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Bacterial Profile and Antimicrobial Susceptibility Pattern of External Ocular Infections in Jimma University Specialized Hospital, Southwest Ethiopia

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Abstract Bacteria are the major etiologic agents causing external ocular infections. Resistance of ocular bacteria to antimicrobial agents is a worldwide concern. In this study we sought to determine the bacterial profile and antibiotic susceptibility pattern of external ocular infections patients attending Jimma University Specialized Hospital, Southwest Ethiopia. A cross-sectional study design was employed from January 2012 to June 2012 from which a total of 198 patients with external ocular infections were included in the study. The samples thus collected were transported and are microbiologically processed using standard operating procedure (SOP) under standard laboratory conditions. The data acquired was analysed by computer for statistical analysis using SPSS version 16 for Windows. A total of 198 ocular samples were collected for microbiological evaluation, of which 148 (74.7%) had bacterial growth. The gram-positive cocci comprised 52.0% and the predominant isolate was *S. aureus* (28.4%). Gram-negative bacteria accounted for 48.0% and the predominant isolate was *P. aeruginosa* (20.9%). Majority of gram-positive cocci were susceptible to ciprofloxacin (71; 92.2%) and vancomycin (70; 90.9%) and gram-negative isolates to amikacin (67; 94.4%) and ciprofloxacin (57; 91.5%). These findings indicated that gram-positive cocci were the most common bacteria isolated from external ocular infections and were more susceptible to ciprofloxacin and vancomycin, whereas gram-negative isolates were more susceptible to amikacin and ciprofloxacin.

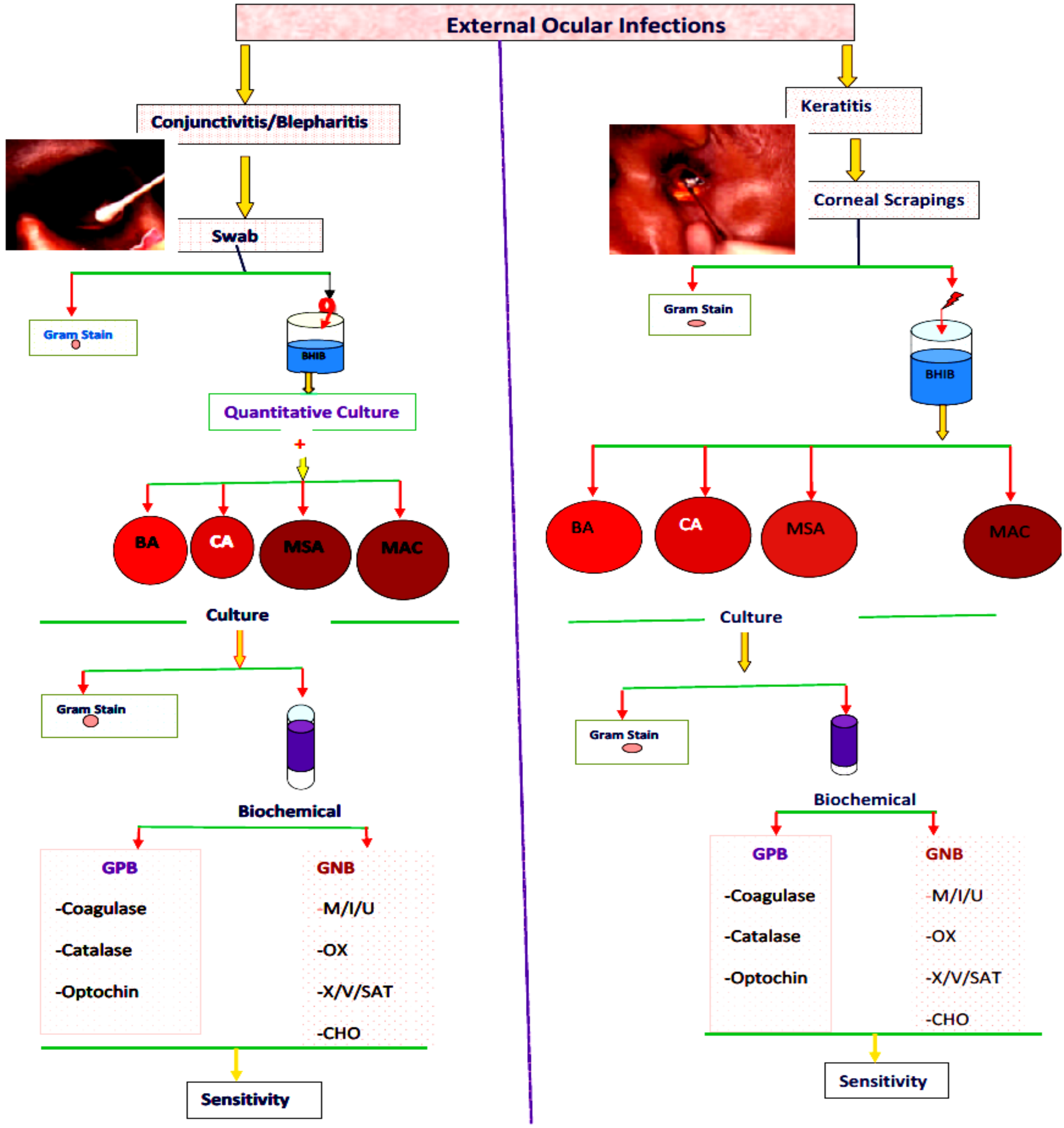
Keywords: *external ocular infections, antimicrobial agents, susceptibility pattern, standard operating procedure (SOP)*

1. Introduction

Pathogenic micro-organisms cause ocular disease due to virulence and host’s reduced resistance because of the factors like personal hygiene, living conditions, socio-economic status, decrease immune status, etc. The areas of the eye that are frequently infected are the conjunctiva, lid and cornea [1,2]. Bacteria are major causative agents that frequently cause infections in eye and possible loss of vision. Hence, there is a need for an immediate treatment for the serious bacterial eye infection that threatens the cornea of eye [2]. For specific antibacterial treatment, isolation and identification of bacterial pathogens along with antibiotic susceptibility spectrum is essential [3]. As there is a worldwide problem regarding the emergence of bacterial resistance towards topical antimicrobial agents which are influenced by characteristics of pathogens, antibiotic-prescribing practises including the use of systemic antibiotics and general healthcare guidelines [4, 5]. This developing resistance increases the risk of treatment failure with potentially serious consequences [6,7].

The bacterial aetiology and their susceptibility as well as resistance patterns may vary according to geographical and regional location [6,7,8]. Therefore, up to date information is essential for ophthalmologists for appropriate antimicrobial therapy [1,4,6,7]. In Ethiopia, the study conducted in Gondar on bacteriology of ocular infections and antibiotic susceptibility patterns reported that there is high incidence of multiple drug resistance problems in ocular pathogens and were observed in 77.3% of bacterial isolates [9], leaving ophthalmologists with a very few choices of drugs for the treatment of ocular infections [6]. Thus, the knowledge of the etiologic agents causing these infections is crucial in proper management of the cases.

The bacterial sensitivity to various antimicrobial agents varies from place to place and in the same place from time to time [6,8]. Therefore, the changing spectrum of microorganisms involved in ocular infections and the emergence of acquired microbial resistance dictate the need for continuous surveillance to guide empirical therapy [6,7,10]. The empirical choice of an effective treatment is becoming more difficult as ocular pathogens are increasingly becoming resistant to commonly used antibiotics [7]. In the study area, there is a scarcity of published data on the spectrum of etiologic agents responsible for external ocular infections. Thus, the purpose of this study was to identify the spectrum of bacterial aetiology of external ocular infections, and to assess the in vitro susceptibility of these ocular bacterial isolates to commonly prescribed antibiotics among patients with external ocular infections in Southwest of Ethiopia (Figure 1).



GPB= Gram positive bacilli, GNB= Gram negative bacilli, M/I/U= Motility/Indole/Urea, Ox= Oxidase test, X/V/SAT= X/V Factors/Satelletism, CHO= Carbohydrate Utilization tests, BA= Blood agar, CA= Chocolate agar, MSA= Manitol salt agar, MAC= MacConkey agar

Figure 1. Flow Chart for Bacterial Identification of External Ocular Infections

2. Materials and Methods

This cross sectional study included 198 ocular samples for microbiological evaluation from patients clinically diagnosed with external ocular infections such as conjunctivitis, keratitis, blepharitis and blepharo-conjunctivitis at Jimma University Specialized Hospital (JUSH), Southwest of Ethiopia, between February 2012 and October 2012. Patients clinically diagnosed with external ocular infections and those who were willing to give informed consent are included in the study. Excluding the patients with trachoma, peripheral ulcerative keratitis, viral keratitis, allergic and viral conjunctivitis, severe ocular trauma, with recent ocular surgery and who took antimicrobial therapy within seven days before requirement.

All the patients were examined on the slit-lamp bio-microscope and infective diseases included in this study were diagnosed clinically by a group of ophthalmologists. After detailed ocular examinations using standard techniques [11], specimens from the eyelid, conjunctiva, and cornea were collected by ophthalmologists. Conjunctival swab was obtained by having the patient look up and wiping a sterile swab moistened with sterile normal saline across the lower conjunctival cul-de-sac of the right eye, from the nasal margin to the temporal margin and back again. A second moistened swab was used to collect specimen from the right eyelid. The swab was placed into a tube containing 1ml sterile normal saline; and was twirled several times. The tube cap was replaced loosely. Similarly, corneal scrapings were obtained with a 20 gauge sterile needle. A topical anaesthetic (tetracaine) was used when taking corneal specimens [12]. The specimen was obtained from the ulcer by scraping the leading edge and base of the ulcer using short, firm strokes. The needle was inserted into a 2ml brain heart infusion broth (Oxoid, Hampshire, UK) tube. The ocular specimen obtained was immediately inoculated into chocolate and blood agar. In cases of conjunctivitis and blepharitis, 100 µl of emulsified sample was dispensed into one chocolate agar plate and two blood agar plates and a chocolate agar plate and a blood agar plate were placed into a candle jar; while one blood agar plate was place into a cold box. The plates and tubes were incubated in appropriate condition.

After vortexing, the tube containing sample from the eyelid and conjunctiva for 60 seconds, 100 µl of emulsified sample with sterile normal saline was inoculated into blood agar and chocolate agar (Oxoid, Hampshire, UK). Colonies were enumerated to determine the culture positivity [13]. Gram stain was done for all ocular specimens. Subcultures was made onto sheep blood agar (5%), chocolate agar, mannitol salt agar and MacConkey agar (Oxoid, Hampshire, UK) using the standard methods. The inoculated media plates were incubated at their respective optimal temperatures; such as blood agar and chocolate agar plates were incubated at 37℃ with 5-10% CO_2. All other media were incubated at 37℃ in aerobic conditions [12]. The plates were examined after 24 and 48 hours. Growth, if any, was identified by standard methods [14]. In case of microbial keratitis, a culture was considered positive when there is growth of the same organism on two or more media, confluent growth of a known ocular pathogen at the site of inoculation on one solid medium, growth in one medium with consistent direct microscopy findings, or growth of the same organism on repeated corneal scraping [15]. In cases with blepharitis and conjunctivitis, a threshold criterion was used for judging culture positive ocular specimens [13].

The bacterial isolates from the ocular specimens were identified up to species level based on different criteria which includes morpho-cultural and biochemical characteristics. Morpho-cultural characteristics including Gram stain to determine the bacterial gram-reaction and colony appearance such as color (e.g. golden colonies indicative of *S. aureus)*, pigment production and haemolysis pattern on blood agar. Bacteria was further characterized by the pattern of biochemical reactions they produce; such as motility, indole, urease, oxidase, catalase, and lactose fermentation and using X and V factors (Oxoid, Hampshire, UK) for *Haemophilus* spp., carbohydrate utilization was performed for identification of gram-negative isolates obtained from ocular samples. Catalase, coagulase test and haemolysis pattern on blood agar was used for identification of gram-positive bacteria. Optochin sensitivity test was also performed to identify *S. pneumoniae [12]*.

In vitro antibiotic susceptibility testing of the bacterial isolates was performed by Kirby-Bauer disc diffusion method [14]. The following antimicrobials was used with their respective concentration: Amikacin (AK, 30µg), gentamicin (CN, 10µg), ceftriaxone (CRO, 30µg), penicillin (P, 10U), ciprofloxacin (CIP, 5µg), tetracycline (TE, 30µg), trimethoprim-sulphamethoxazole (SXT, 1.25/23.75µg), erythromycin (E, 15µg), doxycycline (DO, 30µg), chloramphenicol (C, 30µg), and vancomycin (VA, 30µg) (Oxoid, Hampshire, UK). Using a sterile inoculation loop, 3–5 well isolated colonies of the test organism was emulsified in 4-5ml of Tryptic Soy Broth, incubated at 37℃ for 3 hours, adjusted with sterile saline to achieve a turbidity equivalent to 0.5 McFarland standards. The turbidity of the suspension was also adjusted to 0.5 McFarland using spectrometer at 500nm absorbance in order to standardize the inoculums size [16]. Then the standardized suspension was swabbed on to the Mueller-Hinton agar for non-fastidious organisms and Mueller-Hinton agar with 5% sheep blood for fastidious organisms. Then, the antibiotic discs were placed manually on the medium and incubated at 37℃ for 24 hours and the zones of inhibition diameter was measured using zone scale. The interpretation of the results was according to the Clinical Laboratory Standards Institute (CLSI) methodology as sensitive, intermediate and resistant [14].

Standard Operating Procedures (SOPs) were first prepared by the principal investigator to follow the same procedures during sample collection. Physical diagnosis and sample collection was done by senior ophthalmologists and specimens were collected only from those patients presenting with external ocular infections. SOPs were strictly followed and quality control measures were taken during laboratory processing of the specimens. Culture media was sterilized based on the manufacturer's instruction and the sterility of culture media was checked. The quality of the culture media and antimicrobial susceptibility discs was checked using a standard American Type Culture Collection (ATCC) bacterial reference strain of Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853 were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI). Data obtained was subjected to statistical analysis using SPSS for windows version 16. The study was conducted after getting an approval from the College of Public Health and Medical Sciences Research Ethical Review Committee and the Department of ophthalmology. Patients were informed about the purpose of the study and then written informed consent was obtained from the study participants and any information related to the patient and their clinical history was kept confidential as hospital record. Culture results were sent to the responsible ophthalmologist so that the participants would be benefited from the study.

3. Results

3.1 Socio-demographic Characteristics

The socio-demographic characteristic of study participants is summarized in Table 1. A total number of 198 patients were clinically diagnosed with external ocular infections which were included in this study. The mean age of study participants were 22.7 years with standard deviation of ± 4.2; and the predominant age group was 18-39 years (72; 36.4%). Most of the participants were illiterate (134; 67.7%) in educational status, muslims (112; 56.6%) in religion and urban (110; 55.6%) in residence.

Table 1. Socio-demographic Characteristics of Patients with External Ocular Infections in Jimma University Specialized Hospital, Southwest Ethiopia

Socio-demographic Characteristics	Number	Percent
Age		
0-2	30	15.2
3-11	24	12.1
12-17	30	15.2
18-39	72	36.4
≥40	42	21.2
Sex		
Male	116	58.6
Female	82	41.4
Marital Status		
Married	98	49.5
Others	100	50.5
Residence		
Urban	110	55.6
Rural	88	44.4
Religion		
Muslim	112	56.6
Orthodox	72	36.3
Others	14	7.1

3.2 Bacterial Isolates

A total of 198 ocular samples, 174 swabs eyelid and conjunctiva and 24 corneal scrapings were obtained from patients clinically diagnosed with external ocular infections for microbiological evaluation during the study period. Of 198 ocular specimens subjected to culture, 148 (74.7%) had bacterial growth. The rate of culture-positivity was found to be significantly higher ($P \leq 0.001$) among eyes with conjunctival (87.5%; 35 of 40), than corneal (83.3%; 20 of 24), eyelid and conjunctival (72; 70.6%), eyelids (65.6%; 21 of 32) infections.

The isolated bacteria were shown in Table 2. Out of the 148 isolates, gram-positive cocci accounted for 52.0 % and the predominant isolate was *S. aureus* (54.5%) while gram-negatives comprised of 48.0% and the predominant isolate was *P. aeruginosa* (43.7%). *S. aureus* was the most common isolate in blepharitis (10; 47.6%), conjunctivitis (8; 22.9%), and blepharo-conjunctivitis (19; 26.4%); whereas *P. aeruginosa* was the predominant isolate in keratitis (10; 50.0%). There was a significant variation of bacterial isolates with the clinical features of patients (P = 0.011). Enteric bacteria isolated in this study were *S. marcescens* (9; 6.1%), and *E. coli* (8; 5.4%); together accounted 11.5% of the overall bacterial isolates.

The distribution of bacterial isolates by age is presented in Table 3. *H. influenzae* (10; 34.5%) was the most common bacteria in the age group of newborns to under three years followed by *S. pneumonia* (6; 20.7%); whereas *S. aureus* was found to be the commonest isolate in the age groups of 11-17 years (8; 40.0%), 18-39 years (21; 40.4%), and 40 years and above (10; 35.7%). There was a significant variation of bacterial isolates with the age of patients (P = 0.00).

Table 2. Distribution of Bacterial Isolates of External Ocular Infections in Jimma Specialized Hospital, Southwest Ethiopia

Bacterial isolates	Blepharitis (N=32)	Conjunctivitis (N=40)	Blepharo-conjunctivitis (N=102)	Keratitis (N=24)	Total (N=198)
Gram positive cocci					
S. aureus	10 (47.6)	8 (22.9))	19 (26.4)	5 (25.0)	42 (28.4)
S. pneumoniae	ND	7 (20.0)	11(15.3)	2 (10.0)	20 (13.5)
CoNS	2 (9.5)	4 (11.4)	9 (12.5)	ND	15 (10.1)
Gram negative cocci					
Moraxella Spp.	2 (9.5)	1 (2.9)	3 (4.2)	ND	6 (4.1)
N. gonorrhoeae	ND	2 (5.7)	2 (2.8)	ND	4 (2.7)
Gram negative bacilli					
P. aeruginosa	5 (23.8)	4 (11.4)	12 (16.7)	10 (50.0)	31 (20.9)
H. influenzae	1 (4.8)	5 (14.3)	7 (9.7)	ND	13 (8.8)
S. marcescens	1 (4.8)	1(2.9)	4 (5.6)	3 (15.0)	9 (6.1)
E. coli	0 (0)	3 (8.6)	5 (6.9)	ND	8 (5.4)
Total N (%)	21 (14.2)	35 (23.6)	72 (48.6)	20 (14.2)	148 (100)

CoNS= Coagulase Negative Staphylococcus, ND= Not detected

Table 3. Age wise Distribution of Bacterial Isolates of External Ocular Infections in Jimma University Specialized Hospital, Southwest Ethiopia

Bacterial Isolates	Age (Years)					
	0-2 (N=30)	3-11 (N=24)	12-17 (N=30)	18-39 (N=72)	≥40 (N=42)	Total (N=198)
Gram-positive cocci						
S. aureus	1(3.4)	2(10.5)	8(40.0)	21(40.4)	10(35.7)	42(28.4)
S. pneumoniae	6 (20.7)	5 (26.3)	3 (15.0)	2 (3.8)	4 (14.3)	20 (13.5)
CoNS	3 (10.3)	ND	1(5.0)	10 (19.2)	1(3.6)	15 (10.1)
Gram-negative cocci						
Moraxella spp.	2 (6.9)	2 (10.5)	1(5.0)	1(1.9)	ND	6 (4.0)
N. gonorrhoeae	2 (6.9)	1(5.3)	1(5.0)	ND	ND	4 (2.7)
Gram-negative bacilli						
P. aeruginosa	1 (3.4)	6 (31.6)	3 (15.0)	13 (25.0)	8 (28.6)	31(20.9)
H. influenzae	10 (34.5)	ND	3(13.0)	ND	ND	13 (8.8)
S. marcescens	3 (10.3)	2 (10.5)	ND	3(5.8)	1(3.6)	9 (6.1)
E. coli	1(3.4)	1(5.3)	ND	2 (3.8)	4 (14.3)	8 (5.4)
Total No (%)	29(18.9)	19(12.8)	20(13.5)	52(39.2)	28(18.9)	148 (100)

CoNS= Coagulase Negative Staphylococcus, ND= Not detected

3.3 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility pattern of bacteria was done on eleven antimicrobial agents. Vancomycin was tested only for gram-positives, penicillin for gram-positives and *N. gonorrhoeae*, erythromycin for gram-positives, *H. influenzae* and *N. gonorrhoeae*.

Table 4 illustrate the susceptibility of gram-positive bacteria. More than half of gram-positives showed resistance against penicillin (56; 72.7%); but they were relatively highly susceptible to ciprofloxacin (71; 92.2%), vancomycin (70; 90.9%), doxycycline (63; 81.8%), tetracycline (54; 70.1%), ceftriaxone (51; 66.2%), erythromycin (47; 61.0%), amikacin (42; 63.6%) and, gentamicin (45; 58.4%). *S. aureus*, were coagulase positive *staphylococci*, and was the most common isolates comprising 54.5% of the gram-positive bacteria and showed high rate of susceptibility to ciprofloxacin (37; 88.1%), amikacin (37; 88.1%), gentamicin (36; 85.7%), vancomycin (35; 83.3%), tetracycline (31; 73.8%), ceftriaxone (30; 71.4%), doxycycline (30; 71.4%). They were highly resistant to penicillin (42; 100%), and chloramphenicol (24; 57.1%).

Table 4. Antimicrobial Susceptibility Pattern of Gram Positive Bacteria of External Ocular Infections in Jimma University Specialized Hospital, Southwest Ethiopia

Antibiotic	Gram-positive bacterial isolates (n=77)								
	S. aureus (n=42)			*S. pneumoniae* (n=20)			CoNS (n=15)		
	S	I	R	S	I	R	S	I	R
P	0	0	42 (100)	16 (80.0)	3 (15.0)	1 (5.0)	2 (13.3)	0	13 (86.7)
AK	37 (88.1)	3 (7.2)	2 (4.7)	0	1 (5.0)	19 (95.0)	12 (80.0)	0	3 (20.0)
C	13 (31.0)	5 (11.9)	24 (57.1)	20 (100)	0	0	2 (13.3)	2 (13.3)	11 (73.4)
CN	36 (85.7)	4 (9.5)	2 (4.8)	3 (15.0)	0	17 (85.0)	6 (40.0)	1 (6.7)	8 (53.3)
VA	35 (83.3)	2 (4.8)	5 (11.9)	20 (100)	0	0	15 (100)	0	0
E	21 (50.0)	6 (14.3)	15 (35.7)	18 (90.0)	0	2 (10.0)	8 (53.3)	3 (20.0)	4 (26.7)
CIP	37 (88.1)	3 (7.2)	2 (4.8)	19 (95.0)	0	1 (5.0)	15 (100)	0	0
SXT	23 (54.8)	10 (23.8)	9 (21.4)	6 (30.0)	1 (5.0)	13 (65.0)	4 (26.7)	4 (26.7)	7 (46.6)
TE	31 (73.8)	2 (4.8)	9 (21.4)	14 (70.0)	2 (10.0)	4 (20.0)	9 (60.0)	1 (6.7)	5 (33.3)
DO	30 (71.4)	4 (9.5)	8 (19.1)	19 (95.0)	0	1 (5.0)	14 (93.3)	0	1 (6.7)
CRO	30 (71.4)	2 (4.8)	10 (23.8)	12 (60.0)	5 (25.0)	3 (15.0)	9 (60.0)	2 (13.3)	4 (26.7)

P-Penicillin(10 U), AK- Amikacin (30 µg), C-Chloroamphenicol (30 µg), CN-Gentamicin (10 µg), VA-Vancomycin (30 µg), E-Erthromycin (15 µg), SXT-Trimethoprim-sulphamethoxazole (1.25/23.75 µg), TE- Tetracycline (30 µg), DO-Doxycycline (30 µg), CRO- Ceftriaxone (30 µg), CoNS= Coagulase Negative Staphylococcus, S= Susceptible, I= Intermediate, R= Resistance

S. pneumonia were α-haemolytic and Optochin sensitive, showed high rate susceptibility to chloramphenicol (20; 100%), vancomycin (20; 100%), doxycycline (19; 95.0%), erythromycin (18; 90.0%), penicillin (16; 80.0%), tetracycline (14; 70.0%), and ceftriaxone (12; 60.0%); but more than half of *S. pneumoniae* showed resistance against amikacin (14; 70.0%), and trimethoprim-sulphamethoxazole (13; 65.0%)

The antimicrobial susceptibility pattern of gram-negative bacteria against eight antimicrobial agents was shown in Table 5. More than half of gram-negative isolates showed resistance against trimethoprim-sulphamethoxazole (54; 76.1%). However, more than half were susceptible to amikacin (67; 94.4%), ciprofloxacin (57; 91.5%), gentamicin (56; 72.7%), doxycycline (46; 64.8%), and ceftriaxone (44; 62.0%).

P. aeruginosa in culture media generally produce a sweet grape-like odour, with smooth round colonies having fluorescent greenish colour with non-fluorescent bluish pigment which diffused in the agar with positive oxidase with no fermentation towards carbohydrate. This constitutes 43.7% of the Gram-negative bacteria which were highly sensitive towards amikacin (30; 96.8%), ciprofloxacin (26; 83.9%), ceftriaxone (21; 67.7%), doxycycline (17; 54.8%), and chloramphenicol (16; 51.6%). However, they show a high resistance rate towards trimethoprim-sulphamethoxazole (23; 74.2%), and tetracycline (22; 71.0%). *H. influenzae* constituted 18.3% of gram-negative bacteria and were occur in pairs as coccoid bacilli. They usually occur in short chains and differentiated by their ability to grow on tryptic soy agar containing XV factors. They were found sensitive to gentamicin (13; 100%), ciprofloxacin (12; 92.3%), ceftriaxone (11; 84.6%), erythromycin (11; 84.6%), chloramphenicol (8; 61.6%), and tetracycline (9; 69.2%).

N. gonorrhoeae were gram-negative cocci kidney-shaped occur in pairs and with flat or concave sides. They formed convex glistering elevated transparent or opaque, non-pigmented mucoid colonies; constituted 5.6 % of the gram-negatives, showed resistance against trimethoprim-sulphamethoxazole (3; 75.0%), gentamicin (1; 25.0%), tetracycline (1; 25.0%), doxycycline (1; 25.0%), and penicillin (1; 25.0%); however, it was fully susceptible to amikacin, ceftriaxone, ciprofloxacin, and erythromycin.

Moraxella spp. was non-pigmented with pinkish grey opaque colonies, oxidase positive and was differentiated by its lack of carbohydrate fermentation. They were fully susceptible to ciprofloxacin (6; 100%), and doxycycline (6; 100%); but was highly resistance to trimethoprim-sulphamethoxazole (5; 83.3%).

Table 5. Antimicrobial Susceptibility Patterns of Gram-negative bacteria of External Ocular Infections in Jimma University Specialized Hospital, Southwest Ethiopia

Antibiotic	Gram-negative bacterial isolates (n= 71)																	
	P. aeruginosa (n=31)			*H. influenza* (n=13)			*S. marcescens* (n=9)			*E. coli* (n=8)			*Moraxella* spp. (n=6)			*N. gonorrhoeae* (n= 4)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
AK	30 (96.8)	1 (3.2)	0	10 (76.9)	3 (23.0)	0	9 (100)	0	0	8 (100)	0	0	6 (100)	0	0	4 (100)	0	0
C	16 (51.6)	3 (9.7)	12 (38.7)	8 (61.6)	2 (15.4)	3 (23.0)	7 (77.8)	0	2 (22.2)	2 (25.0)	0	6 (75.0)	6 (100)	0	0	3 (75.0)	0	1 (25.0)
CN	27 (87.1)	4 (12.9)	0	13 (100)	0	0	5 (55.6)	0	4 (44.4)	3 (37.5)	2 (25.0)	5 (37.5)	5 (83.3)	1 (16.7)	0	3 (75.0)	0	1 (25.0)
CIP	26 (83.9)	3 (9.7)	2 (6.4)	12 (92.3)	0	1 (7.7)	9 (100)	0	0	3 (37.5)	0	5 (62.5)	6 (100)	0	0	4 (100)	0	0
SXT	7 (22.6)	1 (3.2)	23 (74.2)	1 (7.7)	0	12 (92.3)	5 (55.6)	0	4 (44.4)	1 (12.5)	0	7 (87.5)	1 (16.7)	0	5 (83.3)	1 (25.0)	0	3 (75.0)
TE	8 (25.8)	1 (3.2)	22 (71.0)	9 (69.2)	1 (7.7)	2 (15.4)	4 (44.4)	0	5 (55.5)	7 (87.5)	0	1 (12.5)	5 (83.3)	0	1 (16.7)	3 (75.0)	0	1 (25.0)
DO	17 (54.8)	0	14 (45.1)	10 (77.0)	2 (15.3)	1 (7.7)	6 (66.7)	3 (33.3)	1 (11.1)	5 (62.5)	1 (12.5)	2 (25.0)	6 (100)	0	0	2 (50.0)	1 (25.0)	1 (25.0)
CRO	21 (67.7)	4 (12.9)	6 (19.4)	11 (84.6)	0	2 (15.3)	2 (22.2)	0	7 (77.8)	1 (12.5)	2 (25.0)	5 (62.5)	5 (83.3)	0	1 (16.7)	4 (100)	0	0
E	NT	NT	NT	11 (84.6)	0 (0)	2 (15.4)	NT	NT	NT	NT	NT	NT	NT	NT	NT	4 (100)	0	0
P	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	3 (75.0)	0 (0)	1 (25.0)

P-Penicillin (10 U), AK-Amikacin (30 µg), C- Chloroamphenicol (30 µg,) CN- Gentamicin (10 µg), VA-Vancomycin (30 µg), E- Erthromycin (15 µg), SXT-Trimethoprim-sulphamethoxazole (1.25/23.75 µg), TE-Tetracycline (30 µg), DO-Doxycycline (30 µg), CRO-Ceftriaxone (30 µg), NT= Not tested

4. Discussion

A total of 198 ocular samples, 174 swabs from eyelid and conjunctiva and 24 corneal scrapings from cornea were obtained from patients clinically diagnosed with external ocular infections for microbiological evaluation during the study period. To judge the culture positivity, quantitative culture was done for the swabs collected from the eyelid and conjunctiva [13]. Of 198 ocular specimens subjected to culture, 148 (74.7%) had bacterial growth. Out of the 148 isolates in this study, gram-positives accounted for 52.0% while gram-negatives accounted for the remaining 48.0% of the total isolates. The most common isolate observed in this study was *S. aureus* (28.4%; 42 of 148) followed by *P. aeruginosa* (21%; 31 of 148). Similar studies conducted in India [1,8], Nigeria [2,17], Gondar [9], and Addis Ababa [16] also reported *S. aureus* as a predominant ocular isolate. However, similar studies reported *E. coli* [9,17], *S. albus* [2], *S. pneumoniae* [1,8] as the second common bacterial isolate.

In this study, *S. aureus* was the most common isolate in blepharitis (10; 47.6%), conjunctivitis (8; 22.9%), and blepharo-conjunctivitis (19; 26.4%). This is consistent with similar studies conducted in India [8] and Nigeria [2], [18]. However, *P. aeruginosa* was found to be the predominant isolate in cases of microbial keratitis which accounted 50% (10 of 20) of the overall bacterial isolates of bacterial keratitis. This finding was supported by similar studies conducted in Australia [19], Sudan [20], Malaysia [21], Thailand [22] and India [32]. As part of the normal flora of the cornea, *Pseudomonas* grow better in the cornea than in any known culture media and causes infection when mechanical trauma of the corneal epithelium occurs. It produces exotoxin A, which causes tissue necrosis leading to corneal ulceration [2,12].

However, studies conducted in India [8,23,24] and Addis Ababa [16] reported *S. pneumoniae* as the most common isolate in microbial keratitis. One study in India [25] reported *P. aeruginosa* and *S. pneumoniae* as a predominant isolates of microbial keratitis with equal frequency. Other studies in California [26], and India [28] reported *Staphylococcus* spp. as a predominant isolates of microbial keratitis. This may be due to the variation with the patient population, health of the cornea, geographic location and climate, and also tends to vary somewhat over time [24].

This study showed fewer isolates of enteric bacteria (11.5%; 17 of 148) when compared to similar study conducted in Nigeria [17], Gondar [9], and Israel [29]. This low number of *enterobacteriaceae* may be due to reduction in hand-faecal contamination and/ or increased access to potable water sources in the study area [2].

In general, the ocular isolates identified in this study were similar to those of many other studies conducted in different areas either nationally or internationally except few differences. Even though the main bacteria which are known to cause external ocular infections is *S. aureus* but the prevalence and degree of occurrence of external ocular pathogens over others are dependent on the geographic location depending on the local population [8]. Resistance and sensitivity based on *in vitro* testing may not reflect true clinical resistance and response to an antibiotic because of the host factors and penetration of the drug [8]. However, these results do provide information that allows a clinician to make rationale-based decision in choosing an initial regimen for treatment of ocular pathogens [1].

Based on results from susceptibility testing in this study, most gram-positive cocci were susceptible to ciprofloxacin (92.2%; 71 of 77) followed by vancomycin (90.9%; 70 of 77). The coverage of vancomycin against CoNS and *S. aureus* was 83.3% (35 of 42) and 100% (15 of 15) respectively. This finding is in agreement with study conducted in India [8]. However, study conducted in Iran [10] reported low coverage of vancomycin against *S. aureus*.

Gentamicin covered against 45(58.4%) of the 77 gram-positive cocci isolates and had high coverage against *S. aureus* (85.7%; 36 of 42). This result is consistent with similar studies conducted in Iran [10], India [8,32] and Nigeria [2]. However, this study showed low coverage of gentamicin against CoNS (40.0%, 6 of 15) as compared to study conducted in Nigeria [2], India (32). The coverage of this antibiotic for *Pseudomonas* spp. was 13 of 17 isolates (76.4%). *P. aeruginosa*, which constitutes 43.7% of the Gram-negative bacteria were highly sensitive towards amikacin (30; 96.8%), ciprofloxacin (26; 83.9%), ceftriaxone (21; 67.7%), doxycycline (17; 54.8%), and chloramphenicol (16; 51.6%). Similar results have been reported for ciprofloxacin from studies done in Saudi Arabia [33] and Nigeria [18]. However, study in India [32] reported low coverage of ciprofloxacin for *P. aeruginosa*.

The coverage of gentamicin against gram-negative bacilli in this study was 72.9% (35 of 48 isolates) and the coverage of this antibiotic for *P. aeruginosa* was 87.1% (27 of 31 isolates). This is comparable with similar studies done in Nigeria [2], and Iran [10]. However, study conducted in India [30] reported low coverage of gentamicin for *P. aeruginosa*. The coverage of tetracycline against gram-positive cocci was 70.1% (54 of 77). This finding is comparable with study conducted in Iran [10].

Majority of the gram-negative cocci (72.7%; 56 of 77) showed resistance to penicillin. However, the coverage of penicillin against *S. pneumoniae* in this study was high (80.0%; 16 of 20). This is comparable with studies conducted in Iran [10] and Nigeria [2]. Amikacin had high coverage against *S. aureus* (88.1%; 37 of 42) and CoNS (80.0%; 12 of 15). This is consistent with studies conducted in Iran [10] and India [8,32].

The emergence of bacterial resistance was due to characteristics of the pathogens, antibiotic-prescribing practices including the widespread use of systemic antibiotics, and health care guidelines [5,6,31]. Other contributing factors may include improper dosage regimen, misuse of antibiotics for viral and other non-bacterial infections, extended duration of therapy and not in the least globalization and migration [6].

In Ethiopia, it is in common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics. This may contribute to the emergence and spread of antimicrobial resistance [9,16]. Other factors may include availability of the suboptimal quality or substandard antimicrobial drugs, increased usage of a particular antimicrobial agent, poor sanitation, contaminated food and cross-contamination from humans or animals [2,5,16]. As a result with susceptibility patterns of bacteria to various antimicrobial agents may vary from place to place and in the same place from time to time [7,8,12].

In this study, gram-positive cocci were the most common bacteria isolated from external ocular infections and were more susceptible to ciprofloxacin and vancomycin, while gram-negative isolates were more susceptible to amikacin and ciprofloxacin. Pharmacists and ophthalmologists should further study on those antibiotics which showed high coverage against the external bacterial isolates before using for the treatment of external ocular bacterial infections in the study area. Continuous large scale studies should also be done in the future in order to monitor the antimicrobial resistance of external ocular bacterial isolates.

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Conflict of Interest

The authors would like to declare no potential conflict of interest.

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