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VERSITA

Susceptibility of *Acanthamoeba* to multipurpose lens-cleaning solutions

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Abstract

The present study investigated the susceptibility of *Acanthamoeba* spp. trophozoites to two multipurpose systems for cleaning and maintenance of contact lenses. Three strains of trophozoites from the ATCC (*A. castellani* T4, *A. castellani* Neff, and *A. polyphaga*) and two *Acanthamoeba* isolates obtained from swimming pools (PT5 and PO1) were placed in monoxenic culture. To test their survival in cleaning solutions for contact lenses, the trophozoites were exposed for 4 and 24 h to two multipurpose solutions (A and B), and were then inoculated into a new monoxenic culture. Amoebic growth on the plates was observed after 72 h of incubation. Trophozoites from all three ATCC strains and one isolate from a swimming pool (PO1) grew in all plates after 4 h of exposure to solutions A and B. After 24 h, the ATCC strains and the PO1 isolate showed growth in most of the plates treated. Only the PT5 isolate showed susceptibility to both solutions over the time intervals tested. The two solutions were not completely effective against most strains and isolates over the time intervals tested. These results are important, since species of *Acanthamoeba* are widely distributed in the environment and are potential agents of eye pathologies.

Keywords

Acanthamoeba, multipurpose solutions, keratitis

Introduction

Species of the genus *Acanthamoeba* are amphizoic free-living amoebae, widely distributed in the environment. The clinical importance of these organisms is due to the fact that they are responsible for cases of human infections, such as encephalitis in immunocompromised patients, and amoebic keratitis in immunocompetent patients, especially users of contact lenses.

Historically, *Acanthamoeba* keratitis (AK) has been associated with corneal trauma and exposure to contaminated water (Cohen *et al.* 1985, Theodore *et al.* 1985). With the growing use of contact lenses, the incidence of AK has increased, and although this has not been precisely determined, it has been estimated to affect 1.65 to 2.09 per million contact-lens wearers in the United States (Schaumberg *et al.* 1998) and 3.06 per million in The Netherlands (Morlet *et al.* 1997). The disease is a potentially severe eye infection caused by progressive inflammation and ulceration in the cornea (Zheng *et al.* 2004), and symptoms include intense pain, photophobia, decreased vision, tearing, and swelling (Marciano-Cabral and Cabral 2003). If during the early stage, inappropriate treatment is instituted, the agent can penetrate deeply into the cornea and other parts of the eye (McClellan *et al.* 1987). Thus, *Acanthamoeba* can invade tissues and settle in the stroma, causing severe infections that can lead to the enucleation of the ocular globe (Khan *et al.* 2002).

The virulence of the amoeba is related to its genotype, which is determined by using the 18S rDNA gene sequence. Evolutionary studies have led to the identification of at least 16 genotypes (T1–T16) based on rRNA gene sequencing (Stothard *et al.* 1998; Gast *et al.* 2001; Booton *et al.* 2005; Maghsood *et al.* 2005; Corsaro *et al.* 2010). Most cases of keratitis are associated with genotype T4 (Yu *et al.* 2004, Molmeret *et al.* 2005).

Although the pathogenesis of AK is not well recognized, the use of contact lenses has been a major risk factor (Zheng *et al.* 2004). Current studies show that over 90% of cases occur in contact-lens wearers. Predisposing factors for keratitis in users of contact lenses are the use of non-sterile solutions, swimming in a pool while wearing contact lenses, and inadequate disinfection of contact lenses (Radfort *et al.* 2002).

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Therefore, considering the growing number of wearers of both corrective and cosmetic contact lenses, and the increasing incidence of infections associated with their use, the purpose of this study was to determine the efficacy of two multipurpose systems that are currently used in Brazil, against ATCC strains and isolates of *Acanthamoeba* spp.

Materials and Methods

Two disinfecting and maintenance solutions for contact lenses (designated A and B), chosen based on their dominance in the market and registered in ANVISA (BRASIL 2011) in the category „health products", and a commercial saline solution, which served as a negative control, were evaluated against three ATCC strains (*Acanthamoeba castellani* T4, *Acanthamoeba castellani* Neff, and *Acanthamoeba polyphaga*) and two environmental isolates from swimming pools (PT5 and PO1) (Caumo and Rott 2011).). The genotype to which each belongs and its source are listed in Table I.

The trophozoites of *Acanthamoeba* spp. were grown in a 50 µL aliquot of PYG (De Carli and Moura 2007) liquid medium (2% proteose peptone, 0.2% yeast extract, and 1.8% glucose) supplemented with penicillin G potassium (400 IU/ml) and streptomycin (400 mL/mL). Aliquots containing approximately 100 trophozoites were added to the surface of 1.5% non-nutrient agar (NNA) (De Carli and Moura 2007), previously coated with a suspension of heat-killed *Escherichia coli*. The plates were sealed with Parafilm® and incubated at 30°C for 72 h. Areas of plates with a confluent amount of trophozoites were delimited by a 2 x 2 cm square (~ 300 trophozoites/square).

Subsequently, the plates were opened under sterile conditions, and the squares were cut and transferred to 50-ml aliquots of the A, B, and saline solutions. All tests were performed in triplicate. After 4 and 24 h of exposure to the test solutions at room temperature, the viability of the trophozoites was tested. Each square, after being removed from the A, B, and saline solutions, was transferred to Page saline solution (De Carli and Moura 2007) for 5 min in order to rinse the squares. Then, the squares were individually placed face down in a monoxenic culture and the plate was sealed with Parafilm. The amoebic growth on plates was inspected after 72 h incubation at 30°C, under a light microscope (100X). Ten random fields were observed, and the number of trophozoites in each field was counted. The mean number of amoebae per field was calculated.

Table I. List of genotypes and sources of the *Acanthamoeba* strains and isolates investigated

Name	ATCC	Genotype	Source
Acanthamoeba castellani T4	ATCC30010	T4	Cornea with keratitis
Acanthamoeba castellani Neff	ATCC50492	T4	Soil
Acanthamoeba polyphaga	ATCC 30461	T4	Cornea with keratitis
Isolate PT5	–	T5	Pool water
Isolate PO1	–	T4	Pool water

Table II. Growth of *Acanthamoeba castellani* T4, *Acanthamoeba castellani* Neff, and *Acanthamoeba polyphaga* strains, and isolates PT5 and PO1 after 4 h and 24 h contact with solutions A and B

Strain/isolate	Solution	Growth after 4 h plates (n/n)	Percentage of growth	Growth after 24 h plates (n/n)	Percentage of growth
Acanthamoeba castellani T4	Solution A	3/3	100%	3/3	100%
	Solution B	3/3	100%	2/3	66.6%
	Saline solution	3/3	100%	3/3	100%
Acanthamoeba castellanii Neff	Solution A	3/3	100%	2/3	66.6%
	Solution B	3/3	100%	3/3	100%
	Saline solution	3/3	100%	3/3	100%
Acanthamoeba polyphaga	Solution A	3/3	100%	3/3	100%
	Solution B	3/3	100%	3/3	100%
	Saline solution	3/3	100%	3/3	100%
PT5	Solution A	0/3	0%	0/3	0%
	Solution B	0/3	0%	0/3	0%
	Saline solution	3/3	100%	3/3	100%
PO1	Solution A	3/3	100%	3/3	100%
	Solution B	3/3	100%	3/3	100%
	Saline solution	3/3	100%	3/3	100%

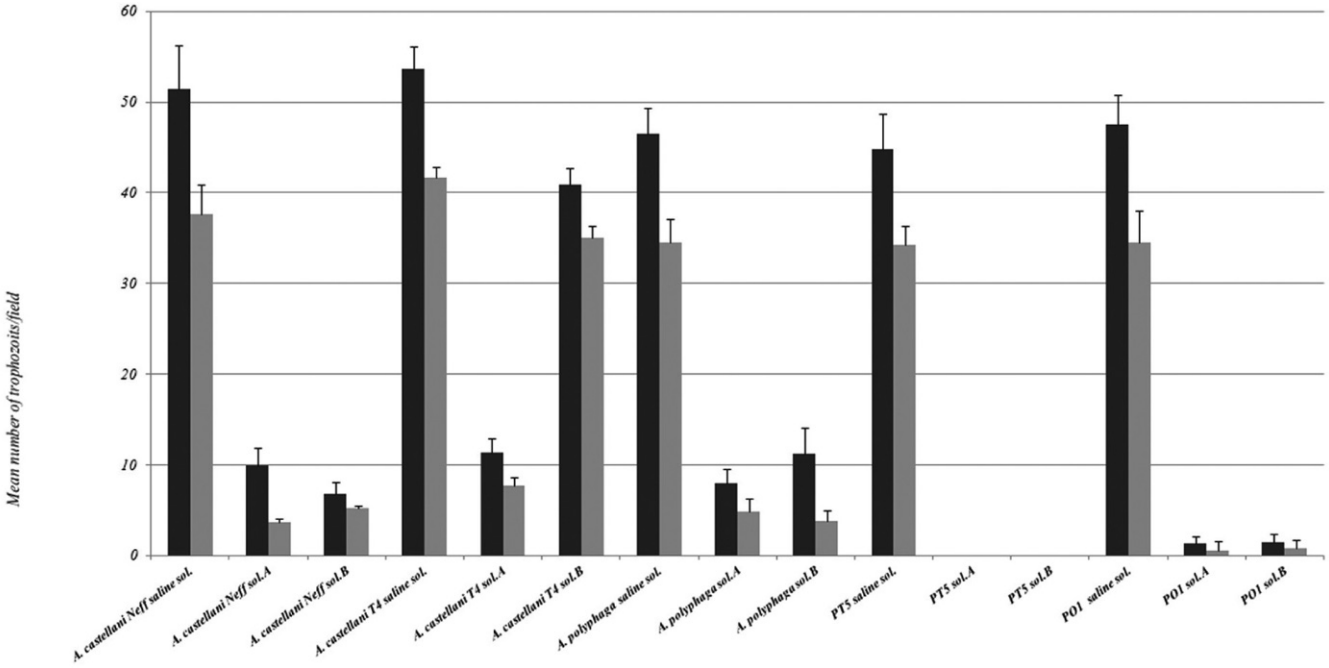

Fig. 1. Number of trophozoites per field after treatment with solutions A and B and a saline solution (negative control), time intervals tested for *Acanthamoeba castellani* Neff, *Acathamoeba castellani* T4, *Acanthamoeba polyphaga*, and isolates PT5 and PO1. Data represent mean ± standard deviation

Results

The two solutions evaluated did not kill off all the amoebae of ATCC strains and the swimming-pool isolate (PO1) of *Acanthamoeba* after 4 h of immersion of the NNA blocks containing protozoan trophozoites, in triplicate testing. The *Acanthamoeba castellani* Neff strain showed growth in all plates after 24 h contact with solution B, and in two of three plates after 24 h contact with solution A. *Acanthamoeba castellani* T4 grew in all plates after 24 h in contact with solution A, and in two of three plates after 24 h with solution B. *Acanthamoeba polyphaga* and isolate PO1 grew in all plates after 24 h of contact with solutions A and B. PT5 isolate showed no growth in solutions A and B over both time intervals tested (4 and 24h) when inspected after 72 h of incubation. Then these plates were following until the tenth day of the incubation and no growth was observed. The results are presented in Table II.

Analyzing the average growth per field, all strains and isolates showed a higher growth rate when in contact with the saline solution, in comparison to the multi-purpose solutions. Comparing the contact periods, the organisms that were in contact for 24 h had a lower growth rate than those that remained for 4 h in all solutions. The exception was strain PT5, which showed no growth in either time interval.

Regarding the efficacy of the solutions, *A. castellani* strain T4 grew more slowly in solution A than in solution B. The other isolates showed no significant differences in growth in the two solutions. The results are shown in Figure 1.

Discussion

Acanthamoeba is a widely distributed organism in the environment, and is commonly found in anthropogenic aquatic environments. Its resistant form tolerates adverse environmental conditions, allowing it to survive in the various physical and chemical treatments, and arousing the interest of many researchers in the study of this amphizoic protozoan. Carlesso *et al.* (2007) isolated and characterized amoebae of the genus *Acanthamoeba* and *Naegleria* in biofilms and dust, from a hospital in southern Brazil. In this study, the most prevalent genus found was *Acanthamoeba*. Caumo *et al.* (2009), evaluating the presence of *Acanthamoeba* in swimming pools, found that some water samples studied were positive for free-living amoebae, all identified as belonging to the genus *Acanthamoeba*. The characterization of *Acanthamoeba* isolates by thermal and osmotolerance assays indicated that some of the isolates were potentially pathogenic. Pens *et al.* (2008), evaluating the presence of bacteria and *Acanthamoeba* spp. in the biofilm and storage liquid of contact-lens cases of volunteers also from a hospital in southern Brazil, found positive samples. In this study, younger users of contact lenses showed more contamination in their contact-lens cases.

In our study, only isolate PT5, which belongs to the T5 genotype, showed susceptibility to the multipurpose solutions. However, studies comparing the T4 and T5 genotypes for resistance to multipurpose solutions, curiously, showed that the T5 genotype has a higher resistance (Hiti *et al.* 2002; Shoff *et al.* 2007). Only isolates of the T2, T3, T4, T6, T11 (Walo-

chnick *et al.* 2000; De Jonckheere 2003) and T5 (albeit rarely) (Spanakos *et al.* 2006; Iovieno *et al.* 2010) genotypes have been associated with keratitis. The T4 genotype is found in 90% of the cases of AK and is the most prevalent genotype found in clinical and environmental samples (Yu *et al*. 2004; Molmeret *et al*. 2005).

In the present study, we observed that the two solutions studied were not completely effective against most strains and isolates over the time intervals tested, and only a swimming-pool isolate (PT5) showed susceptibility. This shows that the recommendation by the product manufacturers that it is possible to dispense with rubbing the lens with the fingers when multipurpose solutions are used for cleaning, is questionable. As discussed in a previous study (Lipener and Ray 2008), several factors can contribute to infections in contact-lens wearers, such as poor maintenance and inadequate hygiene, including in this case by eliminating the digital-rubbing step. In another study (Shoff *et al.* 2008), *Acanthamoeba* trophozoites of different origins, belonging to the T4 genotype, were tested against the hydrogen-peroxide systems and the multipurpose solutions. The results demonstrated the ineffectiveness of multipurpose solutions against *Acanthamoeba* strains, and the two hydrogen-peroxide systems tested were much more efficient. In 2007, the Centers for Disease Control and Prevention (CDC) started a study of 22 national centers for ophthalmology in the United States, to assess whether the possible increase in the number of AK cases found in central Illinois, was also occurring in other U.S. centers. Data received from 13 centers also demonstrated an increase in cases of AK, and the investigation revealed a relationship between cases of keratitis and the use of Complete MoisturePlus multipurpose solution. At that time the manufacturer undertook a voluntary recall of the product (Bryan *et al.* 2010).

In another study (Patel and Hammersmith 2008), a literature review of infections in contact lenses and their relationship to the multi-purpose solutions was performed, which indicated a rise in infections caused by *Acanthamoeba* and *Fusarium* fungus. Moreover, it was found that multipurpose solutions are more effective against bacteria than against fungi and *Acanthamoeba*, and that these formulas are not as effective under real-world conditions.

However, it is very important to inform users about the risks caused by the substitution of solutions for cleaning and disinfection by saline, which does not clean or remove protein deposits from lenses, leaving them dirtier, contaminated, and less comfortable. Moreover, the saline environment can be conducive to the proliferation of microorganisms that can affect eye health. Another problem is crystallization of the serum, in other words, salt (sodium chloride) crystallizes and adheres to the lenses, and may cause discomfort and reduce their useful life. Indeed, the major cause of toxic reactions in contact-lens users is the use of saline, because of the presence of chemical preservatives in its composition (Lipener and Ray 2008). However, a large number of people prefer physiological solutions than other cleaning systems, because of their affordable cost.

The results of this study are similar to those found by most authors who have studied the effectiveness of disinfection and preservation solutions for contact lenses against *Acanthamoeba*. The incidence of *Acanthamoeba* keratitis has been increasing in recent years, related to the higher frequency of contact-lens use, so it is important that the use, cleaning, handling, and storage are done with medical supervision, since with proper maintenance, the risk of developing eye disorders will be much lower. As for manufacturers, larger studies are needed regarding the efficacy of multipurpose solutions against *Acanthamoeba* spp., thus increasing the quality and reliability of their products, and perhaps the return of the recommendation for including digital rubbing in the contact-lens cleaning step.

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