PARASITOLOGY

# Anti-*Acanthamoeba* Activity of OPTICARE Multimate® Contact Lens Disinfecting Solution against Cyst of *Acanthamoeba* from Environmental Isolate

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## ABSTRACT

***Introduction:*** **Keratitis is one of the common causes of visually impaired cases in Malaysia and *Acanthamoeb*a is known to cause severe keratitis leading to blindness if left untreated. Contact lens wear is a major risk factor for *Acanthamoeba* keratitis (AK) and therefore effective contact lens disinfectants are important in preventing the infection.**

***Objective:*** **This study was performed to evaluate the anti-*Acanthamoeba* activity of a locally produced commercial contact lens disinfecting solution (CLDS), OPTICARE Multimate®, towards the cysts of *Acanthamoeba* isolates.**

***Materials and Methods:*** **Two environmental isolates of *Acanthamoeba;* TTT1 & TKT19 were exposed to OPTICARE Multimate® multipurpose CLDS at several soaking time periods of 4, 6 and 8 hours. Following the soaking durations, the cysts were subcultured onto non-nutrient agar plates seeded with heat killed *Escherichia coli* and observed daily for 14 days for any evidence of excystation of the cyst to the trophozoite stage. Successful excystation indicates ineffectiveness of the CLDS and vice-versa.**

***Result:*** **OPTICARE Multimate® multipurpose CLDS with Polyhexamethylene Biguanide (PHMB) as active ingredient, failed to exhibit anti-*Acanthamoeba* activity in all soaking intervals against the 2 environmental isolates of *Acanthamoeba*.**

**Conclusion: This study has proven that though contact lens disinfecting solutions have antimicrobial properties, not all are effective against *Acanthamoeba cysts*. OPTICARE Multimate® multipurpose CLDS may not be able to protect contact lens users from *Acanthamoeba* keratitis but more *Acanthamoeba* strains including the clinical, should further be tested.**

## KEY WORDS

***Acanthamoeba* keratitis, contact lens disinfecting solution, PHMB, Malaysia**

## INTRODUCTION

*Acanthamoeba* keratitis (AK) among contact lens wearers is being reported with increasing frequency in various parts of the world including Malaysia (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 PP *et al*. 2010).

Malaysia has seen significant increased in the number of AK cases since the first case was reported in 1995 (Mohamed Kamel & Norazah, 1995). Most of the cases were contact lens related particularly involving the use of soft lenses (Mohamed Kamel *et al*. 2018). Since AK is largely associated with contact lens wear, the role of contact lens disinfecting agent is crucial in preventing all sorts of microbial infections. Even though a patient may comply with general contact lens wear and care procedures recommended by lens manufacturers and healthcare professionals, a solution that does not kill *Acanthamoeba* may not protect the wearer against ocular infection with this organism (Stehr-Green *et al*. 1987). The most popular lens disinfecting products are multipurpose disinfecting solutions, which are designed for cleaning, disinfecting, rinsing, and storing contact lenses using a single formulation. Most of these solutions also contain polyhexamethylene biguanide (PHMB). For this reason, we need to evaluate the effectiveness of chemical storage/soaking and disinfecting solutions on *Acanthamoeba* cysts which are known to be more resistant than the trophozoites (Ludwig *et al*. 1986).

A number of previous studies disputed the efficiency of some contact lens disinfecting solution towards various isolates and strains of *Acanthamoeba*. Thus, this has raised the doubt on the effectiveness of the currently available contact lens disinfecting solutions. In accordance to it, this study was conducted to evaluate the efficacy of a new locally produced commercial contact lens disinfecting solution in Malaysia towards *Acanthamoeba* of environmental isolates.

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**Table 1: Isolation details of *Acanthamoeba* isolates**

| No. | Strains | Isolates | Source | Sample Type | Morphological group | Place of isolation | Date of isolation |
|---|---|---|---|---|---|---|---|
| 1. | Environmental | TKT19 | Soil | Beach Soil | Group (II) | Teluk Kemang Negeri Sembilan | 19/4/2012 |
| 2. | Environmental | TTT1 | Soil | Beach Soil | Group (II) | Tanjung Tuan Negeri Sembilan | 1/4/2012 |

**Table 2: Contact lens disinfecting solution Properties**

| Contact lens Disinfecting solution (CLDS) | Manfacturer | Active ingredient | System Type | Contact Lens Type | Manufacturers Recommendation Soaking Time (Hours) |
|---|---|---|---|---|---|
| OPTICARE Multimate® | ExcelVision Medical Sdn Bhd, Selangor (M.Y.S) | PolyMed® PHMB (0.0001%) | MPCLS | SOFT | 4 |

**Table 3: Efficacy of OPTICARE Multimate® CLDS against *Acanthamoeba* cyst isolates**

| No. | Strains | Isolates | Soaking period (hours) | | |
|---|---|---|---|---|---|
| | | | 4*hrs. | 6 hrs. | 8** hrs. |
| 1. | Environmental | TKT19 | ( + ) | ( + ) | ( + ) |
| 2. | Environmental | TTT1 | ( + ) | ( + ) | ( + ) |

( + ) Presence of trophozoites (disinfection ineffective).
( - ) Absence of trophozoites (disinfection effective).
* Manufacturer's recommended soaking time.
** Over night soaking time.

**Table 4: Microbiological Control Results (Postive and Negative)**

| No. | Strains | Isolates | (Microbiological positive control) Cyst suspension (parasite viability test) | (Microbiological negative control) NNA media (Contamination test) |
|---|---|---|---|---|
| 1. | Environmental | TKT19 | ( + ) | ( - ) |
| 2. | Environmental | TTT1 | ( + ) | ( - ) |

( + ) Presence of *Acanthameba* spp trophozoites in the media (cysts are viable).
( - ) Absence of *Acanthameba* spp trophozoites in the media (no contamination).

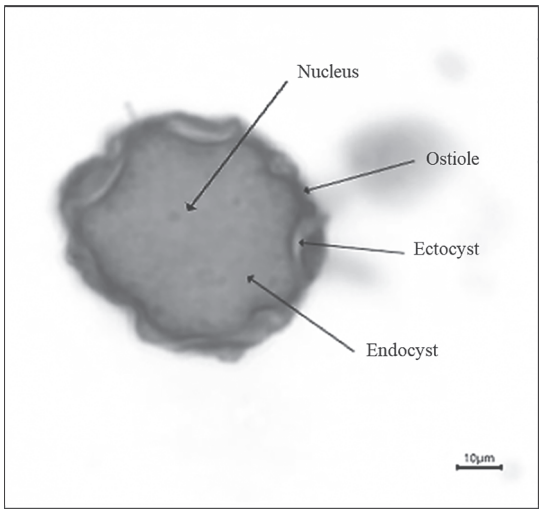


**Figure 1: Morphological group classification of the *Acanthamoeba* cyst using 0.01% Methylene Blue stain**

## MATERIALS AND METHODS

### Source of Acanthamoeba

Two environmental isolates of *Acanthamoeba* were used in this study and obtained from the *Acanthamoeba* Culture Lab, Department of Parasitology, Faculty of Medicine, Universiti Kebangsaan Malaysia. The isolates were coded as TTT1 and TKT19 and the isolation details are shown in Table 1.

### Contact lens disinfecting solution (CLDS)

OPTICARE Multimate® multipurpose CLDS is a new Malaysian local brand manufactured by ExcelVision Medical Sdn Bhd, Selangor and its properties are shown in Table 2.

### Sub-culturing Acanthamoeba

Agar plates containing *Acanthamoeba* were sub cultured onto Non-Nutrient Agar seeded with *E. coli* and allowed to grow and encyst for 11 days following the method by Narasimhan *et al*. 2002. The *Acanthamoeba* cyst suspension used in this study was standardized to a concentration of $10^5$ cysts per ml using a Neubauer Chamber.

### Contact Lens Disinfecting Solution (CLDS) Efficacy Test

The efficacy test for OPTICARE Multimate® multipurpose disinfecting system against the *Acanthamoeba* cysts was adopted from method by Narasimhan *et al*. 2002 with some modifications.

The contact lens disinfecting solution efficacy test was carried out using 24-well microtiter 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 $10^5$ cyst/ml was pipetted into the wells containing contact lens disinfecting solutions. The cyst suspension was vortexed for 30 seconds before pipetted into each well. The microtiter 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 microtiter plates were incubated at room temperature following the time parameters as recommended by the manufacturer (4 hours); 6 hours and 8 hours (representing overnight soaking). Positive and negative controls were run together with the test samples. All tests were run in duplicates.

### Cyst Morphological Test

The *Acanthamoeba* isolates were morphologically classified by studying the internal structure of the cyst as described by Duarte *et al*. 2013; Pussard & Pons 1977. The distinctive features of the cysts have led to classification into three major groups ie; group I or *Astronyxid*, group II or *Polyphagid* and group III or *Culbertsonid*. The cysts were observed under X100 magnification using ZEISS A1 (AX10) compound microscope with LCD attachment and 0.01% Methylene blue dye staining to enhance the morphology of the cyst for better differentiation.

## RESULTS

OPTICARE Multimate® CLDS fails to exhibit anti-*Acanthamoeba*

activity against both *Acanthamoeba* cyst isolates at the manufacturer's soaking time of 4 hours, 6 hours and 8 hours representing overnight soaking as shown in Table 3. Both the positive and negative controls perform as expected as shown in Table 4. The cysts were viable and the was no contamination of the media used.

The result of cysts morphological test under ZEISS Scope A1(AX10) compound microscope with imaging software shows that both environmental *Acanthamoeba* isolates are from morphological group II or *Polyphagid*. Its features include small cyst size with diameter lesser than 18 µm, irregularly wrinkled ectocyst arches and wide polygonal endocyst as shown in Figure 1.

## DISCUSSION

The effectiveness of commercially available contact lens disinfecting solutions is doubted because contact lens disinfecting solutions are not required to demonstrate effectiveness against *Acanthamoeba*. The active ingredient of OPTICARE Multimate® is polyhexamethylene biguanide (PHMB 0.0001%) which is claimed by manufacturer to be both bactericidal and fungicidal. PHMB kills microbial organisms via membrane dysfunction. It however, fails to exhibit anti-*Acanthamoeba* efficacy against two environmental *Acanthamoeba* isolates in this study, for all the soaking periods, including manufacturer's recommended soaking time of 4 hours, 6 hours and 8 hours which represent overnight soaking. Similarly in our previous study (Mohamed Kamel *et al*. 2019), it also failed to inhibit the excystation of 2 clinical isolates of *Acanthamoeba*. These findings were in concert with several previous studies conducted whereby PHMB with concentrations of 0.0001-0.0005% were not effective against various *Acanthamoeba* isolates (Kilvington, 1990, Mohamed Kamel *et al*. 2016; Boost *et al*. 2012; Kilvington *et al*. 2010). This could be attributed to the very low concentration of PHMB used, well below its minimum cysticidal concentration (MCC).

Moon *et al*. 2016, discovered that most of the multipurpose disinfecting solutions with PHMB as active ingredient made in Korea, were ineffective or insufficient to kill *Acanthamoeba*, and the amoebicidal effective solutions also showed cytotoxicity on human corneal epithelial cells. However, our previous study showed partial effectiveness of 0.0005% PHMB towards a few *Acanthamoeba* clinical isolates (Mohamed Kamel *et al*. 2018a). In fact, at higher concentration, PHMB is effective in the treatment of *Acanthamoeba* keratitis (Niszl & Markus, 1996). 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* sp. according to the ISO 14729 (2001) (Microbiological Requirements and Test Methods for Products and Regimens for Hygienic Management of Contact Lenses). In fact, 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*.

## CONCLUSION

OPTICARE Multimate® multipurpose CLDS, with PHMB as its active ingredient for disinfection, was found to be ineffective to inactivate the cysts of *Acanthamoeba* isolates, even at the extended soaking duration. It may not be suitable for use by contact lens users with regards to protection against *Acanthamoeba* keratitis.

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