ORIGINAL ARTICLES

Contamination of contact lens storage cases by *Acanthamoeba* and bacteria

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Abstract

In order to identify possible risk factors for microbial keratitis the storage cases for contact lenses of 102 asymptomatic lens wearers were tested for contamination by bacteria and free-living amoebae. Of this group 43 had significant counts of viable bacteria and only 40 had negligible counts. Seven had contamination by acanthamoebae, of whom six also had significant bacterial counts. These results were categorised according to the type of contact lens worn and the lens disinfection method. The high rates of contamination by apathogenic and pathogenic organisms, in particular *Acanthamoeba*, and the probable support by contaminating bacteria of *Acanthamoeba*, are discussed.

Contact lenses have become an increasingly popular mode of correction of refractive errors, and it is estimated that there are now 1·5 million lens wearers in the United Kingdom (G Jones, Association of Contact Lens Manufacturers, personal communication, 1989). Sight-threatening corneal infection by bacteria[1] or *Acanthamoeba*[2 3] is the most important complication of contact lens wear. Microbial contamination of the storage case is the usual source of infecting organisms.[4] Soft lens wearers are at greater risk of these infections, probably on account of enhanced microbial adherence to the lens material.[5 6] A previous survey by Donzis *et al*[7] of the cases of 100 asymptomatic lens wearers found that 46% were contaminated by bacteria and no cases by *Acanthamoeba* (although 'home-made' saline bottles of two patients were).

A study of the efficacy of lens disinfection was undertaken in which contamination of lens storage cases by bacteria and free-living amoebae (FLA) was ascertained. The study group of 102 included wearers of hard and soft lenses who used a variety of disinfection methods. Particular attention was given to *Acanthamoeba*, because there has been an exponential increase in incidence of reported *Acanthamoeba* keratitis in recent years.[3] This increase is primarily due to infection in contact lens wearers, and among lens wearers those that use home-prepared saline are recognised to be at highest risk.[3 8]

TABLE I *Contamination of hard and soft lens systems*

Lens type	*No.*	*TVBC <10/ml*	*TVBC >10⁴/ml*	*Acanthamoeba contam.*	*Other FLA contam.*
Hard	35	6	22	1	–
Soft	67	34	21	6	2
Total	102	40	43	7	2

The table shows numbers of patients with lens case contamination by bacteria, *Acanthamoeba*, and other free-living amoebae (FLA) categorised according to type of contact lens worn. For the purpose of statistical analysis a total viable bacterial count (TVBC) of <10/ml is considered negligible, and >10⁴/ml is considered significant. Note: the other FLA were one isolate each of *Vahlkamphia* and *Hartmannella*.

Patients and methods

PATIENTS

One hundred and two patients were recruited. They had been wearing daily-wear lenses for at least six months, were cosmetic lens wearers (using lenses only for correction of minor refractive errors), and had no eye disease. All patients were asymptomatic and were recruited from optometry practices at the time of scheduled review visits. Patients were instructed to bring their lens storage cases and disinfecting solutions, but were not informed about the contamination survey. Participating patients' storage cases were exchanged for new cases. The following information was recorded: lens type, lens disinfecting regimen used, and whether saline solutions were home prepared.

LABORATORY METHODS

Cases were shaken and opened under aseptic conditions. All solution was transferred to a sterile universal container. A sterile cotton-wool swab moistened with sterile unpreserved saline was then rubbed over the internal surface of the case and the tip added to the universal container. The contents of the container were then mixed in a vortex mixer for 10 seconds, and divided for bacterial and amoebal studies.

Bacterial isolation. Solution was diluted 1:10 into a disinfectant neutraliser and left to stand for 10 minutes. The medium used was that of Norton *et al*[9] modified here by the inclusion of 0·4 g/l of beef liver catalase for peroxide and sodium thiosulphate 20 g/l for chlorine. 10 µL aliquots of the neutralised sample were cultured on blood agar and on cysteine, lactose, electrolyte-deficient (CLED) plates. After incubation for two days the isolates showing colony morphology typical of *Serratia* and all non-lactose-fermenting organisms were sub-cultured and identified by the API 20E system

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Total viable bacterial counts (TVBC) were made by the pour-plate method using yeast extract agar.[10] Serial 10-fold dilutions to 10^{-6} were made in 1/4 strength Ringer's solution. Plates were incubated in air at 30°C for three days. A total viable count of $>10^6$/ml was considered significant for the purpose of analysis.

Amoebal isolation. Remaining storage case solution in the universal which had been vortexed was centrifuged at 2000 *g* for 10 minutes. The supernatant was aspirated and the pellet resuspended in 100 µl of neutraliser. Previous studies have shown that the neutraliser is not inhibitory to *Acanthamoeba* trophozoites or cysts (data not shown). This was inoculated on non-nutrient agar seeded with *Escherichia coli*[11] and incubated at 30°C. Plates were examined daily by low power microscopy for seven days. Amoebae were characterised to genus level according to cyst and trophozoite morphology.[11]

Pathogenicity of *Acanthamoeba* isolates was tested on African green monkey kidney (Vero) cell cultures.[12] Cell monolayers were inoculated with amoebae, incubated at 32°C, and examined daily for cytopathic effect.

Results

A total of 102 lens wearers participated in the study, 45 male and 57 female. The age range of the patients was 17–73 (mean 32) years. Of these, 35 (34·2%) wore hard or rigid gas-permeable lenses and 67 (65·7%) wore soft lenses. Eighty-two patients used chemical disinfectants, 19 used heat methods, and one used home-prepared saline solution without any recognised disinfecting regimen. In addition to the latter patient four prepared their own saline solution for rinsing after disinfection.

Of the total, the storage case solution of 43 (42%) patients had TVBC/ml of $>10^6$ and 40 had TVBC/ml <10. Nineteen had TVBC/ml between 10 and 10^6. The culture results for the two lens types are shown in Table I. The association between hard lens wear and significant bacterial contamination was statistically significant ($\chi^2=9{\cdot}38$; df=1; $p<0{\cdot}05$).

Culture results according to disinfectant method used are shown in Table II. The association of significant bacterial contamination with chlorhexidine was statistically significant ($\chi^2=5{\cdot}3$; df=1; $p<0{\cdot}05$). This association was not statistically significant for any other disinfection method.

TABLE II *Contamination associated with different lens disinfection systems*

Disinfecting method	*No.*	*TVBC <10/ml*	*TVBC >10⁶/ml*	*Acanthamoeba contam.*	*Other FLA contam.*
Chlorhexidine	35	10	20	–	1
Hydrogen peroxide	20	9	3	–	1
Heat	19	13	4	3	–
Chlorine based	9	1	6	2	–
Other chemical	18	7	9	1	–
None*	1	–	1	1	–

The table shows numbers of patients with lens case contamination by bacteria, *Acanthamoeba*, and other free-living amoebae (FLA) categorised according to contact lens disinfection method.
*Home prepared saline but no recognised disinfectant method used.

A mixed growth was cultured from most contaminated lens cases. The majority of bacteria were environmental 'pseudomonads', being non-lactose-fermenting, oxidase-positive, Gram-negative bacilli. One recognised corneal pathogen, *Serratia marcescens* was isolated from 11 cases. Chlorhexidine was the disinfectant used by 10 of these patients and this association was significant (Fisher's exact test; 2-sided; $p=0{\cdot}04$). *Serratia liquifaciens* was isolated from 10 cases. Other lactose-fermenting organisms isolated include *Acinetobacter spp*, *Klebsiella spp*, *Enterobacter spp*, and *Aeromonas spp*. *Pseudomonas aeruginosa* was not isolated from any lens case.

Nine patients (8·8%) had case contamination by free-living amoebae; seven of these were *Acanthamoeba*, one *Vahlkamphia*, and one *Hartmannella vermiformis*. Of the seven patients with *Acanthamoeba* contamination six were soft lens wearers, six had significant numbers of contaminating bacteria, and six used commercially manufactured disinfecting solutions as instructed. *Acanthamoeba* was isolated from the case of one of the five patients who prepared saline solution at home: this was the patient who used no disinfectant. All patients were asymptomatic.

All seven *Acanthamoeba* isolates were cytopathic to Vero cells, producing complete destruction of the cell monolayer in 2–3 days.

Discussion

We identified a disturbingly high incidence of storage case contamination by bacteria, in accordance with the report of Donzis *et al.*[7] We found that contamination by *Serratea marcescens*, a recognised corneal pathogen,[13] was significantly associated with chlorhexidine disinfection. *S. marcescens* infection due to contamination of chlorhexidine hand washing solution has been reported,[14] and R plasmids probably transfer resistance in a resistant strain.[15] However, we found contamination in the cases of patients using all disinfection methods and solutions. Similar bacteria contaminated hard and soft lens cases. The lower rate of bacterial contamination in soft lens cases reflects the use of hydrogen peroxide or heat disinfection by 38 patients in this group, these methods being more effective in reducing bacterial contamination (see Table II).[13]

It is even more disturbing that *Acanthamoeba* contamination of lens storage cases is more prevalent than expected. Numerically significant bacterial contamination has been found to coexist in most instances. This suggests that bacteria may support *Acanthamoeba* and possibly enhance virulence. Six of the seven patients from whose cases *Acanthamoeba* was isolated complied with lens hygiene instructions; only one patient prepared 'home-made' saline solutions, a widely recognised risk factor for *Acanthamoeba* keratitis.

This study indicates that *Acanthamoeba* contamination of lens storage cases must be far more common than *Acanthamoeba* keratitis. Keratitis

probably arises in only a small proportion of those asymptomatic patients with lens case contamination, and the predisposing factors for development of keratitis have yet to be established.

Tap water rinsing of lenses prior to wear is widely advised by contact lens practitioners. A variety of free-living amoebae, including *Acanthamoeba*, can be isolated from domestic tap water samples (unpublished observations), and tap water might therefore be a source of contact lens case contamination.

It is established that most disinfecting methods are effective against bacteria and *Acanthamoeba* in the laboratory[16]; home disinfection does not reflect this in the majority of lens wearers. All must be aware of possible contamination by dangerous pathogens, and lens disinfectant manufacturers must develop new compounds that kill *Acanthamoeba* cysts. We suggest that tap water rinsing of lenses be strongly discouraged. We further suggest that inexpensive disposable lens storage cases be developed for use and disposal after two weeks. This would reduce the contaminant bacterial population and in some cases abolish *Acanthamoeba* contamination.

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