Etiological agents of corneal ulcer: five years prospective study in eastern Nepal

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ABSTRACT

To identify the most common isolates from the corneal ulcers with antimicrobial pattern of bacterial isolates

All patients with suspected corneal ulceration presenting to BP Koirala Institute of Health Sciences from Jan 2004 – Dec 2008 were evaluated. Corneal scraping was performed and processed for direct microscopy, bacteriological and fungal culture. Bacterial isolates were subjected to antimicrobial susceptibility testing. Of 351 specimens examined, growth of etiological agents were obtained in 278 (79.20%). Of these, 113 (40.65%) had pure fungal growth, 108 (38.85%) had pure bacterial growth and 57 (20.50%) had mixed fungal and bacterial infection. The commonest fungal pathogen was Aspergillus spp 50 (33.33%) followed by Fusarium spp 19 (12.66%). Staphylococcal aureus 57 (44.53%) was isolated as commonest bacterial agent. Coagulase Negative Staphylococci 20 (15.6%) was second in the list. Pseudomonas spp 12 (9.40%) was the most common gram negative bacilli isolated. Most of the bacterial isolates were sensitive to commonly used antibiotics. Corneal Ulcer is a common problem in eastern Nepal. Knowledge of both fungal and bacterial agents associated with this condition is of value for the prevention and management of corneal ulcers and their complications.

INTRODUCTION

Corneal infection is a leading cause of ocular morbidity and blindness worldwide.[1-6] Corneal ulceration is a major cause of monocular blindness in developing countries. Corneal scarring listed second only to cataracts as an important cause of blindness and visual impairment in many developing countries in Asia, Africa and the Middle East.[7]

Corneal stroma can be invaded by almost any microorganism if the normal corneal defense mechanisms, i.e. lids, tear films and corneal epithelium are compromised.[8] A wide spectrum of microbial organism can produce corneal infections and consequently the therapeutic strategies may be variable.[9] The etiology of microbial keratitis varies significantly from country to country and even from region to region within the same country. There should be a proper understanding of microbial etiology that will enable the ophthalmologist to initiate the appropriate antimicrobial therapy.[9]

The purpose of this study was to evaluate the bacterial and fungal agents causing corneal ulceration among patients presenting to a tertiary care hospital in Eastern Nepal over a period of five years (January 2004 to December 2008).

MATERIALS AND METHODS

All patients with suspected microbial keratitis presenting to Ophthalmology Department of B.P. Koirala Institution of Health Sciences (BPKIHS), a tertiary care hospital in eastern Nepal from January 1, 2004 to December 30, 2008 were evaluated. The study had been approved by the Ethical Board of Research Committee of BPKIHS.

Material obtained from corneal scraping was directly inoculated onto sheep blood agar, chocolate agar, brain heart infusion broth and incubated aerobically at 37° C for 24 hrs. Chocolate agar plates were put under candle jar for incubate to ensure 5-10 % CO_2. The plates were examined after 24 and 48hr. Subcultures were made from brain heart infusion broth onto blood agar, chocolate agar and Mac Conkey agar if the primary culture plates were sterile. All the bacterial isolates were identified with the help of their colony morphology, gram staining and standard biochemical tests.[10,11] All bacterial isolates were tested by Kirby Bauer disc diffusion method against Amikacin (30µg), Gentamycin (10µg), Tobramycin (10µg) , Chloramphenicol (10 µg),Ciprofloxacin (10µg) and Cefotaxime (30 µg).

For fungal detection, materials from corneal scrapings were inoculated into two sets of plain Sabouraud's dextrose agar (SDA) and incubated at 25° C. The SDA tubes were examined every day during first week, then every week for 3 weeks to look for growth of any organism. Microscopical examination in lactophenol cotton blue staining were performed from fungal growth in SDA for the identification of fungal species. [10,11]

RESULTS

Out of 351 specimens of corneal scrapings studied, 278 (79.2%) yielded microorganisms. Ratio of male to female patients was 1.4: 1. The predominance of corneal ulceration was most pronounced in patients who were between the age of 21-30 years. Out of 278 culture positive specimens, 113 (40.6%) had pure fungal growth, 108 (38.8%) had pure bacterial growth and 57 (20.5%) had mixed fungal and bacterial growth. Taking the mixed bacterial and fungal growth in account, total bacterial growth was 150 (54%) and fungal was 128 (46%). The commonest fungal pathogen isolated was *Aspergillus* spp 50 (33.3%), followed by *Fusarium* spp 19 (12.6%) and *Curvularia* spp (8.7%) as shown in Table-1. Different species of *Aspergillus* isolated is shown in Figure 1. *Staphylococcus aureus* 57(44.53%), *Coagulase Negative Staphylococci* and *Pseudomonas* spp were the commonest bacterial agents isolated as shown in Table-2. The antimicrobial susceptibility patterns of bacterial isolates to common antibiotics tested are highlighted in Table-3

Table-1: Frequency of fungal agents causing corneal ulcer (n=150)

Organisms	n. (%)
Aspergillus spp	50 (33.33)
Fusarium spp	19 (12.67)
Curvularia spp	13 (8.67)
Sterile hyphae	10 (6.67)
Fonsecaea spp	9 (6.00)
Aureobasidium spp	7 (4.67)
Acremonium spp	6 (4.00)
Alternaria spp	5 (3.33)
Candida spp	5 (3.33)
Penicillium spp	4 (2.67)
Cladosporium spp	3 (2.00)
Exophiala spp	3 (2.00)
Scopulariopsis spp	3 (2.00)
Verticillium spp	2 (1.33)
Bipolaris spp	2 (1.33)
Dermatophytes	2 (1.33)
Sepedonium spp	1 (0.67)
Phoma spp	1 (0.67)
Ustilago spp	1 (0.67)
Dreshleria spp	1 (0.67)
Absidia spp	1 (0.67)
Paecilomyces spp	1 (0.67)
Rhodotorula spp	1 (0.67)

Table-2: Bacterial Isolates from corneal ulcers (n=128)

Organism	n. (%)
Gram Positive Bacteria	
Staphylococcus aureus	57(44.5)
CoNS	20(15.6)
Streptococcus pneumoniae	5(3.9)
Streptococcus spp	5(3.9)
Enterococcus spp	4(3.1)
Diphtheroids	2(1.5)
Other gram positive bacilli	7(5.5)
Total	100(78.1)
Gram Negative Bacilli	
Pseudomonas spp	12(9.4)
Acinetobacter spp	6(4.7)
Klebsiella pneumoniae	4(3.1)
Escherichia coli	4(3.1)
Other gram negative rods	3(2.3)
Total	28(21.9)

DISCUSSION

The present study focuses on to the pattern of bacterial and fungal pathogens causing corneal ulcers and the antibiogram of the bacterial isolates among patients attended in tertiary care hospital in Eastern Nepal. Total isolation rate in the present study was 79.2% which is consistent with other reports from Central Nepal revealing 80% and from Bangladesh revealing 82% culture positivity.[12,13] However, similar studies conducted in the same institution previously, and in other countries like Malaysia and India revealed less culture positivity. [14,15,16] High percentage of culture positivity in the present study could be due to corneal scraping perfomed scientifically taking sterile precaution and before the use of antibiotic therapy.

As far as causative agents for corneal ulcers are concerned, 54% of them were fungal in the present study. Higher rate of fungal isolation had been reported in other studies.[14,17,18] Bacteria had been identified as principal etiological agents of corneal ulcers by researchers from Malaysia and Japan.[15,19] Etiological pattern of corneal ulceration varies significantly from region to region and from country to country depending upon the different occupations, climatic conditions etc.

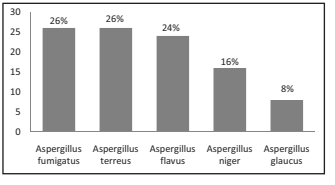

Table-3: Antimicrobial susceptibility pattern of Bacterial isolates from corneal ulcers

Organism	Ak (R%)	C (R%)	Cf (R%)	G (R%)	Tb (R%)	Ce (R%)
Gram Postive Bacteria						
Staphyloccus aureus (57)	52 (5.3)	40 (20.0)	45 (13.3)	47 (17.0)	41 (21.9)	41 (4.8)
CoNS (20)	17 (0)	15 (0)	18 (11.1)	20 (10.0)	9 (22.2)	19 (5.3)
Gram positive rods (9)	9 (0)	7 (0)	6 (16.7)	8(0)	6(16.7)	4 (0)
Streptococcus pneumoniae (5)	4 (0)	3(0)	5 (0)	4 (25.0)	3(66.7)	2 (0)
Streptococcus spp (5)	5 (20.0)	5(0)	5(20.0)	4 (0)	5 (30.0)	4 (0)
Enterococcus spp (4)	4 (50.0)	4(0)	4 (0)	3(66.7)	3(0)	4(0)
Gram Negative Bacteria						
Pseudomonas spp (12)	12 (58.3)	-	12 (16.7)	12(50.0)	12 (16.7)	10(70.0)
Acinetobacter spp (6)	6 (33.3)	5 (60.0)	6 (16.6)	6 (50.0)	4(0)	5(0)
Klebsiella pneumoniae (4)	3 (33.3)	4 (25.0)	2(0)	4(0)	3(33.3)	3(0)
Escherichia coli (3)	3(33.3)	2(100.0)	2(0)	3(66.7)	2(0)	3(0)
Other Gram negative rods (3)	3(0)	3(0)	3(0)	3(0)	3(33.3)	3(0)

*Ak= Amikacin, C= Chloramphenicol, Cf= Cifrofloxacin, G=Gentamycin, Tb= Tobramycin, Ce=Cefotaxime, R% = Percentage of resistance

The most commonly isolated fungal pathogen in the current series were *Aspergillus* spp (33.3%) followed by *Fusarium* spp (12.67%). *Aspergillus* spp had been the commonest fungal agents in many of the studies done by Samar *et al*,[17] Laila *et al*[18] and Rumpa *et al*.[20] *Fusarium* which is one of the most virulent ocular pathogens predominated in studies conducted by other researchers[15,16,21,22] which is the second highest fungal agent in the present study. *Candida* spp was isolated in 3.3% cases in the present series which was less compared to other studies.[12,14,20] Isolation of various dematecious fungi was high (25%) as compared to studies done by Khanal *et al* (15%) in the same institution and by Rumpa *et al* (8%).[14,20] Isolation of two dermatophytes (*Trichophyton tonsurans* and *Trichophyton ajelloi*) were unusual feature of this study as dermatophytes are rare etiological cause of fungal keratitis. Mohammad et al and Shenoy et al had also reported cases of fungal keratitis due to *Trichophyton* spp.[23,24]

Among different species of *Aspergillus*, *A. fumigatus* (26%) and *A. terreus* (26%) were the leading agent followed by *A. flavus* in our study. *Aspergillus fumigatus* had been the most common *Aspergillus* spp reported in various studies[18,21] while *A. flavus* had been isolated in highest rate as noted by other researchers.[14,17,20] This phenomenon may be explained by differences in climate and the natural environment. Seasonal variations have been observed to be important in the incidence of fungal keratitis and in the predominant genera of fungi isolated from such patients. Such variations have been linked to environmental factors such as humidity, annual rainfall, wind and also to harvest.[21]

In our study, 78.12% of bacteria were Gram positive bacteria which is comparable to studies done in West Bengal and Japan.[17,19] A Study on bacterial keratitis in Taiwan, however showed the predominance of Gram Negative bacteria.[25] In the present study, *Staphylococcus aureus* was the most frequently isolated bacterial pathogen (44.5%) which is similar to other studies.[14,17,18,26,27] We found Coagulase Negative *Staphylococci* as the second most common organism accounting for 15.6% of cases. Basak *et al* had also reported *S epidermidis* as second most common pathogen causing corneal ulcer.[17] *Streptococcus pneumoniae* was reported as leading pathogen of bacterial keratitis in studies done earlier in Nepal and South India.[12,16] However, we isolated only 3.9% of *S. pneumoniae*. Diphtheroids are undoubtly a primary cause of corneal ulceration as demonstrated in a report of eight cases by Rubinfeld *et al*.[28] Our reports showed only 1.6% of them.

Pseudomonas aeruginosa is a common inhabitant of soil, water and vegetation and may remain as the main pathogen following vegetation related corneal injury. It has been isolated in highest frequency in a study done in Malaysia.[15] Various studies where major risk factor was contact lens usage had also isolated *Pseudomonas aeruginosa* in highest frequency.[29,30] Although our study does not analyze various risk factors for corneal ulceration, it was the most common gram negative bacilli isolated in the present study (9.38%). Other bacterial agents which were less dominant in the scene in our studies were *Acinetobacter* spp, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterococci* spp.

Antimicrobial susceptibility pattern of bacterial isolates carried out in this study revealed susceptibility to most of the commonly used antibiotics. Chloramphenicol, the frequently used ophthalmic antibiotic was found to be

effective for most of the isolates except for *S. aureus*, *Acinetobacter* spp and *Esch coli* which showed 20%, 60% and 100% resistance respectively. About one fifth of *S. aureus* also showed resistance to Tobramycin. Resistance rate for *Pseudomonas spp* to antibiotics like Amikacin, Gentamycin and Cefotaxime was more than 50%. The excessive and inappropriate use of antibiotics is thought to be one of the most important factors influencing the increased prevalence of antibiotic resistance.

Laboratory confirmation of the etiological agents is the key element in the diagnosis of corneal ulceration. This study was developed primarily to determine the specific pathogens causing corneal ulcer in Eastern Nepal. An attempt was also made to know the antibiogram of isolated bacteria. These information can surely help the ophthalmologists for the prevention and management of microbial keratitis. This study also reveals the changing epidemiology at the same place with time. Thus emphasizes the need for a continuous surveillance of both the aetiology and antibiogram of bacterial isolates.

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