PARASITOLOGY

The Efficacy of Oxysept® 1- Step Contact Lens Disinfecting Solution against Environmental Isolates of *Acanthamoeba* Cysts

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ABSTRACT

Introduction: **Rising number of *Acanthamoeba* keratitis cases globally is a public health concern. Contact lens use is the principal risk factor for contracting *Acanthamoeba* keratitis and hence, the disease may be prevented by the adequate use of an effective disinfection system which possess anti-Acanthamoeba activity.**

Objectives: **This study investigates the cysticidal efficacy of Oxysept® 1-Step lens disinfecting solution against 4 environmental isolates of *Acanthamoeba* sp. from Malaysia.**

Materials and Methods: **Cyst suspensions were prepared using *Acanthamoeba* cysts grown on Non-Nutrient Agar (NNA) for 10 days at 30℃ (± 2℃). Oxysept® 1-Step contact lens disinfecting solution was used to determine its effectiveness as anti-*Acanthamoeba* agent. Cyst suspensions of each isolates were tested based on the manufacturers' recommended soaking time of 6 hours, 4 hours and 8 hours respectively. After the soaking time, 100 µl cyst suspension of each isolate was cultured onto NNA plates seeded with *E. coli* at 30℃ (± 2℃) and observed daily for 14 days under an inverted microscope to detect the presence of trophozoites.**

Results: **Oxysept® 1-Step was successful at inactivating the cysts of all the *Acanthamoeba* spp. environmental isolates tested within all the testing times. Oxysept® 1- Step showed cysticidal efficacy against all *Acanthamoeba* isolates.**

Conclusion: **Contact lens disinfecting solutions using hydrogen peroxide system are probably the best choice for contact lens wearers in preventing *Acanthamoeba* keratitis.**

KEY WORDS

***Acanthamoeba*, Oxysept® 1- Step, Contact lens disinfecting solution, Malaysia**

INTRODUCTION

Acanthamoeba, a common free-living amoeba, is increasingly incriminated as a cause of keratitis and corneal ulceration. *Acanthamoeba* keratitis is one of the most severe and potentially sight threatening ocular parasitic infectious diseases and is recognized as the most challenging among ocular infections because of the protracted painful clinical course and frequently encountered treatment failures. The single most important risk factor for acquiring *Acanthamoeba* keratitis is contact lens wear. Usually this involves a deviation from contact lens wear and care procedures recommended by the lens manufacturer and health professionals. The *Acanthamoeba* cysts are resistant to antimicrobial agents, including current lens disinfection solutions, and may contaminate lens storage cases in asymptomatic users. Over 80% of cases of *Acanthamoeba* keratitis could be prevented by the adequate use of an effective disinfection system (Radford *et al*. 1995).

Commercially available, cold contact lens disinfection solutions did not result in uniform killing of *Acanthamoeba* cysts in an assay system involving adherence to hydrogel lenses. Only those solutions containing chlorhexidine or hydrogen peroxide resulted in complete non-viability of the protozoa; chlorine-generating systems were completely inefficient in terms of their amoebicidal activity.

Globally, *Acanthamoeba* keratitis is a cause for concern with outbreaks occurring in Singapore (Por *et al*. 2009), Chicago, USA from 2003 to 2005 (Joslin *et al*. 2006). The number of cases in Australia has also notably risen with 9 cases reported from March 2006 to March 2007 compared to only 4 cases reported from January 2003 to February 2006 (Jae *et al*. 2009). In Malaysia, the first case of *Acanthamoeba* keratitis was reported in 1995 involving a woman who was a long-term contact lens wearer. Although the patient had to undergo treatment overseas, it was the wake up call to the local medical community on the pathogenicity of *Acanthamoeba* (Mohamed Kamel, A.G. & Norazah, 1995). Since then, this condition is no longer a rarity and is seen with increasing frequency especially among contact lens wearers.

During the period between 2013-2015, a total of 62 suspected cases

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Table 1: Isolation details of *Acanthamoeba* isolates

Isolates	Isolation Date	Source	Isolation Location
TTT8	17/4/2012	Soil	Tanjung Tuan, Melaka
TKA14	17/4/2012	Sea water	Teluk Kemang, Negeri Sembilan
TTT1	17/4/2012	Soil	Tanjung Tuan, Melaka
TTA1	17/4/2012	Sea water	Tanjung Tuan, Melaka

Table 3: Positive Control Results

Isolate(s)	Cyst suspension in PAGE saline	Cyst suspension + 3% H_2O_2
TTT8	+	-
TKA14	+	-
TTT1	+	-
TTA1	+	-

Key:

\+ Trophozoite present (indicating viability of the cysts)

\- Trophozoite absent

Table 2: Results of the effectiveness of Oxysept® 1-Step against *Acanthamoeba* isolates

Isolate (s)	Soaking Time		
	4 hours	6 hours Manufacturers' Recommendation	8 hours
TTT8	-	-	-
TKA14	-	-	-
TTT1	-	-	-
TTA1	-	-	-

Key:

\+ Trophozoite present (disinfection ineffective)

\- Trophozoite absent (disinfection effective)

Table 4: Negative Control Results

Oxysept® 1-Step CLDS only	PAGE saline only
-	-

Key:

\+ Trophozoite present (denoting contamination)

\- Trophozoite absent (denoting no contamination)

of *Acanthamoeba* keratitis had their corneal scrapings sent to the *Acanthamoeba* laboratory from different hospitals across Malaysia. 14 (22.6%) were parasitologically confirmed culture positive cases identified from the total 62 suspected cases. All the 14 positive cases were contact lens wearers (Mohamed Kamel *et al*. 2021)

Contact lens disinfecting system ineffective at killing *Acanthamoeba* sp. cysts and trophozoites has been identified as a major factor of corneal infection (Tzanetou *et al*. 2006). It has also been found that not all multipurpose contact lens disinfecting solutions are successful at killing various strains of *Acanthamoeba* sp. (Borazjani & Kilvington, 2005). The number of contact lens wearers is increasing both locally and globally. Since contact lens wearers are at higher risk of contracting *Acanthamoeba* keratitis compared to non-contact lens wearers, an increase in number of *Acanthamoeba* keratitis cases among contact lens wearers is noted.

Commercially available contact lens disinfecting solutions are not required to prove its effectiveness against *Acanthamoeba* cysts. 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 Oxysept® 1-Step, a hydrogen peroxide disinfection system against *Acanthamoeba* sp.

MATERIALS AND METHODS

Source of *Acanthamoeba*

Four environmental strains of *Acanthamoeba* were used in this study and obtained from *Acanthamoeba* Lab, Faculty of Medicine, Universiti Kebangsaan Malaysia (Table 1). The environmental strains were coded as TTT8, TKA14, TTT1 and TTA1.

Contact lens disinfecting solution

Oxysept® 1- Step contact lens disinfecting solution is manufactured by Abbot Medical Optic Inc. It is a hydrogen peroxide disinfection system and the active ingredient is 3% hydrogen peroxide. The recommended soaking time is 6 hours.

Sub-culturing of *Acanthamoeba*

Agar plates containing *Acanthamoeba* were observed under an inverted microscope and suitable areas for sub-culturing were selected and marked with a marker pen on the outer surface of the agar plate. Then, agar in the marked areas were cut with a sterile surgical knife and transferred onto Non-Nutrient Agar face down. *E. coli* suspension is then dropped onto the agar piece, forming a straight line. The *E. coli* suspension is dropped in the middle of the agar plate to prevent the *Acanthamoeba* from growing near the sides of the petri dish. The *Acanthamoeba* was allowed to grow and encyst for 10 days.

Preparation of *Acanthamoeba* cyst suspension

The method used was modified from Johnston *et al*. (2009). Cyst isolates were obtained after sub-culturing. Mature cysts were obtained by extending the incubation time until 10 days. The agar plates containing *Acanthamoeba* cysts were observed under an inverted microscope. 1 ml of PAGE saline solution was pipetted onto the agar surface and mixed with an L-shaped rod to detach the *Acanthamoeba* cysts from the surface of the agar. This was repeated three times and the cyst suspension was pipetted into a centrifuge tube and centrifuged at 2500 rpm for 10 minutes. The supernatant was discarded and 7 ml of PAGE saline solution was added to the sediment. The number of cysts is counted using a *Neubauer Chamber*. The cyst suspension used in this study was standardized to a concentration of 10^5 cysts per ml.

Testing the efficacy of contact lens disinfecting solution

The testing method used is a modification of the method used by Johnston *et al*. (2009). The contact lens disinfecting solution efficacy test was carried out using 12-well microtitre plate where 1 ml of the contact lens disinfecting solution was placed in each well. 100 µl of the cyst suspension with an approximate concentration of 1 x 10^5 was pipetted into the wells containing contact lens disinfecting solution. The cyst suspension was vortexed for 30 seconds before pipetted into each well. The microtitre plates were covered with aluminium foil to prevent drying out and also to mimic the dark conditions of a contact lens storage case. All the microtitre plates were incubated at room temperature following the time parameters which are: manufacturer's recommended soaking time of 6 hours, 4 hours and 8 hours. Positive and negative controls were run together with the test samples. Two types of positive controls were run. The first positive control is the cyst suspension in one ml of PAGE saline. The second positive control is the cyst suspension with 3% hydrogen peroxide. Two types of negative controls were used comprising PAGE saline solution and contact lens disinfecting solution.

After the incubation, 100 µl of the sample was pipetted onto non-nutrient agar seeded with heat-killed *E. coli*. The agar plates were double-sealed with parafilm to prevent it from drying out. Then, the agar plate was incubated at 30°C for 3 days. The presence of *Acanthamoeba* trophozoites on the agar plate will be determined by viewing it under an inverted microscope daily for 14 days. The results of the efficacy of contact lens disinfecting solution against *Acanthamoeba* cysts were recorded as positive or negative. The presence *Acanthamoeba* trophozoites was recorded as positive while its absence was recorded as negative, reflecting effectiveness of the contact

lens disinfecting solution.

RESULTS

Tables 2 shows the results of the effectiveness of Oxysept® 1-Step contact lens disinfecting solution as anti-*Acanthamoeba* agent when tested on the 4 environmental isolates of *Acanthamoeba* sp. The agar plate that shows the presence of trophozoites after 3 days incubation is recorded as positive. Negative results are recorded for agar plates that do not show the presence of trophozoites after daily observation for 14 days.

Oxysept® 1-Step was found to be effective in inactivating the cysts of all *Acanthamoeba* isolates for all the soaking times tested. The positive control of *Acanthamoeba* cyst suspension for all isolates showed the presence of trophozoites indicating viability of the cysts in used (Table 3). The positive control of cyst suspension in 3% hydrogen peroxide showed the absence of trophozoites (Table 3). PAGE saline and the contact lens disinfecting solution used as negative control showed no contamination (Table 4). The results for the controls were as expected.

DISCUSSION

Acanthamoeba keratitis is a severe, often sight threatening corneal infection, which is challenging to diagnose and manage. Therefore, especially in contact lens users, prevention by proper and strict disinfection using effective contact lens solutions is of significant importance. The improper use of contact lens solutions may lead to lens contamination and thus predispose one to the development of *Acanthamoeba* keratitis. While manufacturers are required to test contact lens disinfection systems for efficacy against specific microorganisms using defined standardized in vitro testing methods (ISO 14729 standalone), they are not required to test against *Acanthamoeba*. The ISO 14729 stand-alone method specifies certain strains of bacteria, fungi and yeast that must be tested against in order to deem the solution efficacious, and it defines the minimum criteria for the amount of log kill required for each microorganism. (International Organization for Standardization (ISO) 14729: 2001).

Oxysept® 1-Step is a one-step hydrogen peroxide system. This disinfecting solution contains 3% hydrogen peroxide (H_2O_2) which is known to be effective against *Acanthamoeba* cysts. The disinfecting properties of hydrogen peroxide against bacteria, fungus and *Acanthamoeba* by oxidation is well known (Hughes & Kilvington, 2001). However, the effectiveness of hydrogen peroxide disinfecting system against *Acanthamoeba* cysts varies according to different isolates tested against (Niszl & Markus, 1998).

The results of this study indicate that disinfecting system using hydrogen peroxide is effective in inactivating all 4 environmental isolates of *Acanthamoeba* cysts. This is consistent with the findings of our previous study where Oxysept® 1-Step inactivates all 4 clinical isolates of *Acanthamoeba* cysts (Mohamed Kamel *et al*. 2019). The findings of this study are also reflected in studies by Johnston *et al*. (2009), Hughes & Kilvington (2001), as well as Hiti *et al*. (2006). Hughes and Kilvington (2001) found that two-step hydrogen peroxide disinfecting system is more effective in inactivating *Acanthamoeba* cysts compared to one-step hydrogen peroxide disinfecting system. They also found that at least 6 hours is required to kill all *Acanthamoeba* cysts. In a more recent study by Yosra & Heba, 2014, the one-step hydrogen system, Tutti, used, was able to destroy the *Acanthamoeba* trophozoites after 2 hours of immersion but failed to destroy the resistant cysts which remained viable after the overnight (8 hours) exposure time.

One-step hydrogen peroxide disinfecting system used in this study was found to be effective in inactivating the *Acanthamoeba* cysts after soaking time of 4 hours only. This is contrary to the findings of Hughes and Kilvington (2001), probably the types of isolates influence the viability of the *Acanthamoeba* cysts (Shoff *et al*. 2008). Shoff *et al*. (2007) found varying sensitivities between environmental isolates of *Acanthamoeba* towards contact lens disinfecting solutions. The study also noted that isolates from different genotypes possess varying resistance whereby cysts from genotype T3, T5, and T11 were more resistant than genotype T4. Studies also indicate that environmental isolates of Acanthamoeba possess the potential to become pathogenic (Nurul Farhana *et al*. 2010, 2011). In our previous study using the same environmental isolates (TTT8, TKA14, TTT1 & TTA1) used in the current research, Opti-Free® Express and Meni Care Plus failed to demonstrate anti-*Acanthamoeba* activity on all 4 isolates (Mohamed Kamel *et al*. 2020a). Similarly, Complete® and Revita Lens Ocutec™ were also ineffective at inactivating the same *Acanthamoeba* isolates within the testing times (Mohamed Kamel *et al*. 2020b).

Discrepancies in the effectiveness of contact lens disinfecting solutions against *Acanthamoeba* cysts in this and other studies could be due to difference in the age of *Acanthamoeba* cysts used in testing. Mature *Acanthamoeba* cysts are more resistant compared to immature *Acanthamoeba* cysts (Beattie *et al*. 2003). According to a study by Hughes *et al*. (2003), *Acanthamoeba* cyst shows increasing resistance towards contact lens disinfecting solutions as it ages.

The differing results with previous studies may also be due to method of cyst production which can affect the efficacy of contact lens disinfecting solution towards *Acanthamoeba* cysts (Kilvington & Lam, 2013). The active ingredients in contact lens disinfecting solutions are more effective when tested on axenically prepared *Acanthamoeba* cysts (Silvany *et al*. 1991). Many studies testing the effectiveness of contact lens disinfecting solutions against *Acanthamoeba* cysts use strains that are prepared axenically. This technique may influence the effectiveness of contact lens disinfecting solution because the strains are not as resistant as environmental strains due to the highly selective laboratory environment. *Acanthamoeba* that grow on contact lenses feed on the biofilm on the surface that includes tear film constituents like protein, mucin and bacteria. This environment is worlds apart from the sterile environment of the axenic system.

The differing techniques used in testing the effectiveness of contact lens disinfecting solution against *Acanthamoeba* is due to the absence of a standardised testing protocol. The ability of *Acanthamoeba* in producing numerous isolates of varying resistance is a limiting factor in the development of a standardised testing protocol.

CONCLUSION

Oxysept® 1- Step representing the Hydrogen peroxide system has shown good cysticidal activity against *Acanthamoeba* and is a choice contact lens disinfecting solution to be used in preventing *Acanthamoeba* keratitis for contact lens wearers.

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