Anti-*Acanthamoeba* Activity of Boston Simplicity™ Contact Lens Disinfecting Solution against *Acanthamoeba* Cysts

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ABSTRACT

Introduction: ***Acanthamoeba*** **keratitis incidence amongst contact lens wearers is increasing. The effectiveness of commercially available contact lens disinfecting solutions (clds) as anti-*Acanthamoeba* is doubtful.**

Objective: **This study investigates the anti-*Acanthamoeba* activity of Boston Simplicity contact lens disinfecting solution against 3 clinical isolates of *Acanthamoeba* and to determine its efficacy based on the soaking times recommended by the manufacturer (4 hours) and soaking times of 8 hours, 24 hours and 48 hours.**

Materials and Methods: **Cyst suspensions were prepared using *Acanthamoeba* cysts grown on Non-Nutrient Agar (NNA) for 10 days at 30℃ (± 2℃). Boston Simplicity™ contact lens disinfecting solution was tested in this study. Cyst suspensions of each isolate were tested against the contact lens disinfecting solution based on the manufacturer's recommended soaking time of 4 hours, 8 hours, 24 hours and 48 hours. 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: **Boston Simplicity does not exhibit anti-*Acanthamoeba* activity after 4 hours of soaking time as recommended by the manufacturer and 8 hours for all three *Acanthamoeba* isolates tested but it shows anti-*Acanthamoeba* activity against all three isolates after immersion for 48 hours. For the 24-hour immersion time, Boston Simplicity™ was only effective against *Acanthamoeba* HTH 67 but not for isolates HTH 73 & HTH 87. Most commercially available contact lens disinfecting solutions are ineffective as anti-*Acanthamoeba* agents.**

KEY WORDS

Acanthamoeba**, Boston Simplicity™ Contact lens disinfecting solutions (clds), Malaysia**

INTRODUCTION

Acanthamoeba is a free-living amoeba found in a variety of environmental niches including soil, water and air. It has also been isolated from contact lens, contact lens storage cases and contact lens solutions (Johnston *et al*. 2009; Mohamed Kamel *et al*. 2013).

Acanthamoeba keratitis is a corneal infection that mostly affects contact lens wearers resulting in corneal ulcer, partial vision loss as well as blindness. Substandard contact lens hygiene is a major risk factor for this condition where contact lens storage cases contaminated with micro-organisms are optimal for *Acanthamoeba* growth. The trophozoites and cysts can then transfer onto the eye leading to keratitis (Hiti *et al*. 2002).

Globally, *Acanthamoeba* keratitis is a cause for concern. 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 & Norazah 1995). Since then, this condition is no longer a rarity and is seen with increasing frequency especially among contact lens wearers. In 2001, 10 cases of *Acanthamoeba* keratitis were diagnosed at Hospital Universiti Kebangsaan Malaysia (HUKM) alone (Mohamed Kamel *et al*. 2005). A national registry of contact lens related corneal ulcers (CLRCU) patients in Malaysia was established in 2007 as a surveillance tool used by Malaysian Ministry of Health ophthalmology departments aiming at detecting outbreaks, identifying pattern of causative organisms, risk factors and monitoring outcome of treatment. The registry found that *Acanthamoeba* was the presumptive causative organism of corneal ulcers at a prevalence of 4.6% and 7.3% in 2007 and 2008 respectively (Goh *et al*. 2010).

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.

According to a study by Tzanetou *et al*. (2006), contact lens disinfecting systems ineffective at killing *Acanthamoeba* cysts and trophozoites is a major factor of corneal infection. 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. This study was carried out due to the doubts that exist over the effectiveness of commercially available contact lens disinfecting solutions as

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Table 1. Contact lens disinfecting solution tested and its properties

Brand	Recommended soaking time	Active ingredient (s)
Boston Simplicity™	4 hours	Polyaminopropyl biguanide 0.0005 % w/v Chlorhexidine gluconate 0.003 % w/v

Table 2. Effectiveness of Boston Simplicity™ against *Acanthamoeba* isolates

Isolate	Soaking time			
	(4 hours)*	8 hours	24 hours	48 hours
HTH 67	x	x	√	√
HTH 73	x	x	x	√
HTH 87	x	x	x	√

Key:
x Ineffective (trophozoites present)
√ Effective (trophozoites absent)
* Manufacturer's recommendation

Table 3. Results for controls of cyst suspension, cyst suspension in 3% hydrogen peroxide and PAGE saline

Isolate	Cyst suspension	Cyst suspension plus 3% H_2O_2	PAGE saline
HTH 67	+	-	-
HTH 73	+	-	-
HTH 87	+	-	-

Key:
+ Trophozoites present
- Trophozoites absent

Table 4. Results for negative control of contact lens disinfecting solution

Contact lens disinfecting solution	Trophozoite
Boston Simplicity™	-

Key:
+ Trophozoites present
- Trophozoites absent

anti-*Acanthamoeba* agents.

MATERIALS AND METHODS

Source of *Acanthamoeba*

Three clinical isolates of *Acanthamoeba* were used in this study which were coded as HTH 67, HTH 73 and HTH 87. The clinical isolates were obtained from corneal scrappings of patients with *Acanthamoeba* keratitis from the Tun Hussein Onn National Eye Hospital, Selangor (HTH).

Contact lens disinfecting solution (clds)

Boston Simplicity™ brand of contact lens disinfecting solution was obtained from a pharmacy. Table 1 shows details of Boston Simplicity™.

Sub-culturing *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. The marked areas were observed again to confirm the presence of *Acanthamoeba* cysts. 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. The method used was adopted from Johnston *et al*. (2009).

Preparation of *Acanthamoeba* cyst suspension

Cyst isolates were obtained after sub-culturing. Mature cysts were obtained by extending the incubation period 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 (clds)

The clds efficacy test was carried out using a 12-well microtitre plate where 1 ml of the clds 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 clds. The cyst suspension was vortexed for 30 seconds before being 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 immersion time parameters recommended by the manufacturer at 4 hours, followed by 8 hours, 24 hours and 48 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 are used which are PAGE saline solution and clds.

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 them 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 emergence of trophozoites from cysts indicates ineffectiveness of the CLDS or lacking anti-*Acanthamoeb*a activity, whereas the absence of trophozoites shows efficacy of clds against *Acanthamoeba* cysts.

RESULTS

Tables 2. shows the results of the effectiveness of Boston Simplicity™ contact lens disinfecting solution as anti-*Acanthamoeba* agents when tested on the 3 isolates of *Acanthamoeba* sp. Boston Simplicity™ does not exhibit anti-*Acanthamoeba* activity after 4 hours of immersion time (recommended time by manufacturer) and 8 hours for all three *Acanthamoeba* isolates tested but it shows anti-*Acanthamoeba* activity against all three isolates after immersion for 48 hours. For the 24-hour immersion time, Boston Simplicity™ was ineffective against *Acanthamoeba* HTH 73 & HTH 87 but was effective against the *Acanthamoeba* HTH 67.

The positive control of *Acanthamoeba* cyst suspension for all 3 isolates showed the presence of trophozoites. The positive control of cyst suspension in 3% hydrogen peroxide showed the absence of trophozoite as shown in Table 3. PAGE saline used as a negative control showed no contamination as well as all the clds used as negative controls as shown in Table 4. The results for the controls were as expected.

DISCUSSION

The increasing number of *Acanthamoeba* keratitis cases amongst contact lens wearers is a public health concern globally. If contact lens disinfecting solutions have anti-*Acanthamoeba* activity, this problem could be minimized or even prevented. The results of this study show that Boston Simplicity™ is effective in inactivating all *Acanthamoeba* cysts during a 48-hour immersion period. It also began to show its

effectiveness during the 24-hour immersion except for isolates HTH 73 and HTH 87. However previous study by Mohamed Kamel *et al*. (2016) showed that Boston simplicity™ had shown anti-*Acanthamoeba* activity after 8 hours. The exposure time of Boston Simplicity™ will affect its effectiveness against the *Acanthamoeba* cyst.

The active ingredient in Boston Simplicity™ is chlorhexidine gluconate (0.003% w/v) and polyaminopropyl biguanide, PAPB (0.0005% w/v). The combination of chlorhexidine with propamidine has been proven to be successful in the treatment of Acanthamoeba's corneal infection (Seal *et al*. 1996). Infact, Kosrirukvongs *et al*. (1999) reported that *Acanthamoeba* keratitis patients were successfully treated with 0.006% chlorhexidine alone.

Boston Simplicity™ containing Polyaminopropyl biguanide 0.0005% w/v and Chlorhexidine gluconate 0.003% w/v showed cysticidal effect in 8 hours of soaking but failed to show that activity at the recommended time by the manufacturer in 4 hours. Chlorhexidine acts as inhibitor to prevent transportation of cation and ATP in pathogen's membrane (IACLE 2000). Chlorhexidine 0.001% and 0.005% are capable of killing *Acanthamoeba* at interval 4 to 24 hours, but at concentration less than 0.001%, it was not effective in killing *Acanthamoeba* (Kilvington 1990; Silvany *et al*. 1991). However, at a concentration of 0.09%, Chlorhexidine showed ability of destroying *Acanthamoeba* cysts but was cornea toxic as well (Neville *et al*. 1986).

Chlorhexidine 0.004% to 0.005% has been reported to be effective in killing cysts and *Acanthamoeba* trophozoites after exposure to 1 hour to 4 hours (Kilvington 1990; Silvany *et al*. 1991). At this concentration, the disinfectant solution is able to reduce the number of *Acanthamoeba* but is unable to completely restrict *Acanthamoeba* activity (Kilvington 1990; Brandt *et al*. 1989).

The results of this study were slightly different from the study by Kilvington (1990) and Silvany *et al*. (1991). This study shows that anti-*Acanthamoeba* activity requires longer exposure time, which is 24 hours compared to the results of their studies which only require exposure time of 1 hour to 4 hours. This may be due to the concentration of chlorhexidine in Boston Simplicity™ being lower (0.003%) than their studies. Furthermore, the efficacy of Chlorhexidine varies depending on the tested *Acanthamoeba* concentration and exposure time to contact lens disinfectant solution (Meisler & Rutherford 1991).

According to Niszl and Markus (1998), Polyaminopropyl biguanide, PAPB with a concentration of 0.0005% w/v is not effective directly against the *Acanthamoeba* cyst. Nevertheless, PAPB with a concentration of 0.0015% was found to be effective after 4 hours of exposure to two trophozoite strains and *Acanthamoeba* cysts in the study conducted by Silvany *et al*. (1991). However, studies by Connor *et al*. (1991) as well as Hugo *et al*. (1991), had shown that 0.0015% PAPB was ineffective against the *Acanthamoeba* cyst.

The varying effectiveness of the contact lens disinfecting solutions against the *Acanthamoeba* isolates could also be due to the differing groups of cysts used in this study. Discrepancies in the effectiveness of contact lens disinfecting solutions against *Acanthamoeba* cysts in this study and other studies could be due to differences 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* cysts show 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

Most commercially available contact lens disinfecting solutions are ineffective as anti-*Acanthamoeba* agents. Soaking time recommended by the manufacturers is found to be ineffective at inactivating most of the *Acanthamoeba* cyst isolates tested.

Further testing needs to be done using other brands of contact lens disinfecting solutions on more *Acanthamoeba* cyst isolates. Besides that, it is also recommended to compare the sensitivity of clinical as well as environmental strains as to provide a clearer picture on the *Acanthamoeba* susceptibility pattern towards contact lens disinfecting solutions in Malaysia.

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