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Potential Transmission of *Acanthamoeba* spp. from Contact Lens Solution and Tap Water in Jakarta, Indonesia

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Abstract

Acanthamoeba spp. can cause corneal infection or keratitis that can be transmitted through contamination of contact lenses and water. The objective of this study was to determine the risk factors, transmission potential of *Acanthamoeba* spp. from the contact lenses cleaning solutions including the household water sources to the contact lens wearers. The study was conducted between January 2019 and May 2019. An examination of *Acanthamoeba* was carried out by collecting contact lenses from 53 graduate students in a college in Jakarta. Their cleaning solutions of contact lenses and household water were also obtained. Each sample was separately examined for *Acanthamoeba* spp. in the Parasitology Laboratory of the Faculty of Medicine, Universitas Indonesia, on page – salt agar culture. The findings show 2 and 3 contact lens samples positive for *Acanthamoeba* spp. and other free-living ameba (FLA), respectively. In addition, from the cleaning solution and tap water samples revealed, there are 5 (9.4%) and 34 (64.1%) cultures positive for *Acanthamoeba* spp. and other FLA, respectively. A positive sample of *Acanthamoeba* spp. originating from the same source of the contact lens and tap water, indicating an association of *Acanthamoeba* spp. from contact lenses and tap water with the cleaning solutions of the contact lenses. Thus, there is might be a risk of transmission of *Acanthamoeba* sp. from household water to the cleaning solutions of contact lenses.

Introduction

Acanthamoeba spp. is a unicellular protozoan that is free-living ameba (FLA) and it can easily be found in soil and water [1]. Other FLAs such as *Balamuthia mandrillaris* and *Naegleria fowleri* are known to cause granulomatous amoebic encephalitis (Soedarto, 2012). *Acanthamoeba* spp. causes eye infection in the form of *Acanthamoeba* keratitis. Although the prevalence of *Acanthamoeba* keratitis is low, it can have adverse effects if timely treatment is not received, ultimately causing blindness. *Acanthamoeba* spp. that are known to cause keratitis include *Acanthamoeba castellanii*, *Acanthamoeba culbersoni*, *Acanthamoeba divionensis*, *Acanthamoeba astronyxis*, *Acanthamoeba rhysodes*, *Acanthamoeba hatchetti*, *Acanthamoeba lugdunensis*, *Acanthamoeba lenticulata*, *Acanthamoeba stevensoni*, *Acanthamoeba griffinii*, and *Acanthamoeba polyphaga* [1], [2].

Acanthamoeba keratitis is associated with cases of trauma/injury in the cornea through exposure to soil or water contaminated by *Acanthamoeba* spp. cysts or trophozoites. *Acanthamoeba* can be present in all forms of water sources such as brackish water, seawater, groundwater, PAM water, river water, wastewater, pool water, and potentially contaminated cleaning solutions of contact lenses [3]. It can also grow in contact lenses that are cleaned with contaminated tap water [4]. The risk factors associated with *Acanthamoeba* keratitis include swimming while wearing contact lenses, washing eyes with tap water during or immediately after wearing contact lenses, contaminants from the ground, water-related activities, wearing contact lenses with unhygienic hands or hands washed with tap water, and using artificial salts or chlorinated disinfectants to clean contact lenses [5]. The risk of *Acanthamoeba* keratitis is higher in individuals who use contact lenses and pay less attention to hygiene while using, washing, or storing contact lenses.

A report of *Acanthamoeba* keratitis in Iran found 16 cases positive for *Acanthamoeba* spp. [6]. The risk factors included a lack of cleanliness while using and storing contact lenses. A study in Italy reported >55 cases of diagnosed *Acanthamoeba* keratitis [7]. Another study in the United States reported >85% of cases of *Acanthamoeba* keratitis among contact lens wearers. The incidence of this disease in developed countries is approximately 1 in 33 cases of contact lens wearers [4].

The number of contact lens wearers in Indonesia is increasing by >15% every year [8]. However, research exploring the risk factors for the transmission of *Acanthamoeba* spp. to humans, especially contact lens wearers and identifying its presence in the cleaning solutions of their contact lenses and tap water sources [9].

The cosmopolitan distribution of *Acanthamoeba* spp. in water, air, and soil facilitates its transmission to humans. Therefore, the study aimed to determine the risk factors and its potential transmission to contact lens wearers from their lens cleaning solutions and their water sources. The identification of *Acanthamoeba* spp. from these samples can elucidate the possibility of *Acanthamoeba* keratitis.

Methodology

This study included 53 students (contact lens wearers) from Universitas Kristen Krida Wacana. The subjects were asked to fill out a questionnaire form and collect a solution that was used to rinse contact lenses and a sample of tap water from their respective homes. The primary inclusion criteria were students who wear contact lenses. Every participating student was asked to fill out questionnaires, collect respective samples of their contact lens cleaning solutions, including solutions used to rinse contact lenses, and household water sources.

The cleaning solution samples were entirely poured into the page – salt agar media for the protozoa to grow. The samples of tap water were collected by collecting 500 ml of water from three water sources that are often used. These samples were filtered using a cellulose nitrate membrane filter with a pore size of 0.2 µl and directly placed inverted on the page – salt agar media. The cultures were stored at 30°C and examined after 10 days using a light microscope (100 × 10) [9].

This study received ethical review permission issued by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia no. 0712/UN2.F1/ETIK/2018. The data collected were analyzed by Microsoft Excel and SPSS.

Results

Characteristics of the research subject

From a total of 53 research subjects, more women (92.45%) were found to wear contact lenses than men (7.55%), and most of them (69.81%) have been using PAM water sources for cleaning purposes (Table 1). Based on the age characteristics, it was found that the most age groups were 18–21 years (42 subjects). The majority of subjects live in West Jakarta (69.8%). The average age of study subjects was 20.43 years with the lowest age of 18 years, the highest age of 31 years and the standard deviation value of 2.043-2.089.

Table 1: Demographic data of research subjects

Characteristics	Total (53)	%
Gender		
Men	4	7.55
Women	49	92.45
Age		
18–21	42	79.24
22–25	10	18.87
26–31	1	1.89
Area of residence		
North Jakarta	1	1.89
West Jakarta	37	69.8
Central Jakarta	4	7.55
East Jakarta	1	1.89
South Jakarta	2	3.77
Tangerang	6	11.32
Bekasi	1	1.89
Banten	1	1.89
Type of tap water source		
PAM	37	69.81
Soil	16	30.19

Attitudes and behaviors of research subjects

Based on the questionnaire (Table 2) regarding the attitudes and behaviors of subjects, most contact lens wearers did not routinely examine the eye to the doctor (55%) but read the contact lens instructions carefully (66%). In addition, most of them always wash hands before and after handling contact lenses (92%).

Table 2: Attitudes and behaviors of research subjects

Question	Yes (%)	Sometimes (%)	No (%)
Routine eye checkup	2 (4)	22 (42)	29 (55)
Read the contact lens usage instructions carefully	35 (66)	13 (25)	5 (9)
Wash hands before and after holding contact lenses	49 (92)	4 (8)	0 (0)
Wash with a special cleaning solution before and after using contact lenses	47 (89)	3 (6)	3 (6)
Routinely clean the place of contact lenses	35 (66)	12 (23)	6 (11)
Routinely replace special solutions of contact lens cleaners	42 (79)	8 (15)	3 (6)
Sleep using contact lenses	0 (0)	18 (34)	35 (66)
Swim while using contact lenses	7 (13)	7 (13)	39 (74)
Taking a shower when using contact lenses	9 (17)	10 (19)	34 (64)
Wash and rinse contact lenses with tap water	0 (0)	2 (4)	51 (96)
Wash your face when using contact lenses	13 (25)	23 (43)	17 (32)

Culture results in FLA and Acanthamoeba spp.

The culture tests for FLA were carried out by placing each of the 53 samples of contact lenses and tap water in the page – salt agar media separately. After that, they were stored in an incubator at 37°C for 10–14 days to obtain a cyst shape. The growth of the FLA was quite good. Morphological features of FLA *N. fowleri* at the cyst stage suggested a round shape with exocyst double walls and one core endocysts (Figure 1). FLA *B. mandrillaris* showed one core with exocyst double walls at the cyst stage (wrinkled outer wall) and endocyst (inner wall) (round shape) (Figure 2). FLA *Acanthamoeba* spp. was round with a single core and double-walled exocyst at the cyst stage (wrinkled outer wall) and endocyst (star-shaped inner

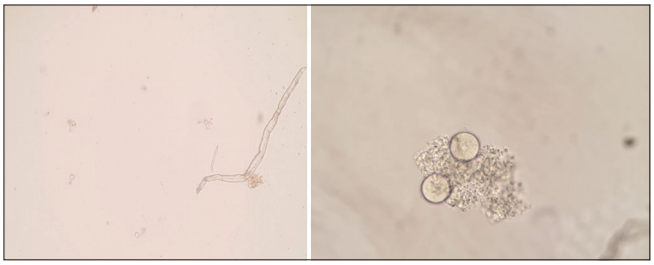

Figure 1: Microscopical examination of 10 × 10 and 40 × 10 of Naegleria fowleri culture taken from S18 AK tap water samples

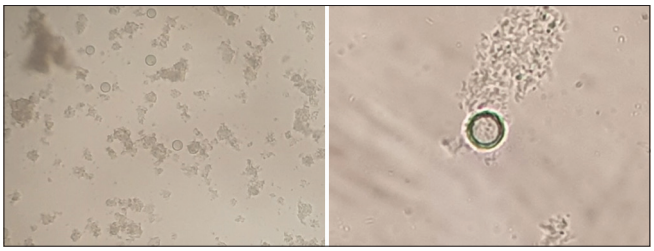

Figure 2: Microscopic examination of 10 × 10 and 40 × 10 of Balamuthia mandrillaris culture taken from S19 AK tap water samples

wall) (Figure 3). The contact lens culture revealed two and three positive results for *Acanthamoeba* spp. and FLA, respectively; the remaining 48 were negative for both. The tap water culture revealed 5 and 34 positive results for *Acanthamoeba* spp. and FLA, respectively; 14 were negative (Table 3).

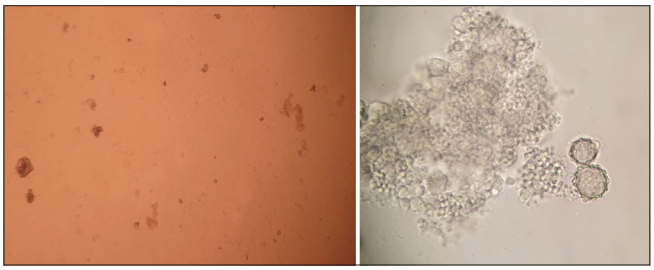

Figure 3: Microscopic examination of 10 × 10 and positive results of Acanthamoeba spp. taken from S48 LK contact lenses samples

Table 3: Culture examination result

Culture results	Total (53)	%
Free-living ameba examination of contact lens culture		
Positive		
Positive for *Acanthamoeba* spp.	2	3.77
Positive for *other* FLAs	3	5.66
Negative	48	90.57
Free-living ameba tap water culture		
Positive		
Positive for *Acanthamoeba* spp.	5	9.43
Positive for other FLAs	34	64.15
Negative	14	26.42

FLA: Free-living ameba.

Discussion

Acanthamoeba keratitis is a corneal infection caused due to the contamination of contact lenses and water by *Acanthamoeba*, which is a type of FLA [1]. FLA can be found in various water sources such as brackish water, seawater, groundwater, drinking water, river water, wastewater, pool water, and contact lens cleaning solutions [3]. A total of 20 FLA genotypes have been identified so far (T1-20 genotype), based on the diagnostic analysis of fragment 3 part of the ribosomal DNA gene in *Acanthamoeba* [10].

The demographic data from 53 research subjects show that a larger number of contact lens wearers are women (92.45%) than men (7.55%). This finding is parallel with the research conducted by Wahyudi and Wahdini [1], which showed a larger number of women (76.3%) using contact lenses than men (23.7%). The main reason for this may be to support physical appearances rather than use as a visual aid. Based on the age-wise characteristics, 79.24%, 18.87%, and 1.89% of users belong to the age groups of 18–21 years, 22–25 years, and 30–33 years, respectively. This is almost similar to the research conducted by Tahir *et al*. [10], which demonstrated that the highest number of contact lens wearers (56.2%) belongs to the age group of 21–25 years, 21.9% belonged to the age group of 15–20 years, and only 3.1% belonging to the age group of >31 years old. Regarding the area of residence, the majority of users in our study (69.8%) lived in the West Jakarta area; therefore, we concluded that three of the six positive samples of *Acanthamoeba* spp. grow in the West Jakarta region, possibly because the water sources of the area have been contaminated by *Acanthamoeba* spp. Based on the type of tap water sources, there were more users of PAM water (69.81%) than groundwater (30.19%). In addition, four of the six samples positive for *Acanthamoeba* spp. originated from PAM water sources, which indicated possible contamination of the PAM water sources by *Acanthamoeba* spp.

The attitude and behavior questionnaires on 53 research subjects revealed that most of the contact lens wearers did not get their eyes routinely checked (55%), whereas only 4% undergo routine checkup. This is probably because of the lack of eye-related complaints that made them unwilling to visit a doctor. Another result demonstrated that a large number of contact lens wearers (66%) read the usage instructions carefully. This is consistent with the research conducted by Tahir *et al*. [10] which showed that 61.5% of contact lens wearers carefully read the usage instructions of the contact lens. In addition, our study revealed that approximately 92% of users wash their hands before and after handling contact lenses. This is almost consistent with the research conducted by Gomes *et al*. [11] which suggested that 79.2% of contact lens wearers wash their hands before using contact lenses. Approximately 89% of contact lens wearers wash their lenses with a special cleaning solution before and after usage. This is contrary to the research conducted by Tahir *et al*. [10] which stated that 24.2% of wearers wash their lenses with a special cleaning solution. The results of our study suggested that 66% of the wearers clean the contact lens area with a proper cleaning solution on a daily basis, whereas 11% did not follow this practice. This is consistent with the research conducted by Gomes

et al. [11] which stated that 70.8% of contact lens wearers cleaned their contact lenses daily, whereas 29.2% of contact lens wearers did not clean their lenses on a daily basis. A total of 79% of contact lens wearers routinely replaced the special cleaning solutions, whereas 15% of them replaced it sometimes and 6% of them did not replace them at all. This is consistent with the research conducted by Gomes *et al*. [11] which stated that 67.9% of the contact lens wearers routinely replaced the cleaning solutions, whereas 32.1% of them did not regularly change them. Our study revealed that 34% of the users sometimes wear contact lenses while sleeping, whereas 66% of the users did not wear them while sleeping. This is compatible with the research conducted by Tahir *et al*. [10] which showed that 5.4% of the users wear contact lenses while sleeping and 94.6% of users do not wear them while sleeping. Further results of our study suggested that 13% of users wore contact lenses while swimming, 13% sometimes wore them while swimming, and 74% did not wear them while swimming. This is consistent with the research conducted by Tahir *et al*. [10] which suggested that 3.1% of users wore contact lenses while swimming and 96.9% did not. Our study showed that 64% of the users wear contact lenses while taking a shower; however, 19% sometimes wore the contact lens and 17% did not wear lenses at all during a shower. This is almost consistent with the research conducted by Gomes *et al*. [11] which showed that 34.5% of users wear contact lenses during showers, 41.1% wear them sometimes, and 24.4% do not wear them. A total of 96% of users in our study do not wash and soak their lenses with tap water, whereas 4% of the users used tap water. This is almost similar to the research conducted by Gomes *et al*. [11] which indicated that 78% of the users do not wash and soak contact lenses with tap water and 22% of users used tap water. Approximately 25% of contact lens wearers in our study washed their faces while wearing contact lenses, whereas 43% of them sometimes washed their faces and 32% did not. These results indicate that it is highly likely that contact lens wearers have implemented a cleaner and healthy lifestyle.

This study examined 53 samples of contact lens and their cleaning solutions including tap water. The culture tests revealed two cultures positive for *Acanthamoeba* spp. (3.77%), three cultures positive for other FLA (5.66%), and 48 cultures were negatives (90.57%). These findings are in accordance with the study conducted by Casero *et al*. [12] which stated that only a few (7 out of 65) samples showed cultures positive for *Acanthamoeba* spp. and 21 of the 65 samples were negative for cultures. This is probably due to the contamination of contact lenses by tap water containing *Acanthamoeba* spp. or other FLA. The culture of 53 tap water samples revealed five results positive for *Acanthamoeba* sp. (9.43%), 34 positives for other FLA (64.15%), and 14 negatives (26.42%). These results are quite the same as the research conducted by Behnia *et al*. [13] which showed that only 12% of water samples cultured were positive for *Acanthamoeba* spp. Even the findings show a few percentages of *Acanthamoeba* spp. and other FLA contamination in tap water samples, it may indicate that there is a risk and the possible contamination of tap water sources by *Acanthamoeba* spp. or another FLA. Therefore, health promotion and counseling regarding the risk of using tap water to clean contact lenses might transmit *Acanthamoeba* spp. infection to their eyes.

Conclusion

A greater number of women wear contact lenses than men. The contamination of PAM water in the West Jakarta region with *Acanthamoeba* spp. was confirmed with its identification in the PAM tap water samples in the region. There is a potential for transmission of *Acanthamoeba* spp. from contact lens cleaning solutions and tap water sources to the users of contact lenses.

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