Rationale and Objectives

Rationale:

Keratitis incidences due to *Fusarium* are increasing worldwide and it is becoming a leading cause of infection. It has developed resistance against several antifungal agents and several strains of *Fusarium* are reported as multidrug resistant strains. FSSC is most common causative organism of mycotic keratitis and has developed multidrug resistance. There might be several factors, which impart resistance to fungi against antifungal agents.

Keratitis is a disease of cornea. Cornea is composed mainly of collagen. In fungal keratitis, corneal perforation could be seen, it has been reported that extracellular proteases are being produced in order to penetrate the cornea and help the fungus to establish itself. Several reports are there which characterize protease and mention their role in plant pathogenesis but in case of keratitis causing *Fusarium*, such reports are sparse. There is need to characterize the proteases from keratopathogenic *Fusarium*.

The cell wall play important role in survival of pathogen in stressed condition. Cell wall is majorly composed of β-glucan. There are no studies which clarify the role of varying levels of β-glucan of *Fusarium spp.* in the pathogenesis of keratomycosis. There is a need to characterize and quantify the β-glucan levels in the clinical isolates and study the factors which regulate synthesis of β-glucan.

To study the disease mechanism of keratitis, several animal models have been developed. The murine model is one of the most commonly used models for studying fungal infections of cornea because of the similarity of murine and human physiology and immune systems. However, ethical and logistical constraints associated with the use of mice in such experiments slow the progress of our understanding in the field of corneal infectious diseases. Hence, it would be beneficial if a convenient model is developed that mimics the physiological condition of an infected cornea with pathogen. There is an urgent need for the development of a simple *ex vivo* model that can be easily used for the detection of virulence factors, studying the progression of infection and finally testing of antifungal drugs.

Extensive studies comparing levels of virulence factors among various isolates have not been reported. Also, comparison of *in vitro* results with infection model are lacking.

With this background, the proposed work focuses on characterization of various virulence factors of pathogenic *Fusarium* spp.; development of an explant corneal culture model for fungal keratitis and to study various virulence factors in an *in vitro* and in an infectious condition for better understanding of *Fusarium* keratitis.

Objectives:

- Morphological and molecular identification of pathogenic clinical *Fusarium* isolates
- Quantification of virulence factors of pathogenic *Fusarium* spp. *in vitro*
- Development of an *ex vivo* infection model for fungal keratitis and testing and expression analysis of fungal virulence factors in different phases of infection