

Possible environmental sources of *Acanthamoeba* spp in contact lens wearers

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Abstract

The water supply and dust samples from the home environment (bathrooms and kitchens) of 50 wearers of contact lenses (CLs) were cultured for the presence of free-living amoebae. CL cases, solutions, and water taps were cultured for bacteria, which amoebae require for growth. *Acanthamoeba* spp were isolated from water drawn from six bathroom cold water taps (tank supplied), five in the presence of limescale, and from one kitchen cold water tap (mains supplied). There was an association between the presence of limescale in water and direct culture for free-living amoebae, suggesting that scale provides a favourable microenvironment for amoebae. *Acanthamoeba* were also found in dust from around one washbasin. Nineteen of 50 CL cases, 12/122 CL care rinsing solutions, and 59/100 cold water taps yielded Gram negative bacteria which could be ingested by amoebae. It is concluded from this study that CLs should not be washed in first-drawn tank-fed cold water, especially if limescale is present, and that soft CLs should be rinsed in manufactured single-use, sterile solutions. Rigid CL and CL cases should only be washed with boiled tap water (preferably hot), or single-use sterile solutions, and stored dry to prevent multiplication of amoebae and Gram negative bacteria.

Keratitis due to the free-living amoebae, *Acanthamoeba polyphaga* and *A castellanii*, occurs mainly in wearers of contact lenses (CLs),¹ but can also follow corneal trauma, often in rural surroundings.² The majority of patients have worn daily wear soft lenses,³ but recent cases have occurred associated with disposable extended wear CLs.^{4,5} The infection is painful, prolonged, difficult to treat, and can result in blindness.⁶

Acanthamoeba spp have been isolated from environmental sources including well water, hot tub and domestic tap water.^{1,7-9} Although *Acanthamoeba* spp are ubiquitous in air, soil, and natural waters the main source of CL contamination in the UK has remained unclear for most patients. This contrasts with the USA, where the use of home-made saline for disinfecting CLs has been associated with *Acanthamoeba* keratitis.¹ A recent study of CL storage case contamination, involving asymptomatic CL wearers, has found *Acanthamoeba* to be present in seven of 102 cases, of which six also had high bacterial counts.¹⁰

To investigate possible sources for CL contamination, which is likely to occur prior to infection, 50 persons who wore CLs were visited at home. Samples were collected for isolation of amoebae from their bathrooms and kitchens

including dust, the cold water supply, and the washbasin drain. These are the areas in the home where CLs are most likely to be handled. Cold water taps were also cultured for bacteria, as were CL storage cases and lens care solutions.

Materials and methods

PATIENTS

Fifty new patients who presented to the accident and emergency department at Moorfields Eye Hospital, wearing CLs for the correction of low refractive errors, were visited at home. Six had been admitted to the hospital with CL associated bacterial keratitis. Lens wearers without a problem associated with lens wear ($n=44$), who attended immediately following a wearer with bacterial keratitis, were identified as a control group.

HOME ENVIRONMENT SAMPLING

Cold water taps in each patient's bathroom and kitchen were initially sampled by inserting two swabs into each tap. Taps were not pre-heated and run to waste for 2 minutes, which is a standard public health technique to decontaminate the tap and to sample water in the supply pipe, as the requirement was to sample the tap flora likely to be deposited in a CL storage case. Dust in the bathroom was sampled with swabs behind the basin and in adjacent areas. Basin drains were sampled by inserting a swab through the plug hole and rubbing against the inside of the pipe. Swabs were processed for amoebae by placing them on non-nutrient agar seeded with live *Escherichia coli* washed in phosphate-buffered saline (PBS). Plates were incubated for 1 week at 37°C and observed for amoebae under low power microscopy. Identification was made on the basis of morphology and the presence of different types of cysts.

Water samples were collected from bathroom and kitchen cold water taps by drawing off 30 ml of first-run water through the tap, into a sterile bottle. Tap water was not run to waste initially. Thiosulphate was not added to the bottle, to neutralise chlorine in the water, again to simulate conditions in the CL storage cases. Water samples were spun at 2500 rpm for 10 minutes and 0.1 ml of the deposit was inoculated onto non-nutrient agar plates seeded with *Escherichia coli*, as above. These were incubated at 37°C for 1 week. Each sample was observed for the presence of limescale by microscopy of the spun deposit. These water samples were then reinvestigated by a more sensitive enrichment technique, which involved prior incubation of the water with live *Escherichia coli* for 48 hours. Samples were then

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plated out onto non-nutrient agar and incubated at 37°C. Different types of amoebae were identified by their morphological appearances.

CL storage cases were sampled with a swab which was rubbed around the inside of the case. These swabs, and others for bacterial culture, plus all lens care materials (3 ml of solutions), were cultured on 10% horse blood, MacConkey, and Sabouraud's agars. Bacteria were identified by standard methods. Patients were questioned as to whether and how CL storage cases were cleaned.

STATISTICAL METHODS

The χ^2 test with Yates correction factor, or Fisher exact test,¹¹ where appropriate, was used to analyse the results.

Results

Table 1 summarises the results for kitchen and bathroom water samples, including the presence of limescale, and culture for amoebae, *Acanthamoeba* spp, and bacteria. There was good association ($p < 0.001$) between isolation of amoebae by direct culture and the presence of limescale in cold water taps for both the kitchen and the bathroom. However limescale was more frequently found in bathroom taps ($p = 0.04$). Amoebae were found more commonly in the bathroom tap water (tank-fed tap) than in the kitchen water (mains-fed tap), by direct culture ($p < 0.05$). This association was lost when increased numbers of amoebae were recovered using the enrichment technique. *Acanthamoeba* spp were isolated more frequently (6/50) from bathroom water by the enrichment technique compared with kitchen water (1/50). This indicates a trend to growth for amoebae and *Acanthamoeba* spp in tank-fed water, a warmer environment, and in the presence of limescale. This difference was not statistically significant for *Acanthamoeba*, probably due to small numbers.

Amoebae and *Acanthamoeba* spp were not significantly associated with the presence of Gram negative bacteria in either water sample

(Table 1) or tap swabs (data not shown), using direct or enrichment recovery techniques.

Amoebae were isolated from bathroom dust, beside the washbasin, on 6/50 occasions. On one occasion, *Acanthamoeba* sp was isolated as well.

Amoebae were isolated from the wash-basin drain on 40/50 occasions. Gram negative bacteria were present in 39 samples, of which amoebae were present on 30 occasions. Interestingly, amoebae were isolated from 10/11 samples when Gram negative bacteria were absent. *Acanthamoeba* spp were isolated on two occasions and Gram negative bacteria were present.

Sixteen CL cases belonging to 44 asymptomatic CL wearers were contaminated with Gram negative 'coliform' bacteria. Two out of five CL cases belonging to the patients who developed keratitis contained *Pseudomonas aeruginosa* while one CL case contained 'coliform' bacteria. Such 'coliform' bacteria were also isolated from 12 out of 122 CL care solutions overall. This contamination included 10 out of 108 CL care solutions from 44 asymptomatic CL wearers and two out of 14 solutions from six patients who had developed keratitis.

Results of questioning 35 asymptomatic CL wearers, who were resident at home, revealed that 13 washed their CL cases in tap water of which 11 used bathroom cold water and two used kitchen water. None ran their tap for 2 minutes prior to use but took off the first-drawn water. Fifteen did not clean their CL storage case while seven washed their cases in detergent.

Discussion

In England the cold water supply to the bathroom is derived from a storage tank in the roof that is rarely drained and cleaned. In contrast, the kitchen cold tap is supplied directly from the mains supply to the house, which later supplies the storage tank. *Acanthamoeba* spp, thought to be similar to pathogenic strains which infect the cornea, were isolated from 6/50 tank-fed bathroom cold water taps and from 1/50 mains-fed kitchen cold water taps. Isolation of amoebae and *Acanthamoeba* spp has been described from the water supply of Strasbourg,¹² which is derived

Table 1 Association of amoebae and bacteria with limescale in kitchen and bathroom tap water

Culture technique	Water samples				p value† ($\chi^2=23$) ($p < 0.05$)‡	p value <0.001 ($\chi^2=16$)
	Bathroom (n=50)		Kitchen (n=50)			
	Limescale		Limescale			
	Present (n=32)	Absent (n=18)	Present (n=21)	Absent (n=29)		
Direct						
Amoebae§	24	0	12	1		
<i>Acanthamoeba</i>	1	0	0	0		
Bacteria¶ (tap swab)	19	13	11	14	NS	NS
Enrichment						
Amoebae§	29	15	17	17	NS	NS
<i>Acanthamoeba</i>	5	1	0	1	NS	-

*=comparison of the association of limescale and different sites

†=comparison of the association of amoebae with limescale

‡=comparison of the growth of amoebae in kitchens and bathrooms.

§=includes *Hartmannella*, *Naegleria*, *Vahlkampfia*, *Vanella*, *Vexillifera*, *Platyamoeba*, *Filamoeba* and *Nuclearia*

¶=Gram-negative rods

NS=not significant

from artesian wells. Free-living amoebae can also be expected to be found in the mains water supply in the UK. Furthermore, cysts of *Acanthamoeba* spp are relatively resistant to chlorine.¹³

Limescale was found more frequently in water samples drawn from bathroom cold water taps (32/50), compared with samples drawn from kitchen taps (21/50), $p=0.04$. By direct culture, there was good association ($p<0.001$) between the presence of limescale and amoebae, but this association was lost when the enrichment technique was used. Use of the enrichment technique, which allows small numbers of cells to multiply in the water sample prior to culture on agar, demonstrated that in 5/6 bathroom waters and in one mains water sample *Acanthamoeba* spp were present in low numbers, which could not be detected by direct culture from 0.1 ml of spun deposit derived from a 30 ml sample. There was no statistically significant correlation between the presence of bacteria and amoebae for either kitchen or bathroom samples, nor between bacteria and limescale. We propose that limescale traps the amoebae, which are known to survive in the absence of bacteria, by forming cysts. Small numbers demonstrated in the supplying water could then multiply in the limescale when bacteria are present. It would appear that free-living amoebae can be used as a marker for the possible presence of *Acanthamoeba* spp but are present from 6–24 times more frequently; *Acanthamoeba* was not isolated in the absence of other amoebae. Our observation that only two cases of *Acanthamoeba* keratitis have been reported in Scotland (population 5 million).^{14,15} which has 'soft' water without limescale, is interesting but needs confirmation and comparison with similar situations elsewhere. Both amoebae and *Acanthamoeba* spp were isolated from the washbasin drains and at a low frequency from the dust in adjacent sites.

Larkin *et al*¹⁰ found that 43 CL storage cases, used by 102 asymptomatic wearers in an optometric practice, were contaminated by large numbers of bacteria and that seven contained *Acanthamoeba* spp. This present study has confirmed that CL storage cases from asymptomatic wearers are similarly contaminated with bacteria (16/44) in our population. This implies that CL disinfection in the community is ineffective. Furthermore, 12/122 commercial lens care solutions in the home were contaminated with Gram negative bacteria, despite being manufactured to remain sterile. In the USA a similar proportion of asymptomatic lens wearers has been reported as having bacterial contamination of their CL storage cases.¹⁶

Washing CL cases with freshly-drawn bathroom cold tap water, in areas using 'hard' water containing limescale, will deposit scale in them which is heavily contaminated with amoebae and bacteria. This scale may inactivate the chemical disinfectant process used. Organic debris has been identified as inactivating chlorine-based CL disinfectant systems.¹⁷ These observations are consistent with the recent findings that six CL disinfecting solutions were not effective in eliminating *Acanthamoeba* cysts, from a starting suspension of 100 000, within a

reasonable time for clinical use.¹⁸ *Acanthamoeba* spp have also been shown to adhere to unworn hydrogel lenses,¹⁹ which may further reduce the efficacy of chemical disinfectants.

Although a potential source of the organism has been from the lens storage case, two recent cases have been documented associated with the use of disposable extended wear soft lenses,^{4,5} without the use of lens solution or a lens storage case. This demonstrates that other possible sources of the organism exist. These may include air,²⁰ dust such as that in the bathroom, shown in this study, swimming pools, and hot tubs.²¹

Amoebae ingest bacteria and thus good disinfection management involves the removal of both bacteria and amoebae. The findings of this study suggest that freshly-drawn bathroom cold tap water, particularly in 'hard' water areas, should *not* be used to wash out CL storage cases or to rinse the CLs themselves because of the high contamination rate. It is suggested that CL cases and hard CLs should be rinsed with freshly boiled and cooled water from the domestic kettle. CLs should be cleaned with a surfactant solution prior to disinfection, which should take place in a previously cleaned and dry case, ideally sterile and disposable. After disinfection the CL cases should be rinsed again with hot, boiled water and stored *dry* to reduce contamination and multiplication of amoebae and Gram negative bacteria. It must be accepted from this study and others^{10,16} that current use of CL care systems by the general public is not as envisaged by the manufacturers nor as recommended by optometrists.

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