

molecular characterization

by Inneke K Susanto

Submission date: 11-Apr-2023 01:35PM (UTC+0700)

Submission ID: 2061352281

File name: molecular_characterization.pdf (193.04K)

Word count: 2099

Character count: 11569

Molecular Characterization of *Acanthamoeba* spp. on Contact Lens and Household Water Sources of Contact Lens Users in Jakarta, Indonesia

Inneke Kusumawati Susanto^{1,2}, Sri Wahdini³, Ika Puspa Sari^{3*}

Masters Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia¹
Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Department of Parasitology,
Faculty of Medicine, UKRIDA²
Department of Parasitology, Faculty of Medicine, Universitas Indonesia³

Corresponding Author: 3*



17. words:

Acanthamoeba spp., keratitis, contact lens user, tap water.

ABSTRACT

Acanthamoeba keratitis is a disease affecting the cornea caused by unicellular protozoa *Acanthamoeba* spp. that live freely in nature. This study aims to determine the potential for transmission and genetic characteristics of *Acanthamoeba* spp. from contact lens cleaning solutions and household water sources for contact lens users. The was a cross-sectional study that was conducted in January-May 2019. The collected samples were contact lens and tap water from 53 lens users in Jakarta. *Acanthamoeba* spp was detected microscopically from culture and molecularly by PCR-sequencing. From 53 contact lenses samples, there were 2 positive samples of *Acanthamoeba* sp and three positive samples of other free-living amoebae. From the culture of 53 tap water samples, there were 5 positive cultures of *Acanthamoeba* spp. and 34 positive cultures of free-living amoeba. From the positive PCR results on contact lenses and tap water, it was found that there was a similarity in 1 sample of contact lenses and tap water from 1 subject. Sequencing analysis found that only 1 contact lens sample was *Acanthamoeba castellanii* genotype T4. Meanwhile, from the tap water sample, there were 5 samples that could be sequenced. The sequencing analysis results showed that 2 samples were *Acanthamoeba lenticulata* genotype T5 and 3 were *Acanthamoeba jacobsi* genotype T15.



This work is licensed under a Creative Commons Attribution Non-Commercial 4.0 International License.

1. Introduction

Acanthamoeba spp. is a protozoan parasite that lives freely in soil and water. It may cause infectious diseases when in contact with the eyes and cause *Acanthamoeba* keratitis. The prevalence of *Acanthamoeba* keratitis is low, but if it is not immediately treated, it can negatively impact clinical symptoms until blindness occurs [1], [2]. *Acanthamoeba* keratitis mostly occurs in individuals who wear contact lenses [3]. Therefore, contact lens hygiene is essential to prevent the transmission of this infection.

Acanthamoeba spp. can be classified molecularly by knowing the genotype to identify pathogen isolates. Several species of the genus *Acanthamoeba* spp. have been known to cause *Acanthamoeba* keratitis. Up to now, 20 genotypes have been identified (genotype T1-20) based on diagnostic analysis of fragment 3 (DF3), part of the ribosomal DNA (rDNA) of the *Acanthamoeba* gene [4]. DF3 encodes a variable to identify the 18S rDNA small subunit gene. *Acanthamoeba* species group II consisting of *A. castellanii* (T4), *A. griffithii* (T3), *A. hatchetti*, *A. polyphaga* and *A. stevensoni* (T11) were reported to be the cause of infection in humans, namely *Acanthamoeba* keratitis or keratitis amoeba and granulomatosa amoebic encephalitis (GAE). Based on the study of [5] in Argentina in 2017, all contact lens samples collected were the T4 genotype. In research by [6] in Tehran, Iran, 6 of 90 (6.7%) samples showed positive results for *Acanthamoeba* spp. from tap water sources. The diagnosis was made based on the morphological characteristics of a double-walled cyst and molecular analysis showed genotypes T4 (83%) and T11 (17%). [7] also reported that the T4 genotype is the most common genotype in the environment and a pathogen that causes eye disease with risk factors for lack of hygiene in contact lens users and contamination of tap water sources in residential homes. Research conducted by [3] on a contact lens cleaning solution found *A. castellanii* genotype T4.

It is known that the distribution of *Acanthamoeba*, which is cosmopolitan so that it can be found anywhere, especially in water, air, and soil causes transmission to humans. A study in Turkey, found that some tap water in Sivas district identified free-living amoeba species such as *A. castellanii*, *H. vermiformis*, and *A. polyphaga* in the collected water samples [8] In Indonesia, contact lens user numbers increased by more than 15% annually [9]. It is important that research is needed to assess the risk factors for transmission of *Acanthamoeba*, such as contact lens cleaning solution and tap water sources in contact lens users by molecular identification [8]. This study was conducted to know whether there is a potential for transmission of *Acanthamoeba* spp. from water sources usually used to clean contact lenses.

2. Material and Method

The contact lens and tap waters samples was collected from 53 contact lens users of Faculty of Medicine of Krida Wacana Christian University (UKRIDA) students. Microscopic examination was performed after 14 days incubation of Page Salt Agar culture samples [10]. The positive *Acanthamoeba* samples were then extracted using Promega Kit (Wisconsin, USA) and amplified with the 18s rRNA gene of JDP1 forward primers (5'-GGCCAGATCGTTTACCGTGAA-3') and JDP2 reverse primers (5'TCTCACAAAGCTGCTAGGGGAGTCA-3') [11]. Amplicon was carried out then visualized on agarose gel with UV light and the results of the amplicon length was expected to be 450 bp. The PCR products were then sequenced for genotyping analysis. Alignment and phylogenetic analysis were generated by MEGA XI software [12].

Research has been reviewed and granted ethical permission by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia No. 0712 / UN2.F1 / ETIK / 2018.

3. Result

3.1 Microscopic examination from Page Salt Agar Culture

Microscopic examination of the Page Salt Agar culture from contact lenses and tap water found two and five positive *Acanthamoeba* spp. cyst respectively. The morphological description of *Acanthamoeba* spp cyst stage is round and has a single core and exocyst double wall (wrinkled outer wall) and endocyst (star-shaped inner wall) as described in figure 1.



Figure 1. Acanthamoeba spp. cysts of S48 LK contact lens sample in 40x magnification

3.2 Molecular Analysis

All positive culture examination were successfully amplified Sequencing analysis such as alignment and phylogenetic analysis ((Figure 2) was using MEGA XI software. The sequencing results were then compared with the reference sequences of *Acanthamoeba* spp. published on GenBank. The strain of the contact lenses samples S25 and S48 are *A. castellani* genotype T4 and *A. lenticulata* genotype T5, respectively (Table 1). Moreover, genotypes of the tap water are *A. lenticulata* genotype T5 for S11 and S48, *A. jacobsi* genotype T15 for S40, S41, and S44.

Table 1. The genotype of Contact lenses and tap water

Sample	Contact lens	Tap water
S11	-	<i>A. lenticulata</i> genotype T5
S25	<i>A. castellani</i> genotype T4	-
S40	-	<i>A. jacobsi</i> genotype T15
S41	-	<i>A. jacobsi</i> genotype T15
S44	-	<i>A. jacobsi</i> genotype T15
S48	<i>A. lenticulata</i> genotype T5	<i>A. lenticulata</i> genotype T5

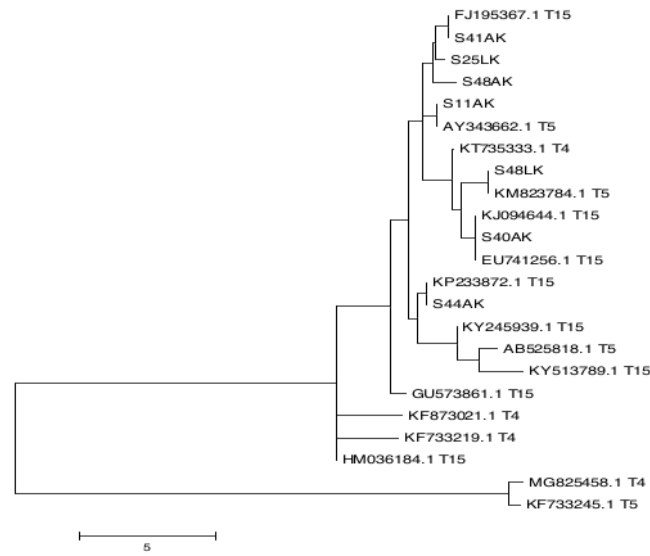


Figure 2. Phylogenetic analysis of the *Acanthamoeba* sample. Phylogenetic tree based on 18SrDNA sequencing was generated by Tamura-Nei method 500 replicates.

The results of the sample identification support the statement from previous studies that the *Acanthamoeba* genotype T4 is the cause of *Acanthamoeba* keratitis. *A. lenticulata* genotype T5 found in the environment can also be pathogenic and potentially cause keratitis. In addition, *Acanthamoeba* genotype T15 is also found in the environment.

8 Discussion

Acanthamoeba keratitis (AK) is a disease caused by one of the free-living amoeba, *Acanthamoeba* spp. It can cause cornea infection due to contamination of the contact lenses used and contact lens waste and tap water [2]. FLA can live in all types of water, such as brackish water, seawater, tap water, groundwater, river water, wastewater, pool water, and contact lens cleaning solutions that are potentially contaminated with *Acanthamoeba* spp. [1].

From the positive PCR results on contact lenses and tap water, it was found that there was a similarity in 1 sample of contact lenses and tap water from 1 subject, namely the S48 sample. This can be due to the possibility of using tap water to clean the contact lenses. Other possible causes were not washing hands before handling contact lenses, not using special contact lens cleaning solutions, or if they are using special contact lens cleaning solutions that are ineffective in killing *Acanthamoeba*. Water that is distributed to the population and used for daily needs has been contaminated by *Acanthamoeba* spp.

From the results of sequencing analysis, it was found that only 1 contact lens sample was *Acanthamoeba tellani* genotype T4. These results are in accordance with the research conducted by [5] in Argentina that the T4 genotype is the most common genotype which is the pathogen that causes eye disease, and similar to the research conducted by [3], the *Acanthamoeba castellanii* genotype T4 was detected in contact lens samples and lens cleaning solutions. Meanwhile, there were 5 samples that could be sequenced from the tap water sample. The results of the sequencing analysis showed that 2 samples were *Acanthamoeba lenticulata* genotype T5 and 3 samples were *Acanthamoeba jacobsi* genotype T15. This is slightly different from the research conducted by [6], which found that *Acanthamoeba* spp. from tap water sources were the T4

genotype (83%) and T11 (17%). There is a possibility that some of the tap water sources in Indonesia have been contaminated by *Acanthamoeba* spp genotypes T5 and T15. One of the 2 subjects had similarities in the results of sequencing between contact lenses and tap water, namely *Acanthamoeba lenticulata* genotype T5. This may indicate transmission and contamination. The source of tap water that has been contaminated by *Acanthamoeba lenticulata* genotype T5 may transmit keratitis in humans. There were no complaints in contact lens users may indicate that *Acanthamoeba* spp. genotype T5 and T15 were not virulent.

5. Conclusion

The finding of similar genotypes in contact lenses and the household tap water of the user may imply that there is potency for transmission of *Acanthamoeba* spp. from the household water sources to the contact lens users and the contact lens used as well.

6. References

- [1] Liu, D. (2017) *Acanthamoeba*, In *Laboratory Models for Foodborne Infections* (Liu, D., Ed.) 1 ed., p 580, CRC Press, New York.
- [2] Wahdini, S., Shanti, A. D., and Sinaga, H. M. (2019) Pengetahuan dan Perilaku Mahasiswa Kedokteran di Sebuah Universitas di Jakarta tentang Penggunaan dan Perawatan Lensa Kontak, *Journal of Medical Science* 13.
- [3] Siddiqui, R., and Khan, N. A. (2012) *Biology and pathogenesis of Acanthamoeba*, *Parasites Vectors* 5.
- [4] Cave D.D, Alfonso R.D, Comlavi KA, Orazi C.D, R, M., and F, B. (2014) Genotyping heterogeneity based on 18SRNA gene sequence among *Acanthamoeba* isolate from clinical samples in Italy, *Experimental Parasitology* 145.
- [5] Rodolfo, D. C., Florencia, M., Laura, L., Fernando, R., Dario, S., Anibal, T., Giovanni, H., and Juan David, R. (2017) Molecular and morphological characterization of *Acanthamoeba* isolated from corneal scrapes and contact lens wearers in Argentina, *Infection, Genetics and Evolution* 54, 170-175.
- [6] Behnia, M., Hatam-Nahavandi, K., Hajjalilo, E., Niyayati, M., Tarighi, F., Bakhtiar Akram, A., Salimi, M., and Rezaeian, M. (2017) Occurrence of *Acanthamoeba* Genotypes in Wastewater Samples in Tehran, Iran *J Parasitol* 12, 516-521.
- [7] Taher, E. E., Méabed, E. M. H., Abdallah, I., and Abdel Wahed, W. Y. (2018) *Acanthamoeba* keratitis in noncompliant soft contact lenses users: Genotyping and risk factors, a study from Cairo, Egypt, *Journal of infection and public health* 11 3, 377-383.
- [8] Coşkun, K. A., Özçelik, S., Tutar, L., Elaldi, N., and Tutar, Y. (2013) Isolation and Identification of Free-Living Amoebae from Tap Water in Sivas, Turkey, *BioMed Research International* 2013, 675145.
- [9] Booton GC, Visvesvara, G., Byers, T., Kelly, D., and Fuerst, P. (2005) Identification and distribution of *Acanthamoeba* species genotypes associated with nonkeratitis infections, *Journal of Clinical Microbiology* 43, 689–693.
- [10] Khan, N. (2001) Pathogenicity, morphology and differentiation of *Acanthamoeba*, *Current Microbiology* 43, 391-395.

[11] Schroeder, J. M., Booton, G. C., Hay, J., Niszl, I. A., Seal, D. V., Markus, M. B., Fuerst, P. A., and Byers, T. J. (2001) Use of subgenomic 18S ribosomal DNA PCR and sequencing for genus and genotype identification of acanthamoebae from humans with keratitis and from sewage sludge, *J Clin Microbiol* 39, 1903-1911.

[12] Tamura, K., Stecher, G., and Kumar, S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11, *Molecular Biology and Evolution* 38, 3022-3027.

molecular characterization

ORIGINALITY REPORT

14%

SIMILARITY INDEX

10%

INTERNET SOURCES

8%

PUBLICATIONS

4%

STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Badan PPSDM Kesehatan Kementerian Kesehatan Student Paper	2%
2	talenta.usu.ac.id Internet Source	2%
3	e-journal.unair.ac.id Internet Source	1%
4	journals.plos.org Internet Source	1%
5	www.azerbaijanmedicaljournal.com Internet Source	1%
6	topsecretapiaccess.dovepress.com Internet Source	1%
7	www.mdpi.com Internet Source	1%
8	jmm.microbiologyresearch.org Internet Source	1%
9	Binod Rayamajhee, Dinesh Subedi, Hari Kumar Peguda, Mark Duncan Willcox, Fiona L.	1%

Henriquez, Nicole Carnt. "A Systematic Review of Intracellular Microorganisms within Acanthamoeba to Understand Potential Impact for Infection", Pathogens, 2021

Publication

10	iwaponline.com Internet Source	1 %
11	jik.fk.unri.ac.id Internet Source	1 %
12	Mahmoud A. Gad, Abdou K. Allayeh, Elmahdy M. Elmahdy, Mohamed N.F. Shaheen et al. "Genotyping and interaction-reality of Acanthamoeba, enteric adenovirus and rotavirus in drinking water, Egypt.", Egyptian Journal of Aquatic Biology and Fisheries, 2019 Publication	<1 %
13	Submitted to UM Surabaya Student Paper	<1 %
14	assets.researchsquare.com Internet Source	<1 %
15	ijpa.tums.ac.ir Internet Source	<1 %
16	repository.essex.ac.uk Internet Source	<1 %
17	"Isolation and identification of free-living amoeba from contact lenses: Thermal and	<1 %

osmotic tolerance in relation to their pathogenicity.", Assiut University Journal of Multidisciplinary Scientific Research, 2022

Publication

18

Nabil Gabr, Rabie Mohamed, Usama S. Belal, Ekhlal Abdel-Hafeez, Mohamed Abdel-Fatah, Rasha Ahmed. "Isolation and identification of pathogenic Acanthamoeba species from different water sources in Minia Governorate, Egypt", Minia Journal of Medical Research, 2020

Publication

<1 %

Exclude quotes On

Exclude matches Off

Exclude bibliography On