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Molecular identification of *Acanthamoeba* sp. Isolates in an *Acanthamoeba* keratitis outbreak

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Objectives: An increase in *Acanthamoeba* keratitis (AK) cases has been documented in the Chicago, Ill., USA area. Epidemiological analysis indicates this is a significant increase in cases compared to historical numbers. It was hypothesized that the increased infections may be due to changes in water treatment. Alternatively, a more pathogenic strain of *Acanthamoeba* may be responsible for the increase in AK cases. Here we use genotypic data to test the hypothesis that a new, or more pathogenic known genotype of *Acanthamoeba*, is the cause of the AK surge.

Methods: Previous sequence analysis of the 18S ribosomal RNA gene (18S rDNA) of *Acanthamoeba* isolates resulted in the placement of *Acanthamoeba* strains into 15 different genotypic classes. Most cases (~97%) of AK are associated with a single genotype (T4) of *Acanthamoeba*. Rarely, AK cases are associated with other genotypes. In this study we determine the genotypes of 23 *Acanthamoeba* sp. isolates from the Chicago AK outbreak by sequencing a highly informative region of the 18S rDNA.

Results: DNA sequencing shows these isolates are predominantly genotype T4 (91%), whereas the remaining isolates were genotype T3 (9%). Both genotypes have previously been observed in AK cases. In addition, DNA sequences are overwhelmingly similar to previously sequenced isolates.

Conclusion: There is no support for the hypothesis that cases of AK in this outbreak are the result of infection by a new *Acanthamoeba* genotype. High sequence similarity between these isolates and the 18S rDNA database does not support the hypothesis that these isolates represent more pathogenic *Acanthamoeba* of known genotypes. Lastly, results lend support to the hypothesis that increased AK cases are due to changes in water treatment, permitting increased bacterial colonization of the water, increased *Acanthamoeba* grazing, and ultimately an increase in AK cases due to an increased abundance of *Acanthamoeba* in the water supply.

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