



اَوْنِوَرَسِيْتِي تِي كُنُو لُو كِي مَارَا
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**PHYSIOLOGICAL TOLERANCE OF
Acanthamoeba GENOTYPE T4
ISOLATED FROM CONTACT LENS
PARAPHERNALIA**

KHAIRUL AMEERA BINTI KAMARUDDIN

Thesis submitted in fulfillment of the requirements for
the degree of
Bachelor of Medical Laboratory Technology (Hons)

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DECLARATION

Project entitled “ Physiological Tolerance of *Acanthamoeba* Genotype T4 Isolated from Contact Lens Paraphernalia” is a presentation of my original research work. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to literature, and acknowledgement of collaborative research and discussions. The project was done under the guidance of Project Supervisor, Assoc. Prof. Dr. Tengku Shahrul Anuar Bin Tengku Ahmad Basri. It has been submitted to the Faculty of Health Sciences in partial fulfilment of the requirement for the Degree of Bachelor in Medical Laboratory Technology (Hons).

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KHAIRUL AMEERA BINTI KAMARUDDIN

950215-10-5344

2016409378

Date:

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ABSTRACT

Amoeba from the genera *Acanthamoeba* is known as a free-living opportunistic protozoan that consumes on other environmental micro-organisms. These ubiquitous pathogen has the ability to survive in very harsh environmental circumstances and have been isolated from different sources including contact lens paraphernalia. Genotype T4 had been acknowledged as the most virulent genotype associated with *Acanthamoeba* keratitis. However, genotyping alone does not provide enough indication of the pathogenicity of an isolate. For this reason, the characterization of *Acanthamoeba* is magnified by the assessment of physiological properties. Physiological tolerance assays comprising osmo- and thermo-tolerance were performed in order to investigate the pathogenic potential of *Acanthamoeba* genotype T4 isolated from contact lens paraphernalia. Overall, a total of 14 contact lens paraphernalia that had been confirmed with *Acanthamoeba* genotype T4 from a previous study were used in this study. Each sample was repeated in duplicate. A clinical strain of *Acanthamoeba castellanii* (ATCC 50492-T4) was used as positive control for both assays. In osmo-tolerance assay, amoeba cysts from 14 positive contact lens samples were directly inoculated (approximately 10^3 cysts) onto a labeled non-nutrient agar plate supplemented with 0.5 M and 1 M mannitol, lawn with 2 mL of *E. coli* suspension. Meanwhile, for the thermo-tolerance assay was performed by incubating the cultivated *Acanthamoeba* into 37°C and 42°C. The number of trophozoites or cysts for each sample were observed (20 mm away from the center of each plate) and counted on the 7th day of incubation in five microscope fields under X40 objectives lens of a light microscope. Demographic and clinical details were collected using pre-tested questionnaire. Of the 14 contact lens samples, all sample isolates (100%) were able to show positive growth at 30°C with 0.5 M mannitol. However, only nine (64%) of them were able to grow at a higher osmolarity concentration, 1 M mannitol at 30°C. Meanwhile, for thermo-tolerance assay, only 13 (93%) of the isolates capable to develop at 37°C, while, nine (64%) of them have the ability to grow up to 42°C. In addition, all of the subjects in this study noted with variation progressive symptoms of the infections that uphold the perceptions for *Acanthamoeba* keratitis. In conclusion, there is a clear need for more detailed knowledge about the distribution of *Acanthamoeba* genotype T4 in different environments and their direct and indirect virulence factors. Thus, the determination of *in-vivo* pathogenicity of the *Acanthamoeba* isolates should be conducted in the future.

Keywords: *Acanthamoeba*, Genotype T4, Keratitis, Osmo-tolerance. Thermo-tolerance