Summary and Conclusion
7. Summary & Conclusion

In this study, the Acanthamoeba genotypes based on the partial *Rns* sequences from 41 isolates from patients diagnosed with Acanthamoeba keratitis have been compiled. Morphological characterization of isolates using the cyst features separated them into the morphological groups II and III. Of the 41 isolates studied, 17 belonged to the MG III, 24 to the MG II and none to the MG I. The amoebae grew luxuriantly on a monoxenic layer of Klebsiella ozenae.

The genus – specificity of the PCR primer pair JDP1 – JDP2 was demonstrated by the amplimer ASA.S1 from the late log phase trophozoites from culture. Depending on the genotype, the primers amplified ~ 423 to 551 bp of the 18S rRNA gene.

Inference from this study highlights that phylogenetic estimation using the partial *Rns* sequences, amplified by the primers JDP1 – JDP2 is very reliable for such studies and is equivalent to studies involving the entire gene.

Not surprisingly, 35 of 41 human keratitis isolates for which partial *Rns* sequences have been characterized belong to the T4 sequence type. Two isolates Acanthamoeba species MA53 and Acanthamoeba species EGM40 showed 94% similarity to the reference genotype T4 of Acanthamoeba castellani (Genbank accession number – U07413). They probably represent new genotypes or belong to the T4 genotype.
It is noteworthy that a single Madurai strain, Acanthamoeba species MA27 belonged to the T12 genotype and it is first report of a T12 isolate associated AK infection. Another strain Acanthamoeba species EGM50 which showed 89% similarity to the T12 genotype could represent a new sequence type or could actually belong to the T12 genotype.

The Acanthamoeba species EGM27 showed 94% similarity to the T3 reference genotype and was consistently found a neighbor to the clade of T3 references. It is highly probable that this isolate belongs to the T3 genotype.

High sequence alignment scores and low e values (0.0) validated the statistical significance of these genotypes.

In the major group of T4 isolates, though a sub-group strains clustered with the reference sequence, another group, instead of over-lapping or clustering together with the reference sequence of T4 - A. castellani (Genbank reference no. U07413), showed a distinct lineage. It was inferred that the observation could have been the result of either a geographical constraint, (i.e. these strains are found only in this part of the world) or it could be due to amoebae that have more than one Rms allele. This probably could be the reason for this separate lineage seen in the inferred trees.

Traditional and Bayesian approaches were used to reconstruct the phylogeny in this data set. Although the Bayesian analysis is slower than MP for a comparable data set, it was much faster than the traditional ML analyses. Bayesian methods also
have the advantage of parameter rich model choices, computational abilities and by inclusion of valid prior information to guide the analyses.

Taxonomic congruence across different methods of data analysis was used as a measure of clade robustness. Inferences were made only on clades which had high bootstrap values, meaning uniform support. The bootstrap values indicated that parsimony jackknifing produced more reliable trees when compared to neighbor-joining. Observations from the BLAST reports and tree topologies indicated that the closest BLAST hit was not always the nearest neighbor perhaps because homology and similarity are different. Homology implies common ancestry whereas similarity might be a consequence of either common ancestry or of chance.

Acanthamoeba Keratitis is a growing problem leading to corneal ulceration and blindness in this country. Unlike the west, non-contact lens wearers, like the agricultural workers and field labourers are prone to occupational hazards and trauma leading to AK. The T4 genotype is the most prevalent genotype in this part of the globe as it is elsewhere. Other types belonging to genotypes T3 and T12 could also be new agents of human eye disease. There may be lineages of Acanthamoebae which are unique to the Indian sub-continent with a distinct phylogeny.

This is first report which associates an isolate belonging to the T12 genotype in the etiology of Acanthamoeba keratitis and this is the study with the maximum number of samples reported from India.