PARASITOLOGY

Effectiveness of AOSept Plus and Biotrue® Contact Lens Disinfecting Solutions against Clinical Isolates of *Acanthamoeba* spp.

Mohamed Kamel AG[1)], Kabithra Raja VR[1)], Anisah N[2)], Noraina AR[2)]

ABSTRACT

Introduction: **The usage of contact lens among the community is increasing due to trend and as an alternative of wearing glasses. However, most ocular diseases and eye infections such as *Acanthamoeba* keratitis are more related to contact lens usage. The contact lens disinfecting solutions (CLDS) are supposed to be able to eradicate the microorganisms that could possibly cause eye infections.**

Objective: **This study was conducted to determine the effectiveness of the contact lens disinfecting solutions against *Acanthamoeba* spp. cysts of clinical isolates based on manufacturer's recommended soaking time and variable soaking times.**

Materials and Methods: **CLDS were tested against two isolates of *Acanthamoeba* spp. in durations recommended by the product manufacturer; 4 and 6 hours and also in extended duration of 8 hours soaking. The disinfecting solutions tested were AOSept Plus and Biotrue® multipurpose solution against two clinical isolates (HKL 55 & HS 19). The *Acanthamoeba* spp. isolates were subcultured onto non-nutrient agar and the cysts produced were mixed with disinfectants separately according to different soaking hours. After the soaking duration, the cysts were cultured onto non-nutrient agar layered with heat-killed *E. coli* and incubated at 30°C. The cultures were observed under inverted microscope for 14 days continuously to see any excystment of the cyst. The failure of the cysts to excyst indicates the efficiency of the disinfectants to kill the cyst successfully.**

Results: **AOSept Plus exhibited good anti-*Acanthamoeba* activity and was able to inhibit the excystment of *Acanthamoeba* spp. cysts in all isolates. Biotrue® Multipurpose solution failed to exhibit anti-*Acanthamoeba* activity.**

Conclusion: **AOSept Plus representing the hydrogen peroxide based contact lens disinfecting solution is a choice for contact lens wearers if *Acanthamoeba* keratitis is to be prevented.**

KEY WORDS

***Acanthamoeba* keratitis, contact lens disinfecting solution, clinical isolates, Malaysia**

INTRODUCTION

Acanthamoeba is a free-living ubiquitous amoeba that can be found in almost all kinds of environments including soil, water and air (Marciano-Cabral & Cabral, 2003). It has even been isolated from the toxic disposal area which has high concentration of chemicals such as pesticides, herbicides, medications and other chemicals like polychlorinated biphenyl (Sawyer & Griffin, 1975). Thus, human exposure to this pathogen is higher than what it is thought to be (Khan & Siddiqui 2009).

Morphologically, *Acanthamoeba* spp. could be differentiated into 3 main groups comprising Group I (Astronyxid), Group II (Polyphagid) and Group III (Culbertsonid) (Marciano-Cabral. & Cabral, 2003). *Acanthamoeba* sp. has two life forms which are the trophozoites and cysts. The infective form is the trophozoite while the dormant and resistant form is the cyst. The cysts could withstand extreme conditions such as high temperature, starvation and presence of chemicals (Byers, 1979).

Acanthamoeba keratitis is increasingly being recognised among people who wear contact lenses (Seal, 1994), and more than 85% of *Acanthamoeba* keratitis occur among contact lens users. Most of these infections occur among those who used soft type contact lenses, swim while using contact lenses and incompletely disinfecting the contact lens (Stehr-Green *et al*. 1987). Besides that, *Acanthamoeba* keratitis can also occur due to eye injury and exposure to contaminated water.

The first ever case of *Acanthamoeba* keratitis has been reported in Britain in the year of 1970's and followed by other western countries like United States (Nagington *et al*. 1974). The first case of *Acanthamoeba* keratitis in Malaysia was reported in 1995 and ever since then, the number of cases has been increasing (Mohamed Kamel & Norazah, 1995, Mohamed Kamel *et al*. 2018). This is directly proportional to the increasing trend of contact lens usage in Malaysia. *Acanthamoeba* keratitis could cause severe consequences and may even cause loss of vision if not treated early. The infection normally occurs in the corneal epitheli-

Received on June 14, 2020 and accepted on September 25, 2020

1) Biomedical Science Programme, Faculty of Health Sciences, Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
2) Department of Parasitology, Faculty of Medicine, Universiti Kebangsaan Malaysia
Jalan Yaacob Latif, 56000, Kuala Lumpur, Malaysia
Correspondence to: Mohamed Kamel AG
(e-mail: mohamedkamela@yahoo.com)

© 2021 Japan University of Health Sciences
& Japan International Cultural Exchange Foundation

Table 1: Isolation details of *Acanthamoeba* isolates.

Isolate and Species	Source	Location of isolation
HKL 55 (*A. culbertsoni*)	Keratitis patient	Hospital Kuala Lumpur
HS 19 (*A. castellanii*)	Keratitis patient	Private hospital in Malaysia

Table 2: Contact lens disinfecting solutions Pharmaceutical Details.

Brand	Active compound	Manufacturer's recommended soaking hours	System
AOSept Plus	3% Hydrogen peroxide	6 hours	Hydrogen peroxide (H2O2)
Biotrue® Multipurpose Solution	0.00013% Polyaminopropyl biguanide and 0.0001% Polyquaternium	4 hours	Multipurpose

Table 3: Results of Positive Controls

Isolates	Cyst suspension in PAS solution	Cyst suspension in 3% H_2O_2
HKL 55	+	-
HS 19	+	-

Key:
+ Presence of *Acanthamoeba* cysts and trophozoites indicating its viability
- Absence of *Acanthamoeba* trophozoites

Table 4: Results of Negative Controls

Solutions	Negative control
Page's amebic saline (PAS)	-
Biotrue® Multipurpose Solution	-
AOSept Plus	-

Key:
+ Presence of *Acanthamoeba* trophozoites and cysts signifies contamination of solution
- Absence of *Acanthamoeba* trophozoites and cysts signifies no contamination of solution

Table 5: Effectiveness of AOSept Plus disinfecting solution on *Acanthamoeba* isolates.

Isolates	Duration of soaking (hours)		
	4	6*	8
HKL 55	-	-	-
HS 19	-	-	-

Key:
+ Presence of *Acanthamoeba* trophozoites (disinfection ineffective)
- Absence of *Acanthamoeba* trophozoites (disinfection effective)
* Manufacturer's recommended duration of soaking

Table 6: Effectiveness of Biotrue® Multipurpose Solution on Acanthamoeba isolates.

Isolates	Duration of soaking (hours)		
	4*	6	8
HKL 55	+	+	+
HS 19	+	+	+

Key:
+ Presence of *Acanthamoeba* trophozoites (disinfection ineffective)
- Absence of *Acanthamoeba* trophozoites (disinfection effective)
* Manufacturer's recommended duration of soaking

um, however certain severe infections could cause invasion of the amoeba into the stromal of the eye and results in eye function impairment.

The cysts of *Acanthamoeba* sp. have been a great challenge in the disinfecting process of contact lens since they are very resistant to chemicals. Many commercially available contact lens disinfecting solutions do not possess anti-*Acanthamoeba* effect because manufacturers are not required to prove their products' efficacy towards *Acanthamoeba*, according to the ISO 14729 (Microbiological Requirements and Test Methods for Products and Regimens for Hygienic Management of Contact Lenses) in which they are only needed to achieve log 3 reduction in *Pseudomonas aeruginosa*, *S. aureus* and *Serratia marcescens* and log 1 reduction in *Fusarium solani* and *C. albicans* (ISO 14729, 2001). Hence, not all multipurpose solutions are successful at killing various strains of *Acanthamoeba* spp. The choice of a highly efficacious lens disinfection system, is one factor that can improve a patient's ability to avoid exposure to *Acanthamoeba* with minimal impact on the convenience or desirability of lens wear. Thus, this study was carried out to evaluate the efficacy of Biotrue® Multipurpose solution and AOSept Plus, a hydrogen peroxide disinfection system against the cysts of *Acanthamoeba* spp.

MATERIALS AND METHODS

Acanthamoeba isolates

Acanthamoeba spp. isolates were obtained from *Acanthamoeba* lab, Parasitology Department, Faculty of Medicine, Universiti Kebangsaan Malaysia. The isolates were from clinical (Table 1) specimens taken from infected patient's corneal scrapping.

Contact lens disinfecting solution (CLDS)

Two commercially available CLDS were used in this study. A hydrogen peroxide (H2O2) disinfecting system solution, AOSept Plus and a multipurpose solution, Biotrue® Multipurpose solution were tested against the two clinical isolates of *Acanthamoeba* spp. (Table 2).

Acanthamoeba sub-cultures

Agar plates containing *Acanthamoeba* spp. were observed under inverted microscope and the area with mature cysts were marked on the back of the petri dish. The marked areas were cut using a sterile knife and transferred onto new Non Nutrient Agar (NNA) plates with the cut surface facing down. E. coli suspension was later dropped on the agar plate in a linear line far from the edges. The cultured plates were incubated at 30°C. This is a monoxenic culture method adopted from Narasimhan *et al*. (2002).

Acanthamoeba cyst suspension preparation

After 10 days of agar plates incubation, 1 ml of PAS solution was pipetted onto agar surface and the *Acanthamoeba* cysts were scrapped and homogenized using L-shaped rod. The cysts suspension was transferred into centrifugal tube using Pasteur pipette. The agar plates were rinsed repetitively using PAS solution to avoid leaving any residue of cysts on the agar plate. The supernatant was later discarded and the cysts were counted using Neubauer chamber and the cysts suspension concentration was standardized to 1 x 10^5 cyst per ml.

Contact lens disinfecting solution Efficacy Test

The contact lens disinfecting solution efficacy test was done on a 24-well microtiter plate where 1 ml of contact lens disinfecting solution was pipetted into each well. 10 µl cyst suspension was pipetted into the wells respectively. The plates were kept closed using aluminium foil to

avoid the solutions from drying up. Besides that, the plates were closed to mimic the dark surrounding of contact lens casing. The microtiter plates were kept in room temperature according to different soaking times ; 4 hours, 6 hours and 8 hours.

Control tests, both positive and negative controls were run together with the efficacy tests. The first positive control contains PAS solution with cysts and the second positive control contains cysts in 3% hydrogen peroxide solution. The cysts in PAS solution were to ensure the viability of the cysts as the cysts were supposed to excyst when cultured. Meanwhile, the cysts in 3% hydrogen peroxide were to ensure the complete killing of *Acanthamoeba* cysts. The negative controls were the PAS solution and contact lens disinfecting solutions without the presence of *Acanthamoeba* cysts. Negative controls were used for the detection of any contaminations.

After the soaking durations, 100 µl of the specimens were transferred onto *E. coli* layered NNA plates. The agar plates were sealed with parafilm and incubated for three days at 30°C. The incubated plates were observed under inverted microscope for 14 days continuously to detect the presence of trophozoites, indicating excystment of the cyst. In the absence of trophozoites, it indicates the total kill of *Acanthamoeba* spp. cysts by the disinfecting solutions. All tests were run in duplicates.

RESULTS

The *Acanthamoeba* spp. cysts tested in this study belong to Group II and Group III. The species from isolate HKL 55 is *Acanthamoeba culbertsoni*, belonging to Group III (Culbertsonid). Isolate HS 19 is *Acanthamoeba castellanii*, belonging to Group II (Polyphagid). Both positive and negative controls have produced valid and expected results (Tables 3 and 4).

Table 5 shows the result of the effectiveness of AOSept Plus contact lens disinfecting solution as anti-*Acanthamoeba* agent when tested on the 2 clinical isolates of *Acanthamoeba* spp. AOSept Plus was found to be effective in inactivating the cysts of all *Acanthamoeba* isolates for all the soaking times tested. Even in the soaking duration shorter than the manufacturer's recommended soaking hours, AOSept Plus effectively inactivates all the *Acanthamoeba* cysts.

Biotrue® Multipurpose solution however, fails to inactivate the *Acanthamoeba* cysts of both isolates in all soaking durations (Table 6). The 8 hours soaking duration is supposed to represent the overnight soaking of contact lens and still Biotrue® Multipurpose solution fails to exhibit anti-*Acanthamoeba* activity.

DISCUSSION

Acanthamoeba keratitis is a severe ocular infection that occurs mainly among contact lens users as a result of unhygienic practice or improper disinfection of contact lenses (Stehr-Green, 1987). The commercially available contact lens disinfecting solutions may not have the ability to kill *Acanthamoeba* spp. effectively as according to ISO 14729 (Microbiology Requirements And Test Methods For Product And Regimens For Hygienic Management Of Contact Lenses). The disinfecting solutions are mainly produced to disinfect bacteria, virus and fungi. The inability and incomplete disinfecting properties of CLDS may cause *Acanthamoeba* spp. to inhabit the contact lens casing and even use it as a reservoir or habitat (Mohamed Kamel *et al*. 2013).

The active compounds in the disinfecting solutions used, influenced the results of the efficacy test. AOSept Plus solution contains 3% hydrogen peroxide which is regarded as gold standard to kill *Acanthamoeba* spp. effectively (Zanetti *et al*. 1995). Hydrogen peroxide is often used as a powerful disinfecting agent to kill microorganisms. Hydrogen peroxide acts through oxidation process where it could take an electron from bacterial or pathogen cell membrane (Hughes & Kilvington, 2001). However, the effectiveness of hydrogen peroxide as disinfecting agent in contact lens disinfecting solution is determined by the method used for disinfection, either 1 step or 2 steps. When compared, the 2 steps method is more effective than 1 step method (Hughes & Kilvington, 2001). This is because the neutralizing tablet used in 1 step method, could inhibit the activity of hydrogen peroxide before disinfection takes place. This study used AOSept Plus disinfectant in 2 steps method and produced good efficacy in killing the *Acanthamoeba* spp. cysts. However, not all disinfecting solutions containing 3% hydrogen peroxide would produce the same efficacy as there might be variation in the ingredients and the pH value of the solutions (Niszl & Markus 1998). Our previous study also showed that a hydrogen peroxide based CLDS, Oxysept®, was effective against four clinical isolates of *Acanthamoeba* (Mohamed Kamel et al. 2019). There are also studies that show AOSept Plus and Oxysept® solutions tested in 1 step method producing only partial anti-*Acanthamoeba* activities (Abjani *et al*. 2017).

Biotrue® Multipurpose solution contains polyaminopropyl biguanide 0.00013% and polyquaternium 0.0001%. Polyaminopropyl biguanide is commonly used as disinfectant in swimming pools and also as a skin antiseptic medication. It acts on cell membrane phospholipid and disrupts the membrane integration that causes the intracellular components and ionic charges to leak out from the cell leading to cell death (Siddiqui, Aqeel & Khan, 2016). However, the concentration of polyaminopropyl biguanide as active compound in Biotrue® Multipurpose solution is lower than the minimal cysticidal concentration of *Acanthamoeba* which is 0.0002%. Thus, it fails to kill the *Acanthamoeba* spp. cysts in this study. A previous study using polyquaternium and polyaminopropyl biguanide in higher concentrations, 0.0011% and 0.0005% respectively has also produced ineffective results in killing *Acanthamoeba* spp. cysts (Zanetti *et al*. 1995). Several studies have also demonstrated the failure of polyquaternium and polyaminopropyl biguanide in the anti-*Acanthamoeba* activity when tested against *Acanthamoeba castellanii* and *Acanthamoeba polyphaga* even when soaking duration was prolonged to 24 hours (Silvany *et al*. 1990). Polyaminopropyl biguanide is better used as a therapeutic agent in healing *Acanthamoeba* keratitis compared to its usage as disinfecting agent (Silvany *et al*. 1990, Kilvington *et al*. 1990).

The results of efficacy test obtained from different studies may differ from one another, based on the isolates being tested and the method of experiment. This study uses *Acanthamoeba* spp. cysts from clinical isolates that has been cultured using monoxenic method which may produce cysts that are more resistant than axenic culture method (Silvany *et al*. 1990). Besides that, the maturity of the cysts, determines its susceptibility as mature cysts are more resistant (Beattie *et al*. 2003). This study uses mature cysts and the maturity of the cysts should be standardized to produce accurate results.

Preventing infections associated with contact lenses is theoretically possible but will be difficult to achieve. It depends on the use of an effective amoebicidal disinfectant. Among the many marketed only two cold chemical disinfectants (hydrogen peroxide and chlorhexidine) are effective against *Acanthamoeba* cysts and most bacteria found in storage cases (Seal, 1994).

CONCLUSION

AOSept Plus disinfecting solution containing hydrogen peroxide as the active ingredient, exhibits good anti-*Acanthamoeba* activity and provides assurance of safety from *Acanthamoeba* keratitis if used hygienically.

REFERENCES

Abjani, F., Khan, N. A., Jung, S. Y. & Siddiqui, R. Status of the Effectiveness of Contact Lens Disinfectants in Malaysia against Keratitis-Causing Pathogens. Experimental parasitology 2017: 183: 187-193.

Beattie, T. K., Tomlinson, A., Mcfadyen, A. K., Seal, D. V. & Grimason, A. M. Enhanced Attachment of *Acanthamoeba* to Extended-Wear Silicone Hydrogel Contact Lenses: A New Risk Factor for Infection? Ophthalmology 2003: 110(4): 765-771.

Byers, T. J. Growth, Reproduction, and Differentiation in *Acanthamoeba*. International Review of Cytology 1979: 61: 283-338.

Hughes, R. & Kilvington, S. Comparison of Hydrogen Peroxide Contact Lens Disinfection Systems and Solutions against *Acanthamoeba polyphaga*. Antimicrobial agents and chemotherapy 2001: 45(7): 2038-2043.

International Organization for Standardization. Ophthalmic optics - contact lens care products—icrobiological requirements and testing methods for production and regimens for hygienic management of contact lenses. ISO 14729 (2001) International Organization for Standardization, Geneva, Switzerland.

Khan, N. A. & Siddiqui, R. *Acanthamoeba* Affects the Integrity of Human Brain Microvascular Endothelial Cells and Degrades the Tight Junction Proteins. International journal for parasitology 2009: 39(14): 1611-1616.

Kilvington, S., Larkin, D., White, D. & Beeching, J. Laboratory Investigation of *Acanthamoeba*

Keratitis. Journal of Clinical Microbiology 1990: 28(12): 2722-2725.

Marciano-Cabral, F. & Cabral, G. *Acanthamoeba* spp. as agents of disease in humans. Clinical microbiology reviews 2003:16(2): 273-307.

Mohamed Kamel AG, Irdawati Azhar, Haliza A. Mutalib, Anisah N, Yusof S, Noraina AR & Norazah Ahmad. *Acanthamoeba* keratitis in Malaysia. Sains Malaysiana 2018: 47(7): 1563-1569.

Mohamed Kamel AG, Kirat Kaur SS, Anisah N, Yusof S, Noraina AR & Norazah A. The Effectiveness of Oxysept® 1-Step Contact Lens Disinfecting Solution against Clinical Isolates of *Acanthamoeba* Cysts. International Medical Journal 2019:26: 3: 196-199.

Mohamed Kamel AG, Majid SA., Abdullah NS, Nordin A., Suboh, Y., Rahim, NA., Mutalib, HA. & Ahmad, N. Isolation of *Acanthamoeba* Spp. From Contact Lens Paraphernalia. International Medical Journal 2013 20(1): 66-68.

Mohamed Kamel, AG & Norazah, A. First Case of *Acanthamoeba* Keratitis in Malaysia. Transactions of the Royal Society of Tropical Medicine and Hygiene 1995: 89(6): 652-652.

Nagington, J., Watson, P., Playfair, T., Mcgill, J., Jones, B. & Steele, A. M. Amoebic Infection of the Eye. The Lancet 1974:304(7896): 1537-1540.

Narasimhan, S., Madhavan, H.N. & Therese, L.K. Development and application of an in vitro susceptibility test for Acanthamoeba species isolated from keratitis to poly-hexamethylene biguanide and chlorhexidine. Cornea 2002: 21(2): 203-205.

Niszl, I. A. & Markus, M. B. Anti-*Acanthamoeba* Activity of Contact Lens Solutions. British Journal of Ophthalmology 1998: 82(9): 1033-1038.

Sawyer, T. K. & Griffin, J. L. A Proposed New Family, Acanthamoebidae N. Fam.(Order Amoebida), for Certain Cyst-Forming Filose Amoebae. Transactions of the American Microscopical Society 1975: 93-98.

Seal DV. *Acanthamoeba* keratitis. A problem for contact lens users that is here to stay. BMJ 1994; 308: 1116-17.

Siddiqui, R., Aqeel, Y. & Khan, N. A. The Development of Drugs against *Acanthamoeba* Infections. Antimicrobial agents and chemotherapy 2016: 60(11): 6441-6450.

Silvany, R. E., Dougherty, J. M., Mcculley, J. P., Wood, T. S., Bowman, R. W. & Moore, M. B. The Effect of Currently Available Contact Lens Disinfection Systems on *Acanthamoeba castellanii* and *Acanthamoeba polyphaga*. Ophthalmology 1990: 97(3): 286-290.

Stehr-Green, J. K., Bailey, T. M., Brandt, F. H., Carr, J. H., Bond, W. W. & Visvesvara, G. S. *Acanthamoeba* Keratitis in Soft Contact Lens Wearers: A Case-Control Study. JAMA 1987:258(1): 57-60.

Zanetti, S., Fiori, P. L., Pinna, A., Usai, S., Carta, F. & Fadda, G. Susceptibility of *Acanthamoeba castellanii* to Contact Lens Disinfecting Solutions. Antimicrobial agents and chemotherapy 1995:39(7): 1596-1598.