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Research Article

EFFECT OF CONTACT LENS SOLUTIONS ON *ACANTHAMOEBA POLYPHAGA* RESPONSIBLE FOR EYE DISEASE AMOEBIC KERATITIS

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ABSTRACT
The free-living protozoan *Acanthamoeba* is recognized as an agent of corneal disease, especially keratitis. Furthermore, this organism is opportunistic in immunosuppressed patients, in whom it causes disseminated fatal diseases like otitis, chronic sinusitis, skin ulcers and a rare form of encephalitis referred to as Granulomatous Amoebic Encephalitis (GAE). Although *Acanthamoeba* Keratitis may result from accidental eye trauma, most cases are associated with contact lens wearers, with soft contact lenses having the highest risk of infection.In the present communication, four cases of *Acanthamoeba keratitis* diagnosed out of 110 patients suffering from eye infections from different hospitals and eye clinics are being reported along with a study on the effect of commercial contact lens cleaning solutions on isolated *Acanthamoeba polyphaga*. Diagnosis in all these patients was based on observation of *Acanthamoeba* in wet mount of corneal ulcer scrapings and its subsequent culture in agar plate. Thermal disinfection killed all trophozoites of *A. castellanii* and *A. polyphaga.* The chlorhexidine solution killed all *A. castellanii* trophozoites and cysts, but only delayed excystation of *A. polyphaga* organisms. The reason for the apparent greater effectiveness of this solution over the other chemical disinfection solution is not clear. It is also unclear that whether different strains of *Acanthamoeba* species would be variably susceptible to this disinfection solution However, results of this study indicate that there may also be strains of Acanthamoeba infecting eyes leading to amoebic Keratitis besides bacteria. Chlorhexidine solution out of all Contact lens cleaning solutions used was more effective overall in killing *Acanthamoeba* trophozoites and cysts as compared to cold disinfection solutions.

Keywords: *Acanthamoeba, Keratitis, Soft Contact Lens, Disinfection*

INTRODUCTION
Acanthamoeba is a free-living, opportunistic protozoan parasite of human being and domestic animals. It can cause a fatal amoebic meningoencephalitis, but its few strains (*Acanthamoeba polyphaga and A.castellini)* are most commonly associated with eye infections i.e. Amoebic Keratitis associated with contact lens use (Anisah *et al.,* 2005). The increasing prevalence of this amoebic keratitis is thought to be linked to the increased use of contact lenses (Kamel *et al.,* 2003). *Acanthamoeba* keratitis is usually diagnosed after viral and bacterial causes have been eliminated. In the absence of delay in proper diagnosis of amoebic keratitis, there is often a significant delay before appropriate treatment is administered. Because of the severity of *Acanthamoeba* keratitis, a significant loss of visual acuity is common and in many cases total loss of sight in the infected eye may occur (Khan, 2003). Current methods of detection involve culture and microscopic identification of *Acanthamoeba* strains responsible for amoebic Keratitis. These methods are time consuming, laborious, and open to error also. The development of a rapid, simple detection method for *Acanthamoeba* is thus very essential.
Acanthamoeba species have been isolated from many different sources, such as freshwater, seawater, chlorinated water and from swimming pools, dental treatment units, and contact lens cases (Anisah *et al.,* 2004, Visvesvara, 2010 and Hong *et al.,* 2014). Most of the strains found are not pathogenic. Some pathogenic strains are known to survive for extended periods in freshwater. Protozoa, in general, become airborne when encysted. The presence of pathogenic *Acanthamoeba* organisms in the atmosphere is an important factor in the prevalence of *Acanthamoeba* keratitis, although this is not its main cause.

Patients with *Acanthamoeba* keratitis usually are users of soft contact lenses users. The use of tap water to clean contact lenses allows deposits of lime scale to accumulate, and this lime scale often contains pathogenic *Acanthamoeba* species. These contact lenses create a corneal abrasion, facilitating entry of *Acanthamoeba* into eyes. The organism can survive in contact lens cases and also in homemade cleaning solutions.

MATERIALS AND METHODS

For the diagnosis of *Acanthamoeba* keratitis sample (tear or corneal scraping) collected aseptically from the patients of different age group ranging from 3year to 17 years. All samples of tear and corneal scrap were collected in sterile screw capped small tubes and brought to the laboratory and tested for culture sensitivity using non nutrients agar plates a pre seeded with *E.coli* as food for amoebae, in accordance with a standard international method (Visvesvara, 1985). Microscopy and culture for amebic, bacterial, mycobacterial, and fungal organisms were performed. Swabs from the cornea were inoculated onto two *Escherichia coli*-seeded, 1% non nutrient agar (ECNNA) plates. In addition; the patient's disposable contact lenses were placed onto two ECNNA plates. Ten milliliters of the patient's contact lens-disinfecting solution was centrifuged at 3,000 rpm for 10 min, and the sediment was inoculated onto two ECNNA plates. All of the ECNNA plates were incubated at 25 and 37°C for 20 days. The disinfecting solution was cultured for bacteria.

The *Acanthamoeba* isolate was cloned by diluting a suspension of cysts in sterile ameba saline, spreading them on agar under a microscope, and selecting individual cysts by using low magnification. A piece of agar bearing the selected cyst was cut out and transferred facing downward to a fresh ECNNA plate. Several plates were prepared in this manner from sequential cultures, and each time the block of agar was carefully examined under the microscope to make sure that only one cyst was present.

RESULTS AND DISCUSSION

Acanthamoeba keratitis is a seldom recorded infection; however, it is a serious condition hence it should not be overlooked or taken lightly. This infection is influential on the eyes and if not treated timely, then it may lead to ocular impairment or blindness on permanent basis (Hammersmith, 2006). It is studied that the infection is caused due to free- living microscopical amoebae, which are also known as *Acanthamoeba*. When these microorganisms, *Acanthamoeba*, infects the cornea of the eye (external transparent layer of the eye) it leads to *Acanthamoeba* keratitis. Human beings are at greater risk of developing this rare eye infection, as this kind of amoeba is frequently found in various water bodies such as lakes, soil air etc. Such an amoebic eye infection was for first time diagnosed in the 1973, wherein approximately 90% of affected individuals were contact lenses users (Bharathi *et al.*, 2007).

There are several factors that may lead to *Acanthamoeba* keratitis. For example, using contaminated water from sources such as tap or well may increase the risk of such infection. Also infected contact lenses may cause such issues (Shoff *et al.*, 2007). Avoid wearing contact lenses while in hot tub or swimming in pool or even while taking shower as these factors may also result in *Acanthamoeba* infection (Shoff *et al.*, 2008). Adopting inappropriate measures for storing contact lenses may cause the virus or bacteria to settle on the lenses and then infect eyes on wearing them.

Four positive cases out of 110examined (3.636) for suspected cases of amoebic keratitis showed positive growth from their eye scrapings /tear drops. Identification and biological characterization of amoebic isolates was done following the patterns of Singh and Hanumaiah (1979) and Levine *et al.*, (1980). All the trophic characters observed were similar to the typical of *Acanthamoeba sp.* strain.

However the cyst was double-walled and polyhedral. The endocyst (inner) was stellate or polygonal and ectocyst was wrinkled with ripples. In Scanning Electron Microscopy study, ridges and groves were present on the surface of cyst and pores were present on the cyst wall. Cysts were uninucleate with a prominent nucleolus.

During excystment trophozoite emerged from the pore that was present on cyst wall. Trophozoites also failed to produce temporary amoebo flagellate stage on repeated efforts. During active locomotion of

amoebae on glass surface, broad, anterior hyaline lobopodium with large number of slender hyaline projections, singly or in groups of two or three were produced. These characteristic projections have been called as "acanthopodia" (Page, 1967b). These were formed in the direction of motion and helped during locomotion. A distinct nucleus and a contractile vacuole were clearly visible in each trophozoite.

Thus, on the basis of above observations of trophozoites and cysts, the strains isolated from eye of suspected patients were also identified as *Acanthamoeba polyphaga* because of their close similarity in the observation of trophic, cystic characters and locomotion, behaviour and in vitro drug testing using different contact lenses cleaning solutions at 37^0c for 2,4,6,8,12 and 24 hours. All contact lens disinfecting solutions were purchased from local retailers. These solutions were taken from the original wrapping and were used before their stated expiry date. Active ingredients of all tested contact lens solution to be used are listed in (Table-1).

Current treatment of *Acanthamoeba* keratitis involved tropical application of mixture of drugs including chlorhexidine, Polyhexamethylene Biguanide (PHMB), Neomycin and Propamidine isethionate. These drugs have been shown to be most effective in killing *Acanthamoeba* trophozoites (Lloyd *et al.,* 2001). Rinsing lenses in tap water before disinfection is often a cause of problem rather than a preventive measure (Dini, *et al.,* 2000).

Experiments were performed in cavity slides. 100µml of the calibrated suspensions containing definite number of amoebae were added to each well of cavity slides respectively and then 2-4ml of respective contact lens solution was added to each well, then these slides were sealed in most chamber and incubated at 37^0C for 2,4,6,8,12 and 24 hours. A contact lens solution was only considered amoebicidal if all amoebae were eradicated in the given time because even single surviving amoebae can grow and multiply. Test was repeated 3-4 times along with controls containing the parasites in normal saline solution were submitted to the same procedure used for the experimental cultures.

Using **Complete multipurpose solution** and **Aqua soft multi-purpose solution** containing PHMB 0.0001%, showed the best amoebicidal effect on *Acanthamoeba polyphaga* trophozoites (after soaking time of 4^{th} hour).The trophozoites were completely destroyed. Using **ReNu multi-purpose** and **All Clean soft solution,** best amoebicidal affect was observed on *Acanthamoeba polyphaga* cysts (after soaking at 12h). The cysts were completely destroyed. The Silk lens was ineffective against *A. polyphaga* cysts (Table-2 & Table-3).

Table 1: List of contact lens cleaning solution used in *In-vitro* test on *Acanthamoeba polyphaga*

S.No.	Contact lens solutions	Active ingredients
1	Complete multi-purpose solution	Polyhexamethylene Biguanide 0.0001%, Poloxamer 2370.05%, Edentate disodium, sodium phosphate monobasic (monohydrate), sodium chloride; potassium chloride, sodium phosphate dibasic (Heptahydrate) and purified water.
2	Renu multi-purpose solution	Polyaminopropyl Biguanide 0.001%, hydroalkylphosphonate 0.03%, Poloxamine 1%, Boric acid, Edentate disodium, Sodium borate and Odium chloride.
3	Silklens multi-purpose solution	Sodium chloride, boric acid, Poloxamer disodium edentate 0.1% w/v and sorbic acid 0.1% w/v.
4	Aqua soft multi-purpose solution	Polyhexanide, Poloxamer, hypomellose, edenated disodium, sodium chloride and potassium chloride.
5	All clean, Soft	Polyhexanide, Poloxamer, EDTA and PVT.

Table 2: Viability of *Acanthamoeba polyphaga* trophozoite after contact lens solution exposures of 2, 4, 6, 8, 12 and 24 hours

S.No.	Contact lens solution	2hr	4hr	6hr	8hr	12hr	24hr
1	Complete multi-purpose solution	+	+	-	-	-	-
2	Renu multi-purpose solution	+	+	+	-	-	-
3	Silklens multi-purpose solution	+	+	+	+	+	-
4	Aqua soft multi-purpose solution	+	+	-	-	-	-
5	All clean, Soft	+	+	+	+	-	-

Table 3: Viability of *Acanthamoeba polyphaga* cysts after contact lens solution exposures of 2, 4, 6, 8, 12, 24 hours

S.No.	Contact lens solution	2hr	4hr	6hr	8hr	12hr	24hr
1	Complete multi-purpose solution	+	+	+	-	-	-
2	Renu multi-purpose solution	+	+	+	-	-	-
3	Silklens multi-purpose solution	+	+	+	+	+	+
4	Aqua soft multi-purpose solution	+	+	+	-	-	-
5	All clean, Soft	+	+	+	+	+	-

Our results demonstrated the necessity of an appropriate concentration of antiamoebic agents in a contact lens disinfecting solution for it to be effective against *Acanthamoeba* cysts and trophozoites. Reasons for the difference in the killing ability and cytotoxic potential of different brands of disinfectants, even though all contained the same concentration of preservative (0.0001% PHMB), are unclear. Another vital point is the need for adequate exposure time for effective killing of *Acanthamoeba* cysts and trophozoites. During testing, trophozoites became rounded, without acanthopodia and a clear decrease in general number of the amoebae appeared. The lack of efficacy against *Acanthamoeba* of all tested solutions upon long storage of soft contact lenses has been observed. Cysts were still viable after overnight (8 hours at 37^0C) exposure time. Surviving cysts in contact lens cases were able to excyst and multiply, and could thus may cause a infection of the eye. Studies have investigated the efficacy of various contact lens disinfectants solutions in eliminating *Acanthamoeba* (Tzanetou, *et al.*, 2006; Borazjani and Kilvington, 2005; Hiti, *et al.*, 2005 and Pinna, 2002). The abandonment of home-prepared saline and use of chlorine-based disinfectants for contact lens cleaning have reduced the prevalence of *Acanthamoeba* keratitis (Borazjani and Kilvington, 2005 and Seal, *et al.*, 1999). The most commonly used contact lens disinfecting systems are the multipurpose solution. The multipurpose solution is a single solution used for cleaning; rinsing, disinfecting and storing contact lenses and it offer continuous antimicrobial protection (Tzanetou, *et al.*, 2006 and Stevenson and Seal, 1998).

Brandt *et al.*, (1989) tested saline solution, cleaning solution and disinfection solution against three species of *Acanthamoebae* recovered from contact lens cases i.e. *A.castellanii*, *A.culbertson*, and *A.polyphaga.* Although solution containing hydrogen peroxide was the most effective, cysts were detected in all solution for at least 6 hours after treatment. Shoff *et al.*, (2008) determined contact lens solution against clinical and tap water isolates strains of *Acanthamoeba* and concluded that hydrogen peroxide were more effective in comparison to multipurpose lens storage solution. The clinical and tap water isolated strains of *Acanthamoeba* strains representing proven human pathogens and of household strains, were highly virulent against contact lens solutions.

Zanettiet *et al.*, (1995) has exposed a corneal isolate of *Acanthamoebae castellanii* to commercial contact lens disinfecting solutions containing hydrogen peroxide, Benzalkonium chloride, Polyaminopropyl Biguanide, Polyquaternium1 and chlorhexidine Thimerosal. They found that solutions containing hydrogen peroxide or chlorhexidine-Thimerosal were active against both trophozoites and cysts. The benzalkonium chloride based solution was effective only against trophozoites but polyaminopropyl Biguanide or polyquaterium1 were completely ineffective. So the need for adequate exposure times must be stressed. Multipurpose solutions have been prepared to clean and store lenses. These multipurpose

solutions contain a detergent with a Polyquaternium or Polyhexamethylene Biguanide (PHMB), in buffered solution (Aguiar *et al.*, 2013).

The results of present study are in conformity with the findings reported by Brandt *et al.*, (1989); Silvany *et al.*, (1990); Kilvington and Anger (2001); Hiti *et al.*, (2002); Polat *et al.*, (2007) and Shoff *et al.*, (2007, 2008). The main risk factor for corneal infection in contact lens wearer is the use of contact lens disinfecting solution being ineffective in killing the *Acanthamoeba* cysts and trophozoites. All commercial solutions examined in this study are not completely effective in eliminating *Acanthamoeba* cysts. Improvement or development of new contact lens disinfecting solution by manufactures is needed to prevent *Acanthamoeba* keratitis infection.

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