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# Research Article

# Clinical and Microbiological Profile of Bacterial and Fungal Suspected Corneal Ulcer at University of Gondar Tertiary Eye Care and Training Centre, Northwest Ethiopia

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Background. A corneal ulcer is a major cause of monocular blindness in developing countries, including Ethiopia. Its etiology varies based on its geographical location and climatic conditions. Therefore, the main objective of this research was to assess the clinical and microbiological profile of suspected bacterial and fungal corneal ulcers at the Tertiary Eye Care and Training Centre at Gondar University. Methods. A cross-sectional hospital-based study of corneal ulcer cases was performed from February to October 2019. Sociodemographic and clinical data were collected using a standardized questionnaire. Corneal scrapings were used to classify bacterial and fungal pathogens. The specimens were inoculated on BHI media and sub-cultured on culture media for the separate cultivation of bacteria and fungi. Biochemical tests have been carried out to classify bacteria. Following CLSI, the antimicrobial resistance pattern of bacterial isolates was carried out. Wet mounting, Lactophenol cotton blue staining, and colony characteristics on SDA were used to classify fungal species. The data were analyzed with version 20 of the SPSS. Results. A total of 30 suspected bacterial and fungal keratitis patients have been enrolled in this study. The visual acuity presented in 90% of the affected eyes was in the category of blindness (<3/60). In 71% of the cases, clinically presumed risk factors were identified. Trauma was the most common risk factor found in 46% of cases, followed by keratitis exposure (13%). Of the corneal scrape tests, 76.6% were positive for bacteria and fungi. Fungi were identified in 53.3% of corneal ulcers followed by 33.3% of bacterial growth. The commonest fungi and bacteria isolated were Aspergillus species (69%) and S. aureus, respectively. The prevalence of Methicillinresistant S. aureus (MRSA) was 2 (40%). The identified Pseudomonas species were susceptible to Gentamicin and Ciprofloxacin but resistant to Ceftriaxone. Conclusion. The primary microbial agents for corneal ulcers were fungi, and trauma was the most significant risk factor associated with corneal ulcers. To avoid chronic ocular morbidity and blindness, early identification of the etiologic agent and the provision of adequate management are recommended.

# 1. Background

A corneal ulcer is discontinuation of necrosis of the underlying corneal tissue on the normal epithelial surface of the cornea [1]. It is a major worldwide cause of corneal blindness that is mostly under-reported but can be responsible every year for 1.5–2.0 million new cases of monocular blindness [2]. In many of the developing nations in Asia, the Middle East, and Africa, it has been consistently identified as the leading cause of blindness and visual disability, ranked next to cataracts [3]. Corneal ulcers tend to exist in epidemic proportions in the developing

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world, ten times more common than in developed countries [4].

Microbial keratitis is an important preventable cause of monocular blindness worldwide. Several studies have evaluated the etiology, management, and outcome of microbial keratitis [5–7]. Demographic and geographic factors affect the distribution of the causative organisms of infectious corneal ulceration [8–10]. They include pre-existing corneal disease as well as other risk factors such as contactlens wear, surgical or non-surgical trauma, and ocular surface disease, contaminated ocular medications, impaired defense mechanisms, and altered structure of the corneal surface [1, 8].

Clinical examination is crucial for the diagnosis of causative microorganisms and the management of microbial keratitis. Patients with the bacterial corneal present with rapid onset of eye pain accompanied by redness, photophobia, and decreased vision [11, 12], and fungal keratitis includes an infiltrate with feathery margins, elevated edges, rough texture, gray-brown pigmentation, satellite lesions, and endothelial plaque [13].

Both fungal and bacterial keratitis predominate in the Asian subcontinent [14]. Relatively little is known about the situation in Africa. Managing severe bacterial keratitis involves treatment with broad-spectrum antibiotics, which is done most of the time before pathogen identification and antibiotic susceptibility tests. This indiscriminate use of antibiotics has contributed to the development of resistance to a variety of widely used antimicrobials. Comprehensive studies of severe sight-threatening infectious keratitis are therefore important as they provide valuable information on the detection of etiology, predisposing factors, the trend of antibiotic resistance of bacterial isolates, and visual outcomes that guide the development of management strategies and protocols.

#### 2. Methods

- 2.1. Study Design and Period. A cross-sectional hospital-based study was conducted at the University of Gondar Tertiary Eye Care and Training Centre from February to October 2019. The research was to determine the clinical and microbiological profile among clinically suspected bacterial and fungal corneal ulcers.
- 2.2. Study Area. The thesis was performed at the University of Gondar Tertiary Eye Care and Training Centre, which is situated in the administrative zone in Central Gondar, Amhara National, Regional State, Northwest Ethiopia. Gondar is about 750 km from Addis Ababa, Ethiopia's capital city. According to the 2007 census, the estimated population of the city is 615,000, and Gondar city has one specialist hospital and five government health centers. The University of Gondar Tertiary Eye Care Centre, founded in 2004, is the only tertiary eye care centre in Northwest Ethiopia. With a total catchment area of 14 million people, it has a referral link from 14 zones in Northwest Ethiopia. The University of Gondar's Tertiary Eye Care and Teaching Unit

has four subspecialty clinics, four senior ophthalmologists, and twenty-four residents working actively. There is a cornea subspecialty clinic run by two cornea sub-specialists.

- 2.3. Population. All patients visiting University of Gondar Tertiary Eye care and Training Centre during the study period will be used as population for this study. However, patients with stromal abscess without epithelial defect, non-infected ulcer, and less than 10 years of age were not part of this study.
- 2.4. Sample Size and Sampling Technique. The research was enrolled in all consecutive patients who visited the University of Gondar Tertiary Eye Care and Training Centre during the study period with clinically suspected infectious corneal ulcer and met the inclusion and exclusion requirements.

#### 2.5. Data Collection

2.6. Sociodemographic and Clinical Data Collection. Data on sociodemographic traits, possible risk factors, and clinical findings from each study participant were obtained by qualified resident physicians using a pretested structured questionnaire.

Clinical data were obtained and the best-corrected visual acuity (BCVA) recorded. Every patient underwent a thorough biomicroscopic slit-lamp examination with a special focus on the cornea. Information of the corneal ulcer including location, size, and type of penetration, ulcer margins, presence of any satellite lesions, corneal vascularization, and hypopyon was examined and registered. B-scan ultrasound examination of the posterior segment was done for those endophthalmitis suspected patients.

- 2.7. Corneal Scrape Collection and Transportation. A corneal scrape was performed by a senior ophthalmologist using standardized sampling techniques. Corneal anesthesia was obtained by applying preservative-free topical proparacaine 0.5% or Tetracaine 0.5%. The sample was directly taken from its base or bulk using a specially designed kimura spatula with an aseptic technique. For patients who were taking antibiotics at the time of presentation to the clinic, treatment was stopped and the investigation was delayed for 24 hours to be part of this study. A medical laboratory technologist and a medical microbiologist conducted the laboratory diagnosis using standard operating procedures. For Gram staining and unstained KOH preparation, a corneal scrape was smeared onto two slides. Another scraping of 0.5 ml brain heart infusion broth has been inoculated and both were transferred to the Department of Microbiology.
- 2.8. Bacterial Culture and Identification Methods. After the brain heart infusion broth was reached in the microbiology laboratory, it was incubated at 37°C for 24–48 hours. The incubated BHI broth was sub-cultured on blood agar plate

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(BAP), chocolate agar plate (CAP), mannitol salt agar (MSA), and MacConkey agar. The BAP, MSA, and MacConkey agar were inoculated at 37°C for 24 to about 48 hours. On the other hand, the CAP was incubated at 37°C with 5% CO2 for 24–48 hrs. At 48 hours, the cultural media that had no visible growth were eventually discarded.

By investigating their colony morphology, Gram stain, and hemolytic reactions on blood agar plates, preliminary identification of bacterial isolates was performed. The discovery of bacteria was carried out via a series of routine biochemical tests. Based on their Gram reaction, catalase, and coagulase test results, Gram-positive cocci have been identified. A suspension of the test organism was prepared by inserting 3–4 colonies of test species in 5 ml of nutrient broth to distinguish Gram-negative bacteria. Indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, urea agar, and motility medium were inoculated with a loop full of the bacterial suspension and incubated at 35–37 OC for 24 hours. Gram-negative organisms have been identified by checking for a shift of color for acid production, gas production, H<sub>2</sub>S production, and medium motility turbidity.

2.9. Antimicrobial Susceptibility Test. The disc diffusion method on Mueller Hinton agar (MHA) was used to conduct an antimicrobial susceptibility test on each identified bacterium. However, the antimicrobial sensitivity test for fastidious bacterial pathogens was conducted on an MHA medium containing 5% defibrinated sheep blood. In brief, 3-5 bacterial colonies of the test organism were picked and emulsified in 5 ml of nutrient broth and mixed thoroughly. To standardize the density of the inoculum for the susceptibility test, a 0.5 McFarland standard solution was used. Then, the plates were inoculated by streaking the swab over the entire agar surface and the antimicrobial impregnated disks were placed using sterile forceps on the agar surface, incubated at 37°C for 24 hours, and the zone of inhibition was determined. The zone diameters were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline as susceptible (S), intermediate (I), or resistant (R).

2.10. Fungal Culture and Identification Methods. The remaining broth sample was inoculated directly by multiple C-shaped streaks on Sabouraud Dextrose Agar (SDA) media for fungi and incubated at 25°C and 37°C aerobically checking for fungal growth every other day. Fungi were identified by examining their cultural characteristics such as topography, texture, rate of growth, pigmentation on the front, and reverse side. Those plates that demonstrated fungal growth were sent to the Ethiopian Public Health Institute (EPHI) for further species identification.

Microscopic identification of fungal species was performed using KOH wet mount by placing pieces of a colony from SDA to clean slides, adding 1 drop of 20% KOH, placing the cover glass on top of the slide, and finally characterizing the fungal structures by microscopic examination starting with low power (10×). In addition to KOH,

wet mount Lactophenol cotton blue staining was used to characterize fungal structures.

2.11. Data Quality Assurance. The reliability of the findings was guaranteed by implementing quality control (QC) measures throughout the whole process of the laboratory work. All materials, equipment, and procedures were adequately controlled. Data on sociodemographic characteristics and eye-related medical history were collected by a trained ophthalmic resident. Pretested questionnaire guided interview was used for the data collection on sociodemographic characteristics and associated factors. All specimens were collected following standard operating procedures (SOPs) for corneal ulcer specimen collection.

Internal quality control materials were included, and tests were performed based on manufacturer instructions.

The sterility of culture media was ensured by incubating un-inoculated media from each batch and the performance of all prepared culture media was checked by inoculating standard strains, such as *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853) obtained from the Ethiopian Public Health Institute, Addis Ababa, Ethiopia. The qualities of biochemical tests were also checked by these reference strains. A double data entry system was used to maintain data entry quality.

2.12. Data Analysis. After completion of each day of data collection, the collected data were checked for accuracy and completeness. The data were coded and entered into EPI-Info version 7, and statistical analysis was made by SPSS version 25. Percentages, means, and frequency were used to describe the results, and they were displayed in tables of frequency, bar graphs, and pie charts.

# 3. Results

3.1. Sociodemographic Characteristics. This research included a total of 30 cases of suspected bacterial and fungal corneal ulcers visiting the University of Gondar Eye Care and Training Centre. The mean age of the participants was 39 years, and most of them were between 18 and 55 years of age (70%). By occupation, the majority of participants were male (90%) and farmers (83.3%). Almost 97% of the cases were from rural areas. Around 87% of respondents had no formal schooling (Table 1). Relatively larger numbers of cases were observed between April and July (63.3%) (Figure 1).

# 4. Clinical Profile of the Study Participants

Around 63.3% of the patients presented within the first two weeks of disease, and 23.3% of cases arrived within 15–30 days. The visual acuity presented in most of the affected eyes (90%) was in the range of blindness (<3/60). The majority of participants (80%) had ulcers larger than 3 mm, and 46% of cases presented with hypopyon. Clinically presumed risk factors were identified in 71% of cases (Table 2). In these,

Table 1: Sociodemographic characteristics of the study participants among bacterial and fungal suspected corneal ulcers at University of
Gondar Tertiary Eye Care and Training Centre, Northwest Ethiopia, 2019 ( $n = 30$ ).

Characteristics	Frequency (n)	Pe	rcent
	10–17	3	10.0
Ago in wooms	18–35	9	30
Age in years  Sex  Residence  Educational status	36–55	12	40
	>56	6	20
S. corr	Male	27	90.0
Sex	Female	3	10.0
Residence	Rural	29	96.7
	Urban	1	3.3
Educational status	No formal education	26	86.6
Educational status	Formal education	3 9 12 6 27 3 29 1	13.4
	<500.00 ETB	16	53.3
Monthly income	500.00-2000.00 ETB	8	26.7
·	>2000.00 ETB	6	20.0
Occupation	Farmer	25	83.3
	Daily worker	1	3.3
	Student	4	13.3

ETB: Ethiopian Birr.

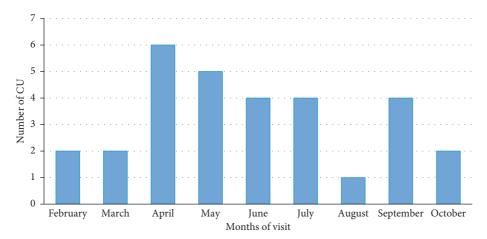


FIGURE 1: The seasonal distribution of the study participants at University of Gondar Tertiary Eye Care and Training Centre, Northwest Ethiopia, 2019 (n = 30).

trauma was the most common risk factor found in 46% of cases followed by keratitis in 13% of cases (Figure 2).

4.1. Correlation between Types of Corneal Ulcers and Duration of Presentation. Concerning the duration of keratitis, 2 (6.7%) corneal ulcer patients presented within 1–3 days, 11 (36.7%) within 4–7 days, 6 (20.0%) within 8–14 days, 7 (23.3%) within 15–30 days, and 4 (13.3%) more than a month at the onset of symptoms at the treatment centre. 60.9% and 39.1% were fungi and bacteria from the total isolates, respectively. Of these, 43.5% and 30.4% were acute fungal and bacterial corneal ulcers, respectively. On the other hand, fungi and bacteria from chronic keratitis were 17.4% and 8.7%, respectively. Among fungal keratitis, most were present 5 (35.7%) within 4–7 days, followed by 4 (28.6%) within 8–14 days, and 3 (21.4%) within 15–30 days at the onset of clinical symptoms. In bacterial corneal ulcers, the majority (5) (71.4%) were between 4 and 7 days of

presentation and 2 (28.6%) more than one month at the treatment centre (Figure 3).

4.2. Microbiological Profile of Corneal Ulcer Cases. Of the corneal scrape tests, 76.6% were culture-positive, indicating the growth of both bacteria and fungi. Fungi were the primary microorganisms found in 53.3% of the total cases of corneal ulcer, followed by bacteria (33.3%). Two patients were co-infected by bacterial and fungal etiology (6.7%). However, 87.5% and 77.8% of patients infected with fungi and bacteria were infected by fungal and bacterial etiology alone, respectively. The commonest fungal isolate was Aspergillus species (69%). The other fungal species identified in this study include two cases of Penicillium species, one case of candida species, and one case of Rhizopus species (Table 3). The majority of bacterial isolates were Gram-positive cocci (70%), in which S. aureus and Streptococcus species accounted for 50% and 20% of bacterial isolates, respectively.

Table 2: Clinical characteristics of the study participants among bacterial and fungal suspected corneal ulcers at University of Gondar Tertiary Eye Care and Training Centre, North West Ethiopia, 2019 (n = 30).

Clinical profile	Frequency (n)	Pe	rcent
	1–7 days	13	43.3
Duration at presentation  Symptom  Visual acuity	7-14 days	6	20.0
Duration at presentation	15-30 days	7	23.3
	>30 days	4	13.3
	Pain	29	96.7
	Blurred vision	28	93.3
Symptom	Tearing	26	86.7
	Redness	25	83.3
	Whitish appearance	23	76.7
	VA<3/60	27	90.0
Visual acuity	3/60 <td>1</td> <td>3.3</td>	1	3.3
	>/6/18	2	6.7
	Central	11	36.7
Symptom  Visual acuity  Location of ulcers  Ulcer size  Hypopyon	Paracentral	6	20.0
Location of dicers	Peripheral	5	16.7
	1-7 days 13 7-14 days 6 15-30 days 7 >30 days 4  Pain 29 Blurred vision 28 Tearing 26 Redness 25 Whitish appearance 23  VA<3/60 27 3/60/6/18 2  Central 11 Paracentral 6	26.7	
	<3 mm	6	20.0
Ulcer size	>3 mm	24	80.0
	Total	30	100.0
Hypopyop	Yes	14	46.7
пуроруоп	No	16	53.3
Endophthalmitis	Yes	4	13.3
Endophthamilus	No	26	86.7

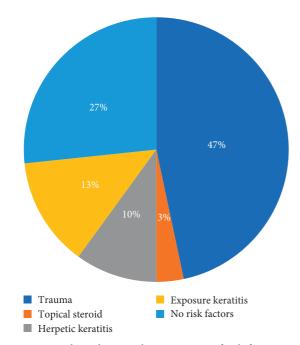


FIGURE 2: Pie chart showing the proportion of risk factors among the study participants at UoG Tertiary Eye Care and Training Centre, North West Ethiopia, 2019 (n = 30).

Pseudomonas species was the commonest Gram-negative rod identified which accounted for 20% of bacterial isolates.

4.3. Antimicrobial Susceptibility Patterns of Bacterial Isolates. The antimicrobial susceptibility pattern was determined for each of the bacterial isolates. This test showed that 7 (100%) of the Gram-positive cocci were sensitive to Vancomycin, Gentamicin, Chloramphenicol, Erythromycin, and Azithromycin. However, the prevalence of Methicillin-resistant S. aureus (MRSA) was 2 (40%), while both Streptococcus species isolates were resistant to Methicillin. The data of the present study showed that 3 (60%) of the S. aureus and 2 (100%) of the Streptococcus species bacterial isolates were susceptible to Ciprofloxacin. However, 60% of the S. aureus and 50% of the Streptococcus species were resistant to Ceftriaxone. The two Pseudomonas species bacterial isolates were sensitive to ciprofloxacin and Gentamicin but resistant to Ceftriaxone (Table 4).

#### 5. Discussion

A corneal ulcer is a significant cause of corneal blindness worldwide. The clinical profile and spectrum of microorganisms responsible for corneal ulceration are variables depending on the patient population, geographic location, and climatic conditions [8–10]. However, to the best of our knowledge, there are limited study reports on bacterial and fungal pathogen burden among patients suffering from corneal ulcers in Gondar and the country of Ethiopia in general. This study was carried out to give an insight into the clinical and microbiological profile among clinically suspected infectious cases at the University of Gondar Tertiary Eye Care and Training Centre.

In this study, we evaluated 30 eyes with clinically presumed bacterial and fungal corneal ulcers. The majority of the participants were male (90%), farmers (83.3%) by occupation, and nearly 97% of the cases came from rural areas. These findings together could be explained because males in rural areas are involved more in agricultural activities that certainly expose them to ocular trauma and subsequent development of corneal ulcers. The risk of agricultural predominance and vegetative corneal injury in bacterial keratitis increases susceptibility to corneal infection [15]. Previous reports documented that the history of corneal injury predisposing to fungal keratitis was frequently recorded among agricultural workers compared to nonagricultural workers [16].

The current study demonstrated that the majority of the study participants were male by sex, rural dwellers, and had no formal education. Previous reports in the same area documented that the majority of the study participants were males (58.8%), illiterate (84.5%), rural dwellers (75%), and farmers (63.3%) by occupation [17]. Investigators had been shown that poor health literacy skills are associated with

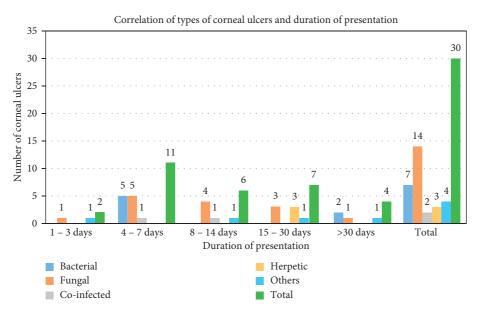


FIGURE 3: The correlation between corneal ulcer types and duration of presentation.

Table 3: Microbiological characteristics of corneal scrape samples for studying clinical and microbiological profile among bacterial and fungal suspected corneal ulcers at University of Gondar Tertiary Eye Care and Training Centre, North West Ethiopia, 2019 (n = 30).

Characteristics	Frequency (n)	Pe	rcent		
	Pure fungi growth	13	43.3		
G	Pure bacterial	7	23.3		
Corneal scrape culture result $(n = 30)$	Mixed bacterial and fungal growth	3	10.0		
	No growth	7	23.3		
Corneal scrape culture result $(n = 30)$ Bacterial isolates $(n = 10)$ Fungal isolate $(n = 16)$	S. aureus	5	50.0		
	Streptococcus species	2	20.0		
	Pseudomonas species	2	20.0		
	Proteus vulgaris	1	10.0		
	Aspergillus species				
	Penicillium and Aspergillus species	2	12.5		
Fungal isolate $(n = 16)$	Candida species	1	6.3		
	Penicillium species	2	12.5		
	Scopulariopsis brevicaulis	1	6.3		
	Rhizopus species	1	6.3		

Table 4: Susceptibility patterns of bacterial isolates for studying clinical and microbiological profile among clinically suspected bacterial and fungal corneal ulcers at University of Gondar Tertiary Eye Care and Training Centre, Northwest Ethiopia, 2019 (n = 30).

Antibiotics	S. aureus $(n=5)$		Streptococcus species $(n=2)$		Pseudomonas species $(n=2)$			Proteus vulgaris (n = 1)				
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Vancomycin (30 µg)	100	0	0	100	0	0	50	50	0	0	0	100
Methicillin (30 µg)	20	40	40	0	0	100	N/A	N/A	N/A	N/A	N/A	N/A
Penicillin (10 µg)	60	0	40	0	0	100	N/A	N/A	N/A	N/A	N/A	N/A
Cloxacillin	0	100	0	0	0	100	0	0	100	0	0	100
Amoxicillin	0	100	0	0	50	50	0	0	100	0	0	100
Ceftriaxone (30 μg)	0	40	60	0	50	50	0	0	100	0	0	100
Ceftazidime $(30 \mu\text{g})$	0	40	60	50	50	0	50	50	0	100	0	0
Ciprofloxacin (5 µg)	60	40	0	100	0	0	100	0	0	100	0	0
Gentamicin (30 µg)	100	0	0	100	0	0	0	0	100	100	0	0
Tetracycline (30 µg)	0	60	40	100	0	0	0	0	100	0	0	100
Chloramphenicol (30 µg)	100	0	0	100	0	0	0	0	100	100	0	0
Erythromycin (30 μg)	100	0	0	0	100	0	0	100	0	100	0	0

S = susceptible, I = intermediate, R = resistant, NA = not analysis.

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poor prescription medication adherence, increased hospital admissions, and increased mortality. Optimizing patient education and improving clinical outcomes require understanding the attributes that the patient brings to the patient-physician relationship, including health literacy [18].

The data of the current study showed that trauma was the significant risk factor identified in 46% of cases. This could be explained by the fact that most of the people in Ethiopia live working on agricultural activities. So, they are highly exposed to trauma by vegetative matter, wooden objects, dengue, etc., and the habit of using protective eyewear is small. Moreover, ocular trauma was a significant risk factor for corneal ulcers in developing countries [19, 20]. Most of the 6 (66.6%) bacterial isolates were between April and May, and most of the 12 (75.0%) fungal isolates were between April and July. The reason might be due to the rains in Ethiopia that started before the summer when the farmer begins planting in the spring season. The reason might lead to trauma, which in this study is a significant risk factor.

Besides, Wong et al. [5] documented that a history of ocular trauma, especially with organic matter, was associated with perforation in corneal ulcers. There are also other study reports that documented that trauma was the commonest risk factor identified as 66% at Pradish in India [21], 51% in Nigeria [22], and 55% in Ethiopia [23]. On the other hand, in a study conducted at Portsmouth, Southern England, contrary reports were documented and showed that contact-lens wear was the main risk factor (31%) for corneal ulcers [24]. This might be due to developing countries involved more in traditional agricultural activities than the developed countries.

Among the entire corneal scrape samples, 76.6% were culture-positive. The data also showed that fungi were the most microbial isolates identified in 53.3% of the total corneal ulcer cases, followed by 33.3% of bacterial growth. A previous study report from Ethiopia showed a 48% prevalence of culture-positive microbial isolates with fungal pathogens being dominant once [23]. Similarly, fungi were the most microbial isolates identified in 71% of culture-positive cases in Bangladesh [25], 72.5% cultures positive in Nepal, and 33.3% of patients with pure fungal infection [26]. This might reveal that in developing countries the majority of infectious corneal ulcers are accompanied by fungal infections.

In the current study, the majority of bacterial isolates were Gram-positive cocci (70%). S. aureus and Streptococcus species accounted for 50% and 20% of bacterial isolates, respectively. The prevalence of *S. aureus* was 64.5% in a study conducted in India, followed by Streptococcus species (12.3%) [27]. In Bangladesh, S. aureus was the commonest bacterial isolate (44.53%) [25]. The commonest Gramnegative bacteria identified in the present study were Pseudomonas species (20%) of the bacterial isolates or 6.6% of the total corneal ulcer cases. Previous studies reported that Pseudomonas species were 10% and 9% of corneal ulcer cases in Bangladesh [25] and Central India [27], respectively. Other reports also documented that, among the Gramnegative bacterial pathogens isolated from corneal ulcer cases, Pseudomonas species was the commonest with a proportion of 9.38% [28].

Gram-positive cocci were 100% sensitive to Vancomycin and Chloramphenicol. However, the prevalence of Methicillin-resistant *S. aureus* (MRSA) was 40%, while both *Streptococcus* species isolates were resistant to Methicillin. Also, Gram-positive cocci were 57% resistant to Penicillin and 28% resistant to Amoxicillin. A previous study report from Northeast Ethiopia revealed that 53.9% and 34.7% of the Gram-positive cocci bacteria isolated from external ocular infections were resistant to Ampicillin and Amoxicillin, respectively [17]. The reduced efficacy of Penicillin might be due to the frequent use of these drugs since they are widely used in patients both with and without prescription.

The data of the present study showed that the two Pseudomonas species were sensitive to both Ciprofloxacin and Gentamicin but resistant to Ceftriaxone. A study conducted at Jimma, Ethiopia, revealed that 85% of Gramnegative bacilli were susceptible to Gentamicin and Ciprofloxacin [29]. Pseudomonas aeruginosa is inherently resistant to many antimicrobial agents, because of the synergy between multidrug efflux system or type1 Amp C  $\beta$ -lactamase and low outer membrane permeability [30]. The irrational and inappropriate use of antibiotics is responsible for the development of resistance of Pseudomonas species to antibiotic monotherapy. Hence, there is a need to emphasize the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. Besides, regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of the resistance patterns. An effective national and state-level antibiotic policy and draft guidelines should be introduced to preserve the effectiveness of antibiotics and for better patient management. The main limitation of the study was the inadequate sample size. Therefore, it was not possible to analyze the statistical association between sociodemographic and clinical variables with the microbiological profile.

#### 6. Conclusions

The most prevalent risk factor observed in infectious corneal ulcers was trauma. From the total corneal ulcer cases accompanied by bacterial growth, fungi were the primary microbial isolate found. Aspergillus species were the commonest fungal isolate. The majority of bacterial isolates were Gram-positive cocci in which *S. aureus* and *Streptococcus* species were the most isolated bacterial pathogens. *Pseudomonas* species was the commonest Gram-negative bacterial isolate of corneal ulcer cases. Around half of *S. aureus* were Methicillin-resistant to *S. aureus*, while both *Streptococcus* species isolates were resistant to Methicillin. Both *Pseudomonas* species isolated were susceptible to Ciprofloxacin but resistant to Ceftriaxone and Gentamicin.

Based on our study findings, future studies should be conducted with a large number of cases, which allows analyzing the association between the factors associated with the microbiological profile. Antibiotic resistance to commonly prescribed antibiotics was observed. Hence, bacterial and fungal identification through culture methods, and

conducting drug susceptibility tests, should be practiced as a routine diagnostic procedure.

#### **Abbreviations**

BCVA: Best-corrected visual acuity EPI-Info: Epidemiological Information

KOH: Potassium hydroxide MHA: Mueller Hinton agar

MRSA: Methicillin-resistant Staphylococcus aureus

OD: Oculus dextra
OS: Oculus sinistra

SPSS: Statistical Package for Social Sciences

UoG: University of Gondar

VRSA: Vancomycin-resistant Staphylococcus aureus

WHO: World Health Organization.

# **Data Availability**

The original data used for this study are available at the corresponding author, so that an interested reader can get the data from the corresponding author with reasonable request.

# **Ethical Approval**

Ethical clearance was first obtained from the ethical review committee of the School of Medicine, University of Gondar. Permission was obtained from the Department of Ophthalmology.

#### **Consent**

During data collection, informed consent was obtained from each study participant. For pediatric patients, guardians or parents were asked their willingness so that their children would be involved in the study.

#### **Conflicts of Interest**

The authors declare that they have no competing interests regarding the publication of this article.

#### **Authors' Contributions**

TW contributed to conceptualization of the idea of the study. TW, MG, AB, WM, and BG participated in the data collection, data curation, analysis, and interpretation of the data. All authors actively participated in the manuscript preparation and finalization of this article. All authors read and approved the final manuscript.

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#### References

- [1] D. P. Khare, K. Kumar, P. D. M. Shrivastava, and D. K. Kumar, "Study of epidemiological characters, predisposing factors and treatment outcome of corneal ulcer patients," *International Journal of Medical Research and Review*, vol. 2, no. 1, pp. 33–39, 2014.
- [2] P. John, M. Whitcher, and S. M. P. Upadhyay, "Corneal blindness: a global perspective," *Bulletin of the World Health Organization*, vol. 79, pp. 214–221, 2005.
- [3] P. Prabhasawat, "Causes of corneal blindness," *The Journal of Medical Association of Thailand*, vol. 90, no. 12, p. 2651, 2004.
- [4] J. S. Titiyal, S. Negi, A. Anand, R. Tandon, N. Sharma, and R. B. Vajpayee, "Risk factors for perforation in microbial corneal ulcers in north India," *British Journal of Ophthal-mology*, vol. 90, no. 6, pp. 686–689, 2006.
- [5] T. Wong, S. Ormonde, G. Gamble et al., "Severe infective keratitis leading to hospital admission in New Zealand," *British Journal of Ophthalmology*, vol. 87, no. 9, pp. 1103– 1108, 2003.
- [6] A. K. Leck, P. A. Thomas, M. Hagan et al., "Aetiology of suppurative corneal ulcers in Ghana and south India, and epidemiology of fungal keratitis," *British Journal of Oph*thalmology, vol. 86, no. 11, pp. 1211–1215, 2002.
- [7] D. Y. Kunimoto, S. Sharma, P. Garg et al., "Corneal ulceration in the elderly in Hyderabad, south India," *British Journal of Ophthalmology*, vol. 84, no. 1, pp. 54–59, 2000.
- [8] A. Shah, A. Sachdev, D. Coggon, and P. Hossain, "Geographic variations in microbial keratitis: an analysis of the peerreviewed literature," *British Journal of Ophthalmology*, vol. 95, no. 6, pp. 762–767, 2011.
- [9] E. S. J. Cury, M. R. Chang, and E. R. J. C. Pontes, "Non-viral microbial keratitis in adults: clinical and laboratory aspects," *Brazilian Journal of Microbiology*, vol. 49, pp. 205–212, 2018.
- [10] A. M. Otri, U. Fares, M. A. Al-Aqaba et al., "Profile of sight-threatening infectious keratitis: a prospective study," *Acta Ophthalmologica*, vol. 91, no. 7, pp. 643–651, 2013.
- [11] S. Wilson UoM. Corneal Ulcers. 2015.
- [12] J. D. Chidambaram, N. Venkatesh Prajna, P. Srikanthi et al., "Epidemiology, risk factors, and clinical outcomes in severe microbial keratitis in South India," *Ophthalmic Epidemiology*, vol. 25, no. 4, pp. 297–305, 2018.
- [13] C. Ranjini and V. Waddepally, "Microbial profile of corneal ulcers in a tertiary care hospital in south India," *Journal of Ophthalmic and Vision Research*, vol. 11, no. 4, pp. 363–367, 2016.
- [14] S. Plianbangchang, "Guideline for the management of cornea ullcer at primary, secondary & tertiary Care health facilities in the South-East Asia Region," WHO Guidline, vol. 26, no. 2, 2014.
- [15] M. J. Bharathi, R. Ramakrishnan, R. Meenakshi, S. Padmavathy, C. Shivakumar, and M. Srinivasan, "Microbial keratitis in South India: influence of risk factors, climate, and geographical variation," *Ophthalmic Epidemiology*, vol. 14, no. 2, pp. 61–69, 2007.

- [16] M. J. Bharathi, R. Ramakrishnan, R. Meenakshi, C. Shivakumar, and R. D. Lional, "Analysis of the risk of factors predisposing to fungus, bacterial and Acanthamoeba keratitis in South India," *Indian Journal of Medical Research*, vol. 130, pp. 749–757, 2009.
- [17] B. Shiferaw, B. Gelaw, A. Assefa, Y. Assefa, and Z. Addis, "Bacterial isolates and their antimicrobial susceptibility pattern among patients with external ocular infections at Borumeda hospital, Northeast Ethiopia," *BMC Ophthalmology*, vol. 15, p. 103, 2015.
- [18] K. W. Muir and P. P. Lee, "Health literacy and ophthalmic patient education," *Survey of Ophthalmology*, vol. 55, no. 5, pp. 454–459, 2010.
- [19] M. Srinivasan, C. A. Gonzales, C. George et al., "Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India," *British Journal of Ophthalmology*, vol. 81, no. 11, pp. 965–971, 1997.
- [20] M. P. Upadhyay, P. C. Karmacharya, S. Koirala et al., "The Bhaktapur eye study: ocular trauma and antibiotic prophylaxis for the prevention of corneal ulceration in Nepal," *British Journal of Ophthalmology*, vol. 85, no. 4, pp. 388–392, 2001.
- [21] K. Oladigbolu, A. Rafindadi, E. Samaila, and E. Abah, "Corneal ulcers in a tertiary hospital in Northern Nigeria," *Annals of African Medicine*, vol. 12, no. 3, pp. 165–170, 2013.
- [22] N. Gupta, R. Tandon, S. K. Sreenivas, and P. Vashist, "Burden of corneal blindness in India," *Indian Journal of Community Medicine: Official Publication of Indian Association of Pre*ventive & Social Medicine, vol. 38, no. 4, pp. 198–206, 2013.
- [23] M. Siraj, G. Teklebirhan, and A. Bitew, "Clinical and microbiological profile of keratitis in menelik II memorial hospital, Addis Ababa, Ethiopia," *Microbiology*, vol. 14, no. 4, pp. 173–180, 2018.
- [24] Y. W. Ibrahim, D. L. Boase, and I. A. Cree, "Epidemiological characteristics, predisposing factors and microbiological profiles of infectious corneal ulcers: the Portsmouth corneal ulcer study," *British Journal of Ophthalmology*, vol. 93, no. 10, pp. 1319–1324, 2009.
- [25] A. Dunlop, E. Wright, S. Howlader et al., "Suppurative corneal ulceration in Bangladesh," Australian and New Zealand Journal of Ophthalmology, vol. 22, no. 2, pp. 105–110, 1994.
- [26] K. Dhakhwa, B. Sanyam, M. Sharma, and A. Dwivedi, "Causative organisms in microbial keratitis, their sensitivity pattern and treatment outcome in western Nepal," *Nepalese Journal of Ophthalmology*, vol. 4, no. 7, pp. 119–127, 2012.
- [27] S. Haridas, M. Palanisamy, A Prithiviraj et al., "Prevalence and antimicrobial susceptibility pattern of staphylococci and streptococci causing ocular infections from a tertiary eye care hospital, South India," *Biomedical Research*, vol. 27, no. 3, pp. 12–17, 2016.
- [28] R. Amatya, S. Shrestha, B. Khanal et al., "Etiological agents of corneal ulcer: five years prospective study in eastern Nepal," *Nepal Medical College Journal: NMCJ*, vol. 14, no. 3, pp. 219–222, 2012.
- [29] T. Gebremariam, T. Alemu, and K. Daba, "Bacteriology and risk factors of bacterial keratitis in Ethiopia," *Health Science Journal*, vol. 9, no. 5, pp. 1–5, 2015.
- [30] R. N. Das, T. S. Chandrashekhar, H. S. Joshi, M. Gurung, N. Shrestha, and P. G. Shivananda, "Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal," Singapore Medical Journal, vol. 47, no. 4, pp. 281–285, 2006.