

## Actinomycetic Ulcerative Keratitis

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### Abstract

**Context:** Number of blind people in the world is 45 million. Out of which 5.4 million blind people are in India. Corneal Ulcer is a major cause of blindness throughout the world. About 10% cases of blindness are due to Corneal Ulcer. Corneal Ulcers can be caused by exogenous infections i.e. by viruses, bacteria, fungi or parasites and sometimes it is allergic in nature or it can be due to endogenous infections. Actinomycosis is a rare cause of ulcerative keratitis. **Objective:** To isolate and identify *Actinomyces* from Corneal Ulcer and study their susceptibility and resistance pattern with various antibiotics. **Materials and Methods:** A total of 100 samples were collected during period of June 2014 to March 2015 from ophthalmology hospital, government hospital and clinical laboratories. Samples were collected in sterile container containing 0.5ml of Brain Heart Infusion Broth (BHI) as enrichment culture medium that supports the growth. Isolation of Actinomycetes has been done by using several selective media. Their identification was confirmed by Morphological, Biochemical and Cultural characteristics. After identification *Actinomycetes* species were subjected to antibiotic resistance and sensitivity pattern have been carried out by using disc diffusion technique. **Results:** The results of present study show that Vancomycin is 100% resistant to *Actinomycetes* and Ciprofloxacin, Ofloxacin were effective against *Actinomycetes* species in the treatment of severe Actinomycosis. **Conclusion:** Ciprofloxacin and Ofloxacin for the initial empirical treatment of Actinomycosis is a good option. Prompt diagnosis of corneal ulcers and treatment with appropriate antibiotics prevent blindness and devastating visual disability.

**Keywords:** Antibiotic Resistance; Corneal Ulcer; *Actinomycetes*.

### Introduction

Cornea is a clear transparent front part of the eye with a smooth shining surface. That covers Iris, Pupil and anterior chamber. The cornea with the anterior chamber and lens reflects light with the cornea accounting for approximately two-third of the eye's

total optical power. "Corneal Ulcer means loss of corneal substances as a result of infection and formation of raw, excavated area [3]"

Number of blind people in the world is 45 million. Out of which 5.4 million blind people are in India. Corneal Ulcer is a major cause of blindness throughout the world. About 10% cases of blindness are due to Corneal Ulcer [6].

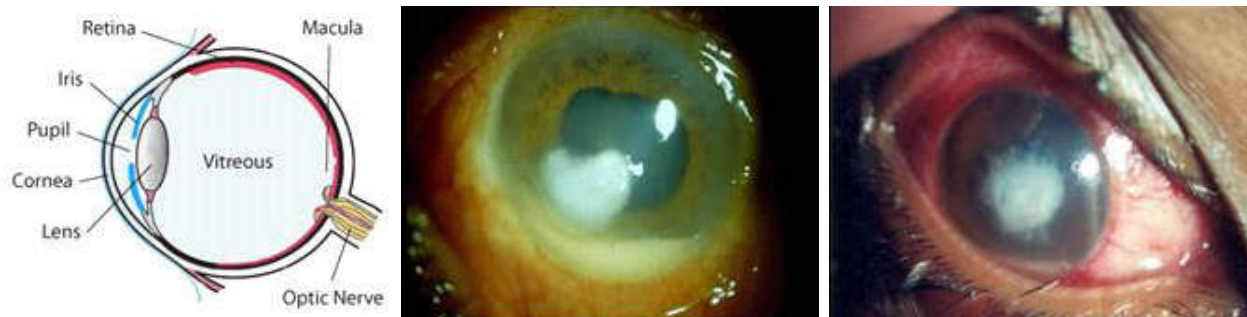


Fig. 1:

Corneal Ulcers can be caused by exogenous infections i.e. by viruses, bacteria, fungi or parasites and sometimes it is allergic in nature or it can be due to endogenous infections. The term keratitis (Corneal Ulcer) had been introduced by “James Wardop” in 1869 in his essay on morbid anatomy of human eye [3,6]. Almost any organism can invade the corneal stroma if the normal corneal defence mechanisms, i.e., lids, tear film and corneal epithelium are compromised [4].

Actinomycosis is a rare cause of ulcerative keratitis. Typical actinomycosis in humans is a chronic disease caused by *Actinomyces spp.* *Actinomyces* is a genus of the actinobacteria class of bacteria. They are all Gram-positive. Actinomycetes are facultative anaerobic. All species grow best under anaerobic conditions. The infection is characterized by persisting swelling, suppuration, and formation of abscesses with draining sinuses. Major types are cervicofacial, thoracic, and abdominal. In addition to the pathogens in actinomycosis some *Actinomyces* species have also been isolated from other mixed anaerobic infections, eye infections, blood and the urinary tract [2].

Early identification of the organism is essential for effective therapy. The infection normally occurs following trivial corneal injury especially when contaminated by vegetable matter and it is therefore more common in outdoor labourers [7].

## Materials and Methods

In assessment to isolate and identify *Actinomyces* from Corneal Ulcer and study their susceptibility and resistance pattern with various antibiotics, present work was under taken.

### Collection of Samples

A total of 100 samples were collected during period of June 2014 to March 2015 from ophthalmology

hospital, government hospital and clinical laboratories.

### Enrichment of Samples

Samples were collected in sterile container containing 0.5ml of Brain Heart Infusion Broth (BHI) as enrichment culture medium that supports the growth of and then transferred immediately to laboratory for further processing [5].

### Isolation and Identification of Actinomycetes

After incubation loopful of each enriched culture was streaked on Nutrient agar plates, the plates were incubated at 37°C for 24 hours. Colonies with different morphological characters and Gram's characters were selected and inoculated on respective selective media viz. Brain Heart Infusion agar and Actinomycetes isolation agar. All the plates were incubated at 37°C for 24 hours.

All the suspicious screened colonies of *Actinomycetes* were then analyzed for their biochemical character viz. Carbohydrate fermentation, IMViC, Enzymes etc. by inoculating into respective media. Further their identification was confirmed by Morphological, Biochemical and Cultural characteristics.

### Antibiotic Resistance Pattern

After identification *Actinomycetes* species were subjected to antibiotic resistance and sensitivity pattern will be carried out by using disc diffusion technique [1].

The Antibiotics were used: Chloramphenicol (30 mcg), Ciprofloxacin (10 mcg), Tetracycline (30 mcg), Gentamicin (10 mcg), Amoxyclav (30 mcg), Cefotaxime (30 mcg), Vancomycin (30mcg), Ofloxacin (2 mcg), Ceftazidime (30mcg), Tobramycin (1.33%). Antibiotic discs were placed on a lawn culture of the isolate under test on Mueller Hinton Agar (MHA).



Fig. 2:

### Result and Discussion

In present study 100 samples were collected during period of June 2014 to March 2015. The patients were of both sex and age groups varying from 20 to 70 years. Out of 100 samples, *Actinomycetes* species were isolated from 24 samples.

*Actinomycetes* is a genus of the actinobacteria class of bacteria. They are all Gram-positive. *Actinomycetes* are facultatively anaerobic. All species grow best under anaerobic conditions. *Actinomycetes* do not require oxygen for growth and are sometimes referred to as anaerobic bacteria. It is actually the requirement for elevated levels of carbon dioxide rather than the negative effect of oxygen that characterizes *Actinomycetes*. Serious ulcers of the cornea of the eye have been caused by contact lens contaminated with saliva containing *Actinomycetes*. Among the *Actinomycetes* there are also pathogenic forms which cause Actinomycosis [8].

The *Actinomycetes* were identified based on the colony morphology and biochemical reaction. *Actinomycetes* were confirmed based on light tan colonies on Brain Heart Infusion agar and good luxuriant growth on Actinomycetes isolation agar.

The sensitivity and resistance pattern of *Actinomycetes* against several antibiotics were

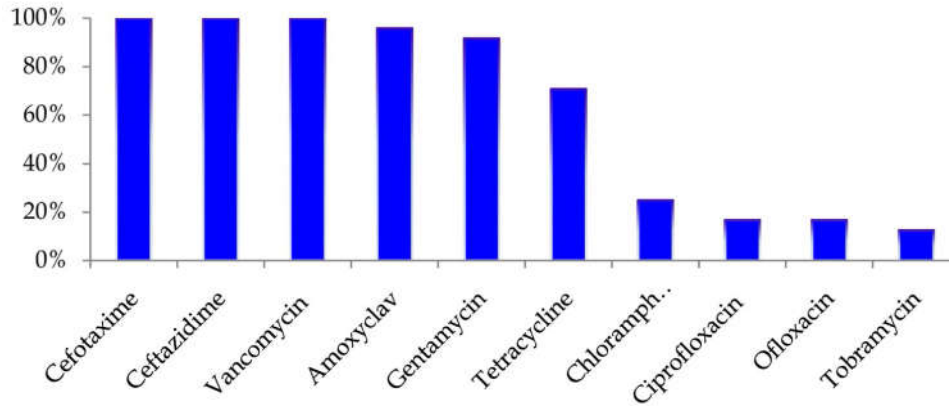
observed by disc diffusion method on Mueller Hinton Agar (MHA- Hi-media) such as Chloramphenicol (30 mcg), Ciprofloxacin (10 mcg), Tetracycline (30 mcg), Gentamicin (10 mcg), Amoxyclav (30 mcg), Cefotaxime (30 mcg), Vancomycin (30mcg), Ofloxacin (2 mcg), Ceftazidime (30mcg), Tobramycin (1.33%). From these antibiotics *Actinomycetes* showed 70% to 100% resistance against Cefotaxime, Ceftazidime, Vancomycin, Tetracycline, Gentamicin, Amoxyclav antibiotics. These four antibiotics such as Tobramycin, Ciprofloxacin, Ofloxacin, Chloramphenicol showed 75% to 80% sensitivity against *Actinomycetes*. (See Table & Graph No.1)



Fig. 3:

**Table 1:** Resistance and Sensitivity Pattern of *Actinomycetes* against several antibiotics

Sr. No.	Antibiotics	No. of Resistance in Percentage
1.	Cefotaxime	100 %
2.	Ceftazidime	100 %
3.	Vancomycin	100 %
4.	Amoxyclav	95.83 %
5.	Gentamicin	91.66 %
6.	Tetracycline	70.83 %
7.	Chloramphenicol	25.00 %
8.	Ciprofloxacin	16.66 %
9.	Ofloxacin	16.66 %
10.	Tobramycin	12.50 %



**Graph 1:** Resistance and Sensitivity Pattern of *Actinomycetes* against several antibiotics

*Actinomycetes* occur in soil. The infection normally occurs following trivial corneal injury especially when contaminated by vegetable matter and it is therefore more common in outdoor labourers [7].

Two genera of the family of Actinomycetaceae produce ulcerative lesions of the cornea. Anaerobic Actinomyces is a relatively more common cause of keratoactinomycosis than aerobic Nocardia. A primary Actinomycetic corneal ulcer always follows an injury [7].

Cycloplegic agents such as atropine sulphate 1%, homatropine 1% or cyclopentolate 1% instilled three times a day reduce ciliary spasm and produce mydriasis, thereby relieving pain and preventing synechiae formation [4].

Our results are in accordance with Bharathi *et al.*, 2002 and Bharathi *et al.*, 2010. They observed these antibiotic treatments such as Ciprofloxacin, Ofloxacin were effective against *Actinomycetes species* in the treatment of severe Actinomycosis. The results of present study show that Vancomycin is 100% resistant to *Actinomycetes* and monotherapy with ciprofloxacin and Ofloxacin for the initial empirical treatment of Actinomycosis is a good option.

### Conclusion

Ciprofloxacin and Ofloxacin for the initial empirical treatment of Actinomycosis is a good option. Prompt diagnosis of corneal ulcers and treatment with appropriate antibiotics prevent blindness and devastating visual disability.

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