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Original Research Article

ISOLATION, IDENTIFICATION OF FUNGAL AGENT CAUSING KERATITIS, ANTIFUNGAL SENSITIVITY TESTING

Venkatalaxmi Rajamanickam *[1], Aruna Sunder [2], Lingam Jaya Lakshmi [3].

[*1] Assistant Professor in Microbiology, Dr. Patnam Mahender Reddy Institute of Medical sciences and hospital, Chevella, Rangareddy, Telangana, India.

[2] Retd. Professor in Microbiology, Sarojini Devi eye hospital, Hyderabad, Telangana, India.

[3] Associate professor in Microbiology, Osmania Medical College, Koti, Hyderabad, Telangana, India.

ABSTRACT

The present study was carried out in the Department of Microbiology Sarojini Davi Eye Hospital, a tertiary care centre. Hyderabad, for a period of six months, with 150 clinically diagnosed keratitis cases were studied for microbial involvement. Incidence of keratitis was higher in males than females. Maximum incidence was found to be in the age group of 41-60 years. Maximum incidence was found in rural residents. Incidence of keratitis was higher in agricultural workers and labourers than in other occupations. Corneal trauma with vegetative matter was identified as the major predisposing factor followed by co-existing ocular conditions. 33 cases yielded pure fungal isolates and 24 cases were of mixed bacterial and fungal etiology. Culture sterile was 42 cases. Aspergillus species was the predominant fungal pathogen isolated followed by Fusarium species. Antifungal susceptibility showed highest sensitivity to Voriconazole followed by Amphotericin – B and Itraconazole.

KEY WORDS: Corneal trauma, Aspergillus, Amphotericin – B and Itraconazole.

Address for correspondence: Dr. Venkatalaxmi Rajamanickam, Assistant Professor in Microbiology, Dr. Patnam Mahender Reddy Institute of Medical sciences and hospital, Chevella, Rangareddy, Telangana, India. **E-Mail**: drlaxmi04@gmail.com

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BACKGROUND

Infectious keratitis is a leading cause of corneal blindness in developing countries [1]. Corneal infections results in 1.5–2 million new cases of corneal blindness annually, posing a major public health problem according to the World Health Organization (WHO) reports [2].

Fungi are the most common etiological agents which account for 30–40% whereas bacteria account for 13–48% of all cases of Suppurative keratitis; this aetiology and epidemiology patterns of corneal ulceration have been found to vary with the patient population, health of the cornea, geographic location, and climate and also tend to vary somewhat over time [3,4].

Invasiveness of a fungal strain is aided by certain properties such as the capacity to adhere to the cells to produce enzymes that destroy anatomical defences and anti microbial proteins, to survive and evade host defense mechanism [5].

The secretion of enzymes such as phospholipases, protease, pseudo collagenase and exotoxins cause coagulative necrosis with the loss of keratocytes and disruption of collagen lamellae [6]. These pathogens lead to corneal

damage directly or by release of toxins and enzymes or by activating the host immune system [7]. An intact corneal epithelium acts as a barrier for the majority of microorganisms. Microorganisms can penetrate through a breach in the epithelium either due to penetrating or perforating ocular trauma or due to surgery. Various risk factors have been implicated for increased incidence of fungal keratitis including widespread use of antibiotics and steroids, use of contact lenses, and postoperative infections [8].

Unfortunately, in the developing world, treatment of these visually disabling infections is often delayed for several weeks or more and patients commonly present with very advanced keratitis. The severity of corneal infection usually depends on the underlying conditions of the cornea and the virulence of the infecting microbes [9]. Emphasizing the importance of corneal ulceration as an important cause of visual loss, many studies have reported the prevalence of microbial pathogens and identified the risk factors [10]. Ocular morbidity such as corneal scarring and subsequent visual loss can be significantly reduced by prompt institution of appropriate therapy guided by the knowledge of the causative agents. The present study is an attempt to identify the prevalence of fungal keratitis in this area and to test for the in vitro antifungal resistance.

MATERIALS AND METHODS

The present prospective study of microbial Keratitis antibacterial and antifungal susceptibility pattern was undertaken from march 2015 to Aug 2015 at a tertiary care centre, Sarojini Devi Eye Hospital Hyderabad, Telangana with a total of 150 outpatient patients of all age groups, of either sex, who were clinically diagnosed as keratitis by the ophthalmologist were included in the study. Patients already on antibacterial and antifungal therapy are excluded from the study. After a detailed ocular examination by an Ophthalmologist, corneal scraping was collected under aseptic conditions from each ulcer by an Ophthalmologist after instillation of 4% lignocaine without preservative using a sterile Bard Parker blade No.15. The procedure was performed under operating microscope.

Corneal scrapings were placed on 2 slides to prepare 10% KOH wet mount and Gram staining / Giemsa staining. In cases of suspected actinomycetes keratits Kinyoun's acid fast staining was performed. The scraping material obtained from leading edge and base of the ulcer was initially inoculated directly on to the surface of solid media such as Blood agar MacConkey agar, and chocolate agar Sabouraud dextrose agar and also on to liquid media such as Brain heart infusion broth. The inoculated media was incubated at 37°C for 24 hours aerobically. CA plates were incubated at 37°c in the presence of 5 to 10 % CO_2 for 24 to 48 hrs. SDA were incubated in BOD incubator at 25°c.

Identification of fungal ocular pathogens: The fungal elements were observed in 10% KOH mount and Gram stain. The fungi were identified based up on the colony character, such as texture, colour, growth rate on observe side of sabouraud dextrose agar slants and presence of pigment on the reverse side of colony and whether the pigment was localized or diffuse. A lactophenol cotton blue mount was done for the observation of microscopic features like mycelium, conidium relationship between hyphae and fruiting bodies. Slide culture in cornmeal agar was used for the observation of conidiogenesis of filamentous fungi.

Antifungal susceptibility testing was performed for isolates of Fusarium spp, and Aspergillus sppaccording to CLSI M 51-A document (11, 12) disc diffusion method. Aspergillus flavus MTCC 1883 was used as the control strain procured from Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology, Chandigarh. In the present study, the susceptibility testing was carried out for the following antifungal agents-Itraconazole, Voriconazole, and Amphotericin-B.

Anti fungal agents

Anti fungal agent	Symbol	Disc con. µg/disc
Itraconozole	IT	10
Voriconozole	VRC	1
Amphotericin - B	AP	100 units

All the antifungals were procured commerically from Hi- Media Laboratories Pvt Ltd Mumbai.

RESULTS

Table 1: Predisposing factors associated with keratitis.

Predisposing factor	Number of cases	Percentage %
Corneal trauma	124	82.66%
Co existing ocular conditions	17	11.33%
Post surgery	9	6%

Table 2: Distribution of culture positive and negative case.

No of cases	No of culture positive cases		No of culture negative cases	
150	108	72%	42	28%

Table 3: Incidence Of Various Microbial Isolates.

Type of Isolate	Number	Percentage (%)
Pure Bacterial	51	34
Pure Fungal	33	22
Mixed (Bacterial & Fungal)	24	16
Culture Sterile	42	28

Fig. 1: Various microbial isolates.

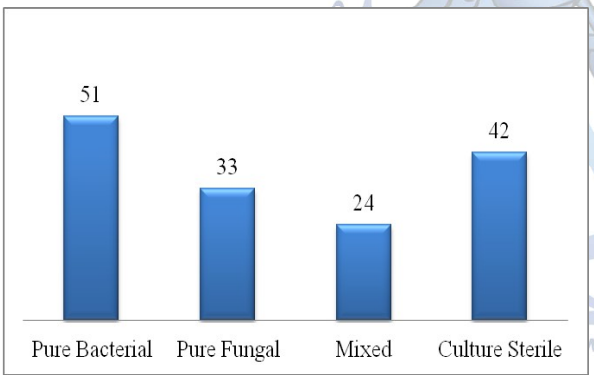


Table 4:- Pure fungal isolates.

Fungal isolate	Number of cases	Percentage (%)
Aspergillus flavus	16	48.48
Fusarium spp.	13	39.39
unidentified	4	12.12
Total	33	100

Fig. 2: Distribution of pure fungal isolates.

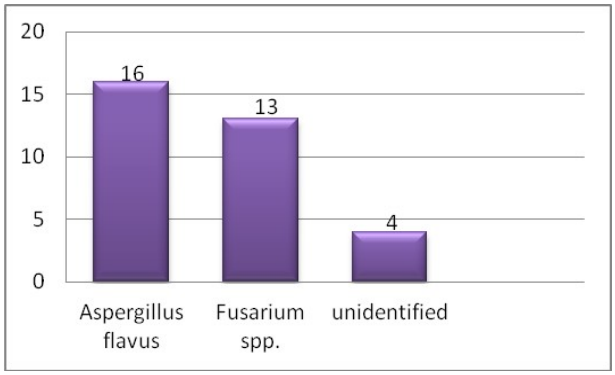
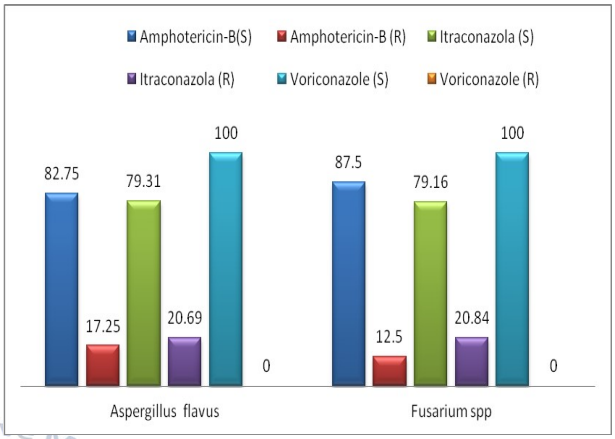


Table 5: Antifungal susceptibility.

Isolate	Amphotericin-B		Itraconazole		Voriconazole	
	S%	R%	S%	R%	S%	R%
Aspergillus flavus	82.75	17.25	79.31	20.69	100	0
Fusarium spp	87.5	12.5	79.16	20.84	100	0

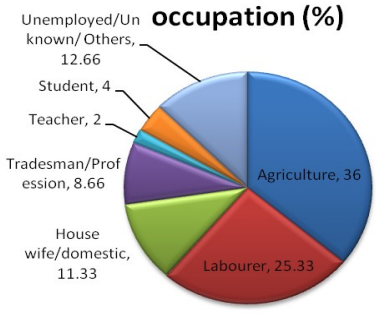
Fig. 3: Antifungal susceptibility.



DISCUSSION

The present study was undertaken on 150 clinically diagnosed as keratitis patients at Sarojini Devi Eye Hospital, Hyderabad. In the present study included 150 cases of clinically diagnosed keratitis, male (66%) subjects were more affected than female (34%) patients which is in agreement with the study done by Tityal et al. [13] The age range of 41–60 years was more affected consistent with the results of Cameron et al [14] in Sydney and Das et al [15] in Kolkata. This could be attributed to the agricultural workers, labourers and domestic workers [Fig. 1] men especially in the agricultural workers, labourers and domestic workers and most of the cases were residents from rural area 54.67% and 45.33% were from urban area. In contrast, in the study done in China, women were more affected and most of them were over the age of 60 (16). This could be due to higher employability of women particularly in the agricultural sector in China.

Fig. 4: Distribution of various occupation profile in keratitis cases.



The most common associated risk factors in our study were trauma [Table 1] followed commonly caused by vegetative matter followed by sand/ stone/dirt [17-20], paddy or its stalk (21), jute followed by steroid(22, 10), and also vitamin A deficiency and acquired external ocular disease as predisposing factors for microbial keratitis [23,24]. In the present study out of 150 corneal scraping 108 (68%) were culture positive, and bacteria were recovered more frequently than fungi (51 Vs 33 eyes, respectively) [Tables 2 and 3]. Srinivasan et al [17] isolated numbers of bacterial (47.1%) agents causing infectious keratitis. Katara et al [25] also reported a culture positivity of 40%, 14% of samples had bacterial etiology.

In our study, out of 150 cases, 108 (72%) were culture positive, and 22 % fungi and 16% Mixed (Bacterial & Fungal) were recovered [Tables 2 and 3]. Srinivasan et al [17] isolated fungal (46.8%) agents causing infectious keratitis with 5.1% cases having mixed infections. Katara et al [25] also reported a culture positivity of 40%, of which 26% were fungal isolates.

Out of total 150 cases studied, pure fungal isolates were 33 cases, among the fungal isolates, Aspergillus flavus was the most isolated species followed by Fusarium spp. [Table 4]. In comparable results were obtained in studied by Leck et al [4] observed a higher incidence of Aspergillus spp in their series. In contrast, Alkatan et al. [26] and Idiculla et al [27] found Fusarium spp. In the current study, most of the fungal isolates (80%) were obtained during the months of March to August. Same as Krishna et al [28] reported maximum incidence of fungal keratitis in Bellary during the harvest months of January, February and June.

The difference in the isolation rates of these fungal pathogens can be explained by the differences in the climate and the natural environment of individual regions. Studies in the South Indian region have shown a higher incidence of Fusarium as compared to studies in the northern or western India. Fusarium keratitis has a more aggressive course and is less responsive to treatment than Aspergillus [29,30]. Katara et al in Gujarat showed Aspergillus as the dominant isolate [25]. The higher incidence of mycotic keratitis due to Aspergillus spp in their study may be due to the high tolerance of their spores to hot and dry weather conditions [29]. Furthermore, Aspergillus spp are more ubiquitous and can almost be found everywhere on every conceivable type of substrate including soil and decaying organic debris while Fusarium species are common plant pathogens and are mostly found in soil [4].

CONCLUSION

In conclusion, routine fungal examination of patients with corneal ulcer is necessary in order to analyze and compare the changing trends of the etiology and their susceptibility patterns which would be beneficial in applying an appropriate antifungal treatment.

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