




# Demography, Risk Factors, and Clinical and Microbiological Features of Microbial Keratitis at a Tertiary Eye Hospital in Nepal

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**Background:** Infective keratitis is the most common corneal pathology in developing countries. Updated knowledge is needed for its control and proper management.

**Methodology:** All cases of presumed microbial keratitis that presented in an 18-month period from October 2013 to March 2015 were enrolled. Data collected were demographic profile, risk factors, clinical features, and organisms isolated and their sensitivities.

**Results:** A total of 602 cases of microbial keratitis were enrolled. Mean age of subjects (598 patients) was 47.9 years with 53.8% male. 64.1% worked in agriculture. 38.3% gave history of trauma followed by history of herpetic eye diseases (17.9%) and topical steroid use (14.2%). A total of 473 who were referred came at an average of 21.5 days of symptoms. 14.9% (n=90) of cases were either perforated or impending to perforate at presentation. 69.6% had infiltrate in the visual axis. A total of 516 (85.7%) underwent diagnostic corneal culture. A total of 256 (49.6%) yielded a positive result. Pure bacterial growth was seen in 111 (43.4%), pure fungal growth in 138 (53.9%), and mixed microbial growth was present in 7 (2.7%) cases. Out of 121 bacterial isolates, 95.0% were Gram positive. *Streptococcus pneumoniae* (45.5%, n=55) was the most common bacterial isolate followed by *Staphylococcus aureus* (20.6%, n=25). Out of 145 fungal isolates, *Aspergillus* and *Fusarium* species were found in equal numbers (n=41, 28.3% each). Over 85% of Gram-positive organisms isolated in the study were sensitive to vancomycin, cefazolin, moxifloxacin, and gatifloxacin. Over 80% of Gram-negative organisms were sensitive to gentamicin, tobramycin, and amikacin.

**Conclusion:** Microbial keratitis and associated risk factors occurring in farmers implies a lack of awareness and prevention programs. Delay in reaching tertiary care is resulting in complicated cases. Training of local health workers for prophylaxis, updated guidelines for treating keratitis, and timely referral to higher centers are all important in a chain to decrease the incidence of microbial keratitis.

**Key words:** microbial keratitis, infective keratitis, corneal ulcer

## Introduction

Diseases affecting the cornea are the major cause of blindness in developing countries and infective keratitis is the main cause for this.<sup>1</sup> According to the Nepal Blindness survey (1981), corneal pathology accounted for 9% of total blindness, which was second to cataract.<sup>2</sup> Factors like lack of awareness and preventive measures, delay in treatment, inaccessibility of eye care, improper

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treatment, and resistant microorganisms all contribute to persistence of infective keratitis in the community.

The burden of corneal ulceration, risk factors, microorganisms, and susceptible antibiotics vary from region to region and also vary with time. So, an update of the information is important especially for a tertiary eye care hospital which treats a huge number of infective corneal ulcers. The current study is a large hospital-based study done for the same purpose. It is being conducted in Tilganga Institute of Ophthalmology (TIO), a tertiary eye care for subspecialty service, located in Kathmandu, Nepal. Comparison with past study of a similar kind is made for any differences in the findings. This study aims to find out the latest sensitivity pattern of the microorganisms to guide in the choice of antibiotics for doctors and paramedics involved in treating corneal ulcers.

## Methodology

This is a descriptive, non-interventional type of study in which all consecutive patients visiting TIO with a clinical or laboratory diagnosis of presumed microbial keratitis from October 2013 to March 2015 (18-month period) were enrolled. Pure viral keratitis like dendritic, geographic, kerato-uveitis, and meta-herpetic ulcers are excluded. Viral keratitis and autoimmune keratitis were included only if they had features of secondary microbial infection. For each patient, a proforma was filled to collect data about demography, risk factors, duration of symptoms, characteristics of ulcer (infiltrate size, location, perforated, or impending to perforate), and microbiological profile. With some exceptions, all microbial keratitis had undergone diagnostic corneal scraping. In our institute, cases do not undergo scraping: 1) when ulcers are perforated or impending to perforate at presentation and if they are intended to manage conservatively; 2) when infiltrate is deep in the cornea without epithelial defect; 3) in ulcers occurring in children and mentally challenging patients; and 4) when ulcer size is less than 2 mm. We do not stop the antimicrobials which patients were using prior to performing corneal scraping (microbial keratitis, infective keratitis, and corneal ulcer are used interchangeably in the text).

## Laboratory Procedures

Specimen collection, inoculation, and identification of the organism had been done through standard protocol. Kimura's spatula was used to obtain material from the leading edge and base of the ulcer. The specimen was

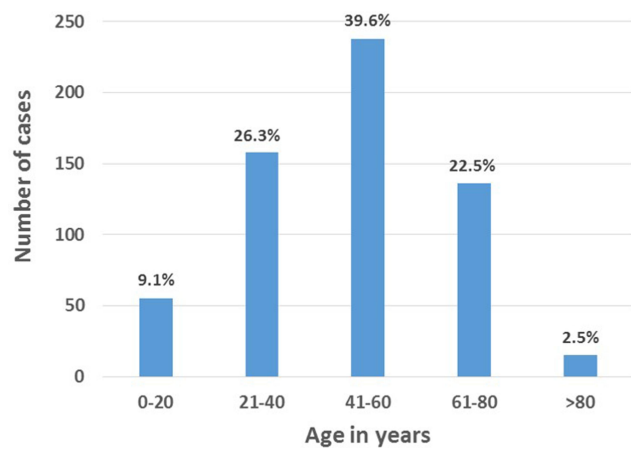
inoculated directly onto blood agar, chocolate agar, Sabouraud dextrose agar (SDA), and thioglycollate broth. Separate glass slides were used for Gram stain, for Giemsa stain, and for potassium hydroxide (KOH) wet mount. For suspected amoebic corneal cases, the specimen was inoculated onto non-nutrient agar overlaid with *Escherichia coli*. All bacterial cultures were incubated aerobically at 37 °C. To ensure 3 to 5% carbon dioxide, incubated chocolate agar plates were put inside a candle jar. Cultures on blood agar, chocolate agar, and broth were evaluated daily up to 72 hours and then discarded if there was no growth. Fungal cultures in SDA were incubated at 25 °C and looked for any growth till 1 week and discarded thereafter. Similarly, cultures on non-nutrient agar overlaid with *E. coli* were discarded at 1 week if there was no growth. Microbial culture was considered significant if any/all of the following criteria were satisfied: 1) growth of the same organism was demonstrated on two or more media; 2) confluent growth was seen at the site of inoculation on one solid medium; 3) growth of one medium was consistent with direct microscopy findings (appropriate staining and morphology with Gram stain); and 4) the same organism was grown from repeated scraping material. Identification of bacteria was based on microscopic morphology, staining characteristics, and biochemical properties. Antimicrobial susceptibility testing was performed using the agar disk diffusion technique according to Clinical Laboratory Standard Institute (CLSI) guidelines using *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) as reference strains. Fungi were identified by their colony characteristics on SDA and by their microscopic appearance in lactophenol cotton blue. Using the culture method as gold standard, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Gram stain and KOH wet mount were determined for bacteria and fungus respectively.

## Ethical Consideration

Approval for the study was taken from the Institutional review board of Tilganga Institute of Ophthalmology. Informed consent was taken from all patients and the study complied with the Declaration of Helsinki. For patients aged less than 18 years, a parent or legal guardian provided informed consent on the patient's behalf.

## Results

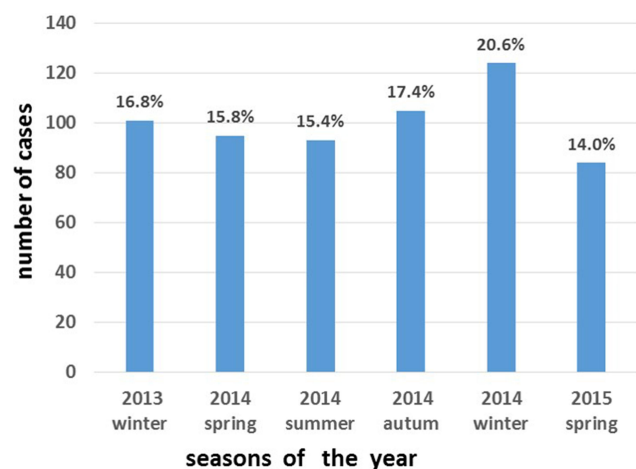
A total of 602 new corneal ulcer cases in 598 patients were registered in the study period of 18 months (average of 33.4



**Figure 1** Age distribution of the subjects with microbial keratitis.

new cases per month). Two patients had bilateral infiltrate and two patients had infection in the same eye twice. Average age of patients was 47.9 years (Figure 1) with 53.8% male patients. The majority of the subjects (386, 64.1%) were engaged in agriculture, followed by business (73, 12.1%), housewife (43, 7.1%), service (40, 6.6%), and students (19, 3.0%). Overall, 332 (55.1%) were illiterate, 81 (13.5%) were just literate, 14.9% had primary or secondary level education, and 9.6% had high school or above level of education. 41 (6.8%) subjects were children aged  $\leq 15$  years. (Education and occupation were not taken into account for the pediatric age group.)

A total of 477 (79.2%) cases were from rural areas, 87 (14.4%) from cities, and 38 (6.3%) from the neighboring country India. Microbial keratitis cases presented at TIO throughout the year but slightly more in winter (Figure 2)



**Figure 2** Distribution of cases of microbial keratitis with respect to seasons of the year.

53.1% of 602 ulcers were first treated by either a trained eye health worker in primary eye care or by an ophthalmologist in an eye hospital (including TIO); 33.8% got treatment from pharmacy shop, 6.1% from general health post, and 3.6% were seen by traditional healers at first hand. Overall, people had sought medical help at an average of 5.6 days of onset of symptoms, but those who were referred to TIO (n=473) came at an average of 21.5 days.

69.6% of 602 ulcers had infiltrate partly or fully covering the visual axis (central 4 mm of the cornea). 67% of the eyes with ulcer had best corrected vision of less than 3/60. 26% (n=156) of cases had infiltrate size more than one quadrant of the corneal surface. 278 (46.1%) had hypopyon. 90 cases (14.9%) were either already perforated (n=57, 9.4%) or impending to perforate (n=33, 5.5%), most of which would necessitate emergency surgical intervention. In total, there were 11 cases of endophthalmitis associated with microbial keratitis.

A total of 459 (76.2%) had at least one risk factor for corneal ulcer. History of corneal trauma was present in 231 (38.3%) of ulcers (Table 1). 88.3% (n=204) of trauma was due to organic matter.

Among total cases, 516 (85.7%) had undergone diagnostic corneal culture. 14.3% did not undergo corneal culture for reasons as mentioned in "Methodology". 49.6% (256 out of 516) of culture showed microbial growth. Pure bacterial growth was seen in 111 (43.4%) cases, pure fungal growth in 138 (53.9%) cases, and mixed microbial growth was present in 7 (2.7%) cases (Table 2). A total of 266 microorganisms (121 bacterial and 145 fungal) were isolated from 256 culture-positive cases (Tables 3 and 4).

**Table 1** Risk Factors of Microbial Keratitis

Risk Factors*	Number (%)
Trauma	231 (38.3)
Herpetic eye disease	108 (17.9)
Steroid use	86 (14.2)
Dacryocystitis	39 (6.4)
Past ocular surgery	30 (4.9)
Diabetes mellitus	29 (4.8)
Corneal edema bullous	20 (3.3)
exposure of cornea	15 (2.4)
Systemic illness <sup>#</sup> (other than diabetes mellitus)	10 (1.6)
Dry eyes	5 (0.8)
Others <sup>###</sup>	37 (5.3)
No risk factors	143 (23.7)

**Notes:** \*109 subjects had more than one risk factor. #Illness that causes immunosuppression like patients on chemotherapy or immunosuppressive drugs or HIV patients. ##Contact lens, entropion, meibomitis, corneal epithelial dystrophy, conjunctivitis, band shaped keratopathy.

**Table 2** Microbial Growth Pattern in Culture-Positive Cases

Growth Pattern	No. of Cases (Subtotal)	Percentage
Pure bacterial growth (single species of bacteria) (two species of bacteria)	111 (109) (2)	43.4
Pure fungal growth (single species of fungus)	138	53.9
Mixed microbial growth (single species of bacteria with a single species of fungus) (two species of bacteria with a single species of fungus)	7 (6) (1)	2.7
Total	256	100.0

Out of 121 bacterial isolates, 115 (95.0%) were Gram-positive bacteria (GPB) and 6 isolates were Gram-negative bacteria (GNB) (Table 3). The most common bacterial isolate was *Streptococcus pneumoniae* (45.5%, n=55), followed by *Staphylococcus aureus* (20.6% n=25) and *Streptococcus viridians* (11.6%, n=14).

**Table 3** Bacterial Isolates

Bacteria	Pure Isolates	Mixed with Other Bacteria	Mixed with Fungus	Total (%)
<b>Gram-positive organism</b>				
<i>Streptococcus pneumoniae</i>	51	1	3	55 (45.5)
<i>Staphylococcus aureus</i>	21	2	2	25 (20.6)
<i>Streptococcus viridians</i>	12	–	2	14 (11.6)
<i>Corynebacterium</i> species	9	1	–	10 (8.2)
<i>Bacillus</i> species	4	–	1	5 (4.1)
<i>Nocardia</i> species	3	–	–	3 (2.5)
<i>Staphylococcus epidermidis</i>	3	–	–	3 (2.5)
<b>Gram-negative organism</b>				
<i>Pseudomonas aeruginosa</i>	3	–	–	3 (2.5)
<i>Moraxella</i> species	3	–	–	3 (2.5)
Total (%)	109	4	8	121 (100.0)

**Table 4** Fungal Isolates

Fungi	Pure Isolates (subtotal)	Mixed with Bacteria (subtotal)	Total (%)
<i>Aspergillus</i> species	39	2	41 (28.3)
<i>A. flavus</i>	(23)	(1)	
<i>A. fumigatus</i>	(14)	(1)	
<i>A. niger</i>	(1)	–	
<i>A. terreus</i>	(1)	–	
<i>Fusarium</i> species	41	–	41 (28.3)
<i>Curvularia</i> species	16	2	18 (12.4)
<i>Exserohilum</i> species	4	–	4 (2.7)
<i>Bipolaris</i> species	3	–	3 (2.1)
<i>Acremonium</i> species	3	–	3 (2.1)
<i>Cladosporium</i> species	2	–	2 (1.4)
<i>Scedosporium</i> species	1	–	1 (0.7)
<i>Colletotrichum</i> species	–	1	1 (0.7)
<i>Candida albicans</i>	1	–	1 (0.7)
Unidentified dematiaceous fungus	9	–	9 (6.2)
Unidentified hyaline fungus	19	2	21 (14.4)
Total (%)	138	7	145 (100.0)

Out of 145 fungal isolates, 99.3% were filamentous type. *Aspergillus* and *Fusarium* species were found in equal numbers, each isolated in 41 cases (28.3%), followed by *Curvularia* species (12.4%) (Table 4). There was only one case of yeast (*Candida albicans*) and no cases of *Acanthamoeba*.

The sensitivity of Gram stain in the detection of bacteria was 82.2% and its specificity was 96.5%. Similarly, for KOH wet mount preparation they were 80.0% and 93.5% respectively in the detection of fungus (Table 5). PPV and NPV of Gram stain and KOH were as shown in Table 5. The antimicrobial sensitivity test for all bacterial isolates is shown in Tables 6 and 7.

## Discussions

In our study, mean age of patients was 47.9 years which corresponds to the working age group (Figure 1). This was similar to the average age, 40 to 55 years, reported by studies within Nepal and neighboring countries.<sup>3–6</sup> Developed countries had a bimodal age distribution in

**Table 5** Correlation Between Direct Microscopic (Gram Stain and KOH Wet Mount) Findings and Culture-Based Diagnosis

S. No.	Direct Microscopic Findings	Results	Number of Cases	Culture		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV% (95% CI)	NPV% (95% CI)
				Positive	Negative				
1	Detection of bacteria in Gram-stained smear	Positive	111	97	14	82.2 (75.3 to 89.1)	96.5 (94.7 to 98.3)	87.4 (81.2 to 93.6)	94.8 (92.7 to 97.0)
		Negative	405	21	384				
		Total	516	118	398				
2	Detection of fungal filament in KOH wet mount	Positive	140	116	24	80.0 (73.5 to 86.5)	93.5 (91.0 to 96.0)	82.8 (76.6 to 89.1)	92.3 (89.6 to 95.0)
		Negative	376	29	347				
		Total	516	145	371				

**Abbreviations:** PPV, positive predictive value; NPV, negative predictive value.

the incidence of microbial keratitis; first peak in young adults and second peak in the old age group.<sup>7-9</sup> This was because of use of contact lenses by young people.<sup>7-9</sup> Like in our study, other studies had also shown a male predominance of 52 to 64%.<sup>3-6,10</sup> This could be because males are involved in outdoor or labor-based jobs. Female predominance was seen in Hong Kong<sup>8</sup> and the ratio of males and females was equal in France.<sup>9</sup> This was because of more females using contact lenses.<sup>8,9</sup> In our study, the majority of patients were farmers (64.1%) compared to 37 to 56% in Bangladesh and South India.<sup>3,11</sup> 55.1 % of our subjects were illiterate, correlating with the occupation. Like in our study, Sitoula et al<sup>5</sup> also found a higher number of keratitis in winter which is the harvesting season of crops.

43.5% of our patients had their initial treatment from people (pharmacist, general nurse, or traditional health worker) who are not trained primary eye caretakers. A significant number (33.8%) presented to the pharmacy in the

beginning. This might have been due to convenience, proximity to location of injury or patient's home, lack of eye care facility, or expected cost or waiting time in eye hospital. Late presentation in tertiary care hospital (average 21.5 days) was the reason why certain ulcers are larger and complicated. In other eye hospitals of Nepal, 76 to 82% of cases had presented after 7 days.<sup>5,13</sup> In South India, 60% of ulcer patients presented to an ophthalmologist within 7 days which was a better situation there.<sup>3</sup> In France, ulcers due to contact lens and trauma cases had consultation done at an average of 48 hours.<sup>9</sup> In our study, perforation was seen in 9.4% at presentation. Lavaju et al<sup>12</sup> reported perforated ulcers in 11.3% in their study done in Nepal. Bourcier et al<sup>9</sup> mentioned perforation in 0.6% in France.

In the present study, 38.3% gave history of ocular trauma, most of which were from vegetative matter (Table 1). Other studies in Nepal reported trauma in 60 to 82.7%.<sup>13,14</sup> In South India and Thailand, trauma was reported as 65% and 43.9% respectively.<sup>3,15</sup> In developed countries, trauma

**Table 6** Antimicrobial Susceptibility Test: Percentage of Gram-Positive Bacteria Sensitive to the Listed Antibiotic

	<i>S. pneumoniae</i> (n=55)(%)	<i>S. aureus</i> (n=25)(%)	<i>S. viridans</i> (n=14)(%)	<i>Corynebacterium</i> (n=10)(%)	<i>Bacillus</i> (n=5)(%)	<i>S. epidermidis</i> (n=3)(%)	<i>Nocardia</i> (n=3)(%)	Total (n=115)(%)
Vancomycin	100	100	100	100	80	100	100	99.1
Gatifloxacin	100	84	92.8	100	80	100	100	94.8
Cefazolin	100	92	100	100	40	100	33.3	93.9
Moxifloxacin	98.2	72	85.7	100	80	66.7	100	89.6
Chloramphenicol	100	76	100	80	40	66.7	100	89.6
Ofloxacin	98.2	60	78.6	90	80	33.3	66.7	83.5
Ciprofloxacin	92.7	44	78.6	90	80	33.3	66.7	77.4
Tobramycin	20	92	42.8	100	100	66.7	100	52.2
Amikacin	7.3	96	14.3	100	100	100	100	44.3
Gentamycin	5.4	88	14.3	100	80	100	100	40.9



**Table 7** Antimicrobial Susceptibility Test: Percentage of Gram-Negative Bacteria Sensitive to the Listed Antibiotic

	<i>Pseudomonas</i> (n=3)(%)	<i>Moraxella</i> (n=3)(%)	Total (n=6)(%)
Gentamycin	100	100	100
Tobramycin	100	100	100
Amikacin	66.7	100	83.3
Gatifloxacin	100	33.3	66.7
Moxifloxacin	100	33.3	66.7
Ofloxacin	100	33.3	66.7
Ciprofloxacin	100	33.3	66.7
Chloramphenicol	0	100	50
Ceftazidime	33.3	66.7	50

accounted for 10 to 16% as a risk factor.<sup>7-9,16</sup> In our study, 17.9% of the patients had underlying herpetic eye disease (HED). HED can predispose to secondary microbial infection through decreased sensation, neurotrophic ulcer, dry eye, presence of epithelial defect like dendrite, and topical use of steroids for treatment.<sup>17</sup> Sometimes HED with stromal necrosis can mimic microbial keratitis.<sup>17</sup> In our study, HED as a risk factor was solely on clinical grounds with past history of recurrent keratitis, significant decrease in corneal sensation, or from past documents.<sup>18</sup> Polymerase chain reaction (PCR) test not being done was the limiting factor in our study. Studies from South India and Hong Kong reported HED as a risk factor in 2 to 7%.<sup>3,8</sup> There had been mentioning of OSD (which included dry eyes, HED, and others) as a risk factor in 18 to 20% of microbial keratitis in Japan, France, and Australia.<sup>7,9,16</sup> In another study in Sydney, HED accounted for 17.4% as a risk factor, of which 7.9% showed a positive herpes simplex viral PCR test.<sup>19</sup> There had never been mention of HED disease as a risk factor in any studies in Nepal. Prevalence of herpes simplex virus (HSV) is all over the world and the HSV seropositivity could be as high as 67 to 90% in the population.<sup>18</sup> In Nepal, HED as a risk factor was probably undervalued in previous studies or herpes simplex stromal keratitis might have been incorporated into the microbial keratitis group due to mimicry. In our study, 14.6% of patients were using topical steroids during presentation. The drug had been advised in the local medical shop or had been prescribed in the past for conditions like HED, post-operative keratoplasty, and vernal keratoconjunctivitis. In the studies in India, use of steroid ranged from 7 to 19%.<sup>3,4,20</sup> A report<sup>5</sup> from Nepal mentioned 2%.

Sitoula et al<sup>5</sup> and Puri and Shrestha<sup>13</sup> reported 65 to 76.1% cases with vision less than 3/60 which is similar to

our study. Our Gram stain and KOH stain sensitivity and specificity were as shown in Table 5. From other reports, the range of sensitivity of Gram stain had been 50 to 100% and for specificity from 77 to 97.6%.<sup>4,7,21,22</sup> Similarly for KOH, the reported range of sensitivity had been from 54 to 99% and specificity from 94 to 99%.<sup>4,7,21,22</sup> The PPV and NPV for bacteria were greater in our study than those reported by Rai et al<sup>22</sup> but for fungus both values were less compared to the same study (Table 5). Bharathi et al<sup>4</sup> reported PPV and NPV of more than 97% for bacterial as well as fungus.

In this study, culture was positive in 49.6% of scrapings. In various studies, the rate of positive growth had been 32 to 76%.<sup>5,8,9,23</sup> Our study showed slightly more fungus (53.9%) than bacteria. In most studies from India and Nepal the proportion of fungus was usually higher than bacteria (62 to 70%),<sup>3,5,20</sup> but fungal ulcers were quite low in developed countries (6 to 17%).<sup>8,16,23</sup> In the present study, *Streptococcus pneumoniae* (45.5%) was the most common bacteria isolated (Table 3). In Nepal, some studies had reported *Streptococcus pneumoniae* whereas others reported *Staphylococcus aureus* or *Staphylococcus epidermidis* as the predominant bacteria.<sup>6,10,14,24</sup> In neighboring countries also, these bacterial isolates were obtained as the commonest.<sup>3,11,20,21</sup> But in Australia,<sup>7</sup> Taiwan,<sup>23</sup> and Thailand,<sup>15</sup> *Pseudomonas* were the most common cause of bacterial keratitis due to contact lenses being the main risk factor. In our study, *Aspergillus* and *Fusarium* were found in equal proportion, 28.3% each (Table 4). A decade back, *Aspergillus* closely followed by *Fusarium* were the commonest fungi isolated in TIO.<sup>6</sup> *Aspergillus* was also the commonest fungus in Eastern Nepal<sup>10</sup> and Bangladesh.<sup>11</sup> But in Western Nepal and South India, *Fusarium* was the commonest.<sup>3,24</sup>

In our study, vancomycin, gatifloxacin, and cefazolin had >90% sensitivity towards GPB. It also showed that moxifloxacin and gatifloxacin were more potent than other fluoroquinolones for *Streptococcus* and *Staphylococcus* (Table 6). Fluoroquinolones were found potent for *Pseudomonas* (100%), but not for *Moraxella* (33.3%) (Table 7). In our past study, sensitivities of gentamycin, tobramycin and amikacin to GNB were 40%, 67% and 100% respectively but the current study showed sensitivities of gentamycin and tobramycin to GNB as 100% and that of amikacin as 83.3%.<sup>6</sup> Difference in sensitivities in the two studies cannot be inferred due to small number of GNB in past as well as present studies.<sup>6</sup>

## Conclusion and Recommendation

Persistence of the disease in the past through the present indicates inadequate prevention programs. It has been important to train the first line of defense – the community eye workers and pharmacists who see these patients after their initial trauma – to make sure the patients get correct prophylactic treatment. Focus should be on geographic regions from where many keratitis cases are referred. This study will guide selection of antimicrobials. Facility for culture of corneal specimens should be set up in secondary eye care centers, which should make timely referral for difficult cases. Emphasis should be given for proper management and adequate counseling for patients with recurrent viral keratitis and other conditions needing long-term treatment with topical steroids. These measures would decrease the incidence of microbial keratitis as well the need for more costly keratoplasty which requires long-term follow-up care.

## Disclosure

The authors report no conflicts of interest for this work.

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