

**Acanthamoeba keratitis: confirmation of the UK outbreak
and a prospective case control study identifying
contributing risk factors**

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5 ***Acanthamoeba* keratitis: confirmation of the UK outbreak and a prospective case**
6 **control study identifying contributing risk factors**
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Competing interest statement

CooperVision UK, one of whose products has been identified by this study as a risk factor contributing to the development of *Acanthamoeba* keratitis, funded the recruitment of the second set of controls. This was to verify the findings of the first set as there was a perception that the selection of these may have been biased. This did not prove to be the case and the company has since phased out the product. CooperVision did not influence the study design. Representatives of CooperVision reviewed the manuscript but made no changes other than clarifications and grammatical changes.

Contributorship

Nicole Carnt submitted the research and ethics applications, recruited the cases, co-ordinated recruitment of the first set of controls, prepared the incidence dataset from 2000-2014, and contributed to the planning and execution of all aspects of the study. Jeremy Hoffman collected the incidence data for 2015/6. Seema Verma facilitated the identification of controls and cases in the Moorfields A&E Dept., Cherry Radford assisted questionnaire design, the dataset collection and recruited the second set of controls. Darwin Minassian contributed to study planning and carried out the statistical analysis. John Dart planned the study, assisted with the data collection and co-ordinated the preparation of the manuscript. All authors contributed to the manuscript.

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Synopsis

There has been a 3-fold increase of *Acanthamoeba* keratitis amongst contact lens users in South-East England since 2010-11. Risk factors include Oxipol disinfection and lens contamination by water. Public health education could reduce the incidence.

Confidential: For Review Only

Abstract

Background/Aims

Acanthamoeba keratitis (AK) is a chronic debilitating corneal infection principally affecting contact lens (CL) users. Studies were designed to test claims that the UK incidence may have increased in 2012-14, and to evaluate potential causes.

Methods

Annualised incidence data was collected from January 1984-December 2016. Case control study subjects were recruited between 14-04-2011 and 05-06-2017. Reusable CL users with AK were recruited retrospectively and prospectively. Controls were reusable CL users, recruited prospectively, with any disorder other than AK. Multivariable analysis of questionnaire data measured independent risk factors for AK.

Results

The current outbreak of AK started in 2010-11 with an incidence 3-fold higher than in 2004-9. Risk factors for AK were: Oxipol disinfection, CLs made of Group IV CL materials, poor CL hygiene, deficient hand hygiene, use of CLs whilst swimming or bathing, being white British, and for those in social classes 4-9.

Conclusion

AK is a largely preventable disease. The current outbreak is unlikely to be due to any one of the identified risk factors in isolation. Improving CL and hand hygiene, avoiding CLs contamination with water, and use of effective CL disinfection solutions, or daily disposable CLs, will reduce the incidence of AK. In the longer-term water avoidance publicity for CL users can be expected to reduce the incidence further. Ongoing surveillance of AK numbers by will identify changes in incidence earlier. Evaluation of *Acanthamoeba* contamination in end-user drinking water would contribute to our understanding of regional variations in the risk of exposure.

Introduction

Acanthamoeba spp. are free-living cyst-forming protozoans, ubiquitous in air, soil, dust, and water, to which 50% - 100% of us develop antibodies. However, infections are uncommon, rarely involving the brain, but more often the cornea. *Acanthamoeba* exist as a vegetative trophozoite, usually feeding on other micro-organisms, whereas in the cornea they probably feed on keratocytes. In adverse environments, including the nutrient deficiency and noxious treatments that the organisms are exposed to in keratitis, trophozoites encyst. The cysts are extremely resilient and are the form of the organism responsible for persistent relapsing keratitis. (1)

Despite its comparative rarity, and status as an orphan disease, AK is of concern because of its severe and prolonged morbidity in the young and economically active contact lens (CL) users who constitute 90% of affected patients in the UK. The most severely affected quartile require more than: 10 months treatment, 38 months follow up, 31 hospital visits, have less than 6/24 vision after resolution, and require corneal transplants.(2) Established independent risk factors for developing the disease have been exposure to water; in the context of CL use this risk has been related to exposure to domestic tap water both in the home(3, 4), and whilst swimming or bathing when wearing CLs.(4, 5) Others have been poor contact lens hygiene,(4-6) orthokeratology use of rigid CLs,(6) and lens disinfection solution failures which have resulted in previous outbreaks of AK both in the UK and USA.(7, 8) Following the last UK outbreak in 1991-5 a national incidence study reported a mean of 53 cases per annum of which 88% were in CL users(4); less than half the annual incidence at the peak of that outbreak.(7)

Since then no increase in annual AK incidence has been noticed until concerns expressed in 2012-14 (9, 10). As a result, we initiated the two studies reported here. Our results are likely to have relevance to the whole UK given that in 1997-9 Moorfields Eye Hospital (Moorfields) treated >75% of AK cases in the South East, and >35% of UK cases(4). The incidence study was to measure the annualized incidence at Moorfields. The case control study was designed to identify potential causes of AK, which we hypothesized might be largely due to ineffective CL solutions given the findings from previous outbreaks. (7, 11) The

Methods

The studies were approved by the National Research Ethics Service Committee London-Hampstead, REC Reference 13/LO/0032 and the Moorfields Eye Hospital Research Governance Committee.

Incidence study of *Acanthamoeba* keratitis at Moorfields 1984-2016

The data collection methodology for the numbers of cases of AK cases seen at Moorfields from January 1984-December 2016 has differed for different periods and are not directly comparable. The methodology is described in the Legend to Figure 1.(4, 7, 12, 13)

Case control study

Patients using CLs and attending Moorfields Accident and Emergency (A&E) Department completed a self-administered questionnaire based on those used in previous studies(7, 14), providing data on demographics, lens type, wear schedule, lens care and frequency of disposal, lens wear experience, frequency of practitioner aftercare, showering and bathing when wearing CLs, and smoking.

Cases were reusable daily wear CL users diagnosed with *Acanthamoeba* keratitis having an initial attendance in the Moorfields A&E service. These included both self-referrals, secondary (general practitioner and optometric) and tertiary (other ophthalmology centres). These were identified between 14-04-2011 and 28-08-2014. Cases diagnosed before ethics approval was given on 18-2-2013 were recruited after diagnosis following which cases were recruited at the time of diagnosis. Inclusion criteria for *Acanthamoeba* keratitis cases were the same as those used for the Incidence study and are described in the Figure 1 Legend c, e.

Controls were recruited prospectively. Like the cases, these were attending Moorfields A&E. Inclusion criteria were daily wear CL wearers using CL solutions as part of the hygiene routine for reusable CL's having any disorder other than AK. Controls were identified in 2 separate periods. This definition excluded users of true daily disposable and overnight (also known as continuous- or extended-) wear soft lenses for which no contact lens solutions were used. A first set of controls was collected between 17-02-2014 and 11-06-2015 and a second between 22-11-2016 and 05-06-2017. A diagnosis for each questionnaire respondent was

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3 derived from the hospital notes. Disorders in the control CL user populations were classified
4 into 2 subsets: those with CL related diseases and those with diseases thought to be unrelated
5 to CL wear. These conditions are listed in Supplementary Table 1. CL related diseases
6 included all contact lens associated keratitis, other than AK, defined using criteria from
7 previous studies.(14-16)
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12 *Contact lens solutions* were classified and analysed by the principal active ingredient using
13 information derived from the packaging or manufacturers where possible or retailers when
14 we could not establish the formulation from the first two sources. The classification is in
15 Supplementary Table 2.
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20 *Contact lenses* were classified and analysed by their material using a simplification of the
21 American National Standards Institute Terminology for Contact Lenses Reference Z80.20-
22 2016 and checked for each contact lens brand, or rebranded lens, against the UK Association
23 of Contact Lens Manufacturers (ACLM) Annual Handbook for the years of the study. These
24 data are summarised in Supplementary Table 3.
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28 Statistical methods

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30 The main analysis sample included all eligible controls collected during 2016-2017. A
31 sample size of 60 cases and 180 controls was expected to provide 85% power (alpha 0.05) to
32 detect a minimum odds ratio of 2.7% assuming 20% of the controls were exposed. Initial
33 assessment of potential risk factors was carried out one at a time using logistic regression
34 without adjustment for confounding and is described in Supplementary Table 4. Contact lens
35 hygiene compliance is an important potential risk factor and the methodology for calculating
36 the score used for this analysis is described in Supplementary Table 5. Following this initial
37 assessment multiple logistic regression models were constructed to obtain estimates of odds
38 ratios (OR) adjusted for confounding effects. Odds ratios are reported in this study as
39 estimates of relative risk. Variables selected initially for inclusion in the model building
40 process were those having p-values of <0.2 from the unadjusted analysis together with
41 variables thought *a priori* to be risk factors for AK: hygiene score, hand washing before lens
42 handling, and the principal active ingredient of the disinfection solution. The distribution of
43 cases and controls for these variables is shown in Table 1. In constructing each final
44 multivariate (MV) model, one exposure variable was considered as the "exposure of main
45 interest", and all others as "auxiliary factors" (potential confounders). The final MV model
46 was thus optimized to estimate the adjusted OR for the "exposure of main interest"; covariate
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3 adjustment is described in Supplementary Table 6. The performance of logistic models -
4 goodness-of-fit, and discriminatory ability - were assessed by calculating the Hosmer-
5 Lemeshow p-value, and area under the ROC curve respectively. The statistical package used
6 was Stata v.14.2 (StataCorp, Texas, USA).
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10 **Results**

11 Incidence study:

12 Figure 1 shows the numbers of cases from 1985 to 31-12-2016. Annual numbers were 8 to 10
13 per annum (p.a.) from 2000-2003, after which there was an annual increase from between 15
14 and 23 p.a. from 2004-2009, rising from 2010 to the current level of between 36-65 cases p.a.
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18 Estimates of CL wear prevalence are given for datapoints throughout this period.
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22 Case control study:

23 There were 63 confirmed *Acanthamoeba* keratitis cases in reusable CLs wearers, with no
24 exclusions: 18 cases, diagnosed up to 22 months before 18-2-13 (when ethical approval was
25 obtained), completed the study questionnaire retrospectively. The remaining 45 AK cases and
26 all the controls completed the questionnaires at the time of diagnosis. Cases were compared
27 independently with the two different control datasets; one additional case was excluded when
28 the initial clinical diagnosis of AK was not confirmed on follow up. There were 56 eligible
29 controls from the first control dataset from which CL using controls were excluded if they
30 had CL related disease. Following the analysis of this dataset concern was expressed by an
31 external advisor about a potential bias in the selection of these controls. As a result, we re-
32 opened the study to obtain the second, and fourfold larger control dataset of 213; exclusions
33 were true daily disposable and overnight wear lens users, CL users with AK, 15 with missing
34 CL solution or CL brand information (despite 3 contact attempts), 1 who left the Hospital
35 before being seen for diagnosis, and 2 whose records could not be retrieved to confirm the
36 outcome. The findings from the analysis of the first dataset were similar to those of the
37 second. The second dataset was chosen for the analysis reported here as giving a more
38 conservative estimate of some odds ratios. Differences between the two datasets made their
39 combination inappropriate. The results of the assessment of putative risk factors for AK,
40 carried out independently for each exposure without adjustment for confounding, are shown
41 in Supplementary Table 4 for the 63 AK cases compared with the entire second control
42 dataset of 213. This control dataset includes both subsets with (n=109) and without (n=104)
43 CL related diseases. Both of these subsets of controls were included in the analysis as being
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likely to give a conservative estimate of the relative risk of exposures for AK, given that these may be shared with the risks for some of the non-*Acanthamoeba* CL related keratitis conditions listed in Supplementary Table 1 which made up 64/109 (59%) of the diagnoses in this subset. The mean age in the whole sample was 33.9 (standard deviation 12.26). The median age was 30 (range 13-76), with 25th & 75th percentiles of 25 - 40 (inter-quartile range). The distribution was similar in cases and controls: median of 30 in both, and inter-quartile range 25-49 and 25-38 respectively. 33/63 (52%) of the cases, and 152/213 (71%) of the controls were female.

Multivariate analysis for the control dataset of 213 controls

The results of the analysis for reusable soft contact lenses, adjusted for confounding, are shown in Table 2. These show significantly increased risks of AK as a result of the following:

- The use of Oxipol disinfection of 4.74 (CI 1.83-12.30, p 0.001)
- Wearing Group IV (high water content, ionic hydrogel lenses) of 6.71 (CI 1.31-34.29 p 0.022)
- Poor CL hygiene practice of 3.34 (CI 1.52-7.38 p 0.003)
- No, or uncertain, hand washing before lens handling of 3.65 (CI 1.52-8.77 p 0.004)
- Wearing contact lenses in swimming pools or hot tubs of 3.49 (CI 1.51-8.04 p 0.003);
- White British ethnicity of 4.82 (CI 1.61-14.46 p 0.005)
- Occupation other than professional/director/senior official of 3.51 (CI 1.52-8.11 p 0.003).

Rigid gas permeable CL solutions were included in the lens disinfectant analysis and these solutions were associated with a higher risk of keratitis compared to the referent: this does not imply a higher risk for AK associated with rigid lens use per se.

Discussion

The current outbreak of *Acanthamoeba* keratitis in South East England and the UK

The incidence study has confirmed a current UK outbreak in South East England starting in 2010/11, resulting in a mean of 50.3 per annum (range 36-65) treated at Moorfields for the years 2011-2016 compared to the most accurate estimate of the numbers between outbreaks, of 18.5 pa at Moorfields from the prospective national audit carried out over 2 years in 1997-9.(4) Given the limitations of the data collection methodology, particularly for the earlier years of the period 2000 to 2012, it is possible that the numbers of cases between 2000-2003

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3 are underestimates. On the other hand, the apparent rise in cases in 2004, that was
4 subsequently maintained until a further rise in 2010-2011, may relate to the outbreak in the
5 USA due to the failure of the CL disinfectant AMO Complete Moisture Plus. The latter
6 resulted in a 17-fold rise in cases in the USA but, following withdrawal of the solution,
7 numbers have remained at similar levels for reasons that are unclear.(11) The industry data
8 on CL user numbers in Figure 1 suggests that this current UK outbreak is independent of
9 changes in the prevalence of CL wear.
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14 15 16 Risk factors for *Acanthamoeba* keratitis

17 Between 80-90% of AK cases are potentially avoidable if effective disinfection systems are
18 used, good CL hygiene practice followed, and exposure to water whilst using lenses is
19 avoided.(4, 7) This current study confirms these previous risk factors and identifies new
20 independent risk factors including deficient hand washing, race and occupation associated
21 risks (which are probably surrogates for safe CL lens use either from not receiving or
22 following appropriate instruction). It has also confirmed our initial hypothesis that a contact
23 lens solution may have been associated with the outbreak given that Oxipol based
24 disinfection was also an independent risk factor (Odds ratio 4.74, CI 1.83-12.30, p 0.001).
25 This risk is relatively low compared to that of the previous outbreaks of AK associated with
26 CL solution failures: a 40-fold higher risk of AK for users of chlorine based disinfection
27 systems in the UK(7) and 17-fold higher for users of AMO Complete Moisture Plus in the
28 USA (8, 11). The occurrence of AK in an individual patient who has used Oxipol cannot be
29 attributed only to the Oxipol disinfection because: AK develops in some patients who do not
30 use Oxipol (not a necessary cause), and also many patients who use Oxipol do not develop
31 AK (not a sufficient cause). The same applies to the other risk factors. Since the time of the
32 investigation Oxipol solution has been phased out by the manufacturer.
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45 Although most manufacturers test their solutions for activity against *Acanthamoeba* this is
46 not mandatory, largely because there is no standard methodology for reproducible *in vitro*
47 sensitivity testing; this has resulted in a wide range of results for different solutions
48 depending on the strains tested, their age, and the storage and encystment methodology
49 used.(17) Independent testing, using rigorous methods, has shown that most multipurpose
50 CL disinfection solutions *in vitro* may be ineffective against *Acanthamoeba*.(17) The issue of
51 mandatory testing of contact lens disinfection solutions for activity against *Acanthamoeba* is
52 being actively addressed in the USA(17) and an international testing standard is being
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3 developed by the ISO TC 172/SC 7/WG 9 committee for Contact Lens Care Products.
4 However, disinfection is not sterilisation, and current anti-microbial test standards demand
5 log reductions not elimination. It is also important to understand that CL disinfection solution
6 efficacy is not just related to the principal active ingredients, which is how the analysis in this
7 study was performed, but is the sum of a complex interaction of the disinfectants with the
8 excipients; storage bottle; CL case; debris in the CL case; and the CL material. All of these
9 may affect disinfection capability, a topic beyond the scope of this report but relevant to the
10 fact that in this study we found polyhexanide based disinfection systems to be the most
11 effective whereas polyhexanide was also the disinfectant in AMO Complete Moisture Plus;
12 the principal cause of the last USA outbreak, but for which the disinfectant failure was
13 attributed to other components.(8)
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22 Another novel independent risk factor identified in this study was the association with the use
23 of Group IV contact lenses of 6·71 (CI 1·31-34·29 p 0·022). This had been identified as a
24 borderline risk in our study of the previous UK outbreak.(7) Etafilcon A is one of many
25 Group IV materials and is one of the most widely used in CL manufacture. However, in a
26 previous study on the risks of CL associated keratitis Etafilcon A, when used as a daily
27 disposable lens, was found to be less associated with (predominantly bacterial) keratitis than
28 other lens types.(14) We think that the potential reduction in the risk of the much more
29 common problem of bacterial keratitis is likely to offset a possible increase in the risk of
30 *Acanthamoeba* keratitis associated with Group IV CL materials. Therefore, we suggest that
31 Group IV lens material users, rather than changing lens material, should optimize their lens
32 hygiene, and avoid wearing CLs when exposed to water to minimize their risk of developing
33 AK (which is already very small). Lens cleaning has the capacity to remove adherent
34 *Acanthamoeba*.(18) This paradoxical finding for Group IV lens materials, associated with
35 potentially higher risks for AK but lower risks for bacterial keratitis, may be explained by
36 differences in adhesion of *Acanthamoeba* and bacteria to different CL materials. Although
37 not always consistent, and also strain dependent, bacteria generally adhere less to Etafilcon A
38 than to hydrophobic lens surfaces (on Group III and many Group V silicone hydrogels).(19)
39 Conversely, *Acanthamoeba* adherence has been shown to be greater to high water content and
40 ionic hydrogel CLs (Group IV lenses) compared to low water content hydrogel CLs (Groups
41 I and III)(20) although this may also be strain dependent.(18) On the other hand
42 *Acanthamoeba* adhesion was shown to be higher to first and second generation Group V CLs
43 than to Etafilcon A (Group IV) in another study which demonstrates some of the limitations
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3 of the *in vitro* investigation of microorganism adherence to unworn CL surfaces given that
4 this difference was reduced by the effects of lens wear deposits and bacterial biofilm (both of
5 which enhance *Acanthamoeba* trophozoite adhesion).(21)
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9 Research findings that support the risks of exposure to contaminated water are numerous.
10 Deficient hand washing (including drying after washing), and water activities whilst using
11 contact lenses have been associated with AK in this and other studies. Showering in CLs was
12 not a statistically significant independent risk factor in this study but, given known risks of
13 exposure to contaminated water whilst wearing lenses in other contexts, this should be
14 avoided. Contact lens case contamination by *Acanthamoeba* spp. has been found in 1-7% of
15 asymptomatic CL users.(22) Most water that CL users are exposed to may be contaminated
16 by *Acanthamoeba*; domestic tap water in Turkey(23), South Korea(24), Hong Kong(22) and
17 the UK(3) as well as swimming pool, hot tub and lake water.(25) Furthermore, in a UK study
18 genetically identical organisms were isolated in the domestic water supply of 6 patients
19 having AK, where the disease is more common in hard water areas, (3) probably because
20 lime-scale in taps provides an optimal environment for the organism.(4) A seasonal
21 association with AK has been related to increased participation in swimming in the summer
22 months(11). To establish whether there might have been a “London Olympic effect” since
23 2012, resulting from increased participation in swimming by CL users we compared control
24 data from a 2004/5 study(14) with this current data, but have shown no increase in swimming
25 (Supplementary Table 7).
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38 Given the importance of contaminated domestic water supplies in these studies it follows that
39 small changes in the disinfection of domestic water supplies, that might lead to an increase
40 the exposure of the population to water contaminated by *Acanthamoeba*, could have a
41 substantial effect on incidence of AK. The incidence of AK amongst CL users has
42 historically been 5 to 15-fold higher in the UK than in other countries, probably as a result of
43 contaminated domestic tank stored tap water.(3, 4) In the USA implementation of US
44 Environmental Protection Agency (EPA) legislation in 2002, designed to reduce the chlorine
45 generated, potentially carcinogenic, disinfection by-products, was temporally related to an
46 outbreak of AK in the Chicago suburbs. This legislation had resulted in treatment plants
47 introducing a number of measures to minimize disinfection by-products including a reduction
48 in the amount of chlorine used and a switch to the use of chloramine, a less potent
49 disinfectant than chlorine. Although these measures may have resulted in increased microbial
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3 contamination of the water delivery pipes, providing a larger food source for *Acanthamoeba*
4 and resulting in increased *Acanthamoeba* contamination at end user sites(26) this was not
5 shown to have occurred in Chicago; a preliminary analysis of the USA 2004 national
6 outbreak of AK could not find an association of AK cases with the use of chloraminated
7 water supplies.(8) In the UK the 1998 European Council Directive 98/83/EC shared similar
8 aims. Although the directive was not introduced formally into UK national law until January
9 2010 (27), coinciding with the start of the current outbreak of AK, informal discussion with
10 three of four major suppliers of water to the South East, and scrutiny of the Drinking Water
11 Inspectorate website(27), has not identified any changes in disinfection procedures in 2010-
12 11 which might have led to increased end user exposure to contamination by *Acanthamoeba*,
13 above what are probably historically high levels due to the widespread use of domestic tank
14 stored water in the UK.
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24 A potential weakness of this study is the difference in recruitment periods for cases and
25 controls. We do not think that this is likely to have resulted in significant bias, due to changes
26 in exposure to contact lens solutions, as there was overlap between the recruitment periods
27 for the cases and that for the first control group for which findings were confirmed.
28 Unfortunately changes in market share of contact lens disinfection solutions are proprietary to
29 the manufacturers who have not felt able to make these available to us. On the other hand,
30 one of the strengths of this study is our proven rationale for the choice of both cases and
31 controls from patients attending Moorfields A&E service. This minimizes potential
32 recruitment bias by recruiting both from a similar catchment area. We have followed this
33 practice in previous studies. (7, 14, 28) In the 2008 study we used both community derived
34 CL controls chosen from the postal code areas in which the cases were living for comparison.
35 However, the analysis was no different using these controls (both time consuming and costly
36 to recruit) compared to the controls derived from the A&E Department, as in the current
37 study. (14)
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48 This study has confirmed a persisting outbreak of AK in the south east of England, starting in
49 2010-11. This probably reflects the situation elsewhere in the UK, at least for hard water
50 areas. AK should be a largely preventable disease with over 90% of CL users developing AK
51 having identifiable, avoidable risk factors.(4) We think it unlikely that the current outbreak
52 results from any of these current risk factors in isolation. However, if the following measures
53 are taken the risk of developing AK is likely to be very low: improving CL and hand hygiene
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when inserting lenses, avoiding the contamination of CLs with water including water activities whilst using CLs, avoiding the use of CL solutions by switching to daily disposable CL use or, for those continuing to wear re-usable lenses, maintenance of optimal lens care with effective solutions. Publicity for these measures, as in the 1991-95 UK outbreak(29), can be expected to rapidly reduce the incidence of AK. Water avoidance publicity for CL users (by water companies, water sports facilities, the CL industry, and Eye Care Professionals) can be expected to reduce the incidence in the longer term. The addition of “no water” labelling on all CL packaging(30) should become mandatory. Ongoing surveillance of AK incidence by major UK ophthalmic units will identify future changes in incidence more rapidly than for this current outbreak. Surveillance of end-user domestic water contamination by the water companies is currently limited to measuring fecal bacterial contamination: the addition of *Acanthamoeba* to this panel can be expected to contribute to our understanding of local variations in the risk of exposure and make CL users aware of the importance of maintaining preventive measures.

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Figure 1 Legend

The total number of cases from 1984-2016 (33 years) totalled 709.

The data collection methodology varied for different periods (labelled a-f). The colours identify the periods for which the same data collection methodology was used. The references retain the numbering they have in the text:

- (a) 1984-1996 data (blue bars) was published in a letter as a bar chart¹² and was collected using the clinical and/or microbiological (culture or histology) criteria used in a previous study.⁷
- (b) 1997-1999 data (red bar) was available from a national survey⁴ for a 24 month period 01-10-1997 to 30-09-1999 for which annual figures are not available: there were 37 cases in this two year period.
- (c) From 2000 to April 2012 (green bars) cases were identified from our current microbiology laboratory electronic database, and an electronic letter search of our electronic patient database, both of which started in 2000. Criteria for inclusion were a positive *Acanthamoeba* culture, histopathological confirmation of trophozoites and/or cysts, culture-negative cases shown to have *Acanthamoeba* cysts on confocal microscopy, and those with a typical clinical course and response to treatment.⁴
- (d) From March 2012 to December 2013 (yellow bars) cases were identified prospectively as part of studies being carried out on AK using the criteria described for the (c) period.
- (e) From 01-01-2014 to 31-12-2016 (black bars) cases were identified by retrospective audit, using the same criteria as for (c) but with the addition of *Acanthamoeba* DNA identification by polymerase chain reaction (PCR) as an additional inclusion criterion.

* <https://www.statista.com/statistics/429790/wearers-of-contact-lenses-united-kingdom-ireland/> [data from the Association of Contact Lens Manufacturers market report 2014: technical summary]

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Table 1

Distribution of cases and controls for the variables that were potential risk factors for AK chosen for inclusion in the MV analyses.

Variable	Control	Case	Total	Odds Ratio *	p-value	95% CI for Odds Ratio	
Lens disinfectant: classified by the principal active ingredient (PAI) see Note 1							
1: Polyhexanide (0.00005-0.0001)	90	11	101	<i>Referent</i>			
2: PQ +ALDOX/PQ + Alex**	26	9	35	2.83	0.038	1.06	7.57
3: Hydrogen peroxide 3%	12	5	17	3.41	0.048	1.01	11.51
4: Polyquad-1(0.0001%) + Polyaminopropyl biguanide	11	2	13	1.49	0.633	0.29	7.60
5: Oxipol	55	29	84	4.31	< 0.001	2.00	9.33
6: Rigid gas permeable lens solution	11	5	16	3.72	0.036	1.09	12.71
Unknown	8	2	10				
Total	213	63	276				
CL Materials classification: simplified from the ANSI classification see Note 2							
Groups I + II + III	18	6	24	1.95	0.233	0.65	5.83
Group IV	13	7	20	3.15	0.039	1.06	9.37
Group VA	76	13	89	<i>Referent</i>			
Groups VB+VC	73	29	102	2.32	0.023	1.12	4.81
Rigid gas permeable materials	11	5	16	2.66	0.113	0.79	8.91
Unknown	22	3	25				
Total	213	63	276				
Hygiene Score: categories split between the top (worst) quartile of the sample and the rest see Note 3							
1: Good-Moderate (score 1.75-5.08)	174	30	204	<i>Referent</i>			
2: Poor (score 5.09-8.08)	39	33	72	4.91	< 0.001	2.68	8.98
Total	213	63	276				
Hand washing before handling CLs							
1: No/unsure	27	21	48	3.71	< 0.001	1.90	7.22
2: Yes	186	39	225	<i>Referent</i>			
Unknown	0	3	3				
Total	213	63	276				
Showering when wearing CLs							
1: No	141	25	166	<i>Referent</i>			
2: Yes	72	38	110	2.98	< 0.001	1.67	5.31
Total	213	63	276				
Water activity using CL's: categories combined							
1: None	114	20	134	<i>Referent</i>			
2: Ocean/Sea/River/Lake	42	9	51	1.22	0.649	0.52	2.89
3: Public or Private Pool/Hot tub	57	31	88	3.10	0.001	1.63	5.91
Unknown	0	3	3				
Total	213	63	276				
Ethnic group: for categories see Note 4							

4: British (white)	141	54	195	<i>Referent</i>			
6: Other	72	6	78	0.22	0.001	0.09	0.53
Unknown	0	3	3				
Total	213	63	276				
Occupation: for categories see Note 5							
1-3 Professional/Director/Manager/Associate professional & technical/senior officials	163	36	199	<i>Referent</i>			
4-9 combined	50	22	72	1.99	0.029	1.07	3.70
Unknown	0	5	5				
Total	213	63	276				

* Odds ratios (OR) are **not adjusted** for confounding effects of other variables

** PAI category 2: PQ +ALDOX/PQ + Alex = Polyquad-1 (0.001%) + ALDOX (0.0005%) or Polyquad-1 (0.0003%) + Alexidine (0.00016%) - the latter for 4 controls and 1 case only.

Notes

1. Classification of CL solutions is given in Supplementary Table 2
2. American National Standard Institute (ANSI) standard Z80.20-2016. American National Standard for Ophthalmics - Contact Lenses - Standard Terminology, Tolerances, Measurements and Physicochemical Properties. (Ophthalmic)
3. Hygiene scores were calculated using the following rules: scores for different categories of each variable are listed in Table-H1 (Supplementary Table 3). Maximum & minimum scores for any category were 10 & 1 respectively. Some of the closely related original variable pairs were combined into a single analysis variable (see Table-H1). All the analysis variables were given equal importance (no weighting). For each patient, the scores were summed across all the variables, then divided by the number of observations (number of variables with score data), to arrive at a mean score for each patient. The objective was to maximise the difference in scores between those who completely deviated from "good hygiene practice" and those who were totally compliant with "good hygiene practice" (scores 10 & 1 respectively). Partial deviations were scored 5.
4. Ethnic categories (UK Census categories): 1: Asian or Asian British (Bangladeshi, Indian, Pakistani) 2: Black or Black British (African, Caribbean, Other) 4: British (white) 6: Other
5. Occupation: 1. Managers, directors and senior officials 2. Professional occupations 3. Associate professional and technical occupations 4. Administrative and secretarial occupations 5. Skilled trades occupations 6. Caring, leisure and other service occupations 7. Sales and customer service occupations 8. Process, plant and machine operatives 9. Occupations requiring no specific training or skills & Student: categorized by parents' occupation.

Table 2.

Independent risk factors for AK from multiple logistic regression models using the Main Analysis Sample (213 Controls and 63 AK Cases). Odds ratios are adjusted for possible confounding.

Exposures	Adjusted Odds Ratio (OR) *	P value	95% CI for OR
Lens disinfectant: classified by principal active ingredient (PAI) see Note 1			
Polyhexanide (0.00005-0.0001%)	<i>(Referent)</i>		
PQ +ALDOX/PQ + Alex**	2.32	0.184	0.67 - 8.04
Hydrogen peroxide3%	1.77	0.534	0.29 - 10.83
Polyquad-1(0.0001%) + Polyaminopropyl biguanide (0.00013%)	0.80	0.851	0.08 - 8.03
Oxipol	4.74	0.001	1.83 - 12.30
Rigid CL solutions	7.34	0.011	1.57 - 34.24
CL Materials classification: simplified from the ANSI classification see Note 2			
Groups 1+2+3	2.39	0.313	0.44 - 12.98
Group 4	6.71	0.022	1.31 - 34.29
Group 5A	<i>(Referent)</i>		
Groups 5B+5C	2.3	0.107	0.84 - 6.32
Hygiene Score: categories split between the top (worst) quartile of the sample and the rest see Note 3			
Good-Moderate (1.75-5.08)	<i>(Referent)</i>		
Poor (5.09-8.08)	3.34	0.003	1.52 - 7.38
Hand washing:			
Yes	<i>(Referent)</i>		
No/unsure	3.65	0.004	1.52 - 8.77
Shower wearing CLs:			
No	<i>(Referent)</i>		
Yes	1.81	0.150	0.81 - 4.07
Water Activities wearing CLs:			
None	<i>(Referent)</i>		
In Ocean/Sea/River/Lake	1.45	0.519	0.47 - 4.45
In public pool/private pool/hot tub	3.49	0.003	1.51 - 8.04
Ethnic Group: for categories see Note 4			
Other	<i>(Referent)</i>		
White Caucasian	4.82	0.005	1.61 - 14.46
Occupation: for categories see Note 5			
Class 1 + 2 + 3: Professional/Director/Senior Official	<i>(Referent)</i>		
Classes 4-9:	3.51	0.003	1.52 - 8.11

* In constructing each final MV model, one exposure variable was considered as the "exposure of main interest", and all others as "auxiliary factors" (potential confounders). The final MV model was thus optimized to estimate the adjusted odds ratio for the "exposure of main interest" (see Supplementary Table 6 Covariate adjustment)

** PAI category 2: PQ +ALDOX/PQ + Alex = Polyquad-1 (0.001%) + ALDOX (0.0005%) or Polyquad-1 (0.0003%) + Alexidine (0.00016%) - the latter for 4 controls and 1 case only.

Notes

1. Classification of CL solutions is given in Supplementary Table 2
2. American National Standard Institute (ANSI) standard Z80.20-2016 American National Standard for Ophthalmics - Contact Lenses - Standard Terminology, Tolerances, Measurements and Physicochemical Properties (Ophthalmic)
3. Hygiene scores were calculated using the following rules: scores for different categories of each variable are listed in (Supplementary Table 4). Maximum & minimum scores for any category were 10 (poorest) & 1 respectively. Some of the closely related original variable pairs were combined into a single analysis variable (see Supplementary Table 4). All the analysis variables were given equal importance (no weighting). A mean score was calculated for each patient, and the patients were classified into 2 groups: using the top (worst) quartile of the mean score for the sample (5.09)
4. Ethnic categories (UK Census categories): 1; Asian or Asian British (Bangladeshi, Indian, Pakistani) 2; Black or Black British (African, Caribbean, Other) 4; British (white) 6; Other
5. Occupation: 1. Managers, directors and senior officials 2. Professional occupations 3. Associate professional and technical occupations 4. Administrative and secretarial occupations 5. Skilled trades occupations 6. Caring, leisure and other service occupations 7. Sales and customer service occupations 8. Process, plant and machine operatives 9. Occupations requiring no specific training or skills, & Student: categorized by parents' occupation

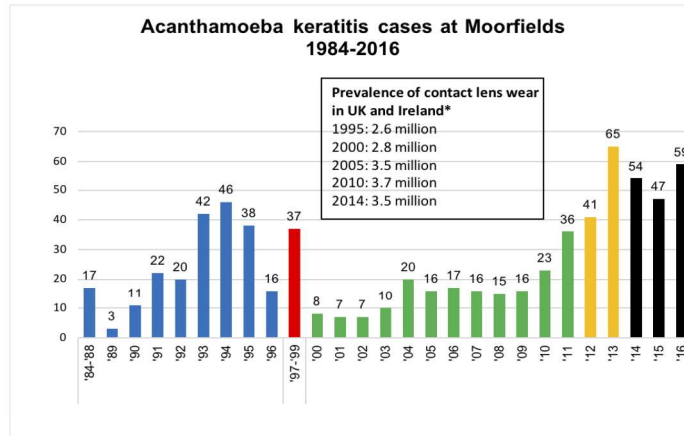


Figure 1

209x296mm (300 x 300 DPI)



Supplementary Table 1

Control contact lens users: diagnoses for the 213 Controls

Diagnosis	Frequency
Diseases classified as unrelated to contact lens wear: listed by frequency	
Viral conjunctivitis	17
Dry eyes	10
Chalazion	5
Posterior vitreous detachment	4
Sub-tarsal foreign body	4
Conjunctivitis	3
Allergic conjunctivitis	3
Blepharitis & dry eye	3
Marginal keratitis & bilateral blepharitis	3
Conjunctival abrasion (non-lens related)	2
Ocular migraine	2
Recurrent corneal erosion syndrome	2
Chalazion & bilateral blepharitis	3
Corneal foreign body	2
Blepharitis	2
Blepharitis & dry eyes	2
Inflamed chalazion	1
Corneal scar	1
Acute anterior uveitis and right chalazion	1
Acute anterior uveitis	1
Meibomian gland dysfunction & dry eyes	1
Corneal foreign body & conjunctival abrasion	1
Lattice degeneration	1
Sub-tarsal foreign body and corneal abrasion	1
Ametropia	1
Nothing found (given lid hygiene and/or lubricants)	2
Allergic reaction (lids)	1
Blunt trauma	1
Conjunctival retention cyst	1
Eczematous lids	1
Ocular hypertension	1
Nothing found (possibly a systemic viral illness)	1
Allergic reaction (lids) & dry eye	1
In-growing eyelash	1
Episcleritis	1
Blepharitis and right acute hordeoleum	1
Limbitis	1
Dry eyes following viral conjunctivitis	1
Chalazia	1
Vitreous floaters	1
Blepharo-conjunctivitis	1
Marginal keratitis	1
Optic neuritis	1
Marginal keratitis & bilateral dry eyes	1
Retrolbulbar optic neuritis	1
Optic disc drusen	1
Preseptal cellulitis and bilateral blepharitis	1
Preseptal cellulitis and chalazion	1

Diseases classified as unrelated to contact lens wear: listed by frequency continued	
Diagnosis	Frequency
Marginal keratitis and blepharitis	1
Scleritis	1
Chemical keratitis	1
Nothing found	1
Total unrelated to Contact Lens wear	104

Diseases classified as Contact Lens (CL) related: listed by frequency	
Corneal infiltrative event (CIE)	44
CL overwear	23
CL associated - microbial keratitis (MK) - moderate	11
CL associated - microbial keratitis - mild	8
CL associated - uncertain whether a CIE or MK	1
Corneal abrasion (1 in mild keratoconus patient)	8
Toxic (chemical keratitis)	2
CL related punctate corneal erosions (possibly fit related)	1
CL overwear & R conjunctival abrasion	1
CL overwear & dry eyes	1
CL overwear & corneal abrasion	1
Conjunctival abrasion: CL related	1
Scatched CL's & CL related dry eyes	1
CL related punctate epitheliopathy	1
CL related corneal epitheliopathy	1
Epithelial oedema: probably secondary to a tight CL	1
Epitheliopathy: probably CL solution related	1
Retained CL with associated punctate corneal erosions	1
CL associated conjunctival hyperaemia	1
Total CL-Related	109

Supplementary Table 2

Contact lens (CL) solution information table (listed alphabetically by manufacturer (where known) and product name)

SOFT CL DISINFECTANT SOLUTIONS Unless stated data from ACLM handbooks; internet; solution packaging (Boots and Mesmeryez) and online						
Manufacturers name	Rebranded name	Active disinfectant(s)	Excipients (non-disinfectant ingredients)	Availability at 01 2011	Formulation source	Verified
AMO ⁺ Oxysept 1 step	Not applicable (NA)	Hydrogen peroxide 3%	Neutralized with enzyme neutralizing tablet containing catalase, hydroxypropyl methylcellulose, and cyanocobalamin (vitamin B12) as color indicator, with buffering and tabletting agents. Solution also contains sodium stannate, sodium nitrate, buffered with phosphates and purified water	Yes		Y
AMO Complete Revitalens	NA	Polyquaternium-1 0.0003%, Alexidine dihydrochloride 0.00016%	EDTA, Tetronic 904, boric acid/borate [1]	Yes	Kilvington, 2010	Y
Alcon OPTI-FREE EXPRESS	NA	polyquaternium-1 (POLYQUAD®) 0.001% and myristamidopropyl dimethylamine (ALDOX®) 0.0005%	Sodium citrate, sodium chloride, boric acid, sorbitol, aminomethylpropanol, TETRONIC® 1304 ^d , with edetate disodium 0.05%	Yes	Package insert	Y
Alcon OPTI-FREE PureMoist	NA	polyquaternium-1 (POLYQUAD®) 0.001% and myristamidopropyl dimethylamine (ALDOX®) 0.0006%	Sodium citrate, sodium chloride, boric acid, sorbitol, aminomethylpropanol, disodium EDTA, two wetting agents (TETRONIC® 1304 ^d and Hydraclyde® Moisture Matrix [EOBO-41® polyoxyethylenepolyoxybutylene])	No from June 2011	Package insert	Y
Alcon OPTI-FREE RepleniSH	NA	polyquaternium-1 (POLYQUAD®) 0.001% and myristamidopropyl dimethylamine (ALDOX®) 0.0005%	Sodium citrate, sodium chloride, sodium borate, propylene glycol, TEARGLYDE® proprietary dual action reconditioning system (TETRONIC® 1304 ^d , nonanoyl ethylenediaminetetraacetic acid)	Yes	Package insert	Y
ASDA All in One contact lens solution	Uncertain whether this is own manufacture or rebranded	Polyhexanide (PHMB) (concentration unstated on packaging)	Poloxamer, HPWC, sodium hyaluronate, sodium phosphate	Uncertain	Phone interview with store staff	Y
B&L ⁺ Biotrue	NA	Polyaminopropyl biguanide (PAPB) 0.00013% & polyquaternium 0.0001%. PAPB and PHMB are interchangeable terms ^e	Hyaluronan, sulfobetaine, poloxamine, boric acid, sodium borate, edetate disodium & sodium chloride	Yes	Package insert	Y
B&L EasySept	NA	Hydrogen peroxide 3% with catalytic disc	Phosphonic acid, phosphate buffers & sodium chloride	Yes	Package insert	Y
B&L Renu MPS for sensitive eyes	NA	Polyaminopropyl biguanide (PAPB, DYMED®) 0.00005%	Boric acid, edetate disodium, poloxamine, sodium borate & sodium chloride	Yes	Package insert	Y

B&L ReNu Multipius	B&L ReNu Fresh	Polyaminopropyl biguanide (PAPB, DYMED®) 0.0001%	Hydroxyalkylphosphonate (HYDRANA TE®), boric acid, edetate disodium, poloxamer, sodium borate & sodium chloride	Yes	Package insert	Y
Boots CL solution		Polyaminopropyl biguanide (PAPB) 0.0001% PAPB and PHMB are interchangeable terms ⁶	Hydroxyalkylphosphonate 0.03%, poloxamine 1%, boric acid, disodium edetate, sodium borate & sodium chloride	Same formulation as B&L ReNu Multipius/Fresh	Package insert	Y
Boots All In One		Polyaminopropyl biguanide (PAPB) 0.00005% PAPB and PHMB are interchangeable terms ⁶	Poloxamine 1%, boric acid, disodium edetate, sodium borate & sodium chloride	Same formulation as B&L ReNu MPS for sensitive eyes	Package insert	Y
Ciba Vision AOSept Plus	NA	Hydrogen peroxide 3% with catalytic disc	Phosphonic acid, phosphate buffers, poloxamer & sodium chloride	Yes	Package insert	Y
Ciba Vision Solocare Aqua ⁶	Menicon Solocare Aqua	PHMB 0.0001%	Dexpanthanol (provitamin B5), Tromethamine, Sodium dihydrogen phosphate, Poloxamer 407, Ethylenediaminetetraacetic acid (EDTA), Sorbitol	Yes	Product brochure	Y
CooperVision Options Multi	NA	PHMB 0.0001%	Borate buffers	Yes	ACLM Handbook	Y
CooperVision Sautlon ComfortVue	Procis Premium All in One (Optic Actuel, Germany).	PHMB 0.0001%	Poloxamer, phosphate buffer, hydroxypropyl methylcellulose	Uncertain	ACLM Handbook	Y
CooperVision Sautlon Synergi	easyvision all purpose (Specsavers, UK) AND Irista Silicone Hydrogel multipurpose solution (France marketed by Generale d'Optique)	Oxipol, Oxxychlorite complex (sodium chlorite and hydrogen peroxide)	Phosphate, poloxamer, Hydroxypropyl methylcellulose (HPMC), Polyvinylpyrrolidone (PVP) [2]	Yes	Garcia-Porta, 2015	Y
CooperVision Sautlon Multi	easyvision peroxide (Specsavers, UK)	Hydrogen peroxide 3% with platinum catalytic disc		Yes	ACLM Handbook	Y
CooperVision Sautlon All in One Light	easyvision multipurpose (Specsavers, UK)	PHMB 0.0001%	Phosphate, Poloxamer, Disodium edetate [3]	Yes	Beattie, 2003	Y
	easyvision ultrapurpose	PAPB 0.0013 and Polyquaternium 0.001%	Hyaluronan, Sulfobetaine (detergent that may have antimicrobial effects), boroc acid, sodium borate, EDTA, sodium chloride	Uncertain	Phone interview with store staff	Y
CooperVision CycClean		PHMB 0.0001%	Phosphate, Poloxamer, HPMC (Bipol), EDTA	Yes	Manufacturer	N
Menicon MeniCare Soft multipurpose solution	NA	PHMB 0.0001%	Marogolglycerol, hydroxystearate 60, EDTA, propylene glycol, tonicity agents (2)	Yes	Kilvington, 2010	Y
MesmerEyz multipurpose soft lens solution	Unknown no response from UK distributor	PHMB (concentration not on bottle or website) No response	EDTA, Poloxamer, Boric acid, Sodium borate, sodium chloride, hydroxypropylmethylcellulose	Yes	Bottle label	Y

Morrison's All in One contact lens solution	Not clear whether this is own brand or rebranded	to email to company on 30 1 16	Sodium and potassium chloride, EDTA, poloxamer, HPMC, sodium hyaluronate, sodium phosphate	Uncertain	From website	Y
Optical Express Ciel All in One contact lens solution	Own manufacture	Polyhexamide (PHMB) 0.0001%	The active ingredient is the only one listed on the packaging	Uncertain	Phone Interview with store staff	
Sainsbury's all in one contact lens solution	Proprietary to Sainsbury's (East Midlands Pharmaceuticals)	PHMB 0.00015%	Disodium Edetate, Hypromellose, Poloxamer, Sodium & Potassium Chloride	No from August 2012	Product brochure	Y
Tesco all in one contact lens solution	Proprietary to Tesco's (East Midlands Pharmaceuticals)	PHMB 0.0002%	Disodium Edetate, Sodium Hyaluronate, Hypromellose, Poloxamer, Sodium & Potassium Chloride	Yes	Product brochure	Y
SOFT CL CLEANING SOLUTIONS						
EYEE crystal cleaning solution now rebranded as Oté Clean 40	Oté Pharma Sol BV Netherlands 5406	PHMB 0.002% as preservative	Non-ionic cleaning ingredients	Yes	From bottle	Y
RIGID CL DISINFECTION SOLUTIONS						
AMO Total Care disinfecting, storing and wetting solution	NA	PHMB 0.0001%	Purified water, tromethamine, sodium chloride, hydroxypropylmethylcellulose, EDTA and tyloxapol	Yes	Website	Y
B&L Boston Advance	NA	chlorhexidine gluconate (0.003%), PAMB (0.0005%)	Cationic cellulose derivative polymer, a cellulosic viscosifier, polyvinyl alcohol and a derivatized polyethylene glycol, edetate disodium (0.05%),	Yes	Package insert	Y
B&L Boston Simplus	NA	chlorhexidine gluconate (0.003%), PAMB (0.0005%)	Poloxamine, hydroxyalkylphosphonate, boric acid, sodium borate, sodium chloride, hydroxypropylmethyl cellulose, Glucan	Yes	Package insert	Y
RIGID LENS CLEANERS						
B&L Boston Cleaner	NA	PHMB 0.0005% Chlorhexidine 0.003%	Alkyl ether sulfate (9.8% w/v), ethoxylated alkyl phenoI, sodium chloride, silica suspension, sodium phosphate dibasic and triquatarnary cocoa base phospholipids	Yes	Bottle	Y
EYEE crystal cleaning solution now rebranded as Oté Clean 40	Oté Pharma Sol BV Netherlands 5406	PHMB 0.002% as preservative	Non-ionic cleaning ingredients	Yes	Bottle	N

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^aAbbot Medical Optics

^bBausch & Lomb

^cPAPB and PHMB are interchangeable terms: the term quoted is the one given on the packaging.

^dTRITONIC® is a registered trademark of BASF.

^eSystem contains antibacterial contact lens case

1 Kilvington S, Huang L, Kao E, Powell CH. Development of a new contact lens multipurpose solution: comparative analysis of microbiological, biological and clinical performance. *Journal of Optometry* 2010; 3(3):134-142

2. Garcia-Porta N, Rico-del-Viejo L, Ferreira-Neves H, Peixoto-de-Matos SC, Queiros A, Gonzalez-Mejome JM: Performance of three multipurpose disinfecting solutions with a silicone hydrogel contact lens. *BioMed research international* 2015, 2015:216932.

3. Beattie TK, Seal DV, Tomlinson A, McFayden AK, Grimason AM: Amoebicidal activity of multipurpose contact lens solutions using a most probable number (MPN) enumeration technique. *J Clin Microbiol* 2003, 41:2992-3000.

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Supplementary Table 3

Contact lens types

CL Brand/Name = alternative name	Water content	Oxygen permeability	Material name	Material Group
ACUVUE 2 colours (Johnson & Johnson)	58	21	Etafilcon A	IV
ACUVUE 2/ BIFOCAL (Johnson & Johnson)	58	21	Etafilcon A	IV
ACUVUE ADVANCE / ASTIGMATISM (Johnson & Johnson)	47	60	Galyfilcon A	VA
ACUVUE OASYS/ASTIGMATISM/ PRESBYOPIA (Johnson & Johnson)	38	107.4	Senofilcon A	VA
AIR OPTIX AQUA/ ASTIGMATISM/ MULTIFOCAL (CIBA Vision)	33	110	Lotrafilcon B	VC
AIR OPTIX Night and Day (CIBA Vision)	24	140	Lotrafilcon A	VC
Avaira (CooperVision)	46	100	Enfilcon A	VC
Biofinity /Toric/ Multifocal (CooperVision)	48	128	Comfilcon A	VC
Biomedics 55 Evolution/ Toric (CooperVision)	55	19	ocufilcon D	IV
Boots Night and Day (Boots)	24	140	Lotrafilcon A	VC
Boots Monthly (Boots) = Premium-(Lotrafilcon B)	33	110	Lotrafilcon B	VC
easyvision Monthly Classic/Aspheric/Toric/XR (Specsavers) = Coopervision Frequency 55	55	19	Methafilcon A	II
easyvision Irisian /Toric/Multifocal (Specsavers) = Air Optix for Astigmatism Monthly Toric (Ciba Vision)	33	110	lotrafilcon B	VC
Easy Vision Irisian Sphere = Air Optix Aqua, monthly (Ciba Vision)	33	110	lotrafilcon B	VC
easyvision Opteyes/Toric (Specsavers) = Biofinity Toric Monthly Toric (CooperVision)	48	128	Comfilcon A	VC
easyvision Aquaeyes (Specsavers) = AirOptix Night and Day Aqua	24	140	Lotrafilcon A	VC
EASYVISION UVICIA (silicone hydrogel) = Avaira	46	100	Enfilcon A	VC
EASYVISION LACRIMA PLUS (silicon hydrogel) = clariti elite	56	60	somofilcon A	VB
Expressions Colors (CooperVision)	55	19	Methafilcon A	IV
Frequency 55 / Xcel Toric (CooperVision)	55	19	Methafilcon A	IV
FreshLook Colorblends / Colors / Dimensions (CIBA Vision)	55	16	Phemfilcon A	IV
Proclear/ Toric/ EP (CooperVision)	62	27	omafilcon B	II
PureVision/ Toric/Multi-focal (Bausch & Lomb)	36	91	Bala filcon A	III
PureVision 2 HD/ 2 HD for Astigmatism (Bausch & Lomb)	36	91	Bala filcon A	VA
SofLens 38 (Bausch & Lomb)	38	6	Polymacon	I
SofLens 59 (Bausch & Lomb)	59	17	Hilafilcon b	II
SofLens Toric /Multifocal (Bausch & Lomb)	66	24	Alphafilcon a	II
CRYSTAL (Ocuflcon D 45%)	45	19.6	ocufilcon D	III
GEO COLOUR (from Korea) = 'Circle' SCLs (large diameter)	42	9	polymacon	I
CLARITI ELITE (silicon hydrogel)	56	60	somofilcon A	VB
DESIO (Desiolens.com). Coloured, 62% polymacon, 38%WC	38	9	polymacon	I
SEQUENCE 55	38	9	polymacon	I
Sauflon clariti	56	60	Somofilcon A	VB
Ascend Premier = Biofinity	48	128	Comfilcon A	VC
IWear XRT Supreme = Biofinity Toric (available in LATAM)	48	128	Comfilcon A	VC
Irisia care (monthly)	33	110	Uncertain	VC

Lens material classification

Group I: (low water (< 50%), non-ionic)

Group II: (high water (>= 50%), nonionic)

Group III: (low water, (< 50%), ionic)

Group IV: (high water, (>= 50%), ionic)

Group V: Materials with Dk > 40 Dk units (in mmHg) and greater than expected on the basis of water content

Group VA: Group 5 materials containing an ionic monomer or oligomer at pH 6-8

Group VB: Group 5 non-ionic material containing > 50% water

Group VC: Group 5 non-ionic material containing < 50% water

Supplementary Table 4

Potential risk factors for AK, initially selected for inclusion in the model building process.

Results from logistic models without adjustment for confounding.

Variable	Control	Case	Total	Odds		95% CI for	
				Ratio ¹	p-value	Odds Ratio	
Disinfectant classified by Principal Active Ingredient (PAI)							
1: PHMB (0.00005-0.0001)	90	11	101	<i>Referent</i>			
2: Polyquad-1(0.001%)+ALDOX (0.0005%) ²	26	9	35	2.83	0.038	1.06	7.57
3: Peroxide3%	12	5	17	3.41	0.048	1.01	11.51
4: Polyquad 1(0.0001%)+Polyaminopropyl biguanide	11	2	13	1.49	0.633	0.29	7.60
5: Oxipol	55	29	84	4.31	< 0.001	2.00	9.33
6: Rigid Gas permeable lens solutions	11	5	16	3.72	0.036	1.09	12.71
Unknown	8	2	10				
Total	213	63	276				
Frequency of contact lens CL wear: days per week							
Up to 4 days	34	4	38	<i>Referent</i>			
> 4 days	179	57	236	2.71	0.070	0.92	7.95
Unknown	0	2	2				
Total	213	63	276				
CL materials classification: based on FDA/ANSI standards listed in Supplementary Table 3							
Group 1	3	0	3	(empty)			
Group 2	13	6	19	2.70	0.086	0.87	8.37
Group 3	2	0	2	(empty)			
Group 4	13	7	20	3.15	0.039	1.06	9.37
Group 5A	76	13	89	<i>Referent</i>			
Group 5B	3	1	4	1.95	0.576	0.19	20.2
Group 5C	70	28	98	2.34	0.023	1.12	4.87
RGP	11	5	16	2.66	0.113	0.79	8.91
Unknown	22	3	25				
Total	213	63	276				
CL grouping simplified:							
Groups I + II + III	18	6	24	1.95	0.233	0.65	5.83
Group IV	13	7	20	3.15	0.039	1.06	9.37
Group VA	76	13	89	<i>Referent</i>			
Group VB + VC	73	29	102	2.32	0.023	1.12	4.81
Rigid gas permeable CL's	11	5	16	2.66	0.113	0.79	8.91
Unknown	22	3	25				
Total	213	63	276				

Variable	Control	Case	Total	Odds Ratio ¹	p-value	95% CI for Odds Ratio	
Hygiene Score (HS) Mean	4.30	5.09	4.47	----	< 0.001 ²	----	----
Hygiene Score (HS) Categories³ based on top (worst) quartile of the sample							
1: Good-Moderate (score 1.75-5.08)	174	30	204	<i>Referent</i>			
2: Poor (score 5.09-8.08)	39	33	72	4.91	< 0.001	2.68	8.98
Totals	213	63	276				
Hand wash before handling CLs							
1: No/unsure	27	21	48	3.71	< 0.001	1.90	7.22
2: Yes	186	39	225	<i>Referent</i>			
Unknown	0	3	3				
Total	213	63	276				
Shower wearing CLs							
1: No	141	25	166	<i>Referent</i>			
2: Yes	72	38	110	2.98	< 0.001	1.67	5.31
Total	213	63	276				
Water activity wearing CLs							
1: None	114	20	134	<i>Referent</i>			
2: in Ocean/Sea/River/Lake	42	9	51	1.22	0.649	0.52	2.89
3: in Public pool	46	23	69	2.85	0.003	1.43	5.68
4: in Private pool	8	7	15	4.99	0.005	1.63	15.29
5: in Hot tub	3	1	4	1.90	0.586	0.19	19.19
Unknown	0	3	3				
Total	213	63	276				
Water activity: 3,4,5 combined							
1: Nowhere	114	20	134	<i>Referent</i>			
2: in Ocean/Sea/River/Lake	42	9	51	1.22	0.649	0.52	2.89
3: Swimming Pools/Hot tub	57	31	88	3.10	0.001	1.63	5.91
Unknown	0	3	3				
Ethnic group							
1: Asian	29	2	31	<i>Referent</i>			
2: British Other Black	16	1	17	0.91	0.938	0.08	10.79
4: White Caucasian	141	54	195	5.55	0.022	1.28	24.08
6: Other	27	3	30	1.61	0.616	0.25	10.39
Unknown	0	3	3				
Total	213	63	276				

Variable	Control	Case	Total	Odds		95% CI for	
				Ratio *	p-value	Odds Ratio	
Ethnic group: 1,2,6 combined							
4: White Caucasian	141	54	195	<i>Referent</i>			
6: Other	72	6	78	0.22	0.001	0.09	0.53
Unknown	0	3	3				
Total	213	63	276				
Occupation⁵							
1-3 Professional/Director/Manager/Associate Professional Technical occupations	163	36	199	<i>Referent</i>			
4 Administrative and secretarial occupations	16	6	22	1.70	0.302	0.62	4.64
5 Skilled trades occupations	6	4	10	3.02	0.100	0.81	11.25
6 Caring, leisure and other service occupations	8	4	12	2.26	0.201	0.65	7.93
7 Sales and customer service occupations	13	7	20	2.44	0.077	0.91	6.54
8 Process, plant and machine operatives	5	0	5				
9 Occupations requiring no training	2	1	3	2.26	0.509	0.20	25.65
Unknown	0	5	5				
Occupation: categories 4-9 combined							
1-3 Professional/Director/Manager/Associate Professional Technical occupations	163	36	199	<i>Referent</i>			
4-9 Categories 4 to 9 combined	50	22	72	1.99	0.029	1.07	3.70
Unknown	0	5	5				
Total	213	63	276				
Education level							
1: up to A level	44	19	63	<i>Referent</i>			
4: Degree	96	28	124	0.68	0.260	0.34	1.34
5: Higher Education	73	11	84	0.35	0.013	0.15	0.80
Unknown	0	5	5				
Total	213	63	276				

- 1 Odds ratios (OR) are **not adjusted** for confounding effects of other variables
- 2 PAI category 2 Includes Polyquad-1(0.001%) + ALDOX (0.0005%) AND (as used by 4 controls and 1 case) Polyquad-1 (0.0003%) + Alexidine (0.00016%)
- 3 Hygiene score categories based on worst (top) quartile of the mean hygiene scores in the sample.
- 4 p-value of 0.0004 from non-parametric Mann-Whitney test
- 5 Listed by categories

Supplementary Table 5

Contact lens hygiene compliance assessment methodology

Contact lens hygiene compliance was assessed by patient responses to 14 multiple choice questions. The responses to each question (or composite pair of questions) from each patient were assigned a score of 1 for full compliance, 5 for partial non-compliance, and 10 for complete non-compliance. An average score was then calculated for the patient. A single variable was created to hold all the mean scores. The questions were given equal importance (no weighting). Patients were then classified according to the *quartiles* of the mean score for the sample. A simpler binary classification was derived for MV analysis, based on the top (worst) quartile: "Good-Moderate" (mean score 1.75 - 5.08), and "Poor" (mean score 5.09 - 8.08). Hand washing before handling CLs and showering while wearing CLs were kept as separate variables and analysed as such.

Category	Hygiene question Variables	Score	Variable ID
	Q23: How often do you use disinfecting solution		1
1	Always	1	
2	Uses extended wear disposable CLs, dispose on removal, no disinfectant (excluded from main analysis sample)*	1	
3	Sometimes	10	
4	Never (excluded from main analysis sample)#	10	
	Q28: How long had the bottle of solution been open		2
1	1 to 30 days	1	
2	31 to 59 days	5	
3	60 or more days	10	
	Q29: Did you transfer your solution into another container		3
	Q30: Did you use this transferred solution the last time you rinsed or stored the lenses		4
1	Q29=No	1	
2	Q29=Yes, Q30=No	1	
3	Q29=yes, Q30=Unsure	5	
4	Q29=Yes, Q30=Yes	10	
	Q31: Did you rub your lenses the last time before you STORED them		5
1	No	10	
2	Yes	1	
99	Unsure	Blank	
	Q32: Did you rinse your lenses before you STORED them		6(a)
	Q32n If Yes, rinsed with what?		6(b)
1	Q32=No	10	
2	Q32=Yes, Q32n=with Water	5	
3	Q32=Yes, Q32n=with disinfectant solution / Saline	1	
	Q33: Did you rinse your lenses the last time before you inserted them into your eyes		7(a)
	Q33n: If Yes, with what?		7(b)
1	Q33=No	10	
2	Q33=Yes, Q33n= disinfectant solution / Saline	1	
3	Q33=Yes, Q33n= Hot water	5	
4	Q33=Yes, Q33n= Warm water / Water	10	

	Q34: Did you rub your lenses the last time before you inserted them into your eyes		8
1	No	10	
2	Yes (all responses: "with disinfectant solution")	1	
	Q35: Did you replace all the disinfecting solution in your case		9
1	No, topped it up	10	
2	Yes	1	
99	Unsure	Blank	
	Q36: After you took your contact lenses out, did you rinse your case		10
	Q37: What did you rinse your case with		11
1	No rinse	10	
2	Yes, with Saline	1	
3	Yes, with Water	5	
4	Yes, with disinfectant solution	1	
99	Unsure	Blank	
	Q38: Did you empty your case and leave it to dry		12
1	No	10	
2	Yes	1	
99	Unsure	Blank	
	Q39: How old was your case when the symptoms started		13
1	One to 90 days old	1	
2	More than 90 days	10	
	Q57: Where did you LAST carry out contact lens insertion and removal		14
1	Bathroom	1	
2	Kitchen	5	
3	Bedroom	5	
4	Other	10	
99	Unsure	Blank	

* The categories of lens are not reusable daily wear CL's and irrelevant to the analysis

This category was for 7 controls using saline only

Supplementary Table 6

Covariate adjustment

Confounders (covariates) adjusted for in the final regression models constructed to estimate the odds ratios for a particular risk factor with optimal adjustment for confounding.

Risk factor of 'main interest'	Covariates adjusted for:	Final Model #
Lens Disinfectant (PAI)	Hygiene Score; Hand washing; Shower wearing CLs; Water Activities wearing CLs; Ethnic Group; Occupation	1
CL Materials	As for Model-1, but excluding Rigid CL solutions	2
Hygiene Score	As for Model-1, but excluding "Shower wearing CLs"	3
Hand washing	As for Model-3	3
Shower wearing CLs	As for Model-1	1
Water Activities wearing CLs	As for Model-3	3
Ethnic Group	As for Model-3	3
Occupation	As for Model-3	3

Supplementary Table 7

Swimming whilst wearing contact lenses.

Comparison of current dataset (collected 12-12-2013 to 05-06-2017 with dataset from a similar case control study of microbial keratitis (all causes) in contact lens users for data collected at Moorfields 03-12-2003 to 02-12-2005 (Reference 14 in the Text: Dart JK, Radford CF, Minassian D, Verma S, Stapleton F. Risk factors for microbial keratitis with contemporary contact lenses: a case-control study. *Ophthalmology* 2008; **115**(10): 1647-54).

Dataset and category	Total number	Users swimming in contact lenses	
		Number	Percent
Current study 12-12-2013 to 05-06-2017			
<i>Acanthamoeba</i> keratitis cases	60	40	66.67
Control dataset 22-11-2016 to 05-06-2017	213	99	46.48
Cases and controls combined	273	139	50.92
Dataset from 03-12-2003 to 02-12-2005 study			
Microbial keratitis cases	366	224	61.20
Population controls	637	326	51.18
Hospital controls	1921	1103	57.42
All cases and controls combined	2924	1653	56.53