



Detection and identification of *Acanthamoeba* spp. in corneal scrapings by real-time PCR and microbiome analysis (16S/18S analysis)



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Background

- Free living amoeba found in soil and water, 20 genotypes.
- One of several causes of keratitis, with also include bacteria, fungi and viruses such as *Herpes simplex*.
- Differential diagnostics is clinically difficult.
- Early diagnosis is critical to ensure a good prognosis.
- All testing for *Acanthamoeba* spp. in Denmark is performed at SSI.

Objectives:

To evaluate the sensitivity of a diagnostic platform based on microbiome analysis for the diagnostics of *Acanthamoeba* spp infections and its capacity to detect other relevant pathogens

Materials and methods

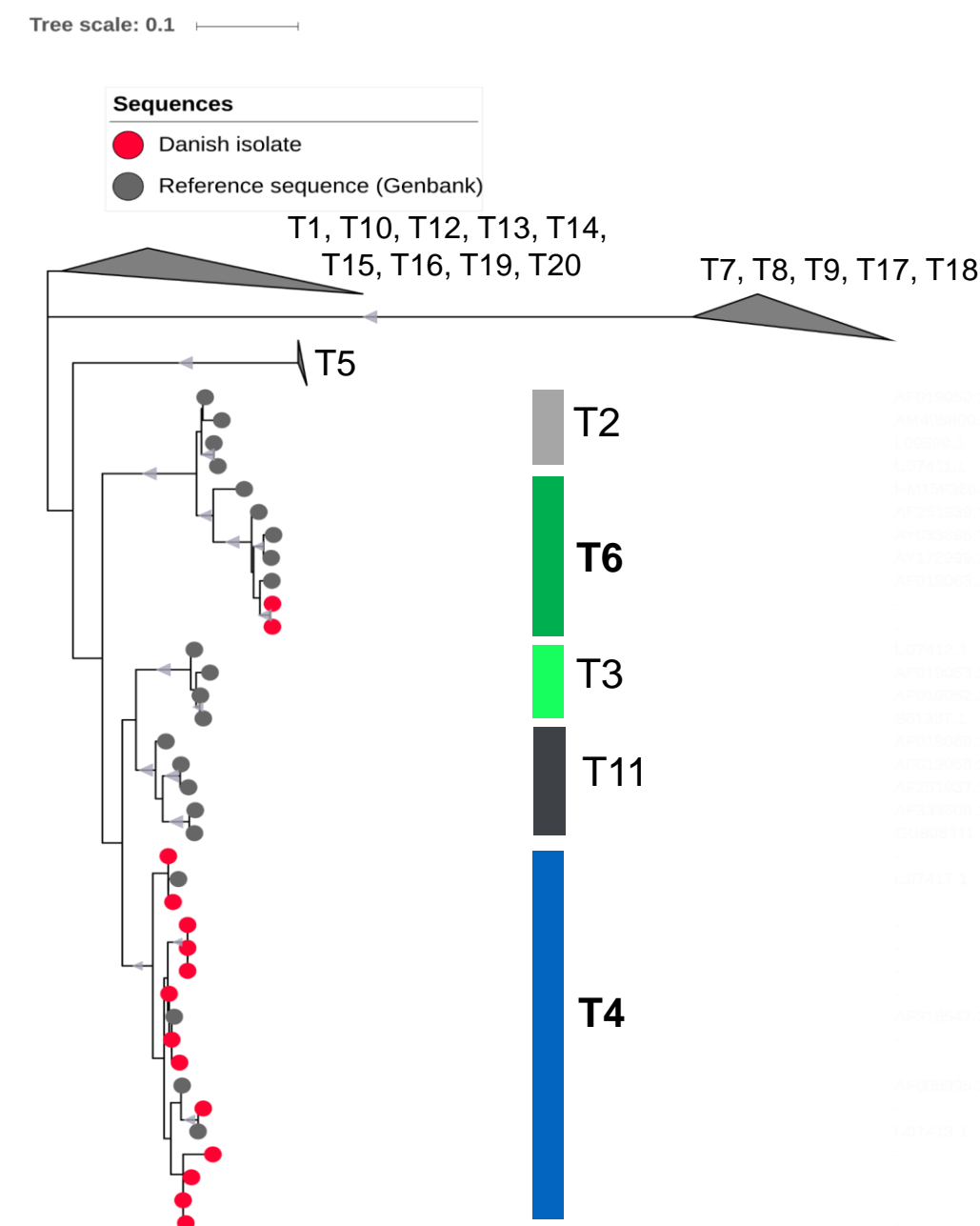
- 100 corneal scrapings, collected in 2015-16.
- Taqman real-time PCR for detection of *Acanthamoeba* spp.
- Amplification of the 18S (eucaryotes) and 16S (bacteria) rRNA using nested multiplex PCRs. Data analysis using the BION software.
- Sequences alignment with MAFFT, tree building in MEGA7 and annotation with iTOL.

Results: Microbiome analysis

Detection and characterisation of *Acanthamoeba* spp:

- Sensitivity = 79%
(15/19 qPCR *Acanthamoeba* spp positive samples detected – 4 false negative samples, Ct > 38)
- Specificity = 100%

Figure 1. Maximum likelihood phylogenetic tree of *Acanthamoeba* sp. 18S sequences

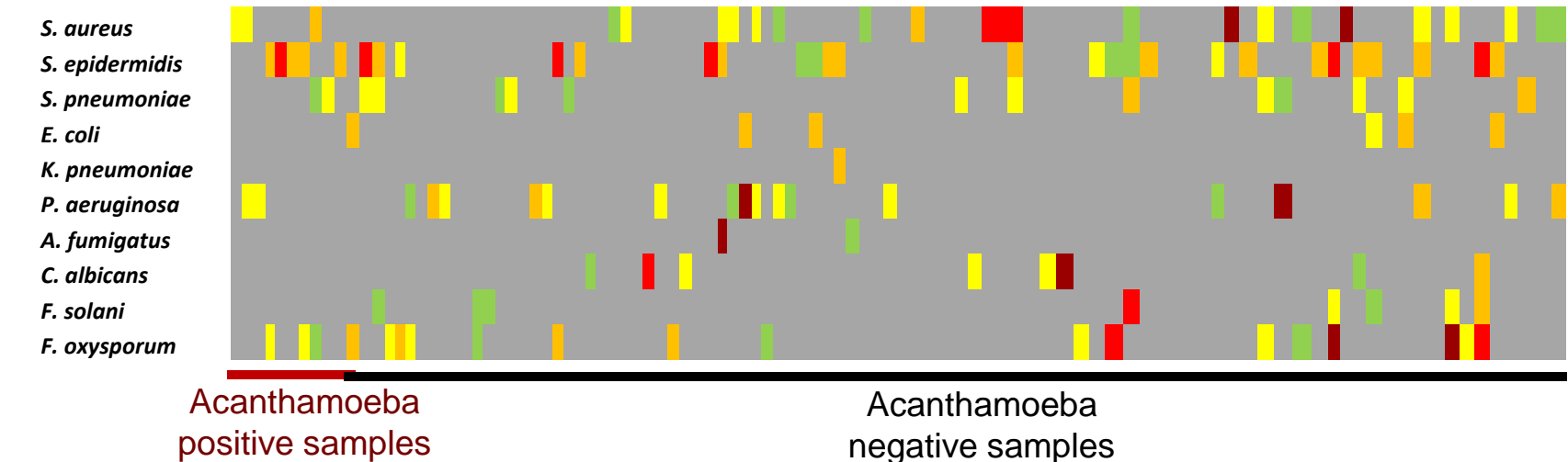
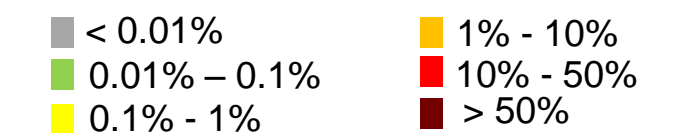


Detection and characterisation of other pathogens:

Multiple organisms were identified in all samples.

Figure 2. Relative abundance of microorganisms known to cause eye infections

Proportion of microorganism reads among 16S or 18S classified reads (including mammals):



Acanthamoeba positive samples

Acanthamoeba negative samples

Results summary and conclusions

- Adequate sensitivity
- Dominant genotype = T4
- 10 potential pathogens (bacteria and fungi) identified in *Acanthamoeba* negative and positive samples.

The microbiome diagnostics platform shows adequate sensitivity for the detection of *Acanthamoeba* infections and allows simultaneous characterisation of other bacteria and fungi causes of keratitis, which will result in better care of patients.