Resistance of *Acanthamoeba* Cysts to Disinfection in Multiple Contact Lens Solutions[▽]

Stephanie P. Johnston,^{1*} Rama Sriram,¹ Yvonne Qvarnstrom,¹ Sharon Roy,¹ Jennifer Verani,¹ Jonathan Yoder,¹ Suchita Lorick,^{2,3} Jacquelin Roberts,¹ Michael J. Beach,¹ and Govinda Visvesvara¹

Division of Parasitic Diseases,¹ Division of Immunization Services,² and Epidemic Intelligence Service Program,³ Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia

Received 20 March 2009/Accepted 20 April 2009

Acanthamoebae are free-living amoebae found in the environment, including soil, freshwater, brackish water, seawater, hot tubs, and Jacuzzis. *Acanthamoeba* species can cause keratitis, a painful vision-threatening infection of the cornea, and fatal granulomatous encephalitis in humans. More than 20 species of *Acanthamoeba* belonging to morphological groups I, II, and III distributed in 15 genotypes have been described. Among these, *Acanthamoeba castellanii*, *A. polyphaga*, and *A. hatchetti* are frequently identified as causing *Acanthamoeba* keratitis (AK). Improper contact lens care and contact with nonsterile water while wearing contact lenses are known risk factors for AK. During a recent multistate outbreak, AK was found to be associated with the use of Advanced Medical Optics Complete MoisturePlus multipurpose contact lens solution, which was hypothesized to have had insufficient anti-*Acanthamoeba* activity. As part of the investigation of that outbreak, we compared the efficacies of 11 different contact lens solutions against cysts of *A. castellanii*, *A. polyphaga*, and *A. hatchetti* (the isolates of all species were genotype T4), which were isolated in 2007 from specimens obtained during the outbreak investigation. The data, generated with *A. castellanii*, *A. polyphaga*, and *A. hatchetti* cysts, suggest that the two contact lens solutions containing hydrogen peroxide were the only solutions that showed any disinfection ability, with 0% and 66% growth, respectively, being detected with *A. castellanii* and 0% and 33% growth, respectively, being detected with *A. polyphaga*. There was no statistically significant difference in disinfection efficacy between the 11 solutions for *A. hatchetti*.

Acanthamoebae, which are free-living amoebae, occur worldwide in soil and water. It has been isolated from ponds, lakes, brackish water, and seawater; filters of heating, ventilating, and air-conditioning units; medical equipment, such as gastric wash tubing, dental irrigation units, contact lenses, and contact lens solutions; as well as vegetables, cell cultures, and even human and animal tissues (7, 23, 39). It has also been isolated from toxic waste dumpsites with high levels of pesticides, herbicides, pharmaceuticals, heavy metals, and polychlorinated biphenyls (35). *Acanthamoeba* species have two stages in their life cycle: a vegetative or trophozoite stage that reproduces by binary fission and that feeds voraciously on the bacteria and detritus present in the environment and a nondividing, cyst stage that is resistant to environmental stress. *Acanthamoeba* amoebae cause different types of human disease, including central nervous system infections (granulomatous amebic encephalitis, cutaneous infections) *Acanthamoeba* dermatitis, and ocular infections (*Acanthamoeba* keratitis [AK]). Granulomatous amebic encephalitis and cutaneous infections principally occur in immunocompromised individuals, including patients with human immunodeficiency virus infection or AIDS (17, 23, 37, 43). In contrast, AK principally occurs in immunocompetent individuals.

AK is a painful vision-threatening infection, which, if it is not treated promptly, may lead to ulceration of the cornea, a loss of

visual acuity, and, eventually, blindness (7, 15, 16). AK is associated with trauma to the cornea and with contact lens wear as a result of poor lens care and hygiene. When introduced into the eye by a contaminated contact lens, *Acanthamoeba* amoebae may adhere to the corneal surface and subsequently infiltrate the stroma and cause tissue damage (10). Both *Acanthamoeba* cysts and trophozoites can be isolated by culture from corneal scrapings or biopsy specimens and from contact lens paraphernalia (23, 43). Confocal microscopy has been used as an aid for the diagnosis of AK (29). Molecular techniques such as real-time PCR assays have been developed for the identification of *Acanthamoeba* species (32, 33). Sequencing analysis of the 18S rRNA gene has been used to identify as many as 15 genotypes of *Acanthamoeba*, of which the T4 genotype appears to be the most common in the environment and in patients with AK (2, 23).

The first documented case of AK in the United States occurred in 1973 in a south Texas rancher following trauma to his right eye (15, 40, 42). Both trophozoite and cyst stages of *Acanthamoeba polyphaga* were demonstrated in corneal sections. Between October 1985 and August 1986, Stehr-Green et al. (41) conducted a case-control study to investigate an outbreak of AK in the United States. The majority of case patients wore contact lenses, and illness was most commonly associated with the use of homemade saline solutions and lens care practices, such as the disinfection of the lenses less frequently than recommended and swimming while wearing contact lenses (8, 41). Contact lens use is now considered an important risk factor for AK in the United States. AK cases have continued to be diagnosed since the 1986 outbreak, but because AK is not a reportable disease in the United States, the actual number of cases occurring each year is not known.

* Corresponding author. Mailing address: Division of Parasitic Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, NE, MS F-36, Atlanta, GA 30341-3724. Phone: (770) 488-7044. Fax: (770) 488-3115. E-mail: sjohnston@cdc.gov.

[▽] Published ahead of print on 29 April 2009.

TABLE 1. Contact lens solutions tested and their ingredients

Contact lens Solution	Active ingredient(s)	Other ingredients
Alcon Opti-Clean II Alcon Opti-Free Express	PolyQuad (0.001%) PolyQuad (0.001%), Aldox (0.0005%)	Tween 21, MicroClens, edetate disodium (0.1%) Sodium citrate, sodium chloride, boric acid, sorbitol, AMP-95, Tetronic 1304, edetate disodium (0.05%)
Alcon Opti-Free RepleniSH	Propylene glycol, PolyQuad (0.001%), Aldox (0.0005%)	Sodium citrate, sodium chloride, sodium borate, TearGlyde, Tetronic 1304, nonannoyl ethylenediaminetriacetic acid
AMO Complete MoisturePlus	Polyhexamethylene biguanide (0.0001%), Poloxamer 237	Hydroxypropyl methylcellulose, propylene glycol, phosphate, taurine, edetate disodium, sodium chloride, potassium chloride, water
AMO UltraCare ^a	Hydrogen peroxide (3%)	Sodium stannate, sodium nitrate; buffered with phosphates and water
Bausch & Lomb Boston Simplus	Chlorhexidine gluconate (0.003%), polyaminopropyl biguanide (0.0005%)	Poloxamine, hydroxyalkylphosphonate, boric acid, sodium borate, sodium chloride, hydroxypropylmethyl cellulose, Glucan
Bausch & Lomb ReNu MoistureLoc	Alexidine (0.00045%)	Boric acid, sodium chloride, sodium phosphate, hydranate, poloxamine, MoistureLoc
Bausch & Lomb ReNu MultiPlus	Dymed (polyaminopropyl biguanide; 0.0001%)	Hydranate, boric acid, edetate disodium, poloxamine, sodium borate, sodium chloride
Ciba Vision Clear Care ^a	Hydrogen peroxide (3%)	Sodium chloride (0.79%), phosphonic acid, phosphate-buffered system, Pluronic 17R4
Ciba Vision AQuify	Polyhexanide (0.0001%)	Sorbitol, tromethamine, pluronic F127, sodium phosphate, dihydrogen, dexpantenol, edetate disodium dehydrate
Kirkland Signature Multipurpose Solution	Polyaminopropyl biguanide (0.0001%)	Poloxamer 237, edetate disodium, sodium chloride, potassium chloride, water

^a Hydrogen peroxide-containing solution.

A recent study indicated a dramatic increase in the number of AK cases in the Chicago, IL, area (16). An investigation conducted by the Centers for Disease Control and Prevention (CDC) revealed that this increase in the number of AK cases was occurring nationwide, starting in 2004 and continuing through 2007 (7). A subsequent investigation identified the use of Advanced Medical Optics (AMO) Complete MoisturePlus multipurpose contact lens solution as the primary risk factor, leading to an international recall of this product by the manufacturer (7, 16). We therefore decided to examine this and other frequently used major contact lens solutions for their efficacies against *Acanthamoeba* species isolated from clinical samples collected during the 2007 AK outbreak investigation.

MATERIALS AND METHODS

Isolation of *Acanthamoeba*. During the 2007 AK outbreak investigation, 94 specimens from patients were collected and cultured on nonnutrient agar plates coated with a layer of *Escherichia coli*. In the 24 plates that were positive, the amoebae consumed the bacteria, multiplied, and encysted after most of the bacteria were gone. Both trophozoites and cysts were examined microscopically and were assigned to morphological group II. In addition, cyst morphology was used for identification of the amoebae to the species level (28, 34). One isolate each of *A. castellanii* (isolate CDC:V568), *A. polyphaga* (isolate CDC:V572), and *A. hatchetti* (isolate CDC:V573) were selected for genotyping, as these species of *Acanthamoeba* are commonly found in the United States. All three isolates were found to be genotype T4 by sequencing analysis of the 18S rRNA gene, as described previously (2, 22, 36).

Contact lens solutions. Eleven different contact lens solutions were tested: Alcon Opti-Clean II, Alcon Opti-Free Express, Alcon Opti-Free RepleniSH, AMO Complete MoisturePlus, AMO UltraCare, Bausch & Lomb Boston Simplus, Bausch & Lomb ReNu MoistureLoc, Bausch & Lomb ReNu MultiPlus, Ciba Vision Clear Care, Ciba Vision AQuify, and Kirkland Signature Multipurpose Solution. These 11 solutions were selected for this study because they were brands used by case patients in the 2004 to 2007 AK outbreak. Ten of the 11 solutions were purchased from retail stores in the Atlanta, GA, area. The remaining solution, Bausch & Lomb ReNu MoistureLoc, was removed from the

market in 2006 because it was the brand of multipurpose contact lens solution associated with an outbreak of *Fusarium* keratitis (9). This solution was provided by colleagues at CDC who had kept a supply of this solution following the investigation of the *Fusarium* keratitis outbreak. The solutions used in this study, along with their active ingredients and disinfectant properties, are listed in Table 1.

To study the effects of various contact lens solutions against the cysts of the three species of *Acanthamoeba*, the amoebae were grown on agar plates for 3 weeks with *E. coli*. When most of the bacteria were consumed, trophozoites began to differentiate into cysts, and by the third week, the agar plates were covered with cysts. Cysts were harvested from the agar plates, washed three times with 50 ml of amoeba saline, counted in a hemacytometer, and adjusted to yield 100 cysts per 10 μ l.

The lens cases used with the nine non-hydrogen peroxide-containing solutions hold 1 ml of contact lens solution. Therefore, 10 μ l of the cyst-containing solution was added to 1 ml of each contact solution (Alcon Opti-Clean II, Alcon Opti-Free Express, Alcon Opti-Free RepleniSH, AMO Complete MoisturePlus, Bausch & Lomb Boston Simplus, Bausch & Lomb ReNu MoistureLoc, Bausch & Lomb ReNu MultiPlus, Ciba Vision AQuify, and Kirkland Signature Multipurpose Solution) in 15-ml tubes, in triplicate, and incubated at 24°C for either 4 or 6 h (according to the manufacturers' contact lens soaking time recommendations) and for 24 h.

The two hydrogen peroxide-containing solutions (AMO UltraCare and Ciba Vision Clear Care) require the use of lens cases, provided in the box, that need to be filled with the contact lens solution to the fill line (approximately 5 ml) of the case. Therefore, 10 μ l of the cyst-containing solution was added to the contact lens cases that had already been filled with the contact lens solutions (along with the neutralizing tablet provided with AMO UltraCare), in triplicate, and incubated at 24°C for either 6 or 24 h. AMO UltraCare includes a neutralizing tablet that must be added to the contact lens solution in the contact lens case, while Ciba Vision Clear Care has a built-in neutralizing disc within the contact lens case.

After incubation, the cysts were washed by centrifugation at $1,500 \times g$ for 10 min, inoculated on agar plates coated with *E. coli*, and incubated at 24°C. The plates were examined daily for 2 weeks with an inverted microscope for the presence of trophozoites, and the efficacies of the solutions were recorded as positive or negative.

Statistical analysis. The Cochran-Mantel-Haenszel test was used to test for the overall association between the number of positive plates and the contact

TABLE 2. Two contact lens solutions containing hydrogen peroxide tested with *A. castellanii*, *A. polyphaga*, and *A. hatchetti* at 6 h and 24 h of contact

Contact lens solution (manufacturer-recommended contact time)	No. (%) of plates positive for the following amoebae at the indicated times:					
	<i>A. castellanii</i>		<i>A. polyphaga</i>		<i>A. hatchetti</i>	
	6 h	24 h	6 h	24 h	6 h	24 h
AMO UltraCare (6 h)	2/3 (66)	2/3 (66)	1/3 (33)	2/3 (66)	2/3 (66)	1/3 (33)
Ciba Vision Clear Care (6 h)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	1/3 (33)	0/3 (0)

lens solutions, controlling for the three *Acanthamoeba* species, at 4 to 6 h and 24 h of incubation. Fisher's exact test was used to compare the number of plates positive for each species. All analyses were performed with SAS (version 9.1) software (SAS Institute Inc., Cary, NC). Statistical significance was set at an alpha level of 0.05.

RESULTS

Of the 11 contact lens solutions that were examined for their efficacies in inactivating cysts of the three *Acanthamoeba* species, one of these solutions that contained hydrogen peroxide (Ciba Vision Clear Care) demonstrated the greatest inactivation of cysts of all three species of *Acanthamoeba* (Table 2). Overall, there were no statistically significant differences in the susceptibilities of the three *Acanthamoeba* species to the contact lens solutions tested. All three species were the most responsive to the Ciba Vision Clear Care solution, which was the only solution that prevented excystation under the experimental conditions used in this study.

Considering all *Acanthamoeba* species together, there were statistically significant differences in the efficacies of the different brands of contact lens solutions at both 4 to 6 h ($P < 0.0001$) and 24 h ($P < 0.0001$) of incubation. At 4 to 6 h of incubation, there were statistically significant differences in disinfection efficacy between the 11 solutions for *A. castellanii* ($P = 0.008$) and *A. polyphaga* ($P = 0.0014$). Specifically, the Ciba Vision Clear Care and AMO UltraCare solutions, both of which contained hydrogen peroxide, were the only solutions that showed any disinfection ability, showing 0% and 66% growth, respectively, for *A. castellanii* and 0% and 33% growth, respectively, for *A. polyphaga*. There was no statistically significant difference in disinfection efficacy between the 11 solutions for *A. hatchetti*. Overall, the differences in the efficacies of the solutions between species at 4 to 6 h incubation were not

significant with *A. castellanii*, *A. polyphaga*, and *A. hatchetti*, for which 87.9% (29/33), 84.9% (28/33), and 90.9% (30/33) of the plates were positive, respectively.

At 24 h of incubation, there were statistically significant differences in disinfection efficacies between the 11 solutions for *A. castellanii* ($P = 0.0081$) and *A. hatchetti* ($P = 0.0264$) but not *A. polyphaga*. In addition to the Ciba Vision Clear Care and AMO UltraCare solutions, several non-hydrogen peroxide-containing solutions also showed some disinfection ability at 24 h of incubation (Tables 2 and 3). Overall, the differences in the efficacies of the solutions against the species at 24 h of incubation were not significant for *A. castellanii*, *A. polyphaga*, and *A. hatchetti*, for which 81.8% (27/33), 69.7% (23/33), and 78.8% (26/33) of the plates were positive, respectively.

DISCUSSION

Contact lens wear is the most common risk factor for the development of AK in the United States; 85% of cases occur in contact lens wearers (30). Studies demonstrate that nearly all rigid and soft contact lens solutions sold in the United States have inadequate *Acanthamoeba* disinfection efficacy (1, 3, 4, 5, 12, 13, 14, 16, 19, 20, 25, 26, 37, 39).

The two most common types of solution used for contact lens disinfection are (i) the multipurpose solution, in which a single solution is used for cleaning, disinfecting, and storing the lenses, and (ii) the hydrogen peroxide-based system, in which either a single solution or multiple products are used for disinfecting and storing the lenses (13, 39). Hydrogen peroxide is known to be very effective at contact lens disinfection due to its broad activity against bacteria, fungi, and *Acanthamoeba* species and its ability to destroy these pathogens by oxidation (13). It is active against *Acanthamoeba* cysts when a concentration of

TABLE 3. Nine non-hydrogen peroxide-containing contact lens solutions tested with *A. castellanii*, *A. polyphaga*, and *A. hatchetti* at 4 to 6 h and 24 h of incubation

Contact lens solution (manufacturer-recommended contact time)	No. (%) of plates positive for the following amoebae at the indicated times:					
	<i>A. castellanii</i>		<i>A. polyphaga</i>		<i>A. hatchetti</i>	
	4–6 h	24 h	4–6 h	24 h	4–6 h	24 h
Alcon Opti-Clean II (4 h)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)
Alcon Opti-Free Express (6 h)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)
Alcon Opti-Free RepleniSH (6 h)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)
AMO Complete MoisturePlus (4 h)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)
Bausch & Lomb Boston Simplus (4 h)	3/3 (100)	1/3 (33)	3/3 (100)	2/3 (66)	3/3 (100)	2/3 (66)
Bausch & Lomb ReNu MoistureLoc (4 h)	3/3 (100)	3/3 (100)	3/3 (100)	2/3 (66)	3/3 (100)	2/3 (66)
Bausch & Lomb ReNu MultiPlus (4 h)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)
Ciba Vision AQuify (4 h)	3/3 (100)	3/3 (100)	3/3 (100)	1/3 (33)	3/3 (100)	3/3 (100)
Kirkland Signature Multipurpose Solution (6 h)	3/3 (100)	3/3 (100)	3/3 (100)	1/3 (33)	3/3 (100)	3/3 (100)

3% and an exposure time of at least 6 h are used (13). Currently, only two hydrogen peroxide-based contact lens disinfection systems are available in the United States. Only one of these, Ciba Vision Clear Care solution, is based on a single-step hydrogen peroxide solution and does not require a separate neutralization step. This solution disinfects and cleans the lenses if they are soaked for 6 h or overnight. AMO UltraCare solution is also a hydrogen peroxide-based contact lens system that is available in the United States, but it includes a neutralization tablet that is added to the solution while the lenses are being disinfected. Other two-step hydrogen peroxide solutions that use a separate neutralization step are no longer available in the United States (39).

The results of this study indicated that Ciba Vision Clear Care solution containing 3% hydrogen peroxide was 100% effective at killing cysts of *A. castellanii* and *A. polyphaga* at both 6 and 24 h. For *A. hatchetti*, it was 66% effective at killing cysts at 6 h but 100% effective at 24 h, although this difference was not statistically significant. Surprisingly, AMO UltraCare solution, which also contains 3% hydrogen peroxide, did not show the same disinfection efficacy. Of the nine non-hydrogen peroxide-containing solutions tested in the current study, only four solutions, Bausch & Lomb Boston Simplus (used for gas-permeant contact lenses and not soft lenses), Bausch & Lomb ReNu MoistureLoc, Ciba Vision AQuify, and Kirkland Signature Multipurpose Solution, had any effect on *Acanthamoeba* cysts (Table 3). We tested the efficacy of Bausch & Lomb ReNu MoistureLoc solution, even though the production of this product ceased after the *Fusarium* outbreak of 2006 (9), because that contact lens solution was very popular before it was pulled from the market. The solutions without hydrogen peroxide had various degrees of activity against *Acanthamoeba* amoebae, but none had activity at 4 to 6 h of incubation. Although the four contact lens solutions mentioned above had some activity against particular species of *Acanthamoeba* after 24 h of incubation, these differences were not statistically significant and most contact lens wearers do not soak lenses longer than 8 to 12 h (overnight).

Current International Organization for Standardization (ISO) and Food and Drug Administration (FDA) regulations do not provide guidelines for testing of the efficacies of contact lens solutions against *Acanthamoeba* species (3, 16, 30). Without an accepted standard for testing, the procedures used and reported in studies that test contact lens solutions are highly variable. Strains differ and the methods of cultivation and cyst production vary, thus clouding the interpretation of the results (1, 3, 5, 11, 12–14, 19, 20, 25, 26, 31, 38, 39). Shoff et al. (39) used five different *Acanthamoeba* strains, all of which belonged to genotype T4 but which were isolated from different sources (including AK patients and tap water), and found differential responses among the various isolates to the different contact lens solutions. They found an overall survival of 54.4% for Ciba Vision Clear Care solution and 25.5% survival for AMO UltraCare solution (39). One isolate recovered from Chicago tap water was the most resistant strain; it survived in all solutions tested at 24 h of incubation except the AMO UltraCare solution. The reason for the variance in the results between studies is unclear but might be due to inherent differences that exist in strains isolated from different geographic areas, possibly be-

cause of the development of resistance after exposure to different toxic chemicals in the environment.

In one study by Borazjani and Kilvington (3), existing ISO and FDA guidelines for the testing of the efficacies of contact lens solutions against bacteria and fungi were modified to test for *Acanthamoeba* species. A 3-log-unit reduction in the number of *Acanthamoeba* amoebae was required to establish efficacy by the use of these guidelines. Of the four no-rub/rinse solutions tested, Bausch & Lomb ReNu MoistureLoc achieved a ≥ 3 -log-unit reduction in the numbers of trophozoites and cysts of the *Acanthamoeba* species; the Alcon Opti-Free Express solution was also highly effective and achieved a ≥ 3 -log-unit reduction of trophozoites within 6 h.

In another study, it was determined that certain commercial products that contain propylene glycol induce *Acanthamoeba* encystment (20). However, a reduction or absence of encystment has been observed with other commercial solutions containing propylene glycol, suggesting that additional factors, such as buffering systems, may be involved (20).

Testing standards need to be developed to evaluate the efficacies of contact lens solutions against *Acanthamoeba* cysts. To date different strains and species of *Acanthamoeba* have been used by various investigators, and this presents several challenges. First, most investigators have used strains that were isolated many years ago and that have thus continuously grown axenically for many years. Hence, these strains are highly selected and may not truly represent the isolates that are currently causing AK in patients. In a recent paper, Köhler et al. (21) demonstrated that *Acanthamoeba* strains, especially those that have been in axenic cultivation for a number of years, not only lose their ability to encyst synchronously but also experience a decline in their encystment potential. This is in part because of the downregulation of certain genes that are essential for the survival of strains under inhospitable conditions. Amoebae grown continuously in axenic medium are provided with abundant nutrition and a constant temperature and, hence, do not need to develop strategies for survival. In contrast, newly isolated strains from AK patients have been subjected to inhospitable conditions, including desiccation and contamination with toxic substances in their milieus. Furthermore, it has been shown that continuous cultivation in an axenic medium makes the amoebae lose their virulence (24, 37).

A second challenge is the way in which the amoebae are processed for testing. Most of the researchers have used axenically grown amoebae that have been induced to produce cysts by nutrient deprivation in the presence of Mg^{2+} (11, 27). Encystment in such media may not always produce 100% mature cysts, which may in turn affect the biocide resistance of the cysts. A mature cyst has two layers in the cyst wall: an outer wrinkled ectocyst that is made of protein and an inner thick, stellate, polygonal, triangular or round endocyst largely consisting of cellulose which is very resistant to physical and chemical agents. Any interference in the maturation process will unduly affect the resistance of the endocyst because resistance to biocides develops during the cellulose synthesis phase of encystment. Previous studies have shown that inadequate aeration and improper control of pH may also hamper encystment (e.g., 8% encystment versus $>80\%$ encystment with aeration and no pH control [6, 27]), leading to imperfect cyst wall synthesis. Variation in buffers and the inclusion of a chelating

agent (EDTA) or the use of dimethyl sulfoxide in the test solutions may also adversely affect the efficacies of the biocides (18, 42).

Hughes et al. (14) showed that strain age, the number of passages in axenic culture, and the method of encystment have great influences on the efficacies of therapeutic agents used to kill cysts. Kilvington and Anger (19) also suggested that these differences may be due to the different methods of cyst production, which may explain the discrepancies in the cysticidal efficacies of disinfectants reported by many investigators. Another important factor to consider is the time that the cysts were stored prior to their use in testing.

Because of all these challenges, we elected to use amoebae that were directly isolated from patient specimens and then grown with *E. coli*. Since encystation in starvation medium does not always produce synchronized cyst formation, we used cysts that were generated by growing the amoebae on agar plates coated with bacteria, a process that occurs in nature (19, 21, 26).

The prevention of future cases of AK will require contact lens solutions that are effective against *Acanthamoeba* species and continued emphasis on proper lens care hygiene. Educating contact lens wearers about the risk factors for AK, including the improper use of contact lens solutions, is important; but a systematic method for evaluating contact lens solutions will reduce the chance that inefficacious solutions are available. We strongly urge the adoption of standardized procedures for determining the efficacy of contact lens solutions for the disinfection of *Acanthamoeba* amoebae in order to reduce the incidence of AK associated with the use of inefficacious contact lens solutions. FDA held an initial meeting in June 2008 to begin addressing the need for standardizing procedures for determination of the efficacy of contact lens solutions for the disinfection of *Acanthamoeba* amoebae (www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfadvistory/details.cfm?mtg=699). Subsequently, FDA held a workshop in Silver Spring, MD, in January 2009 titled Microbiological Testing of Contact Lens Care Products, during which it was decided to include cysts and trophozoites of *Acanthamoeba* species in manufacturer's testing of contact lens solutions (http://www.jcahpo.org/clmw/pdf/FDA_PostMeeting2.pdf). These meetings are the first steps toward improving the testing of the efficacies of contact lens solutions against *Acanthamoeba* amoebae and AK disease in an area that has not been well standardized.

ACKNOWLEDGMENTS

The findings and conclusions in this journal article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the Agency for Toxic Substances and Disease Registry.

The use of trade names is for identification only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

REFERENCES

- Beattie, T. K., D. V. Seal, A. Tomlinson, A. K. McFadyen, and A. M. Grimsom. 2003. Determination of amebicidal activities of multipurpose contact lens solutions by using a most probable number enumeration technique. *J. Clin. Microbiol.* **41**:2992–3000.
- Booton, G. C., D. J. Kelly, Y. W. Chu, et al. 2002. 18S ribosomal DNA typing and tracking of *Acanthamoeba* species isolates from corneal scrape specimens, contact lenses, lens cases, and home water supplies of *Acanthamoeba* keratitis patients in Hong Kong. *J. Clin. Microbiol.* **40**:1621–1625.
- Borazjani, R. N., and S. Kilvington. 2005. Efficacy of multipurpose solutions against *Acanthamoeba* species. *Contact Lens Ant. Eye* **28**:169–175.
- Buck, S. L., R. A. Rosenthal, and R. L. Abshire. 1998. Amoebicidal activity of a preserved contact lens multipurpose disinfecting solution compared to a disinfection/neutralization peroxide system. *Contact Lens Ant. Eye* **21**:81–84.
- Buck, S. L., R. A. Rosenthal, and B. A. Schlech. 2000. Methods used to evaluate the effectiveness of contact lens care solutions and other compounds against *Acanthamoeba*: a review of literature. *CLAO J.* **26**:72–84.
- Byers, T. J., R. A. Akins, B. J. Maynard, R. A. Lefken, and S. M. Martin. 1980. Rapid growth of *Acanthamoeba* in defined media; induction of encystment by glucose-acetate starvation. *J. Protozool.* **27**:216–219.
- Centers for Disease Control and Prevention. 2007. *Acanthamoeba* keratitis—multiple states, 2005–2007. *MMWR Morb. Mortal. Wkly. Rep.* **56**:1–3.
- Centers for Disease Control and Prevention. 1987. *Acanthamoeba* keratitis in soft-contact lens wearers. *MMWR Morb. Mortal. Wkly. Rep.* **36**:403–404.
- Chang, D. C., G. B. Grant, K. O'Donnell, K. A. Wannemuehler, J. Noble-Wang, C. Y. Rao, L. M. Jacobson, et al. 2006. Multistate outbreak of *Fusarium* keratitis associated with use of a contact lens solution. *JAMA* **296**:953–963.
- Clark, D. W., and J. Y. Niederkorn. 2006. The pathophysiology of *Acanthamoeba* keratitis. *Trends Parasitol.* **22**:175–180.
- Hirukawa, Y., H. Nakato, S. Izumi, T. Tsuruhara, and S. Tomino. 1998. Structure and expression of a cyst protein of *Acanthamoeba castellanii*. *Biochim. Biophys. Acta* **1398**:47–56.
- Hiti, K., J. Walochnik, C. Faschinger, E. M. Haller-Schober, and H. Aspöck. 2005. One- and two-step hydrogen peroxide contact lens disinfection solutions against *Acanthamoeba*: How effective are they? *Eye* **19**:1301–1305.
- Hughes, R., and S. Kilvington. 2001. Comparison of hydrogen peroxide contact lens disinfection systems and solutions against *Acanthamoeba polyphaga*. *Antimicrob. Agents Chemother.* **45**:2038–2043.
- Hughes, R., W. Heaselgrave, and S. M. Kilvington. 2003. *Acanthamoeba polyphaga* strain age and method of cyst production influence the observed efficacy of therapeutic agents and contact lens disinfectants. *Antimicrob. Agents Chemother.* **47**:3080–3084.
- Jones, D. B., G. S. Visvesvara, and N. M. Robinson. 1975. *Acanthamoeba polyphaga* keratitis and *Acanthamoeba* uveitis associated with fatal meningoencephalitis. *Trans. Ophthalmol. Soc. UK* **95**:221–232.
- Joslin, C. E., E. Y. Tu, M. E. Shoff, G. C. Booton, P. A. Fuerst, T. T. McMahon, R. J. Anderson, M. S. Dworkin, J. Sugar, F. G. Davis, and L. T. Stayner. 2007. The association of contact lens solution use and *Acanthamoeba* keratitis. *Am. J. Ophthalmol.* **144**:169–180.
- Khan, N. A. 2006. *Acanthamoeba*: biology and increasing importance in human health. *FEMS Microbiol. Rev.* **30**:564–569.
- Khunkitti, W., D. Lloyd, J. R. Furr, and A. D. Russell. 1996. The lethal effects of biguanides on cysts and trophozoites of *Acanthamoeba castellanii*. *J. Appl. Bacteriol.* **81**:73–77.
- Kilvington, S., and C. Anger. 2001. A comparison of cyst age and assay method of the efficacy of contact lens disinfectants against *Acanthamoeba*. *Br. J. Ophthalmol.* **85**:336–340.
- Kilvington, S., W. Heaselgrave, J. M. Lally, K. Ambrus, and H. Powell. 2008. Encystment of *Acanthamoeba* during incubation in multipurpose contact lens disinfectant solutions and experimental infections. *Eye Contact Lens* **34**:133–139.
- Köhler, M., D. Leitsch, U. Färnkranz, M. Duchêne, H. Aspöck, and J. Walochnik. 2008. *Acanthamoeba* strains lose their abilities to encyst synchronously upon prolonged axenic culture. *Parasitol. Res.* **102**:1069–1072.
- Ledee, D. R., G. C. Booton, M. H. Awwad, S. Sharma, R. K. Aggarwal, I. A. Niszl, M. B. Markus, P. A. Fuerst, and T. J. Byers. 2003. Advantages of using mitochondrial 16S rDNA sequences to classify clinical isolates of *Acanthamoeba*. *Investig. Ophthalmol. Vision Sci.* **44**:29–36.
- Marciano-Cabral, F., and G. Cabral. 2003. *Acanthamoeba* spp. as agents of disease in humans. *Clin. Microbiol. Rev.* **16**:273–307.
- Mazur, T., E. Hadas, and I. Iwanicka. 1995. The duration of the cyst stage and the viability and virulence of *Acanthamoeba* isolates. *Trop. Med. Parasitol.* **46**:106–108.
- McBride, J., P. R. Ingram, F. L. Henriquez, and C. W. Roberts. 2005. Development of colorimetric microtiter plate assay for assessment of antimicrobials against *Acanthamoeba*. *J. Clin. Microbiol.* **43**:629–634.
- Mowrey-McKee, M., and M. George. 2007. Contact lens solution efficacy against *Acanthamoeba castellanii*. *Eye Contact Lens* **33**:211–215.
- Neff, R. J., S. A. Ray, W. F. Benton, and M. Wilborn. 1964. Induction of synchronous encystment (differentiation) in *Acanthamoeba* sp. *Methods Cell Physiol.* **1**:55–83.
- Page, F. C. 1988. A new key to freshwater and soil *Gymnamoeba*, p.119. Freshwater Biological Association, Ambleside, United Kingdom.
- Parmar, D. N., S. T. Awwad, W. M. Petroll, R. W. Bowman, J. P. McCully, and D. Cavanagh. 2006. Tandem scanning confocal corneal microscopy in the diagnosis of suspected *Acanthamoeba* keratitis. *Ophthalmology* **113**:538–547.
- Patel, A., and K. Hammersmith. 2008. Contact lens-related microbial keratitis: recent outbreaks. *Curr. Opin. Ophthalmol.* **19**:302–306.

31. Polat, Z. A., A. Vural, and A. Cetin. 2007. Efficacy of contact lens storage solutions against trophozoite and cyst of *Acanthamoeba castellanii* strain 1BU and their cytotoxic potential on corneal cells. *Parasitol. Res.* **101**:997–1001.
32. Qvarnstrom, Y., G. S. Visvesvara, R. Sriram, and A. J. da Silva. 2006. A multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. *J. Clin. Microbiol.* **44**: 3589–3595.
33. Riviere, D., F. M. Szczbara, J. M. Berjeaud, J. Frère, and Y. Hechard. 2006. Development of a real-time PCR assay for quantification of *Acanthamoeba* trophozoites and cysts. *J. Microbiol. Methods* **64**:78–83.
34. Sawyer, T. K., G. S. Visvesvara, and B. A. Harke. 1977. Pathogenic amoebas from brackish and ocean sediments, with a description of *Acanthamoeba hatchetti*, n. sp. *Science* **196**:1324–1325.
35. Sawyer, T. K., T. A. Neard, P.-M. Daggett, and S. M. Bodammer. 1987. Potentially pathogenic protozoa in sediments from oceanic sewage-disposal sites, p. 183–194. In J. M. Capuzzo and D. R. Kester (ed.), *Oceanic processes in marine pollution*, vol. 1. Robert E. Krieger Publishing Co., Malabar, FL.
36. Schroeder, J. M., G. C. Booton, J. Hay, I. A. Niszl, D. V. Seal, M. B. Markus, P. A. Fuerst, and T. J. Byers. 2001. Use of subgenetic 18S rDNA PCR and sequencing for generic and genotypic identification of *Acanthamoeba* from human cases of keratitis and from sewage sludge. *J. Clin. Microbiol.* **39**: 1903–1911.
37. Schuster, F. L., and G. S. Visvesvara. 2004. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. *Int. J. Parasitol.* **34**:1001–1027.
38. Shoff, M., A. Rogerson, S. Schatz, and D. Seal. 2007. Variable responses of *Acanthamoeba* strains to three multipurpose lens cleaning solutions. *Optom. Vis. Sci.* **84**:202–207.
39. Shoff, M. E., C. E. Joslin, E. Y. Tu, L. Kubatko, and P. A. Fuerst. 2008. Efficacy of contact lens systems against recent clinical and tap water *Acanthamoeba* isolates. *Cornea* **27**:713–719.
40. Stehr-Green, J., T. M. Bailey, and G. S. Visvesvara. 1989. The epidemiology of *Acanthamoeba* keratitis in the United States. *Am. J. Ophthalmol.* **107**: 331–336.
41. Stehr-Green, J., T. M. Bailey, F. H. Brandt, J. H. Carr, W. W. Bond, and G. S. Visvesvara. 1987. *Acanthamoeba* keratitis in soft contact lens wearers. *JAMA* **258**:57–60.
42. Turner, N. A., J. Harris, A. D. Russell, and D. Lloyd. 2000. Microbial differentiation and changes in susceptibility to antimicrobial agents. *J. Appl. Microbiol.* **89**:751–759.
43. Visvesvara, G. S., H. Moura, and F. L. Schuster. 2007. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. *FEMS Immunol. Med. Microbiol.* **50**:1–26.