

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.607.196>

## Common Etiological Agents Causing Keratitis: A Study from a Tertiary Care Hospital in South India

P. Sreenivasulu Reddy\*, P. Vasundhara Devi and G. Avinash

Department of Microbiology, Narayana Medical College, Nellore, Andhra Pradesh, India

\*Corresponding author

### ABSTRACT

The aim of the study is to isolate the bacteria and fungi causing keratitis in and around the Nellore district and to do antibiotic sensitivity testing especially to identify the MDR strains. Corneal scrapings were collected from 150 patients who attended to the department of Ophthalmology, Narayana Medical College, Nellore during the period February 2016 to December 2016 with corneal ulcers. A specific proforma was prepared to record the case history. After a detailed case history and clinical examination the corneal scrapings were collected by the ophthalmologist under slit – lamp examination. Three different samples were collected under strict aseptic precautions by using 26 gauge sterile needle, one for microscopy on Gram staining and KOH mount, another for culture and sensitivity on blood agar, chocolate agar and Mac Conkey's agar for pyogenic bacteria and third sample for fungal culture on SDA medium. Out of 150 cases studied 82% (79) of cases belongs to 11-50 years age group. Among the study group 62.6% were males and 37.4% were females. 45.3% of agricultural workers and 13.3% of house wives were recognized as major sufferers. 57.3% of corneal ulcer cases were found during October to December months. Corneal trauma accounted as major predisposing factor accounting for 78% of cases. Vegetable matter was responsible for corneal trauma in 39.3% of cases. Blood agar showed growth with 30 % (45) of cases chocolate agar with 29.3% (44) cases, Mac Conkey with 3.3% (5) cases and SDA with 13.3% (20) cases. 32 (21.3%) fungal isolates and 18 (12%) bacterial isolates were identified from the study group. Among the Gram positive bacteria *Streptococcus pneumoniae* 10(55.5%), *Staphylococcus aureus* 2 (11.1%) *Pseudomonas aeruginosa* 4 (22.2%) and *Klebsiella* 2 (11.1%) were identified. *Fusarium* spp. constitutes 46.8 % (15) *Aspergillus* spp. with 43.75% (14) and *Curvularia* 9.3 % (3) among the fungal isolates.

#### Keywords

Keratitis,  
*Aspergillus* spp.  
and *Curvularia*  
spp

#### Article Info

Accepted:  
19 June 2017  
Available Online:  
10 July 2017

### Introduction

Corneal blindness is considered to be a serious public health problem worldwide. It is estimated that 1.2–2 million cases of corneal blindness occur globally in a year and 90% of them are from developing countries (Chirambo *et al.*, 1986; Whitcher *et al.*, 1997). A survey by the Government of India estimates that corneal lesions are responsible for significant blindness cases (National

Survey on Blindness, 1999 – 2001). Infectious agents are predominantly responsible for corneal blindness due to keratitis by bacteria and fungi among which fungi occupies highest place in the test (Allan *et al.*, 1995). Corneal ulcers are responsible for 9% cases of blindness in India. Prompt isolation, identification and treatment are mandatory in case of infections Keratitis

especially with fungi. Fungal keratitis remains a challenging and often elusive diagnosis in geographic regions where it is endemic. Microorganism invasion occurs secondary to alterations of the corneal surface, resulting in potential spaces for organisms to track deeper into underlying layers (Zubair *et al.*, 2013). Incidence of infectious keratitis varies from region to region in the same country and also on the type of occupation engaged by the people. So it is mandatory to determine the etiology within a specified region.

### **Exclusion criteria**

Study group included both inpatients and outpatients of the department. Patients with typical viral dendrite ulcers, healing ulcers, marginal ulcers, interstitial keratitis, neurotropic ulcers, Mooren's ulcers and ulcers with the suspicion of autoimmune etiology were excluded from the study group.

### **Materials and Methods**

The present study was conducted from February 2015 to December 2015 among the patients attending to the department of Ophthalmology at Narayana Medical College, Nellore, AP, Presenting with suspected bacterial and fungal Keratitis. Total of 150 samples were collected and processed. A standard protocol was prepared with data related to socio demographic features, occupation, predisposing factors, history of corneal trauma, systemic diseases, treatments received prior to presentation to ophthalmologist at our hospital and relevant clinical findings.

Clinical samples were collected by ophthalmologist under slit-lamp bio microscope examination. Prior to the sampling ocular examination was done by using standard techniques. Few drops of 4%

Lignocaine was instilled into the eye and waited for few minutes. Eyelids were retracted and cleared for discharges and debris from the ulcer zone. Corneal scrapings were collected from base and margins of ulcer by the applications of multiple unidirectional strokes and slit- lamp illumination by using 26 gauge sterile needle.

Corneal scrapings were collected by using three separate sterile needles to perform aerobic bacterial and fungal cultures also to do staining. First sample was inoculated on Mac Conkey's agar, Blood agar, Chocolate agar and Sabouraud's Dextrose Agar (SDA). Second sample was spread onto a sterile, grease free glass slides to do grams staining and third sample was used to prepare 10% KOH mount. Strictly followed the aseptic precautions while collection, processing and culturing the samples. All the inoculated plates to isolate bacterial pathogens were incubated at 37<sup>0</sup>C. SDA plates were incubated at 25<sup>0</sup>C after application of a sterile cover slip on the site of inoculation of corneal scrapings Grams stained smears were examined for pus cells and bacteria (Table 1) and 10% potassium hydroxide (10% KOH) wet mounts were observed under 10 X and 40 X magnification for fungal elements.

Inoculated plates were observed regularly for three consecutive days and considered positive in accordance with following criteria a) Same type of organisms on minimum solid media. b) Association between clinical signs and type of organism's pathogenesis. c) Correlating smear results with cultures to meet the above criteria. Isolated colonies were subjected to suitable biochemical tests and Gram staining. Growth for fungi on SDA plates with cover slip and slopes were checked for further two weeks. SDA plates were discarded after 4 weeks of inculcation in BOD incubator as negative if there is no growth. If growth was seen cover slip was

removed and placed on to a sterile glass slide with a drop of Lacto phenol cotton Blue (LPCB) (Chander, 2008; Davis *et al.*, 2002). All the fungal isolates were isolated and identified by using a standard manual based on the types of spores, hyphae and colony morphology on culture media.

## Results and Discussion

Corneal scraping were collected from 150 patients attending to ophthalmology department of Narayana Medical College, Nellore during the period February 2015 to December 2015 with suspected bacterial and fungal keratitis, study group in divided the age group between 11 to 80 years old and both sexes were included. Distribution of age and sex of study group is presented in the out of 150 patients 94 (62.6%) were males and 56 (37.4%) were females with male: female ratio 1.7:1 (Table 2 and Fig. 1).

Occupation wise distribution of ulcers is presented in table 3. People engaged with agriculture and building works, house wives, welders and carpenters constituted 81.33% (122) patients with corneal ulcers in the present study.

Occurrence of corneal ulcer was observed in all seasons which is shown in table 4 and figure 2. From the present study 57.3% (86) of the cases were found during October to December months since it is vigorous and active cultivation period in the region. Also observed a high incidence during February to May since it is harvesting season which may leads to corneal trauma. The predisposing factors for corneal ulceration in the present study were shown in table 5. Most common predisposing factor identified is corneal trauma, constitutes 78% (118 cases) of the study group due to foreign body and vegetable matter. Even usage of steroids and antihistamine drops are associated with 12%

(18 cases) of corneal ulcers. Gram stained smear examination of corneal scrapings is shown in table 6. This result showed that 80 cases (53.3%) seen with '0' grade, 46 cases (30.6%) with 1+ grade, 12 cases (8%) with 2+ grade, 4 cases (2.6%) with 3+ grade and 8 cases (5.3%) with 4+ grade. Gram positive cocci, Gram negative bacilli and Gram positive fragments of mycelia were observed by Gram stained smears. 29 cases (19.3%) were positive for fungal mycelia by KOH mount. Among them 24 were detected by Gram stain and 5 by KOH only. Culture plates were observed for growth and identified that 45 (30%) cases on blood agar, 44 (29.3%) cases on chocolate agar, 5 (3.3) cases on Mac Conkey agar and 20 (13.3%) cases on SDA were positive. These results are showed in table 7. Commonest organisms isolated were *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Fusarium spp.*, *Aspergillus spp* and *Curvularia spp* (Table 8, Figs 3 and 4).

Blindness due to corneal scaring is a burdensome health problem worldwide. Lot of studies were done in India on this kind of blindness and estimated that it would constitutes around 9% of blindness cases. Fungal and bacterial etiology occupies predominant position in the occurrence of corneal ulcers.

The present study was done over a period of eleven months *i.e.* February 2016 to December 2016. We observed that majority of the patients belongs to the age group between 21- 50 years. Previous studies conducted in India showed that majority of corneal ulcers were seen in 16–60 years (Anusuya *et al.*, 2013; Srinivasan *et al.*, 1997) and suggesting its association with outdoor activity of the individuals. Gender distribution of corneal ulcers in India shows the male predominance with male: female sex ratio 2:1 (Gopinathan

*et al.*, 2002). In the present study this ratio is 1.7:1 where 94 male patients and 56 female patients. These findings are similar to a report from south Karnataka (Anusuya *et al.*, 2013).

**Table.1** Microscopic observation of corneal scraping

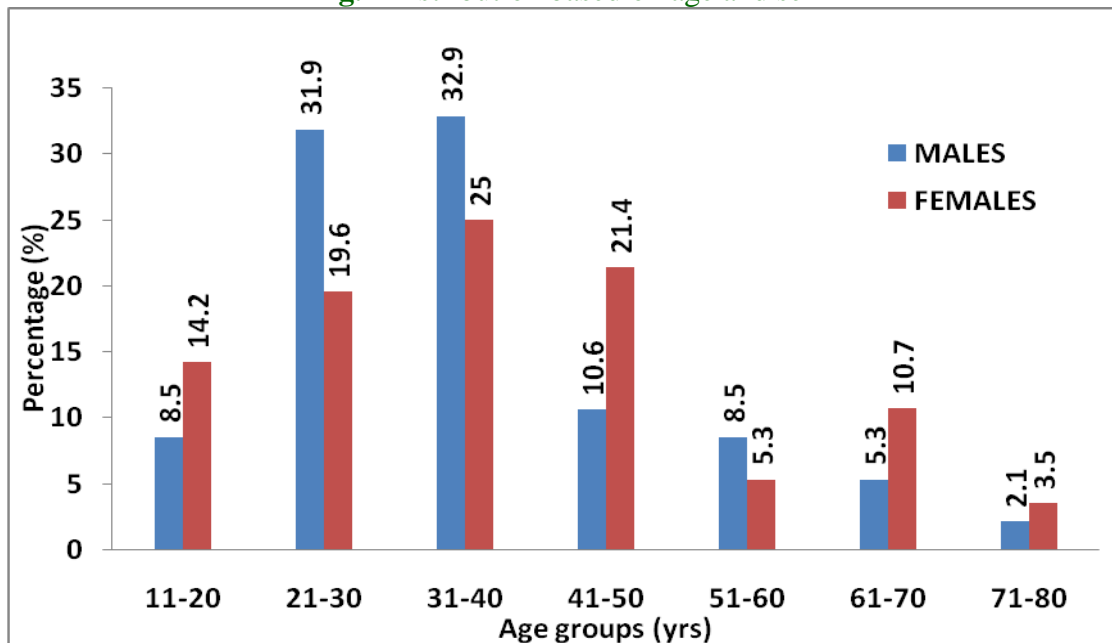
Grade	Description	Cells/ Oil. imm. Field	Bacteria / Oil. imm. Field
0	No pus cells & organisms	0	0
1+	Rarely organisms are seen	<1	<1
2+	Few bacteria are seen	1-5	2-10
3+	Moderate number of bacteria	6-10	11-50
4+	Many bacteria	>10	>50

**Table.2** Distribution based on age and sex

Age	Males (n: 94)		Females (n: 56)		Total (n:150)	
	No	%	No	%	No	%
11-20	8	8.5	8	14.2	16	10.66
21-30	30	31.9	11	19.6	41	27.3
31-40	31	32.9	14	25.0	45	30.0
41-50	10	10.6	12	21.4	22	14.6
51-60	8	8.5	3	5.3	11	7.3
61- 70	5	5.3	6	10.7	11	7.3
71-80	2	2.1	2	3.5	4	2.6

Out of 150 patients 94 (62.6%) were males and 56 (37.4%) were females with Male: Female ratio is 1.7:1.

**Fig.1** Distribution based on age and sex



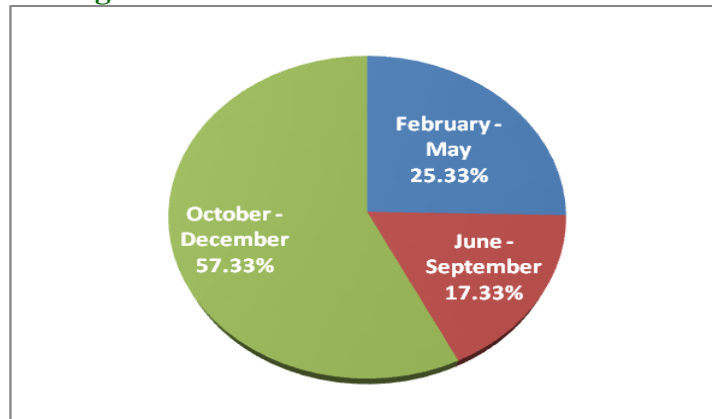
**Table.3** Occupation wise distribution of corneal ulcer

Occupation	Number (N: 150)	%
Agriculturists Building workers	68	45.3
House wife	20	13.3
Carpenters	18	12.0
Welding workers	16	10.6
Stone grinders	12	8.0
Mechanical workers	8	5.3
Students	8	5.3

**Table.4** Season wise distribution of corneal ulcer

Season	Number of cases	%
February – May	38	25.3
June – September	26	17.3
October – December	86	57.3

**Fig.2** Season wise distribution of corneal ulcer



**Table.5** Predisposing factors for corneal ulcer

Predisposing factors	n: 150	%
Corneal trauma	118	78
Steroids and antihistamine drops	59	39.3
Vegetable matter	31	20.6
Welders carpenter	26	17.3
Wind Dust	2	1.3
Animal tail hit	18	12
Not exposed to any of the above	14	13

**Table.6** Gram staining results of corneal ulcer smears

Grading	No. of cases Positive	%
0	80	53.3%
1+	46	30.6%
2+	12	8%
3+	4	2.6%
4+	8	5.3%

**Table.7** Culture results of corneal scrapings on different media

Culture medium	Growth	%
Blood Agar	45	30
Chocolate Agar	44	29.3
Mac Conkey's Agar	5	3.3
Sabouraud's Dextrose Agar	20	13.3

**Table.8** Types of microbial isolates from corneal scrapings

Bacterial isolates (n:18)			Fungal isolates (n:32)		
Type	Number Positive	%	Type	Number Positive	%
<i>Streptococcus pneumoniae</i>	10	55.5	<i>Aspergillus spp.</i>	14	43.75
<i>Staphylococcus aureus</i>	2	11.1	<i>Fusarium spp.</i>	15	46.80
<i>Klebsiella pneumoniae</i>	2	11.1	<i>Curvularia spp.</i>	3	9.30
<i>P. aeruginosa</i>	4	22.2			

**Fig.3** Fungal isolates from corneal scrapings

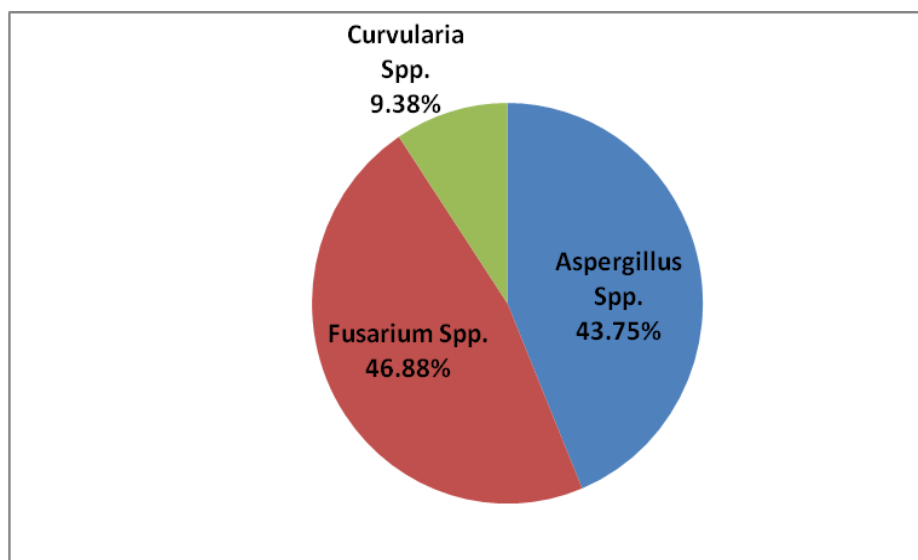
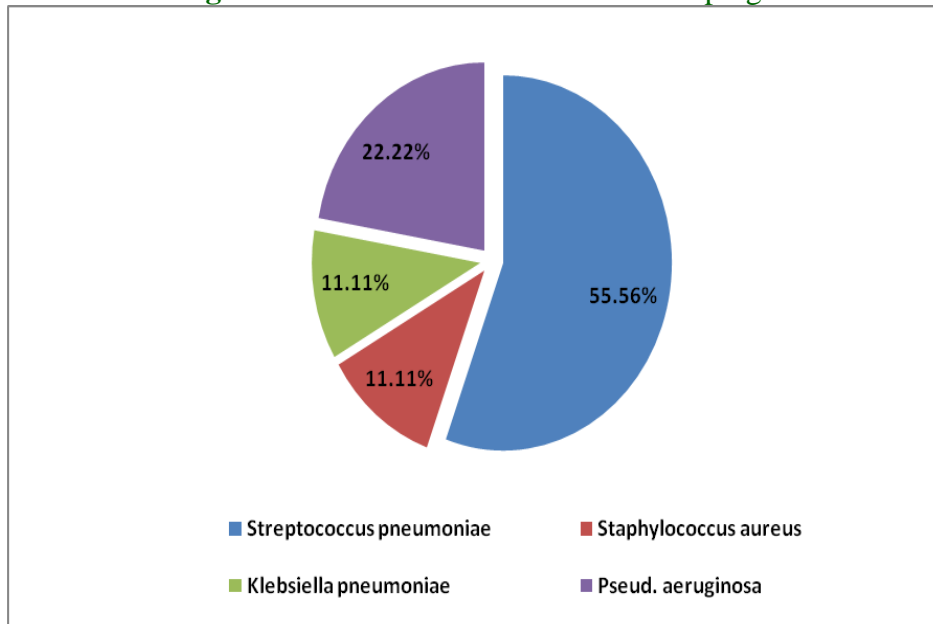


Fig.4 Bacterial isolates from corneal scrapings



In our study 70.6 % of patients with corneal ulcers were engaged with agricultural activities, building works and carpenter works. These results were correlated with scientific publications from southern parts of Tamilnadu (Srinivasan *et al.*, 1997; Bharathi *et al.*, 2003). House wives constitute 13.3% of corneal ulcer cases in this study.

Agricultural works like clearing bushes and plants as well as harvesting were mostly associated with corneal trauma. Gopinadhan *et al.*, (2002) from Hyderabad reported bimodal distribution of fungal keratitis during October to January and June to September. In this study we observed that occurrence of more cases (86) during October to December followed by February to May also harvesting season in this area where study was conducted. This observation is slightly differing with other studies from other parts of South India.

Various studies from west Bengal, Tirunelveli and Madurai showed that corneal trauma was predominant factor for corneal ulceration ranging from 35 to 95% of patients. Present study showed that 118 cases (78%) met with

corneal trauma. Usage of corticosteroid drops and antihistamine plus antibiotic drops associated with development of fungal keratitis along with associated with development of fungal keratitis along with different studies from west Bengal (19.3%) 7 and Benjamin (26%) proved its occurrence ranging from 5-35%. Our study showed that 19.3% of cases positive for Potassium Hydroxide (KOH) mount and 16% (Davis, 2002) of cases were positive by culture on SDA. Percentage of Gram positive isolates among the bacterial isolates were 66.6% (12 cases) and Gram negative isolates were 33.3% (6 cases).

Streptococcus pneumonia was isolated from majority of the cases. No pathogens were isolated from the smears with grade 'O'. But majority of the organisms were isolated from grade 3-4+ smears, so role of gram stain grading (Church *et al.*, 2000) is considerable in the stained smears of corneal scrapings. When compared to KOH and gram stained smears. Stained smears showed significant results in picking up the fungal elements. In the present study commonest bacterial isolate was *Streptococcus pneumoniae* with (55.5%)



cases, followed by *Staphylococcus aureus* 2(11.1%) cases, *Klebsiella pneumoniae* 2(11.1%) cases and *Pseudomonas aeruginosa* in 4(22.2%) cases. These observations on bacterial isolates were correlated with a study done by Bharathi *et al.*, (2002) with 41.85% of *Streptococcus pneumoniae* and 21.25% of *Pseudomonas aeruginosa*. Even though it is rare presentation two *Klebsiella* isolates were detected from the present study. This finding is in accordance with a study done by Gicquel *et al.*, (2007).

Incidence of fungal keratitis varies from region to region in India which is more in North India when compared to other parts of the India. It is around 32–40% in south India and 55.5% in North India (Ragini *et al.*, 2010; Bharathi *et al.*, 2003). The present study showed 21.3% which is slightly low when compared to those studies. This may be due to environmental conditions and the awareness of safety. The predominant fungal isolate among the keratitis patients from South India is *Fusarium spp.* in contrast to *Aspergillus spp.* from North India. We observed in the present study that 15 (46.8%) fungal isolates were *Fusarium spp.* 14 (43.8%) were *Aspergillus spp.* and 3 (9.3%) were *Curvularia spp.* Review of literature is showing the occurrence of *Colletotrichum spp.* and Dematiaceous fungi among the fungal keratitis patients. But no such fungi were isolated from the present study group. Speciation was not done for any of these fungal isolates except *Aspergillus spp.* by colony morphology and identified as *Aspergillus flavus* as the predominant species.

### Conclusion and Summary

- Corneal ulcers are common among 11-50 year age group
- Male and female ratio in the present study group is 1.7:1

- Most of the patients with corneal ulcers were agricultural workers and housewives.
- Corneal trauma with vegetable matter will be considered as major predisposing factor.
- It is possible to isolate bacterial and fungal etiological agents from 33.3% cases of corneal ulcer patients.
- Commonest fungal isolates would be *Fusarium Spp.*,
- *Aspergillus spp.* and *Curvularia spp.* predominantly with *Fusarium spp.* are responsible for fungal keratitis.
- Most common bacterial isolates included are *S. pneumoniae*, *S. aureus*, *Pseudomonas aeruginosa* and *Klebsiella spp.*

No attempt was made to do antibiotic and antifungal sensitivity since the number of Gram negative isolates is less in number to estimate the burden of multi drug resistance. *S. pneumoniae* is usually sensitive to penicillin.

### References

- Albert, D.M., Miller, J.W., Azar, D.T. Albert and Jakobiec's Principles and Practice of Ophthalmology: Expert Consult – Online and Print 3<sup>rd</sup> edn.
- Allan, B.D., Dart, J.K. 1995. Strategies for the management of microbial keratitis. *Br. J. Ophthalmol.*, 79: 777 -86.
- Anusuya devi, D., Ambica R., Nagarathnamma, T. 2013. The epidemiological feature and laboratory diagnosis of keratomycosis, *Int. J. Biol. Med. Res.*, 4(1): 2879- 2883.
- Basak, S.K., Basak, S., Mohanta, A., Bhowmick, A. 2005. Epidemiological and microbiological diagnosis of Suppurative Keratitis in Gangetic West Bengal, Eastern India. *Indian J. Ophthalmol.*, 53: 17-22.
- Bharathi, M.J., Ramakrishnan, R., Vasu, S., Meenakshi, Palaniappan, R. 2002.



- Aetiological diagnosis of microbial keratitis in South India – a study of 1618 cases. *Indian J. Med. Microbiol.*, 20(1): 19-24.
- Bharathi, M.J., Ramakrishnan, R., Vasu, S., Meenakshi, Palaniappan, R. 2002. Aetiological diagnosis of microbial Keratitis in south India. – A study of 1618 cases. *Indian J. Med. Microbiol.*, 20: 19-24.
- Bharathi, M.J., Ramakrishnan, R., Vasu, S., Meenakshi, R., Palaniappan, R. 2003. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three – year study. *Indian J. Ophthalmol.*, 51: 315 -21.
- Bharathi, M.J., Rmakrishnan, R., Vasu, S., Meenakshi, R., Palaniappan, R. 2003. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three year study. *Indian J. Ophthalmol.*, 51: 315-21.
- Bharathi, M.J., Rmakrishnan, R., Vasu, S., Meenakshi, R., Palaniappan, R. 2003. Epidemiological Characteristics and laboratory diagnosis of fungal keratitis: A three – year study. *Indian J. Ophthalmol.*, 51: 315 -21.
- Chander, J. 2008. Text Book of Medical Mycology Oculomycosis, 3<sup>rd</sup> Ed. New Delhi: Mehta Publishers; p. 400-17.
- Chirambo, M.C., Tielsch, J.M., West, K.P., Katz, J. 1986. Blindness and Visual impairment in southern Malawi. *Bull. WHO*, 64: 567- 72.
- Church, D., Melnyk, E., Unger, B. 2000. Quantitative gram stain interpretation criteria used by microbiology laboratories in Alberta, Canada. *J. Clin. Microbiol.*, 38: 4266 – 8.
- Davis, H., Larone. 2002. Medically important Fungi, 4<sup>th</sup> edn, Washington DC USA: Asm Press.
- Gicquel, *et al.* 2007. Amniotic membrane Transplantation in severe bacterial Keratitis. *J. Cornea External Dis.*, 26(1): 27-33.
- Gopinathan, U., Garg, P., Fernandes, M., Shirma, S., Athmanathan, S., Rao, G.N. 2002. The epidemiological features and laboratory results of fungal keratitis. A 10 year review at a referral eye care center in south India. *Cornea*, 21(6): 555-559.
- Govt. of India. National Survey On Blindness. 1999 – 2001. Report 2002.
- Ragini Tilak, Abhishek Singh, Om Prakash Singh, Abhishek Chandra, Vijay Tilak, Anil Kumar Gulati. 2010. Mycotic keratitis in India, A five year retrospective study. *The J. Infections in Developing Countries*, 4(3): 126-131.
- Srinivasan, M., Gonzales, C.A., George, C., Cevallos, V., Mascarenhas, J.M., Asokan, B., *et al.* 1997. Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India. *Br. J. Ophthalmol.*, 8: 965 -71.
- Whitcher, J.P., Srinivasan, M. 1997. Corneal ulceration in developing world: A silent epidemic. *Br. J. Ophthalmol.*, 81: 622 -3.
- Zubair Ansari, Darlene Miller, and Anat Galor. 2013. Current Thoughts in Fungal Keratitis: Diagnosis and Treatment. *Curr. Fungal Infect. Rep.*, 7(3): 209–218.

#### How to cite this article:

Sreenivasulu Reddy, P., P. Vasundhara Devi and Avinash, G. 2017. Common Etiological Agents Causing Keratitis: A Study from a Tertiary Care Hospital in South India. *Int.J.Curr.Microbiol.App.Sci*. 6(7): 1625-1633. doi: <https://doi.org/10.20546/ijcmas.2017.607.196>