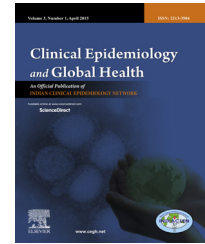


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Original Article

Changing trends in fungal and bacterial profile of infectious keratitis at a tertiary care hospital: A six-year study



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ABSTRACT

Background: Bacteria and fungi are important aetiological agents of infectious keratitis in India. This retrospective study was done to know causative fungal and bacterial species and associated risk factors in patients with suspected fungal or bacterial keratitis. The study included all cases (400) of corneal ulcer investigated and treated at a tertiary care hospital over 6 years.

Methods: Gram staining and 10% KOH wet mount were performed on corneal scrapings. Further identification of fungal and bacterial agents was done using routine methods.

Results: Fungal and bacterial pathogens were identified in 94 (23.50%) and 44 (11.00%) cases, respectively. Keratitis was significantly associated with fungal aetiology. Most frequent agent of fungal keratitis was *Fusarium* species (26, 27.66%). Fungal keratitis was associated with young and middle-aged agriculturists and labourers, and corneal injury with vegetative matter. Most frequent agent of bacterial keratitis was *Staphylococcus aureus* (22, 50.00%). Bacterial keratitis was associated with co-existing ocular diseases, diabetes mellitus, previous ocular surgery, contact lenses and metallic foreign bodies in eyes. Fungal keratitis cases had worse overall outcome compared to bacterial keratitis.

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Conclusions: Knowledge of predisposing factors and microbiological confirmation of keratitis is indispensable to initiate appropriate therapy. We found continuous rise in incidence of *Fusarium* keratitis, which is disturbing due to multidrug-resistant nature of the fungus. Routine surveillance of fungal and bacterial keratitis is needed to know the existing and emerging pattern of pathogens.

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1. Introduction

Corneal blindness is a major public health problem worldwide and infectious keratitis is an important cause.¹ While viral infections are the leading cause of corneal ulcer in the developed countries, bacteria and fungi are important aetiological agents in the developing world.² Though studies on bacterial and fungal keratitis have been carried out in various parts of India, there is scarcity of recent data on bacterial and fungal agents and the underlying factors leading to keratitis published from eastern part of Delhi. Hence this retrospective analysis was undertaken to know the various fungal and bacterial species identified as aetiological agents of keratitis, along with the associated risk factors and outcome in patients attending a tertiary care hospital in East Delhi.

2. Materials and methods

This retrospective study comprised all cases of corneal ulcer investigated and treated in the Departments of Microbiology and Ophthalmology of a 1000-bedded tertiary care hospital in East Delhi from 1st January 2010 to 31st December 2015. The patients' age, gender, residence, occupation, information on the history of the infectious process, as well as the use of topical steroids, antibiotics or antifungals, predisposing conditions like trauma, previous eye surgeries, use of cosmetic or therapeutic contact lens were meticulously recorded.

In all cases, corneal scrapings for analysis were collected directly from the base and margin of ulcers aseptically using Kimura's spatula under direct vision through slit lamp after instillation of anaesthetic eye drops (4% xylocaine). Direct microscopy was performed in 10% KOH wet mount. Smears were prepared from each sample for Gram staining for the demonstration of bacteria, hyphae, pseudohyphae and yeasts cells. For fungal cultures, samples were inoculated on plain Sabouraud's dextrose agar (SDA), as well as SDA with the antibiotics cycloheximide (500 mg/l), chloramphenicol (50 mg/l) and gentamicin (20 mg/l), and maintained at 25 and 37 °C separately over a period of four weeks. All culture media and antibiotics were obtained from Hi-media Laboratories, Mumbai, India. The characteristics considered for fungus identification were macroscopic aspects of texture, colour, growth rate and microscopic aspects, such as mycelium and conidium types, and relationship between hyphae and fruiting bodies by lactophenol cotton blue mount. Microculture on slides was the technique used for observation of filamentous fungi. The yeast isolates were identified by standard tests like germ tube, different spore production on

corn meal agar (CMA), urease production, sugar fermentation and assimilation tests.³ For bacterial cultures, samples were inoculated on Blood agar and MacConkey agar and incubated for 24 h at 37 °C. Bacteria were identified by using routine biochemical tests.⁴

The cultures were considered positive for growth if at least one of the following criteria was fulfilled:

- Same organism growing on one or more solid media.
- The growth on one medium is consistent with direct microscopic findings.²

2.1. Statistical analysis

Frequency distributions were obtained and percentages were calculated accordingly. GraphPad Inc. statistical software (2236 Avenida de la Playa, La Jolla, CA 92037, USA) was used for calculation of P value using Fisher's exact test. Statistical significance was defined as P value lesser than 0.05.

3. Results

A total of 400 cases were tested for suspected fungal and bacterial corneal ulcer during the study period. Fungal and bacterial pathogens were identified in 94 (23.50%) and 44 (11.00%) cases, respectively (Table 1). However, in none of the cases were both fungal and bacterial agents present together. Keratitis was strongly associated ($P = 0.0005$) with fungal aetiology in the present study.

3.1. Fungal corneal ulcer

Out of the total 400 cases of corneal ulcers investigated (239 males and 161 females), fungal aetiology was associated in 72 (76%) males and 22 (24%) females, with a male to female ratio of 3.27:1, which was statistically significant ($P = 0.0073$) (Table 2). The most common age group affected was between 31 and 40 years in both sexes.

Of the 400 cases studied, direct microscopic examination for fungal elements was positive in 147 (36.75%), and in 94 (23.50%) cases, fungi were isolated by culture. Fifty-three (13.25%) cases revealed sterile culture for fungus in spite of positive direct microscopic findings (of which 51 were positive for hyphae and 2 were positive for yeast), in 91 cases both direct microscopic examination and also culture were positive and in 3 cases only culture was positive.

Among the identified fungi, most were hyaline. Of the 94 positive specimens, the most frequent agent isolated was

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