, $n=107$). The isolates of *S. aureus* were 92.3% ($n=36$) resistant to tetracycline; nearly 61.5% ($n=24$) of them were methicillin-resistant too. At the same time, 84.6 ($n=33$) and 76.7% ($n=32$) were susceptible to gentamicin and chloramphenicol, respectively. Isolates of CoNs were resistant to both tetracycline (73.9%, $n=17$) and erythromycin (69.6%, $n=16$). Methicillin resistance was also observed in the case of 65.2% ($n=15$) of CoNs.

In the case of Gram-negative Enterobacteriaceae, the entire isolates were resistant to ampicillin and amoxicillin-clavulanic acid ($n=28$) (Table 4). They have also exhibited resistance to ceftriaxone (71.4%, $n=28$) and meropenem (40.8%, $n=20$). Interestingly, these isolates were highly susceptible to gentamicin (100%, $n=49$) as well as ciprofloxacin (92.4%, $n=49$). The predominant isolates of Enterobacteriaceae, *P. aeruginosa*, showed considerable resistance to cefepime (66.7%, $n=14$), whereas they were only moderately resistant to piperacillin (52.4%, $n=11$). At the same time, they were highly susceptible to gentamicin, norfloxacin, ciprofloxacin, and amikacin, and the susceptibility ranged between 100 and 80.9%. The isolates of *E. coli* showed somewhat higher resistance to ceftriaxone (66.7%, $n=10$) and only a medium level of resistance to amikacin and meropenem

Variables	Categories	Frequency (*n*)	Percentage (%)
Type of infections	Conjunctivitis	100	38.6
	Blepharitis	28	10.8
	Keratitis	64	24.7
	Blepharo-conjunctivitis	67	25.9
Trauma	Yes	82	31.7
	No	177	68.7
History of surgery	Yes	24	9.3
	No	235	90.7
Presence of chronic diseases*	Yes	16	6.2
	No	243	93.8
Previous corticosteroid usage	Yes	24	9.3
	No	335	90.7
Previous antibiotic usage (Ophthalmological)	Yes	120	46.3
	No	139	53.7
Face washing habit with tap water and soap	More frequently**	103	39.8
	Frequently	46	17.8
	Less frequently	110	42.4
Previous hospitalization	Yes	76	29.3
	No	183	70.3
History of eye infection	Yes	171	66.0
	No	88	34.0

Table 2. Clinical characteristics of study participants. *-Diabetes mellitus, tuberculosis and HIV, **-Frequency mean number of washing per day (Twice or more a day- More frequently, one to two times per day-Frequently, sometimes or not at all- Less frequently).

Isolates	Conjunctivitis ($n=100$)	Blepharitis ($n=28$)	Keratitis ($n=64$)	Blepharo-conjunctivitis ($n=67$)	Total ($n=259$)
Bacterial isolates n (%)					
E. coli	5(8.2)	1(5.3)	5(11.4)	4(12.1)	15(9.5)
K. pneumoniae	3(4.9)	3(15.8)	3(6.8)	-	9(5.7)
Proteus spp.	-	-	-	4(12.1)	4(2.5)
P. aeruginosa	15(24.6)	-	6(13.6)	-	21(13.4)
N. gonorrhea	-	-	-	4(12.1)	4(2.5)
S. aureus	16(26.2)	4(21)	2 (4.5)	17(51.5)	39(24.8)
CoNs	8(13.1)	1(5.3)	12(27.3)	2(6.1)	23(14.6)
Total bacteria, ($n=115$)	47 (77)	9 (47.4)	28 (63.6)	31 (93.9)	115 (73.2)
Fungal isolates n (%)					
Rhizopus spp.	4(6.5)	-	-	-	4(2.5)
Aspergillus spp.	4(6.5)	5(26.3)	4(9.1)	2(6.1)	15(9.5)
C. albicans	2(3.3)	5(26.3)	8(18.2)	-	15(9.5)
Fusarium spp.	4(6.5)	-	4(9.1)	-	8(5.1)
Total fungi ($n=42$)	14 (23)	10 (52.6)	16 (36.4)	2 (6.1)	42 (26.8)
Total ocular pathogens	61(61)	19(67.9)	44(68.8)	33(49.3)	157(60.6)

Table 3. Frequency and distribution of ocular microbial isolates.

(46%, $n=7$ each). In contrast, they were fully susceptible (i.e., 100% each) to tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, and gentamicin. The isolates of *K. pneumoniae* showed higher levels of resistance against a couple of antibiotics, namely ceftriaxone and meropenem (i.e., 77.8%, $n=7$ and 66.7%, $n=6$, respectively). On the other hand, these isolates were completely susceptible to tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol and gentamicin (i.e., 100% each). The isolates of *N. gonorrhoea* were fully susceptible to tetracycline, ciprofloxacin, and cefoxitin (i.e., 100% each) and, on the contrary, were completely resistant to penicillin (100%, $n=4$).

Multi-drug resistance patterns of ocular bacteria

The current set of results revealed that the majority of isolates are multi-drug resistant. The overall prevalence of MDR was 71.3% ($n=82$), of which 46% ($n=53$) were Gram-positives. Among them, an extreme level of multi-drug resistance (89.7%, $n=35$) was noted in *S. aureus*. A higher percentage of MDR among the Gram-negatives was detected in the case of *K. pneumoniae* (88.9%, $n=8$), *E. coli* (80%, $n=12$), and *Proteus* species (100%, $n=4$) (Table 5). Among the Gram-negative isolates, those with resistance to four classes of drugs comprise 44%, and particularly, they showed resistance to combinations of drugs involving penicillin/ fluoroquinolones/ third-generation cephalosporins/ aminoglycosides/ tetracycline.

ESBL and carbapenemase - producers

Out of the 53 isolates of Gram-negative bacteria, 22.6% ($n=12$) were found to be ESBL producers in the screening test, and carbapenemase production was detected in the case of 15.1% ($n=8$). The production of ESBL was confirmed in the case of 66.6% ($n=6$) of *K. pneumoniae* isolates and 40% ($n=6$) of *E. coli* isolates. On the other hand, a higher percentage of carbapenemase production was seen in the case of *K. pneumoniae* (55.5%, $n=5$), followed by *E. coli* (20%, $n=3$). The co-existence of ESBL and carbapenemase producers was observed only in the case of 11.3% ($n=6$) isolates (Table 5).

Factors associated with ocular infections

Bivariate logistic regression analysis showed that sex, residence, age, occupational status, family income, type of eye infections, previous eye infections, and consumption of ocular antibiotics correspond to P-values less than or equal to 0.25 ($P\leq0.25$) in association with ocular infections and were further analysed via a multivariate logistic regression model; in which only age ($P=0.03$; AOR = 2.61, 95% CI = 1.08, 6.34) and lower monthly income ($P=0.02$; AOR = 2.51, 95% CI = 1.13, 5.53) were found statistically associated (Table 6).

Discussion

External ocular infections are major contributors to the group of non-fatal disabling conditions in both high and low-income countries[24]. In order to develop a comprehensive strategy for diagnosis and treatment, associated factors linked to infections and causative ocular pathogens must be elucidated. The results obtained from our study can be the vantage point for outlining proper guidelines for treating ocular infections. Conjunctivitis was found to be the most prevailing ocular clinical manifestation. Out of 259 patients, 44.4% were found to be positive for bacterial pathogens, suggesting the role of these infectious agents in patients suffering from ocular infections in the study setting. And by and large, this resembles the results of a couple of previous studies conducted in Ethiopia (Hawassa, 48.8%, and Dilla, 46.1%[12,18]) and also India (46.3%)[25].

However, the prevalence was noticeably below the extent reported in other cities of the country, including Addis Ababa, Bahirdar, Debre Markos, and Jijiga (ranging between 54.5 and 62.8%)[15,16,26,27], and also Nigeria (88.6%)[28]. The prevalence can flatulate significantly due to differences in diagnostic criteria applied and

Antibiotic	P	Bacterial isolates							
		E. coli ($n=15$)	K. pneumoniae ($n=9$)	Proteus spp. ($n=4$)	P. aeruginosa ($n=21$)	N. gonorrhea ($n=4$)	S. aureus ($n=39$)	CoNs ($n=23$)	Total ($n=115$)
		n (%)							
PEN	S	NT	NT	NT	NT	-	-	-	-
	R					4(100)	39(100)	23(100)	66(100)
AMP	S	-	-	-	NT	NT	NT	NT	-
	R	15(100)	9(100)	4(100)					28(100)
AMC	S	-	-	-	NT	NT	NT	NT	-
	R	15(100)	9(100)	4(100)					28(100)
PEP	S	NT	NT	NT	10(47.6)	NT	NT	NT	10(47.6)
	R				11(52.4)				11(52.4)
CTR	S	5(33.3)	2(22.2)	-	NT	NT	NT	NT	7(25)
	R	10(66.7)	7(77.8)	4(100)					21(75)
CFP	S	NT	NT	NT	7(33.3)	NT	NT	NT	7(33.3)
	R				14(66.7)				14(66.7)
FOX	S	NT	NT	NT	NT	4(100)	15(38.5)	8(34.8)	27(40.9)
	R					-	24(61.5)	15(65.2)	39(59.1)
MER	S	8(53.3)	3(33.3)	4(100)	14(66.7)	NT	NT	NT	29(59.2)
	R	7(46.7)	6(66.7)	-	7(33.3)				20(40.8)
CHL	S	15(100)	9(100)	-	NT	NT	32(76.7)	18(64.3)	74(82.2)
	R	-	-	4(100)			7(23.3)	5(35.7)	16(17.8)
TET	S	15(100)	9(100)	4(100)	NT	4(100)	3(7.7)	6(26.1)	41(43.6)
	R	-	-	-			36(92.3)	17(73.9)	45(56.4)
AK	S	8(53.3)	5(55.6)	4(100)	17(80.9)	NT	NT	NT	34(69.4)
	R	7(46.7)	4(44.4)	-	4(19.1)				15(30.6)
GEN	S	15(100)	9(100)	4(100)	21(100)	NT	33(84.6)	20(87)	102(91.9)
	R	-	-	-	-		6(15.4)	3(13)	9(8.1)
CIP	S	15(100)	9(100)	4(100)	17(80.9)	4(100)	35(86.7)	23(100)	107(93.0)
	R	-	-	-	4(19.1)	-	4(13.3)	-	8(7.0)
NOR	S	NT	NT	NT	20(95.2)	NT	NT	NT	20(95.2)
	R				1(4.8)				1(4.8)
SXT	S	15(100)	9(100)	-	NT	NT	25(60.0)	19(71.4)	68(75.6)
	R	-	-	4(100)			14(40.0)	4(28.6)	22(24.4)
ERY	S	NT	NT	NT	NT	NT	31(79.5)	7(30.4)	38(62.3)
	R						8(20.5)	16(69.6)	24(38.7)
DA	S	NT	NT	NT	NT	NT	39(100)	23(100)	62(100)
	R						-	-	-

Table 4. Antimicrobial susceptibility patterns of ocular bacterial isolates. PEN-penicillin, AMP-ampicillin, PEP-piperacillin, AMC-amoxicillin-clavulanic acid, FOX-cefoxitin, CFP-cefepime, CTR-ceftriaxone, MER-meropenem, CHL-chloramphenicol, TET-tetracycline, AK-amikacin, GEN-gentamicin, CIP-ciprofloxacin, NOR-norfloxacin, SXT-sulfamethoxazole-trimethoprim, ERY-erythromycin, DA-clindamycin. NT corresponds to a change in the denominator (total number of isolates tested).

gradations in the characteristics of study populations and also the study design. The isolation of a causative organism depends very much on various factors, including the number of inocula, the time and site from which it is taken, the culture media, and the conditions provided for its growth. The culture-negative cases observed in this study can be attributed to a number of factors, like the involvement of viral pathogens, the anaerobic or fastidious nature of microbes, viability shown by non-culturable pathogens, or clinical conditions created by non-infectious eye allergens. Even though bacterial and fungal pathogens caused the majority of external ocular infections detected in this study, some of the suspected culture-negative cases might be attributed to viral infections, and the clinical diagnosis may not fully identify the etiologic agents. The clinicians may have missed the opportunity or were unable to clinically detect those ocular infections exclusively caused by viral pathogens.

In fact, bacteria are opportunistic pathogens inhabiting the mucous membrane of conjunctiva. Bacterial conjunctivitis is the most frequently diagnosed ocular manifestation, and this result is backed up by the findings from earlier studies done in Ethiopia itself[12,17,27,29]. On the other hand, blepharitis was the most prevailing type of manifestation in recent studies conducted in other parts of the country[15,18,26]. These fluctuations could be linked to differences in population size, methods of analysis, and patient related factors (behavioural, physiological, and immunological variables) existing among different categories of study participants.

Bacterial isolates	MDR	ESBL	CRE	ESBL and CRE
	n (%)			
E. coli (*n* = 15)	12(80)	6 (40)	3 (20)	2 (13.2)
K. pneumoniae (*n* = 9)	8 (88.9)	6 (66.6)	5 (55.5)	4 (45.5)
Proteus spp. (*n* = 4)	4 (100)	-	-	-
P. aeruginosa (*n* = 21)	5 (23.8)	-	-	-
S. aureus (*n* = 39)	35(89.7)	-	-	-
CoNs (*n* = 23)	18(78.3)	-	-	-
Total (*n* = 115)	82(71.3)	12 (22.6)	8 (15.1)	6 (11.3)

Table 5. Multi-drug resistance patterns of ocular bacterial isolates. MDR: Multi-drug resistance, MRSA: methicillin-resistant *S. aureus*, ESBL: extended-spectrum beta-lactamases producers, CRE: carbapenem-resistant Enterobacteriaceae.

All cases of ocular infections, as per our study, were caused by only one aetiology, belonging to the Gram-positive bacteria, which is *S. aureus*, and this is similar to the outcome of a series of previous studies from the country[15,17,26,27]. It is envisaged that patients' skin flora can be a probable source of infections. However, reports from some other studies revealed CoNs as the predominant isolate[15,18,30]. A couple of previous studies[18,31] have shown that *P. aeruginosa* and *E. coli* were the most common Gram-negative bacteria causing ocular infections. The higher extent of isolation rate of *P. aeruginosa* in our study is worrisome as it is resistant to multiple antibiotics and disinfectants and is also termed as an emerging recalcitrant nosocomial pathogen. The isolation of *P. aeruginosa* and *E. coli* revealed that the source of infections could be exogenous as well. The most intriguing part of our study is the detection of gonococcal conjunctivitis[32], and it implies that the responsible pathogen would have been transmitted by manual contact with infected urine or genital secretions.

It was found that there are variations in frequencies associated with bacterial and fungal ocular infections. Keratitis is the most predominant manifestation of mycotic ocular infections[33]. Isolates of *Aspergillus* spp., followed by *Candida albicans* and *Fusarium* spp., were the common causative agents, and these are in line with the results of a set of previous studies reported from Ethiopia[34], Iran[35] and India[36]. Fungal keratitis is mostly prevalent in tropical and subtropical regions, accounting for 37.7–81.5% of the entire culture-positive cases of corneal infections[33]. Recently, the WHO has proposed the inclusion of infectious keratitis as one of the many neglected tropical diseases[37,38]. The enhanced rate of ocular candidiasis is another important finding since it is believed to cause complications, including retinal detachment, vision loss, intraocular haemorrhage, and glaucoma.

Updated information pertaining to the possible susceptibility profile of bacterial aetiology is a prerequisite for rational usage of antibiotics, particularly when implementing an empirical therapy without culture and susceptibility data. The diagnosis of ocular infections in our study setting is often clinical, and the treatment is exclusively empirical, with the application of topical ophthalmic antibiotics such as aminoglycosides, amphenicol, and macrolides, which are the first line of antibiotics. In contrast, fluoroquinolones are reserved for refractory cases only. The Gram-positive bacteria showed a higher to moderate extent of resistance to the beta-lactam and macrolide drugs in the present work and is also indicated in some previous studies[14,27,29,39]. In contrast to other studies, Gram-negative bacteria showed pronounced resistance against amoxicillin, ampicillin and third-generation cephalosporins[18,31]. This disparity could be due to differences existing in the regional prescription policies and antibiotic stewardship practices.

On the other hand, aminoglycoside (gentamicin) and fluoroquinolones (ciprofloxacin) are the most effective antibiotics against Gram-negatives, whereas lincosamide (clindamycin) and fluoroquinolones (ciprofloxacin) are the most active drugs against their counterparts, which are in unison with the national and global trends in antimicrobial resistance[29,31,40]. The reduction of drug resistance could be attributed to the rational use of antibiotics in our study setting. The increasing rate of piperacillin resistance observed among *P. aeruginosa* isolates is a significant issue that warrants special attention.

The presence of MDR ocular bacteria was detected in the case of 71.3% of isolates, substantiating the previous reports of higher incidence in Dilla and Gondar, Ethiopia[18,41]. This alarming rise in drug resistance can be attributed to several factors, including the overuse of antibiotics for eye and other syndromic infections, improper dosage/ regimen, misuse of antibiotics for viral and other non-bacterial infections, and an extended duration of therapy[8]. The higher resistance observed among bacteria can limit the effectiveness of empirical treatment with the existing antibiotics. Eventually, patients are more likely to experience a prolonged course of infections. The results of the antibiograms obtained in our study can serve as guidelines to clinicians, curtailing the prescription of various syndromic antibiotics. Moreover, the WHO still has not proposed any threshold level for antibiotic resistance aiming at changing the empirical therapy in regard to ocular infections.

Another disturbing finding in the present study was the detection of WHO-prioritised pathogens such as ESBL producers, CRE, and MRSA; ESBL producers were numerous among the isolates of K. pneumoniae. This hints at a menace (treatment failure and increased morbidity) that our study setting is going to face in the future. In other words, clinical implications must be taken into consideration to come up with acceptable practical solutions for this significant challenge.

So far, no report exists on the prevalence of ESBL and carbapenemase-producing ocular isolates in the country. The presence of ESBL and CRE in this study can be correlated to the frequent and inappropriate usage

Variables	Categories	Prevalence n (%)		Odds Ratio			
		Positive	Negative	COR (95%CI)	P value	AOR (95%CI)	P value
Sex	Male	111(64.2)	62(35.8)	0.64(0.38–1.08)	0.09*	0.78(0.41–1.46)	0.44
	Female	46 (53.5)	40(46.5)		1		1
Residence	Urban	69(53.5)	60(46.5)		1		1
	Rural	88(67.7)	42(32.3)	1.82(1.10–3.01)	0.02*	0.957(0.47–1.96)	0.90
Age	≤ 25	60(54.1)	51(45.9)		1		1
	26–49	41(58.6)	29(41.4)	0.61(0.29–1.29)	0.20*	2.61(1.08–6.34)	0.03**
	≥ 50	56(71.8)	22(28.2)	0.43(0.22–0.83)	0.01*	1.04(0.47–2.28)	0.92
Marital status	Married	79(63.2)	46(36.8)	1.23(0.74–2.03)	0.41	-	-
	Unmarried	78(58.2)	56(41.8)		1		
Educational level	Illiterate	78(60)	52(40)	1.16(0.57–2.36)	0.66	-	-
	Primary school	51(60)	34(40)	1.16(0.55–2.47)	0.68		
	Secondary school and above	28(63.6)	16(36.4)		1		
Occupational status	Farmer	67(78.8)	18(21.2)	0.26(0.14–0.48)	0.00*	0.17(0.06–0.49)	0.00**
	Employee	16(66.7)	8(33.3)	0.48(0.19–1.20)	0.12*	0.49(0.17–1.50)	0.21
	Unemployed	74(49.3)	76(50.7)		1		1
Family monthly income (in ETB)	< 2000	110(57.3)	82(42.7)	1.75(0.96–3.18)	0.06*	2.51(1.13–5.53)	0.02**
	≥ 2000	47(70.1)	20(29.9)		1		
Type of infections	Conjunctivitis	61(61)	39(39)	1.35 (0.55–3.28)	0.50	0.96(0.24–1.98)	0.49
	Blepharoconju-nctivitis	33(49.3)	34(50.7)	2.17 (0.86–5.49)	0.10*	1.42(0.49–4.12)	0.51
	Keratitis	44(68.8)	20(31.2)	0.96 (0.37–2.48)	0.93	0.48(0.15–1.47)	0.20
	Blepharitis	19(67.9)	9(32.1)		1		1
Trauma	Yes	52(63.4)	30(36.6)	0.84(0.49–1.44)	0.53	-	-
	No	105(59.3)	72(40.7)		1		
Chronic diseases	Yes	10(62.5)	6(37.5)	0.92(0.32–2.61)	0.87	-	-
	No	110(60.5)	96(39.5)		1		
Corticosteroid drug usage	Yes	12(50)	12(50)	1.61(0.69–3.74)	0.26	-	-
	No	145(61.7)	90(38.3)		1		
Facing washing habit	Daily	58(56.3)	45(43.7)		1	-	-
	Often	30(65.2)	16(34.8)	0.68(0.33–1.41)	0.30	-	-
	Sometimes	69(62.7)	41(37.3)	0.76(0.44–1.32)	0.34	-	-
Surgery	Yes	15(62.5)	9(37.5)	0.91(0.38–2.18)	0.84	-	-
	No	142(60.4)	93(39.6)		1		
Previous eye infection	Yes	113(66.1)	58(39.9)	0.51(0.30–86)	0.01*	0.63(0.32–1.23)	0.17
	No	44(50)	44(50)		1		1
Hospitalization	Yes	50(65.8)	26(34.2)	0.49(0.41–1.27)	0.27	-	-
	No	107(58.5)	76(41.5)		1		1
Antibiotic usage	Yes	87(72.5)	33(27.5)	0.38(0.22–0.64)	< 0.00*	0.54(0.28–1.07)	0.07
	No	70(50.4)	69(49.6)		1		1

Table 6. Bivariate and multivariate analysis of external ocular infections. Note: *Statistically significant at $P \leq 0.25$ in bivariable analysis; ** Statistically significant at $P < 0.05$; AOR: Adjusted odd ratio; COR: Crude odds ratio, 1: reference group, CI: Confidence interval.

of third-generation cephalosporins and carbapenem. Some earlier studies in India reported a higher prevalence of ESBL-producing ocular bacteria[42,43]. As per some studies done in Ethiopia and Uganda, MRSA isolates were also detected[26,27,44]. Besides, methicillin-resistant strains demonstrated concurrent multi-drug resistance in our study[27]. The increasing prevalence of WHO-prioritised pathogens observed should be taken into consideration while treating these infections.

The WHO clinical treatment guideline development protocols emphasise the importance of local resistance patterns in fixing the empirical therapy, as resistance can vary significantly across different geographical zones or even within different healthcare settings in the same zone or country. Therefore, clinicians must consider seriously the available data when prescribing the empirical regimen for ocular infections. Besides, a sound periodic antibiotic surveillance program has to be initiated to reduce the aggravation and spread of drug-resistant bacteria. In this context, the results of this study can assist clinicians in the study setting, especially in fixing an empirical regimen based on the updated antibiogram profile of common bacterial pathogens. Nevertheless, the increment of drug resistance among the isolates may limit the practice of empirical regimens to some extent. Hence, continuous surveillance and antimicrobial stewardship programs appear essential.

Realising the risk factors at the individual as well as population level can lead to an informed policy, decision-making, planning and timely interventions. Eye infections are prevalent medical conditions affecting individuals irrespective of age. The results revealed that participants in the age group of 26–49 years ($P=0.03$) were 2.61 times more prone to develop ocular infections than any other age group[26]. Increased outdoor activities of individuals of certain age groups associated with their respective occupations enhance the chances of contracting ocular infections. Income can indeed be an indicator of a higher level of awareness and access to health-associated knowledge sources and services. Ocular infections were also significantly associated ($P=0.02$) with economic status (monthly income); the odds of having infections among participants with lower monthly income was 2.5 times higher compared to their richer counterparts. The probability of infection is significantly higher among individuals in the low-income group, largely due to inadequate personal and environmental hygiene. Lack of awareness and ignorance of symptoms can worsen the scenario. This could also be attributed to the fact that they often engage in daily labour, exposing them to occupational hazards. Policymakers and healthcare professionals must recognise the importance of this issue and work towards creating sustainable solutions to tackle it.

Limitations

Our study has several limitations, as it is based on a single institution and hence may not necessarily represent the exact image of the epidemiology of ocular infections. Only a few variables were analysed in the study. We did not isolate fastidious, anaerobic bacteria and viral pathogens. Besides, molecular characterisations of bacterial and fungal isolates were not conducted, and also the antifungal susceptibility patterns were not analysed due to lack of facilities.

Conclusions

Results of the present study revealed that conjunctivitis is the most common clinical manifestation, and one out of three bacterial ocular infections is caused by *S. aureus*. Ocular infections had a statistical association with age and family income. The detection of WHO-prioritised multi-drug resistant bacteria such as MRSA, ESBL, and CRE is quite disturbing, warranting further stewardship connected to antibiotic prescription/consumption. The response to treatment in regard to the detected MDR requires further studies. Interestingly, gentamicin, chloramphenicol and ciprofloxacin are still effective for the treatment of both Gram-negative and -positive bacterial ocular pathogens. The prevalence of drug-resistant bacteria observed in this study necessitates the testing of ocular samples for antimicrobial resistance to the maximum possible, aiming at curbing its further aggravation and spread.

Data availability

Data will be available from the corresponding author upon reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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