

Genotyping of Israeli *Acanthamoeba* Isolates.

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Introduction: *Acanthamoeba* is a free-living protozoan. Some strains are pathogenic, causing such diseases as *Acanthamoeba* Keratitis (AK) and Granulomatous Amoebic Encephalitis (GAE). Current research focuses on improving the classification scheme used to categorize *Acanthamoeba*, with the trend being to divide the genus into fifteen evolutionary lines (T1-T15) based on 18S small ribosomal subunit DNA. Our goal was to use 18S rDNA to classify clinical isolates collected at Hadassah Hospital.

Methods: Seventeen *Acanthamoeba* isolates collected from corneal scrapings of AK isolates at Hadassah Hospital between 2002 and 2008 were grown on non-nutrient agar plates pre-coated with *Escherichia coli* strain JM 109. The plates were left at room temperature and examined at 48 hours and afterward as necessary. Once trophozoites (after two days) or cysts (three or more days) were visible, whole-cell DNA was extracted using either the Qiagen Buccal Swab Spin Protocol or a boiling protocol (1000 μ L PBS was pipetted onto the *Acanthamoeba* plates and stirred with a quadloop to resuspend the microorganisms. 500 μ L of this solution was pipetted out, heated at 98 $^{\circ}$ C for two minutes, and then vortexed for two minutes.) Extracts were then stored at -20 $^{\circ}$ C. The 18S rRNA gene was amplified by PCR using *Acanthamoeba*-specific primers JDP1 and JDP2, which amplify a region of the 18S rRNA gene containing a fragment diagnostic for genotype. The PCR program was 98 $^{\circ}$ C for 30sec, 95 $^{\circ}$ C for 40sec, 60 $^{\circ}$ C for 40sec, 72 $^{\circ}$ C for 35sec, repeat 38 times, 72 $^{\circ}$ C for 2min. PCR products were visualized via 1% agarose gel electrophoresis and sent to Hylabs for sequencing. Sequences were edited using ContigExpress and aligned using AlignX and 65 published sequences for T types 1-15.

Results: Thirteen out of the seventeen *Acanthamoeba* isolates were determined to be of genotype T4. (These were isolates Had_0644, Had_0946, Had_1293, Had_2902, Had_4955, Had_5130, Had_307, Had_UCI1, Had_UCI2, Had_UCI6, Had_3A, Had_144, and Had_1811. Two isolates (Had_008 and Had_UCI5) were of genotype T13, one (Had_3243) was type T11, and one (Had_3075) was type T3.

Conclusion: The majority of the isolates were of genotype T4. This supports current consensus that T4 is the predominant genotype as well as the genotype most commonly isolated from AK infections. It was also logical that genotypes T3 and T11 were isolated. Together with T4 they constitute a monophyletic group, and they have been previously isolated from AK infections. Genotype T13 is less closely related to T4, and since pathogenicity of *Acanthamoeba* correlates with phylogeny, the finding of Had_UCI5 and Had_008 is particularly interesting.