

A preliminary survey of *Acanthamoeba* spp. contamination in contact lens storages from health science students of Rangsit University, Thailand

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Abstract

Introduction: This study aimed to evaluate the contamination of *Acanthamoeba* spp. in contact lens storage cases and to study the hygiene habits among contact lens users in health science faculties at Rangsit University.

Method: The cross sectional study was conducted and samples were collected between April and October, 2013. *Acanthamoeba* spp. contamination was determined using culture method in non-nutrient agar (NNA), which was coated with non-active *Escherichia coli*. A total of 116 questionnaires and 102 contact lens storage cases were submitted.

Result: The results were no contaminants *Acanthamoeba* spp. for all contact lens storage of participant students. However, the questionnaires have revealed poor hygiene behavior where almost all students had not taken care of their lens correctly. Fifty percent of contact lens users lack of an eye check-up before wearing contact lens and improper wash hands before touch contact lens, 44.8% absence to see a doctor when experiencing eye problems, 40.5% wore contact lens while swimming and lens storages unchanged every 1-2 months.

Discussion and Conclusion: The poor hygiene behavior might be risk factors contributing to the cause of *Acanthamoeba* keratitis and still found in the surrounding environment. This necessity to be aware of using contact lens in which to reduce the risk factor of infection.

Key words: *Acanthamoeba* spp., Contact lens, Keratitis, Risk factor, Hygiene habit

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Introduction

Acanthamoeba is a free-living amoeba which cause opportunistic infections in humans with severe clinical symptoms including a central nervous system disease known as granulomatous amoebic encephalitis (GAE), an eye disease known as *Acanthamoeba* keratitis (AK) and disseminated infection of skin lesions associated with immunocompromised individuals, such as AIDS patients^{1, 2}. *A. keratitis* affects both contact lens users and non-contact lens users. This disease was described severe clinical causes around corneal surface³. In the UK, the incidences of *A. keratitis* have increased, which can arise from accidental trauma to the cornea. Contact lens users have the most risk factor of infection from 62-71% of *A. keratitis* cases. Lack of good hygiene when handling the lens and storage cases, notably using non-sterile saline rinsing solutions, were not following the hygiene routine practices as recommended in cleaning and disinfection steps⁴.

The first reported GAE case of *Acanthamoeba* spp. in Thailand was recorded by Jariya et al¹, *Acanthamoeba* spp. was found (4.8%) in stagnant water from a survey in the northeast region⁵. The prevalence of *Acanthamoeba* spp. in contact lens cases was reported 2.4% (2/87). There were 6 cases of *A. keratitis* which were treated with chlorhexidine successfully⁶. The first diagnosis that fully identified two keratitis were sequenced by Jongwutives et al⁷. In the environment, *Acanthamoeba* spp. that can survive in aquatic habitats was found in five parts of Thailand, which were isolated from soil and water samples at rates of 48% and 36%, respectively⁸. Also, molecular technique related to the surveying of pathogenic marker of *Acanthamoeba* isolated city soils determined a prevalence rate of 35.9% (37/103) and 7% (10/150) and prevalence of *Acanthamoeba* spp. in contact lens cases was reported⁹⁻¹¹. We requested the participation of the health science students as test subjects because a lot of them used contact lens and the contact lens storage cases were a potential source and reservoir of many microorganisms included *Acanthamoeba* spp.

This study was a preliminary survey of *Acanthamoeba* prevalence in contact lens storage cases from health

science students at Rangsit University, who have been using contact lens and also to study routine hygiene habits and practices. Our goal was to remind our students to take care of their hygiene while they were using contact lens to decrease causes of eye infection or eye vision problems.

Methods

The cross sectional study was conducted between April and October, 2013. A preliminary survey of *Acanthamoeba* spp. contamination in contact lens storage from nine faculties of health science students of Rangsit University, Thailand were showed in proportion.

Ethical issue and sample size

The Ethical Review Committee for Research in Human Subjects of Rangsit University has approved (RSEC 16/2555). All subjects had signed an informed consent form to participate in this study. The sample size of 9 faculties of health science student population was calculated from a total of 167 health science students who had been using contact lens for longer than one month (the data recorded on May, 2013). The proportion used a prevalence of *A. keratitis* 6.7% in a previous study by Lek-Uthai et al¹². The calculation of sample size was using the formula¹³.

$$n = \frac{Z_{\alpha/2}^2 PQN}{Z_{\alpha/2}^2 PQ + Nd^2}$$

The estimation of subjects had included 67 samples. All students had no conflict of interest in this study.

Questionnaires and sample collections

The questioning information was inserted in plastic zip bags. A set of questionnaires and collection steps information in easy to follow guides was sent out to all health science student faculties. The questionnaires enquired about routine health habits and hygiene practices when using contact lens. The contact lens samples were sent back in plastic zip bags in the collected box from each faculty. Contact lens used for samples must have been in use for more than 1 month. The samples and questionnaires were separated and proceed in the medical laboratory.

Contact lens cases for amoeba cultivation

The lens storage cases were opened under aseptic conditions. In the storage cases with solution: Samples

solutions were collected from 2 sides of the cases and centrifuged at 2,000 g for 5 mins¹². The supernatant were discarded then 100 µl of the pellet were examined under light microscope for simple screening. Another drop of 100 µl of the pellet was subjected to culture. The cases without solution were moistened with a 1 ml drop of amoeba Page's Saline buffer before centrifugation and process. The storage cases were wiped with a sterile cotton swab soaked with amoeba Page's Saline to scrape over the internal surface of the contact lens cases then subjected to culture. The pellet and the swab were inoculated onto non-nutrient agar plates which were seeded with non-activate *E.coli*, incubated at 35.5°C for 7 days for the presence of trophozoites and/or cysts¹⁴. *A. castellanii* ATCC 30234 strain was cultured as positive control which cultured in the same conditions. In order to isolate *Acanthamoeba* from contact

lens cases, The *Acanthamoeba* cysts were examined and groups identified on the plate by their encysted stage size and morphological features according to the criteria of Pussard and Pons and Visvesvera^{7, 9, 15}.

Results

General information of contact lens users

A total of 116 returned questionnaires were 82 females and 34 males. Age groups were between 19-23 years. The general information of contact lens users was detailed in table 1. The use of soft, monthly contact lens was 85.3%. The most popular solution for contact lens was Renu[®] (41.4%) followed by Duna[®] (21.6%) and Alcon[®] (18.1%), respectively. About 75.9% of health science students were using contact lens due to their eye problems.

Table 1 General information of contact lens users of health science students at Rangsit University

Contact lens used	N = 116	
	No.	%
Types of contact lens		
Soft, monthly	99	85.3
Soft, yearly	9	7.8
Rigid gas, monthly	5	4.3
Rigid gas, yearly	2	1.7
> 1 type	1	0.9
Manufacturer of lens solution		
Alcon [®]	21	18.1
Renu [®]	48	41.4
Duna [®]	25	21.6
Solo care aqua [®]	4	3.4
Dream eye one [®]	9	7.8
Can not remember	7	6.0
> 1 type	1	0.9
No detail	1	0.9
The reason for using contact lens		
Longsighted (Presbyopia)	4	3.4
Shortsighted (Myopia)	46	39.7
Astigmatic	38	32.8
Fashion	27	23.3
Not specific	1	0.9

Table 1 General information of contact lens users of health science students at Rangsit University (cont.)

Contact lens used	N = 116	
	No.	%
Eye check up before using contact lens		
Yes	47	40.45
No	59	50.2
Not specific	10	8.6
Disease during wear contact lens		
No	106	91.4
Pinguecular	1	0.9
Dry	2	1.7
Allergy	3	2.6
Not specific	4	3.4
Eye symptom at present		
No	96	82.8
Irritate eye	9	7.7
Red eye	2	1.7
> 1 symptom	7	6.1
Not specific	2	1.7

Hygienic habits and practices of participants were getting an eye examination from an ophthalmologist (40.5%), lacking eye disorders (91.4%), and lacking ocular symptoms (82.8%). Percentage of contact lens users washing with soap before touching the contact lens (48.3%), use on a daily basis (62.9%), meeting the doctor when

having an eye disorder (55.2%). Unhygienic habits practices of participants were wearing contact lens during swimming (40.5%), wearing contact lens at bedtime (2.6%), not totally cleaning hands before touching contact lens (7.8%), when having eye problems still using and buying medicine without prescription (35.5%) detailed in table 2.

Table 2 Hygiene habits and practices of contact lens users of health science students

Hygiene habits practices	N = 116	
	No.	%
Wear contact lens during swimming		
Not swimming	57	49.1
Yes	47	40.5
No	10	8.6
Not specific	2	1.7
Wear contact lens during sleeping		
Take it off before	101	87.1
Still wear	3	2.6
Not certain	1	0.9
Not specific	11	9.5
Hand cleaned status during wear contact lens		
Unclean hand	9	7.8
Clean with water	19	16.4
Clean with soap	56	48.3
Depend situation	29	25.0
Not specific	3	2.6
Frequency of using contact lens		
Daily	73	62.9
Uncertain	39	33.6
Seldom	2	1.7
Not specific	2	1.7
When having an eye problem		
Buy medication without prescription	41	35.5
Visit doctor	64	55.2
Do nothing	10	8.6
Not specific	1	0.9

Maintenance habits and practices of contact lens users were changing the cleaning solution everyday

(73.3%), knowing the expiration date of contact lens solutions (66.4%) detailed in table 3.

Table 3 Maintenance habits and practices of contact lens users of health science students

Maintenance habits practices	N = 116	
	No.	%
Change the cleaning solution		
Everyday	85	73.3
2 - 3 days	20	17.2
Unusual	9	7.8
Not specific	2	1.7
Know of the expiration date		
Yes	77	66.4
No	28	24.1
Not specific	11	9.5
Change the contact lens storage case		
Every 1 - 2 months	69	59.5
Every 3 months	28	24.1
When out of solution	11	9.5
Reuse/Never change	2	1.7
When buying new contact lens new set	1	0.9
Not specific	5	4.3
Renew the contact lens		
Monthly	69	59.5
Every 1 - 2 months	1	0.9
Every 2 - 3 months	3	2.6
Every 6 months	1	0.9
Yearly	4	3.4
Expired	1	0.9
Lens prescription changed	1	0.9
Not specific	36	31.0

Microbial and *Acanthamoeba* contamination

One hundred and two contact lens storage cases out of 167 (61.1%) were submitted to our laboratory. Only the detection under wet smear prepared found bacterial and

fungal hyphae. Also, there were the fibers and tissue in the solution. *Acanthamoeba* was not found in any storage cases in this study (Table 4).

Table 4 Microbial contaminated contact lens storage cases of health science students

Microorganism	N = 102	
	No.	%
<i>Acanthamoeba</i> spp.	0	0
Bacteria	11	10.8
Fungi	3	2.9
Sterile	88	86.3

Discussion and Conclusion

Acanthamoeba spp. was not found in contact lens cases from health science students, Rangsit University. Unfortunately, bacterial and fungus cultivation were not determined which might have been the other risk factors^{16, 18}. A previous study showed the prevalence rate was 2.4% of *Acanthamoeba* contamination in contact lens cases of the patients attending optometry practice at eye clinic in Bangkok, Thailand¹⁰ and 6.7% from contact lens cases of students in Nakhonpathom Province¹². These may be students of health science subjects who have knowledge in health hygiene and personal hygiene. They may be able to afford to buy a proper solution for cleaning and storing the contact lens¹⁰. However, this study has shown the major unhygienic impact among health science students which regularly used contact lens. The results showed that half of contact lens users absence to visit a doctor to have an eye check-up before wearing contact lens and improper wash hands before touching contact lens, 44.8% absence to see a doctor when having eye problems, 40.5% wore contact lens while swimming, 40.5% contact lens cases unchanged every 1-2 months, 26.7% lack of changed lens cleaning solution daily, 24.1% uncheck the expiration date of the contact lens solution, about 6.1% eye problems with other symptoms and continue to use contact lens, 2.6% wore contact lens when sleeping.

Since 75.9% of health science students were using contact lens due to their eye problems, the proper use of contact lens needs to focus on reducing the contamination of microorganism which may cause problems with eye infection. Additionally, we also explored the soil

and water samples inside the campus area, collecting 20 water samples and 7 soil samples. *Acanthamoeba* spp. cultivations were found in 1 sample of water (5%) from pond and 3 samples of soil (42.9%) from side walk dust. This result still needs more research to identify this organism which could be done by using the confirmatory molecular technique to determine the correlated environmental contamination^{3, 17, 19, 20}. This showed *Acanthamoeba* spp. in the environment was everywhere widespread and could be awareness as an important opportunistic organism. Also, the risk factors of infection can be reduced if the reagents of contact lens practice are taken care of properly²¹. *Acanthamoeba* spp. was discovered to be the cause of eye disease, giving patients AK, although this is a very common infection. High *Acanthamoeba* intensity is caused through cornea infection that has spread to the eyeball. As a result, students using contact lenses for a long time should be aware of all pathogens. Also, we sent out the prevention and maintenance of contact lens use to all faculties of health science students in leaflet form to remind the health hygiene habits practices.

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References

- Jariya P, Lerlaituan P, Warachoon K. *Acanthamoeba* spp.: a cause of chronic granulomatous amoebic meningoencephalitis. *Siriraj Hosp Gaz* 1992;44:148-53.
- Niederhorn JY, Alizadeh H, Leher H, McCulley JP. The pathogenesis of *Acanthamoeba* keratitis. *Microbes Infect Inst Pasteur* 1999;1:437-43.
- Alizadeh H, Neelam S, Hurt M, Niederhorn JY. Role of contact lens wear, bacterial flora, and mannose-induced pathogenic protease in the pathogenesis of amoebic keratitis. *Infect Immun* 2005;73:1061-8.
- Awwad ST, Petroll WM, McCulley JP, Cavanagh HD. Updates in *Acanthamoeba* keratitis. *Eye Contact Lens* 2007;33:1-8.
- Lekkla A, Sutthikornchai C, Bovornkitti S, Sukthana Y. Free-living ameba contamination in natural hot springs in Thailand. *Southeast Asian J Trop Med Public Health* 2005;36(suppl 4):5-9.
- Wanachiwanawin D, Booranapong W, Kosrirukvongs P. Clinical features of *Acanthamoeba* keratitis in contact lens wearers and non-wearers. *Southeast Asian J Trop Med Public Health* 2012;43:549-56.
- Jongwutiwes S, Pariyakanok L, Charoenkorn M, Yagita K, Endo T. Heterogeneity in cyst morphology within isolates of *Acanthamoeba* from keratitis patients in Thailand. *Trop Med Int Health* 2000;5:335-40.
- Nacapunchai D, Kino H, Ruangsitticha C, Sriwichai P, Ishih A, Terada M. A brief survey of free-living amoebae in Thailand and Hamamatsu District, Japan. *Southeast Asian J Trop Med Public Health* 2001;32(suppl 2):179-82.
- Lek-Uthai U, Passara R, Roongruangchai K. Morphological features of *Acanthamoeba* causing keratitis contaminated from contact lens cases. *J Med Assoc Thai Chotmaihet Thangphaet* 2009;92(suppl 7):156-63.
- Roongruangchai K, Supadirekkul P. Contamination of contact lens cases by *Acanthamoeba* in Thailand. *J Trop Med Parasitol* 1997;20:25-9.
- Preechawat P, Ratananikom U, Lerdvitayasakul R, Kunavisarut S. Contact lens-related microbial keratitis. *J Med Assoc Thai Chotmaihet Thangphaet* 2007;90:737-43.
- Lek-Uthai U, Passara R, Roongruangchai K, Buddhirakkul P, Thammapalerd N. Rapid identification of *Acanthamoeba* from contact lens case using loop-mediated isothermal amplification method. *Exp Parasitol* 2009;121:342-5.
- Pipat Luksamijarulkul. Research process in health science. 2nded. Bangkok: Charoendee printing; 2003.
- Khunkitti W, Lloyd D, Furr JR, Russell AD. The lethal effects of biguanides on cysts and trophozoites of *Acanthamoeba castellanii*. *J Appl Bacteriol* 1996;81:73-7.
- Walochnik J, Obwaller A, Aspöck H. Correlations between Morphological, Molecular Biological, and Physiological Characteristics in Clinical and Nonclinical Isolates of *Acanthamoeba* spp. *Appl Environ Microbiol* 2000;66:4408-13.
- Wang AG, Wu CC, Liu JH. Bacterial corneal ulcer: a multivariate study. *Ophthalmologica* 1998;212:126-32.
- Joslin CE, Tu EY, McMahon TT, Passaro DJ, Stayner LT, Sugar J. Epidemiological characteristics of a Chicago-area *Acanthamoeba* keratitis outbreak. *Am J Ophthalmol* 2006;142:212-7.
- Stapleton F, Edwards K, Keay L, Naduvilath T, Dart JKG, Brian G, et al. Risk factors for moderate and severe microbial keratitis in daily wear contact lens users. *Ophthalmol* 2012;119:1516-21.
- Init I, Lau YL, Arin Fadzlan A, Foad AI, Neilson RS, Nissapatorn V. Detection of free living amoebae, *Acanthamoeba* and *Naegleria*, in swimming pools, Malaysia. *Trop Biomed* 2010;27:566-77.
- Yaicharoen R, Ngrenngarmert W, Thongmee P, Damsaman W. Survey of *Acanthamoeba* spp. in dust from Bangkok and Suburban areas. *Bull Chiang Mai Assoc Med Sci* 2007;40:46-53.
- Pens CJ, da Costa M, Fadanelli C, Caumo K, Rott M. *Acanthamoeba* spp. and bacterial contamination in contact lens storage cases and the relationship to user profiles. *Parasitol Res* 2008;103:1241-5.

บทคัดย่อ

การสำรวจเบื้องต้นของการปนเปื้อนของเชื้ออะแคนทามีบาในตลับคอนแทคเลนส์ของนักศึกษาวิทยาศาสตร์สุขภาพ มหาวิทยาลัยรังสิต
เฉลิมพล แก้วใจ, ลีริมา กิจวัฒน์ชัย, ศิริพร ไควบุตร

คณะเทคนิคการแพทย์ มหาวิทยาลัยรังสิต จังหวัดปทุมธานี

บทนำ: การศึกษานี้มีวัตถุประสงค์เพื่อประเมินการปนเปื้อนเชื้ออะแคนทามีบาในตลับคอนแทคเลนส์ และศึกษา
พฤติกรรมการใช้การดูแลรักษาคอนแทคเลนส์ของนักศึกษากลุ่มวิทยาศาสตร์สุขภาพ มหาวิทยาลัยรังสิต

วิธีการศึกษา: สำรวจเก็บตัวอย่างแบบภาคตัดขวางจากผู้เข้าร่วมโครงการ ระหว่างเดือนเมษายน - ตุลาคม พ.ศ. ๒๕๕๖
แล้วทำการตรวจเชื้ออะแคนทามีบาในตลับคอนแทคเลนส์ ด้วยวิธีการเพาะเลี้ยงในวุ้นที่ไม่มีสารอาหาร ที่เคลือบ
ด้วยเชื้อแบคทีเรีย เอชเชอริเชีย โคลิ ที่ไม่มีชีวิต โดยมีผู้ตอบแบบสอบถาม ๑๑๖ คน และส่งตลับคอนแทคเลนส์
๑๐๒ คน

ผลการศึกษา: ผลการศึกษาไม่พบเชื้อในตลับคอนแทคเลนส์ที่ส่งตรวจทั้งหมด อย่างไรก็ตามจากแบบสอบถามพบว่านักศึกษา
ยังมีพฤติกรรมการใช้และดูแลคอนแทคเลนส์ไม่ถูกต้องเป็นส่วนใหญ่ ได้แก่ ร้อยละ ๕๐ ไม่ได้รับการตรวจตา
จากจักษุแพทย์ก่อนใช้คอนแทคเลนส์ และสัมผัสคอนแทคเลนส์โดยไม่ล้างมือฟอกสบู่ เมื่อมีความผิดปกติ
เกี่ยวกับตาไม่พบแพทย์ ร้อยละ ๔๔.๘ ใส่คอนแทคเลนส์ขณะว่ายน้ำ และไม่เปลี่ยนตลับแช่คอนแทคเลนส์
ทุก ๑ - ๒ เดือน ร้อยละ ๔๐.๕ เป็นต้น

**วิจารณ์ และ
สรุปผลการศึกษา:** ปัจจัยเสี่ยงเหล่านี้เป็นสาเหตุของโรคกระจกตาอักเสบจากเชื้ออะแคนทามีบา ซึ่งเชือนี้ยังพบมากในสิ่งแวดล้อม
จึงเสนอแนะให้ผู้ที่ใช้คอนแทคเลนส์มีพฤติกรรมการใช้ที่ถูกต้องเพื่อลดปัจจัยเสี่ยงของการติดเชื้อได้

คำสำคัญ: อะแคนทามีบา, คอนแทคเลนส์, กระจกตาอักเสบ, ปัจจัยเสี่ยง, พฤติกรรมรักษาความสะอาด