Scope and Plan
3. SCOPE AND PLAN OF THE STUDY

During the last couple of decades, ophthalmologists became increasingly interested in Acanthamoeba keratitis. This generated concerns about the reliability of methods for identification and classification of Acanthamoeba isolates at the species level. Better criteria than morphology have been in demand for assessing the relatedness of the various isolates for both clinical purposes and for basic biological studies.

Several molecular approaches have increased the reliability of specimen identification, but the use of DNA sequence variation appears to be the most promising. Extensive work on the complete and partial nuclear small sub-unit ribosomal RNA gene (18S rDNA) sequences of this free – living protozoan parasite has revealed high levels of intra-specific sequence variation within the genus. It has also been demonstrated that a PCR primer pair, the JDP1 & JDP2 produces an amplimer that is reliably specific for the genus Acanthamoeba. The amplimer now designated ASA.S1 for Acanthamoeba Specific Amplimer S1, also has inter-strain sequence variation sufficient to distinguish several clusters of 18 rDNA genotypes. Little work along these lines has been published from the Indian sub – continent.

Hence the prime objective of this study was to pursue basic biological research into the molecular systematics of this medically and ecologically important organism, which is now an increasingly encountered pathogen of the human eye and the central nervous system.
The first objective was to determine the genotypes of the Acanthamoeba strains, isolated from cases of Acanthamoeba keratitis in the non-contact lens wearing population, who reported at two major hospitals in South India, and to look for prevalence of new genotypes, if any in this population.

The second objective of this study was to probe into the phylogenetic relationships of the isolates by traditional and novel methods of molecular phylogenetic analysis, and thereby to identify new lineages, if any, and to make a reliable phylogenetic reconstruction of the isolates in this data set.