Ophthalmology

Acanthamoeba keratitis risk factors for daily wear contact lens users: a case control study

--Manuscript Draft--

Manuscript Number:	OPHTHA-D-22-00281R3
Article Type:	Manuscript
Keywords:	Acanthamoeba keratitis; AK; Contact lens; daily disposable contact lens; reusable soft contact lens; rigid contact lens; hard contact lens; risk factor; case control study; epidemiology; Prospective; keratitis
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Abstract:	Objectives This study was designed to establish risk factors for the development of Acanthamoeba keratitis (AK) for daily disposable contact lens (DD) users compared to daily wear (DW) reusable lens users and for risks unique to DD users. This is important because in many major economies CL use is the principal cause of microbial keratitis (MK) of which AK accounts for ≈ 50% of cases with sight loss. Determining these AK risks informs practitioner advice and consumer behaviour. Design Case control study Subjects and controls Cases and controls were recruited from an Accident and Emergency department serving South-East England. Cases were new CL users with AK recruited retrospectively from January 2011 to February 2013 and prospectively thereafter until August 2014. Controls were recruited prospectively from January 2014 to June 2015. Methods Analysis of a self-administered questionnaire. Main outcome measures Independent risk factors and population attributable risk percentage (PAR%) for AK. Results 83 AK cases and 122 controls were recruited; DD use was reported by 20 (24%) cases and 66 (54%) controls. In multivariable analyses adjusted for potential confounders the odds of AK was higher for DW reusable soft (odds ratio [OR] 3.49, 95% confidence limits [CI] 1.75-8.43 and rigid (OR 4.56, 95% CI 1.03-20.19), compared to DD. Within the DD-using subset, AK was associated with the following modifiable risk factors: less frequent professional follow-up visits (OR 10.12, 95% CI 5.01-20.46; showering in lenses (OR 3.29, 95% CI 1.17-9.23); lens reuse (OR 5.41 , 95% CI 1.55-18.89) and overnight wear (OR 3.93, 95% CI 1.15-13.46). The PAR% estimated that 30-62% of cases could be prevented by switching from reusable soft to DD lens use. Conclusions AK risks are increased >3-fold in DW reusable lens users versus DD lens use. AK risks for DD lens users can be minimised by adherence to safe use guidelines (no reuse, overnight wear, or contamination by water). Safe CL use can be improved by increasing the prominence of risk avoidance information from manufacturers and regulators. Because AK accounts for half of severe keratitis in CL users these

	measures can be expected to have public health benefits.
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Cover Letter

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The Editor
Ophthalmology

2nd August 2022

Dear Professor van Gelder,

Re: Manuscript Number: OPHTHA-D-22-00281
Acanthamoeba keratitis: risk factors for daily wear contact lens users: a case control study

Thank you for accepting this manuscript subject to an alteration to the wording in the abstract results section which we have made. The change which is shown in the revised changes marked manuscript and included in the clean manuscript file.

Yours sincerely,
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Ophthalmology Reviews tabulated for:
Manuscript Number: OPHTHA-D-22-00281R2
Acanthamoeba keratitis risk factors for daily wear contact lens users: a case control study

Editorial Board comment	Comments for Editor	Changes in manuscript
The sentence in the abstract is a little confusing: "Within the DD using subset modifiable increased risks after multivariable analysis, were: less frequent professional follow-up visits…" Clearer would be "Within the DD-using subset, AK was associated with the following modifiable risk factors: less frequent professional follow-up visits, …"	Thank you. We agree and have made this change in the manuscript	Lines 83-84 altered as suggested

Précis

This case control study of *Acanthamoeba* keratitis shows that reusable contact lens use carries a 3.9-fold higher risk compared to daily disposable (DD) lens use and demonstrates five avoidable risks for DD lens users.

ABSTRACT

Objectives

This study was designed to establish risk factors for the development of Acanthamoeba keratitis (AK) for daily disposable contact lens (DD) users compared to daily wear (DW) reusable lens users and for risks unique to DD users. This is important because in many major economies CL use is the principal cause of microbial keratitis (MK) of which AK accounts for ≈ 50% of cases with sight loss. Determining these AK risks informs practitioner advice and consumer behaviour.

Design

Case control study

Subjects and controls

Cases and controls were recruited from an Accident and Emergency department serving South-East England. Cases were new CL users with AK recruited retrospectively from January 2011 to February 2013 and prospectively thereafter until August 2014. Controls were recruited prospectively from January 2014 to June 2015.

Methods

Analysis of a self-administered questionnaire.

Main outcome measures

Independent risk factors and population attributable risk percentage (PAR%) for AK.

Results

83 AK cases and 122 controls were recruited; DD use was reported by 20 (24%) cases and 66 (54%) controls. In multivariable analyses adjusted for potential confounders the odds of AK was higher for DW reusable soft (odds ratio [OR] 3.49, 95% confidence limits [CI] 1.75-8.43 and rigid (OR 4.56, 95% CI 1.03-20.19), compared to DD. Within the DD-using subset, AK was associated with the following modifiable risk factors: less frequent professional follow-up visits (OR 10.12, 95% CI 5.01-20.46; showering in lenses (OR 3.29, 95% CI 1.17-9.23); lens reuse (OR 5.41 , 95% CI 1.55-18.89) and overnight wear (OR 3.93, 95% CI 1.15-13.46). The PAR% estimated that 30-62% of cases could be prevented by switching from reusable soft to DD lens use.

Conclusions

AK risks are increased >3-fold in DW reusable lens users versus DD lens use. AK risks for DD lens users can be minimised by adherence to safe use guidelines (no reuse, overnight wear, or contamination by water). Safe CL use can be improved by increasing the prominence of risk avoidance information from manufacturers and regulators. Because AK accounts for half of severe keratitis in CL users these measures can be expected to have public health benefits.

INTRODUCTION

This study was designed both to evaluate whether daily disposable (DD) contact lens (CL) wear was protective for the development of *Acanthamoeba* keratitis (AK) compared to daily wear (DW) reusable lens use and also to identify risk factors for AK with DD lens use. AK is important in the context of sight loss in CL users as, although the incidence is low at 0.31-0.48:10,000 (UK[1] and Netherlands[2] in 2015), half of these (0.16-0.24:10,000) develop sight loss. Thus, AK accounts for a high proportion of cases of sight loss in CL users resulting in substantial impacts on quality of life[3, 4] and disproportionally higher healthcare costs.[5] This is a public health issue both because CL use is the leading cause of microbial keratitis (MK) in patients with otherwise healthy eyes in high per capita income countries where CL use is widespread[6], resulting in an economic burden both to those affected and to the healthcare system[7], and because inexpensive health protection measures against MK can be effective.[8]

The population penetrance of CL wear in these countries varies at 13.9% (45 million) in the USA in 2016[9] and in 2020 was 9% (6.3 million) in the UK rising to 25-30% in the Netherlands and Sweden.[10] A 2017 worldwide user estimate was c.300 million.[11] This is an important market for manufacturers, valued at $8.69 billion in 2019[12], in which economic imperatives may have mitigated against the promotion of preventive information relating to MK. MK is the only sight threatening complication of CL use and despite the introduction of new lens materials and daily disposable (DD) lenses, the incidence has remained unchanged at 2-4 per 10,000 over many decades [13] of whom 0.2-0.6 per 10,000 will have sight loss.[14, 15]

The most widely used lens types are DD (single use) and daily wear (DW) reusable soft (stored overnight and renewed after 2-4 weeks or longer) which together account for >90% of all lenses fitted.[16] DD lenses have steadily increased in popularity[12] and now account for over half the lenses fitted in some countries (61% in the UK)[16]. The widespread use of DD is both because of convenience and because data suggests that the risk for severe MK with vision loss, including that caused by *Acanthamoeba*, is probably reduced for DD compared to reusable CL wear[13, 17-19] although this has not been confirmed for either predominantly bacterial[13] or for *Acanthamoeba* keratitis.[20, 21] Identifying modifiable risk factors for CL users

is important, particularly with regard to AK for which, unlike bacterial keratitis in CL users, 90% of cases are associated with avoidable risks.[22] Given this background to our study we expect our findings to be generalisable to other high per capita income countries where CL use is widespread. This analysis complements our previous publication on risk factors for AK associated with reusable CL.[23]

METHODS

Ethics approval was from the National Research Ethics Service Committee London-Hampstead, REC Reference 13/LO/0032 and the Moorfields Eye Hospital Research Governance Committee 18th February 2013.

Cases were DW reusable or DD lens users diagnosed with *Acanthamoeba* keratitis. These included both self-referrals, secondary (general practitioner and optometric) and tertiary (other ophthalmology centres) referrals between January 2011 and August 2014. Cases diagnosed before ethics approval was given in February 2013 were recruited after diagnosis following which cases were recruited prospectively at the time of diagnosis. Inclusion criteria before January 2014 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.[22] From January 2014 *Acanthamoeba* DNA identification by polymerase chain reaction (PCR) was added to the diagnostic tests as an additional inclusion criterion.

Controls were DW reusable or DD CL users recruited between February 2014 and June 2015 attending A&E as new patients but with a disorder thought to be unrelated to CL wear (listed in Supplementary Table 1), for which the diagnosis was derived from the hospital records.

Both cases and controls completed a 5-part (contact lens wear history, disinfectant solution history, lens use environment, eye care and demographics) self-administered questionnaires with 48 multiple part questions. Case questionnaires included 15 additional questions, encompassing a section about events leading up to the episode of keratitis, and for which the data was not included in the case control study analysis. The questionnaires were modified from those used in a previous study.[17] Cases or controls were excluded if they had insufficient questionnaire data despite attempts to contact them to clarify and/or complete data, had not used a CL during the previous 30 days, had a medical indication for CL wear, or had any previous attendance at Moorfields. The questionnaire data were entered into a database for analysis.

Analysis of the association between Acanthamoeba keratitis and the contact lens type (daily disposable versus daily wear reusable)

The DW reusable lens cases are those already described in our previous analysis of AK risks for reusable CL wearers in which the hygiene scoring methodology (summarised in Supplementary Table 2) is described.[23] The hygiene scores for DD lens users were compared with those for reusable lens users by allocating all the DD users who reused their lenses to the highest score for poor hygiene; none of the other hygiene parameters were relevant to DD users.

Analysis of risk factors for AK among DD lens users

To explore risk factors for AK among DD users, a separate case control analysis was performed restricted to the study population subset who were DD users. DD users who reused their lenses were categorised as DD users as this was considered a behavioural issue (such as overnight wear) that required assessment as a risk factor for DD lens use.

Statistical analysis

The sample size calculation (including all DD and DW reusable subjects) indicated a sample size of 86 AK cases and 111 controls to detect a true odds ratio of 3.0 or more with 80% power, alpha (2-sided) set at 0.05, specifying a control/case ratio of 1.3 assuming an exposure proportion of 10% in controls (larger proportions requiring smaller sample sizes).

Analyses were performed using Stata software version 17 (StataCorp LP, College Station, TX). Variables with more than 3 categories were grouped for analysis. The descriptive and crude (unadjusted) analysis of the characteristics of cases and controls and their association with risk of AK were evaluated one at a time without adjustment for confounding. Logistic regression was used to estimate odds ratios as a measure of association. Odds ratios are regarded as estimates of relative risk throughout.

The main analysis employed multivariable logistic modelling to evaluate odds ratios for a variable of interest, with adjustment for effects of potential confounders (covariates). Variables of interest were chosen both because they were associated with higher odds of having AK in the unadjusted analysis ($p<0.05$) and/or because they had been found to be potential risk factors or confounders in previous studies. Least absolute shrinkage and selection operator (LASSO) inferential logistic models for binary outcome data were fitted via cross-fit partialing out using plugins [note: LASSO was our preferred method for selection of covariates because unlike stepwise procedures it does not tend to produce biased estimates of regression coefficients (away from zero), deals better with problems of collinearity, and

was appropriate for our datasets where the sample sizes were modest and number of potential covariates relatively large]. These models were used for evaluation of adjusted odds ratios for each exposure of interest, taking all other candidate variables as potential confounders. Data on occupation was missing in more than 10% (9/86) of the DD lens users. Since this could be a considerable source of bias, the variable was not included in the main LASSO analyses as a covariate, however, subsequent inclusion of the variable in the modelling process did not result in material change of odds ratios for other variables of interest but did reduce precision of the estimated odds ratios considerably (details not reported but available).

Calculation of population attributable risk% (PAR%) for the potentially remediable AK risk factors was based on the odds ratio estimate and the proportion of cases exposed to the risk factor at issue.

RESULTS

Recruitment

Eighty-three AK cases and 122 controls were recruited. Supplementary Table 3 describes the numbers of cases recruited retrospectively (21) as opposed to prospectively (62) and the differences in contemporaneity of recruitment for cases and controls resulting in 81controls recruited after cases. Table 1 summarises the numbers in the DD and reusable CL datasets. Case recruitment was limited by researcher availability; only 1 case refused to participate whereas a second was unsuitable having no English language use. Control recruitment was limited by both researcher availability and the inclusion criteria requirements and fell behind the recruitment of cases; further recruiter resources were found to recruit the additional controls required for the analysis resulting in an extension of the period of control recruitment for 10 months beyond the recruitment of cases.

Analysis of the association between Acanthamoeba keratitis and the contact lens type (daily disposable versus daily wear reusable)

Supplementary Table 4a shows the characteristics of the cases and controls together with unadjusted odds ratios as crude measures of association with risk of AK. Table 1 shows both unadjusted and adjusted analyses which are similar. Reusable soft CL were associated with higher odds of AK compared to daily disposable CL, as were the rigid lenses. The adjusted analysis includes the covariates (potential confounders) in the LASSO model building process which are listed in the Table footnote. The adjusted odds ratios indicated a significantly higher risk of AK for both reusable soft (odds ratio 3.84, 95% CI 1.75-8.43) and rigid CLs (odds ratio 4.56, 95% CI 1.03-20.19), compared to DD lenses.

Analysis of risk factors for AK among DD lens users

Supplementary Table 4b shows the characteristics of the cases and controls together with unadjusted odds ratios as crude measures of association with risk of AK. Variables included in the multivariable LASSO modelling process are marked by asterisks in the Table.

Multivariable analysis (with adjustment for confounding) findings

Results of the multivariable analysis, with adjustment for the confounding effects of covariates, for identified independent risk factors are shown in Table 2 (see Supplementary Table 5 for full analysis results). Six independent risk factors were identified by the adjusted analysis:

1. White British DD users had a higher risk (approximately 5-fold) of AK (odds ratio 5.07; 95% CI 1.10 - 23.44, p 0.038)
2. Wearing DD lenses for longer periods (12-18 hours) was protective for AK compared to shorter periods of wear (odds ratio 0.22; 95% CI 0.06-0.88, p 0.032)
3. Having a contact lens check more than 30 days before their attendance at the Hospital was associated with a 10-fold higher risk of developing AK (odds ratio 10.12, CI 5.01 - 20.46, p<0.001)
4. Showering whilst wearing CLs was associated with a c. 3-fold increase in odds of having AK (odds ratio 3.29; 95% CI: 1.17 - 9.23; p 0.024).
5. Reuse of CLs increased odds of AK by c.5-fold (odds ratio 5.41; 95% CI: 1.55 - 18.89; p 0.008)
6. Overnight CL wear was associated with a c. 4-fold increase in odds of AK (odds ratio 3.93; 95% CI: 1.15 - 13.46; p 0.030)

Population attributable risk percentage calculations

Population attributable risk percentage (PAR%) were calculated in order to estimate the proportion of AK cases attributable to each of the risk factors. These are shown in Table 3 for the remediable AK risks. These are substantial for most exposures but with wide confidence limits. For reusable soft lenses versus DD lenses 51.7% (95% CI 29.9-61.6%); for rigid GP lenses versus DD lenses 4.7% (CI 0.2% -5.7%). Within the DD lens user subset, the PAR% for a CL check >30 days before 85.4% (95% CI 75.8-90.1); for showering in CL 45.2% (95% CI 9.4-58.0); for CL reuse 48.9% (95% CI 21.3-56.8); overnight CL wear 26.1% (95 CI 4.6 - 32.4).

DISCUSSION

This study has identified DD lenses as protective for AK compared to both reusable soft and rigid lenses with the PAR% suggesting that approximately 30-62% of AK could be prevented

by switching from reusable soft to DD lens use. It has also identified five modifiable factors that increased risk for AK in users of DD lens users: shorter wearing time; not having a recent appointment with a contact lens professional; showering whilst wearing lenses; lens reuse and overnight wear.

The use of DD in comparison to reusable DW lenses has been shown either to increase the risk of predominantly bacterial keratitis 1.56-fold[17] or not to reduce its incidence.[15] However, both of these studies showed a reduction in severe MK for DD users that was significant in univariate analysis[17] probably because of elimination of the lens case. Our findings for AK show DW reusable lens users to have a 3.71-fold higher risk than DD lens users after multivariable analysis and that this was similar for both soft and rigid lens users. This reduction in AK risk for DD users may also relate to the elimination of the lens storage case which commonly harbours *Acanthamoeba* spp. and their bacterial food source.[24] Contact lens solutions are regulated for antibacterial efficacy but not for anti-*Acanthamoeba* efficacy due to the absence of an agreed test standard.[25] This lack of regulation may be responsible for the periodic outbreaks of AK due to disinfection solution failures.[19, 23, 26] Given that this study provides evidence that DD use protects against AK, and the probability that it also protects against severe bacterial keratitis, DD lens wear should be encouraged.

Wearing DD lenses for longer periods per day (12-18 hours) was protective for AK versus shorter periods. This finding is mirrored by a study showing an increased risk of corneal infiltrates in overnight wear lens users unable to adapt to >21 days of wear[27], and might relate to factors like dry eye & microtrauma from insertion and removal difficulty in subjects unable to wear lenses comfortably for longer periods.

The association of AK with the frequency of DD CL follow up appointments is consistent with findings in other studies showing that internet purchase [15] or poor aftercare instruction and recall is associated with predominantly bacterial MK[11, 26] which are all surrogates for education on risks of lens wear. The PAR% CI of 76-90% suggests that improving education could have a substantial effect.

Exposure to contaminated water as a risk factor for AK has been acknowledged since the first case-control study, with limited multivariable analysis, investigated the USA outbreak of AK in 1985-6.[28] Subsequent case reports in both CL users and after corneal trauma have associated AK with contaminated sea, lake, swimming pool, and domestic water.[29-31] However, confirmation of these probable risks for AK, using multivariable analysis, has only been confirmed recently for reusable CL wearers with a 3.5-fold increase in risk whilst

wearing lenses in hot tubs and swimming pools[23] and, in our current study in DD lens users, a 3.3-fold increased risk for showering in lenses (PAR% CI 9-58%). Exposure to any water when using CL is a risk for AK and should be avoided. By contrast, bacterial keratitis due to swimming in lenses, although reported in case series, has not been proven in large epidemiological studies and is probably relatively uncommon.[15, 17] Swimming in lenses is widespread; it is prudent to advise users that the least risk of AK whilst swimming is without lenses and that the advice to use goggles over lenses[32] and renew lenses immediately afterwards may not be safe.

Reuse of DD lenses unsurprisingly increased the risk of AK by 5.4-fold (PAR% CI 21-57%) and probably relates to absent disinfection and the use of nonsterile liquid to maintain lens hydration.

Overnight CL wear is a well-established risk factor for predominantly bacterial keratitis in reusable soft and DD lenses, however it has not been associated with AK prior to this study.

An unmodifiable risk factor was White British ethnicity, associated with a 5-fold higher risk of AK, which may be related to cultural differences such as a greater risk-taking propensity.[33]

Limitations and sources of bias

Due to the comparative rarity of AK the sample size for this study limited the detection of odds ratios ≥ 3.0-fold unless the exposure of controls was high, as for the risk of AK in DD v. reusable CLs, where the exposure of controls to reusable lenses was 56/122 (46%) giving a lowest detectable odds ratio of 2.3-fold. The study was designed to eliminate important sources of potential bias in the selection of cases and controls with little or no subjectivity in ascertainment. Using controls that were referred or self-referred to the same hospital department as the cases can be expected to reduce bias arising from differential referral or attendance patterns since many factors determining attendance are common to both cases and controls; this has held true in a previous and similar study on microbial keratitis in contact lens users where no substantive difference in odds ratio estimates were found when comparing Hospital with Non-Hospital controls which led us to combine these two groups.[17] (see Sources of bias in Supplementary Appendix 2 for a detailed description of this rationale).

There was a difference in the ethnicity of tertiary referral cases with a higher proportion of these being white British. This is a potential source of bias for the ethnicity findings. In Supplementary Appendix; Sources of bias we have shown that the odds ratio for ethnic group

in DD users remains substantial when tertiary AK cases are excluded from the analysis, but with loss of power due to small numbers. As a result, we think it probable that the excess risk in British white subjects is present despite the imbalance in the referral pathway.

The disparity in the timing of enrolment of cases and controls, as well as the fact that some cases but no controls were enrolled retrospectively, could have introduced bias through a variety of factors although we are unaware of any (such as weather, pandemics, and changes in the availability of lenses and disinfection solutions) that would have introduced excessive bias.

Regulatory deficiencies

CL are designated Class IIa (low to medium risk) medical devices in the UK and EU and Class II in the USA (for daily wear lenses) requiring manufacturers to include essential information on safe use and risks. However, CL manufacturers in the UK and EU are currently utilising an exception to this requirement reasoning that CL users will have received this information and training from the regulated professional who dispenses their lenses. Now that lenses are available to consumers on the internet without professional involvement (20/85 in this study) in the EU/UK (but not in the USA) many users may have no training or ongoing education in safe CL use. In the EU/UK, and for soft lenses in the USA, information on lens safety and risk avoidance recommendations are absent in lens packaging where the "do's and don'ts" needed to reduce the risk of keratitis might be reinforced at each purchase. Instead, users are directed to access "Patient information/instruction for use" guides on CL company websites, or from their practitioner; these provide variable information about MK risks and risk avoidance. CL companies have adopted little of the effort that public health (UK National Health Service and USA Centers for Disease Control amongst others) and professional organisations (British Contact Lens Association) have put into campaigning against the use of water with CL wear, apart from advising against this in "Instruction for use" guides on their websites and in social media feeds.[34] That education can reduce keratitis risks has already been discussed above in relation to internet purchase[15] or deficient instruction in use[11, 26] and a recent study on the effect of "no water" stickers on CL cases has shown that water exposure was reduced by this simple measure which could be incorporated into all CL packaging, including the capsules containing individual lenses.[8] This evidence should be used by CL manufacturers, or their regulators, to include both no water symbols on each lens capsule and case, together with a statement on the packaging, in the language used by the markets into which the lenses are sold, regarding keratitis avoidance (see Supplementary Appendix for an example of a risks and precautions statement and graphic).

Given that MK is the only sight threatening complication of lens wear, more accessible and prominent information about MK risks and avoidance should be mandatory.

This paper adds new data confirming previously suspected risk factors for AK in CL users and new avoidable risk factors including showering and/or reuse of DD lenses together with a 3-fold increased risk of AK in reusable lenses compared to daily disposable lenses. The PAR% calculations suggest that avoiding the remediable risks can be expected to substantially reduce the number of AK cases. These results can be expected to encourage more CL users to switch from reusable CL, with their associated storage and solution risks, and to practice safer use of DD lenses (without reuse, overnight wear, or contamination by water). Safe CL use could be improved by the inclusion of clear risk avoidance data on lens packaging by manufacturers and advice in public swimming pools on water avoidance whilst using lenses.

Acknowledgements:

Ruth Lloyd-Williams, Benefit Risk Evaluation Assessor, Medicines and Healthcare products Regulatory Agency, UK for advice on the regulation of Class IIa medical devices in the UK and EU and for comments on the manuscript.

Moorfields Eye Hospital staff: the Accident & Emergency Department Nurses for their help in identifying control contact lens users. Melanie Mason and the Corneal Clinic staff for assistance with recruiting cases. Staff in Research and Development for database management.

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Table 1
Comparison of the risks for the development of *Acanthamoeba* keratitis in daily disposable versus reusable CL wearers: unadjusted analysis and adjusted analyses. The adjusted odds ratio estimates are from LASSO inferential logistic models for the combined dataset of 205 patients. Statistically significant values <0.05 are in bold typeface and shaded cells.

Exposure Variables	Controls n=122	AK Cases n=83	Unadjusted Odds Ratio (95% CI)	*p-value*	Adjusted Odds Ratio (95% CI)	*p-value*
Type of CL						
Daily disposable	66	20	*Referent*		*Referent*	
Reusable	56	63	3.71 (2.00-6.88)	***< 0.001***	4.14 (1.92-8.9)	***< 0.001***
Type of CL: detailed						
Daily disposable	66	20	*Referent*		*Referent*	
Reusable Soft	51	58	3.75 (2.01-7.02)	***< 0.001***	3.84 (1.75-8.43)	***0.001***
Rigid gas permeable	5	5	3.30 (0.87-12.56)	*0.080*	4.56 (1.03-20.19)	***0.046***
Total	122	83				

Covariates included in the LASSO model-building process were: mean hygiene score (described in Supp. Table 2); where CL were purchased (from the internet via a contact lens website versus all optician associated purchases); hand washing before handling CL; showering with CL in; swimming/water activities with CL in; routine CL check-up periods and ethnicity.

Table 2
Adjusted odds ratio estimates for independent risk factors associated with the development of *Acanthamoeba* keratitis in daily disposable contact lens (CL) users from the multivariable analysis (using LASSO inferential logistic models). Statistically significant values <0.05 are in bold typeface and shaded cells.

Exposure variable	Controls n (%)	AK Cases n (%)	Adjusted Odds Ratio (OR)	95% CI for OR	*p-value*
Ethnicity					
British white	31 (48.4)	16 (84.2)	5.07	1.10 - 23.44	**0.038**
Other	33 (51.6)	3 (15.8)	*Referent*		
Unknown	2	1			
Hours of CL wear per day (median=12 hours)					
4 - 11 hours	26 (39.4)	13 (65.0)	*Referent*		
12 - 18 hours	40 (60.6)	7 (35.0)	0.22	0.06 - 0.88	**0.032**
Routine contact lens check					
1-30 days ago	13 (19.7)	1 (5.3)	*Referent*		
>1month ago	53 (80.3)	18 (94.7)	10.12	5.01 - 20.46	**< 0.001**
Unknown	0	1			
Showering with CLs in					
No	41 (62.1)	7 (35.0)	*Referent*		
Yes/unsure	25 (37.9)	13 (65.0)	3.29	1.17 - 9.23	**0.024**
CL reuse					
No	53 (81.5)	8 (40.0)	*Referent*		
Yes	12 (18.5)	12 (60.0)	5.41	1.55 - 18.89	**0.008**
Unknown	1	0			
Overnight CL wear					
Never	56 (88.9)	13 (65.0)	*Referent*		
Sometimes	7 (11.1)	7 (35.0)	3.93	1.15 - 13.46	**0.030**
Unknown	3	0			

See supplementary Table 4 for a list of covariates (potential confounders) included in the LASSO model-building process and supplementary Table 5 for the full results of the adjusted analysis.

Table 3
Population attributable risk percent (PAR%) for the comparison of daily disposable with re-usable contact lenses (CL) in 205 CL users and for the 4 remediable independent risk factors with adjusted odds ratios above 1.00 in 86 daily disposable lens users.

Exposure variable	Adjusted odds ratio (OR)	p-value	PAR% [1]	95% CI for PAR%
Type of contact lens				
Daily Disposable	*Referent*			
Reusable soft	3.84	0.001	51.7	29.9 - 61.6
Rigid gas permeable	4.56	0.046	4.7	0.2 - 5.7
For Daily Disposable lens use				
Routine contact lens check				
1-30 days ago	*Referent*			
>1month ago	10.12	< 0.001	85.4	75.8 - 90.1
Showering when wearing CL				
No	*Referent*			
Yes/unsure	3.29	0.024	45.2	9.4 - 58.0
Contact lens reuse				
No	*Referent*			
Yes	5.41	0.008	48.9	21.3 - 56.8
Overnight contact lens wear				
Never	*Referent*			
Sometimes	3.93	0.030	26.1	4.6 - 32.4

1. Population Attributable Risk% calculation based on odds ratio estimate and the proportion of AK cases exposed to the risk factor.

Supplementary Table 1
Diagnoses for Control contact lens users

Daily disposable contact lens users		**Reusable contact lens users**	
Diagnosis	**Number**	**Diagnosis**	**Number**
Adenoviral keratoconjunctivitis	1	Acute anterior uveitis	1
Allergic blepharoconjunctivitis	1	Allergic conjunctivitis / Dry eyes	2
Allergic conjunctivitis / Dry eyes	2	Allergic conjunctivitis	2
Allergic conjunctivitis	2	Blepharitis	9
Blepharitis	5	Blepharitis / Chalazion	2
Blepharitis / Chalazion	3	Blepharitis / Dry eyes	4
Blepharitis / Dry eyes	1	Chalazion	2
Blepharitis / Keratoconus	1	Chalazion / corneal abrasion	1
Cataract / Glaucoma	1	Conjunctivitis	3
Central Serous Retinopathy	1	Conjunctival foreign body	2
Chalazion	2	Contact lens intolerance	1
Conjunctivitis	1	Corneal abrasion	2
Contact lens stuck in eye	1	Corneal abrasion / Dry eyes	1
Corneal abrasion	1	Corneal foreign body	1
Corneal foreign body	5	Corneal punctate keratopathy	1
Corneal punctate keratopathy	1	Dry eyes	8
Dry eyes	15	Ectropion	1
Episcleritis	1	Migraine with aura	1
Exposure keratopathy	1	No eye abnormality detected	2
Eyelid concretions	1	Optic disc abnormality	1
Follicular conjunctivitis	1	Post-lasik ectasia	1
Migraine with aura	1	Retinal tear	1
No eye abnormality detected	3	Sub-conjunctival hemorrhage	1
Ocular hypertension	1	Viral conjunctivitis	2
Optic disc abnormality	1	Viral keratoconjunctivitis	2
Posterior vitreous detachment	2	Vitreous haemorrhage	1
Preseptal cellulitis / oedema	1	Vitreous syneresis	1
Recurrent erosion syndrome	1	**TOTAL**	56
Subepithelial opacities	1		
Viral conjunctivitis	4		
Vitreous condensation	1		
Vitreous floater	1		
Vitreous syneresis	1		
TOTAL	66		

Supplementary Table 2
Contact lens hygiene compliance assessment methodology

Contact lens (CL) hygiene compliance was assessed in both Cases and Controls by their 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	

Supplementary Table 2 page 2			
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 3
Recruitment contemporaneity for *Acanthamoeba* cases and controls using reusable daily wear (DW) and daily disposable (DD) lenses

First attendance Month	Year	Total recruited per period	Daily disposable lens users		Reusable lens users	
			Cases	Controls	Cases	Controls
Retrospective recruitment						
Jan and Sep	2011	4	2	-	2	-
Jan and Dec	2012	17	5	-	12	-
Prospective recruitment						
Feb to Dec inclusive	2013	43	9	-	34	-
Feb to Aug inclusive for cases	2014	60	4	26	15	15
Feb to Dec inclusive for controls	2014	19	-	11	-	8
Jan to Jun inclusive	2015	62	-	29	-	33
Totals		205	20	66	63	56

Supplementary Table 4a
Characteristics of the cases and controls in the combined dataset of 205 subjects using daily disposable or reusable contact lenses (CL), together with unadjusted odds ratios as crude measures of association with risk of AK. Statistically significant values <0.05 are in bold typeface and shaded cells. Exposure Variables included in the multivariable analysis modelling process are marked with an asterisk*
Abbreviations for both 4a and 4b: sd = standard deviation, IQR = inter-quartile range

Exposure Variables	**Controls n=122**	**AK Cases n=83**	**ALL n=205**	**Unadjusted Odds Ratio (95% CI)**	***p-value***
Age group *					
11 - 28	38	32	70	1.82 (0.89-3.73	*0.104*
29 - 38	41	19	60	*Referent*	
39 - 76	43	32	75	1.61 (0.79-3.27)	*0.192*
Mean Age (sd)	36.3 (12.3)	37.9 (16.6)	37.0 (14.2)		
Median Age (IQR)	32 (28-41)	32 (25-51)	32 (27-44)		
Minimum / Maximum Age	18 / 69	11 / 76	11 / 76		
Highest level of education *					
Higher Degree	38	13	51	*Referent*	
Degree	46	33	79	2.10 (0.97-4.54)	*0.060*
Other: lower	36	31	67	2.52 (1.14-5.56)	***0.022***
Unknown	2	6	8	----	
Ethnicity *					
British White	53	62	115	4.22 (2.23-7.99)	**< 0.001**
Other	65	18	83	*Referent*	
Unknown	4	3	7	----	
Ethnicity Detail					
1:British White	53	62	115	*Referent*	
2:European.White	16	7	23	0.37 (0.14-0.98)	***0.045***
3:British Asian	21	3	24	0.12 (0.03-0.43)	***0.001***
4:Chinese SE Asian	11	0	11	-----	-----
5:British Black	4	1	5	0.21 (0.02-1.97)	*0.173*
6: Other	13	7	20	0.46 (0.17-1.24)	*0.124*
Unknown	4	3	7	----	
Occupation[3]					
Grades: 1:2:3	83	45	128	*Referent*	
Grades: 4:5:6:7:9	29	31	60	1.97(1.06-3.68)	***0.033***
Unknown	10	7	17	----	

Supplementary Table 4a page 2					
Exposure Variables	**Controls n=122**	**AK Cases n=83**	**ALL n=205**	**Unadjusted Odds Ratio (95% CI)**	***p-value***
Occupation grade detail (Students were categorized by their parents' occupation)					
1. Managers, directors, senior officials	20	13	33	1.1 (0.48-2.54)	*0.814*
2. Professional occupations	51	30	81	*Referent*	
3. Associate professional and tech.	12	2	14	0.28 (0.06-1.35)	*0.114*
4. Administrative and secretarial	10	10	20	1.70 (0.63-4.56)	*0291*
5. Skilled trades	3	5	8	2.83 (0.63-12.71)	*0.174*
6. Caring, leisure and other service	8	6	14	1.27 (0.40-4.03)	*0.679*
7. Sales and customer service	6	8	14	2.27 (0.72-7.16)	*0.163*
8. Process, plant and machinists	0	0	0	----	----
9. Occupations requiring no specific training or skills	2	2	4	1.70 (0.23-12.70)	*0.605*
Unknown	10	7	17	----	
Total	122	83	205		
Travel in last 3 months					
No	39	23	62	*Referent*	
Yes	80	57	137	1.21 (0.65-2.24)	*0.548*
Unknown	3	3	6	----	
CL source *					
Internet	24	9	33	*Referent*	
Direct/Prearranged	97	74	171	2.03 (0.89-4.64)	*0.091*
Unknown	1	0	1	----	
Years of CL wear *					
Up to 5yrs	33	22	55	*Referent*	
> 5yrs	89	61	150	1.03 (0.55-1.93)	*0.931*
Years of CL wear detail					
<3years	11	8	19	1.05 (0.39-2.82)	*0.922*
3-5years	22	14	36	0.92 (0.43-1.99)	*0.830*
6-10 years	24	16	40	0.96 (0.46-2.01)	*0.920*
>10years	65	45	110	*Referent*	
Hours of CL wear per day *					
4 -11 hours	51	36	87	*Referent*	
12 - 24 hours	71	46	117	0.92 (0.52-1.62)	0.766
Unknown	0	1	1		
Supplementary Table 4a page 3					
Exposure Variables	**Controls n=122**	**AK Cases n=83**	**ALL n=205**	**Unadjusted Odds Ratio (95% CI)**	***p-value***

Hours of CL wear per day					
Mean hours (sd)	11.5 (3.0)	11.8 (3.9)	11.6 (3.4)		
Median hours (IQR)	12 (10-14)	12 (10-15)	12 (10-14)		
Minimum / Maximum	4 / 24	4 /24	4 / 24		
CL wear frequency per week *					
Up to 4 days	34	10	44	*Referent*	
> 4 days	88	71	159	2.74 (1.27-5.93)	***0.010***
Unknown	0	2	2		
Routine contact lens check *					
1-30 days ago	22	2	24	*Referent*	
>1month ago	99	76	175	8.44 (1.93-37.02)	***0.005***
Unknown	1	5	6		
Up to 6 months ago	70	32	102	*Referent*	
> 6 month ago	51	46	97	1.97 (1.11-3.52)	***0.021***
Unknown	1	5	6		
Up to 12 months ago	102	67	169	*Referent*	
> 12 months ago	19	11	30	0.88 (0.39-1.97)	*0.758*
Unknown	1	5	6		
Swimming with CLs in *					
No	82	41	123	*Referent*	
Yes	40	42	82	2.10 (1.18-3.72)	***0.011***
Showering with CLs in *					
No	78	32	110	*Referent*	
Yes/unsure	44	49	93	2.71 (1.52-4.84)	***0.001***
Unknown	0	2	2		
Hand wash with soap pre-handling CLs *					
yes	95	38	133	*Referent*	
No/unsure	27	42	69	4.31 (1.29-14.43)	***< 0.001***
Unknown	0	3	3		

Supplementary Table 4a page 4					
Exposure Variables	**Controls n=122**	**AK Cases n=83**	**ALL n=205**	**Unadjusted Odds Ratio (95% CI)**	***p-value***
Hygiene Score: *					
Mean (sd)	3.2 (2.8)	5.4 (2.7)	4.1(3.0)	1.31 (1.18-1.46)	***< 0.001***
Median (IQR)	1.9 (1-4.5)	5.4 (3.5-7.1)	3.6(1-5.8)		
Minimum / Maximum	1 (best) - 10	1 (best) - 10	1 (best) - 10		
Unknown	1	0	1		
CL reuse Daily Disposable only					
No	53	8	61	*Referent*	
Yes	12	12	24	6.62 (2.22-19.75)	***0.001***
Unknown	1	0	1	----	
Totals	66	20	86		
Overnight CL wear *					
Never	92	52	144	*Referent*	
Sometimes	27	30	57	1.97 (1.06-3.66)	***0.033***
Unknown	3	1	4	----	
Totals					

* Variables included in the LASSO modelling process. [1]Occupation was not included as a covariate (confounder) in the main LASSO modelling process (see Methods).

Supplementary Table 4b
Characteristics of the cases and controls using daily disposable CLs, together with unadjusted odds ratios as crude measures of association with risk of AK. Statistically significant values <0.05 in bold typeface and shaded cells

Exposure Variables	Controls n=66	AK Cases n=20	ALL n=86	Unadjusted Odds Ratio (95% CI)	*p-value*
Age group *					
11 - 28	16	6	22	1.87 (0.49-7.18)	*0.359*
29 - 38	25	5	30	*Referent*	
39 - 76	25	9	34	1.80 (0.53-6.13)	*0.347*
Mean Age (sd)	36.9 (12.4)	39.1 (17.7)	37.4 (13.7)		
Median Age (IQR)	33 (29-41)	34.5 (27-52)	33 (28-44)		
Minimum / Maximum Age	18 / 68	11 / 76	11 / 76		
Highest level of education *					
HigherDeg	19	2	21	*Referent*	
Degree	25	7	32	2.66 (0.50-14.28)	*0.254*
Other: lower	21	10	31	4.52 (0.88-23.32)	*0.071*
Unknown	1	1	2	----	
Ethnicity *					
British White	31	16	47	5.68 (1.51-21.40)	**0.010**
Other	33	3	36	*Referent*	
Unknown	2	1	3	----	
Ethnicity Detail					
1:British White	31	16	47	*Referent*	
2:European.White	9	1	10	0.22 (0.03-1.85)	*0.162*
3:British Asian	10	1	11	0.19 (0.02-1.65)	*0.133*
4:Chinese SE Asian	4	0	4	-----	-----
5:British Black	3	0	3	-----	-----
6: Other	7	1	8	0.28 (0.03-2.45)	*0.248*
Unknown	2	1	3	----	
Occupation [1]					
Grades: 1:2:3	44	9	53	*Referent*	
Grades: 4:5:6:7:9	15	9	24	2.93 (0.98-8.76)	*0.054*
Unknown	7	2	9	----	

Supplementary Table 4b page 2					
Exposure Variables	**Controls n=66**	**AK Cases n=20**	**ALL n=86**	**Unadjusted Odds Ratio (95% CI)**	***p-value***
Occupation grade detail (Students were categorized by their parents' occupation)					
1. Managers, directors, senior officials	11	4	15	2.11 (0.48-9.33)	*0.325*
2. Professional occupations	29	5	34	*Referent*	
3. Associate professional and tech.	4	0	4	-----	-----
4. Administrative and secretarial	5	4	9	4.64 (0.92-23.48)	*0.064*
5. Skilled trades	1	1	2	5.8 (0.31-108.60)	*0.240*
6. Caring, leisure and other service	4	2	6	2.9 (0.41-20.28)	*0.283*
7. Sales and customer service	3	1	4	1.93 (0.17-22.50)	*0.599*
8. Process, plant and machinists	0	0	0	----	----
9. Occupations requiring no specific training or skills	2	1	3	2.9 (0.22-38.32)	*0.419*
Unknown	7	2	9	----	
Total	66	20	86		
Travel in last 3 months *					
No	22	9	31	*Referent*	
Yes	42	11	53	0.64 (0.23-1.78)	*0.392*
Unknown	2	0	2	----	
CL source *					
Internet	16	4	20	*Referent*	
Direct/Prearranged	49	16	65	1.31 (0.38-4.48)	*0.671*
Unknown	1	0	1	----	
Years of CL wear *					
Up to 5yrs	17	6	23	*Referent*	
> 5yrs	49	14	63	0.81 (0.27-2.44)	*0.708*
Years of CL wear detail					
<3years	8	3	11	1.35 (0.30-6.05)	*0.695*
3-5years	9	3	12	1.20 (0.27-5.29)	*0.810*
6-10 years	13	4	17	1.11 (0.30-4.15)	*0.879*
>10years	36	10	46	*Referent*	
Hours of CL wear per day *					
4 -11 hours	26	13	39	*Referent*	
12 - 18 hours	40	7	47	0.35 (0.12-0.99)	**0.049**
Mean hours (sd)	11.2 (3.2)	9.5 (4.0)	10.9 (3.4)		
Median hours (IQR)	12 (9-14)	10 (7-13)	12 (8-14)		
Minimum / Maximum	4 / 16	4 / 18	4 / 18		

Supplementary Table 4b page 3					
Exposure Variables	**Controls n=66**	**AK Cases n=20**	**ALL n=86**	**Unadjusted Odds Ratio (95% CI)**	***p-value***
CL wear frequency per week *					
Up to 4 days	26	6	32	*Referent*	
> 4 days	40	14	54	1.52 (0.52-4.45)	*0.448*
Routine contact lens check *					
1-30 days ago	13	1	14	*Referent*	
>1month ago	53	18	71	4.42 (0.54-36.16)	*0.166*
Unknown	0	1	1		
Up to 6 months ago	40	9	49	*Referent*	
> 6 month ago	26	10	36	1.71 (0.61-4.77)	*0.306*
Unknown	0	1	1		
Up to 12 months ago	52	14	66	*Referent*	
> 12 months ago	14	5	19	1.33 (0.41-4.31)	*0.639*
Unknown	0	1	1		
Swimming with CLs in *					
No	52	16	68	*Referent*	
Yes	14	4	18	0.93 (0.27-3.22)	*0.907*
Showering with CLs in *					
No	41	7	48	*Referent*	
Yes/unsure	25	13	38	3.05 (1.07-8.66)	**0.037**
Hand wash with soap pre-handling CLs *					
yes	52	11	63	*Referent*	
No/unsure	14	9	23	3.04 (1.05-8.77)	**0.040**
CL reuse *					
No	53	8	61	*Referent*	
Yes	12	12	24	6.62 (2.22-19.75)	**0.001**
Unknown	1	0	1	----	
Overnight CL wear *					
Never	56	13	69	*Referent*	
Sometimes	7	7	14	4.31 (1.29-14.43)	**0.018**
Unknown	3	0	3	----	

* Variables included in the LASSO modelling process. 1. Occupation was not included as a covariate (confounder) in the main LASSO modelling process (see Methods).

Supplementary Table 5
Analysis of 86 daily disposable CL users (20 AK cases and 66 controls). Full results of inferential LASSO logistic regression with adjustment for confounders. Statistically significant values <0.05 in bold typeface and shaded cells.

Exposure variable	Adjusted Odds Ratio (OR)	95% CI for OR	*p-value*
Age group			
11 - 28	1.80	0.38 - 8.45	*0.458*
29 - 38	*Referent*	----	
39 - 76	1.43	0.34 - 5.94	*0.623*
Highest level of education			
Higher degree	*Referent*		
Degree	2.55	0.36 - 18.06	*0.350*
Other: lower	4.44	0.66 - 29.88	*0.125*
Ethnicity			
British white	5.07	1.10 - 23.44	**0.038**
other	*Referent*		
Occupation [1]			
Grade 1:2:3	*Referent*		
Grade 4:5:6:7:-9	2.91	0.77 - 10.97	*0.115*
Travel in last 3 months			
No	*Referent*		
Yes	0.48	0.16 - 1.44	*0.188*
CL source			
Internet	*Referent*		
Direct/Prearranged	1.19	0.27 - 5.35	*0.816*
Years of CL wear			
Up to 5 years	*Referent*		
> 5 years	1.01	0.34 - 3.07	*0.980*
Hours of CL wear per day			
4 - 11 hours	*Referent*		
12 - 18 hours	0.22	0.06 - 0.88	**0.032**
CL wear frequency per week			
Up to 4 days	*Referent*		
More than 4 days	2.00	0.66 - 6.06	*0.222*
Routine contact lens check			
1-30 days ago	*Referent*		
>1month ago	10.12	5.01 - 20.46	**< 0.001**
Routine contact lens check			
Up to 6 months ago	*Referent*		
> 6 months ago	1.83	0.60 - 5.59	*0.288*
Routine contact lens check			
Up to 12 months ago	*Referent*		
> 12 months ago	1.64	0.47 - 5.72	*0.437*

Supplementary Table 5 page 2			
Exposure variable	**Adjusted Odds Ratio (OR)**	**95% CI for OR**	***p-value***
Swimming with CLs in			
No	*Referent*		
Yes	0.81	0.22 - 3.06	*0.760*
Showering with CLs in			
No	*Referent*		
Yes/unsure	3.29	1.17 - 9.23	**0.024**
Hand wash with soap before handling CLs			
Yes	*Referent*		
No/unsure	2.23	0.52 - 9.61	*0.281*
CL reuse			
No	*Referent*		
Yes	5.41	1.55 - 18.89	**0.008**
Overnight CL wear			
Never	*Referent*		
Sometimes	3.93	1.15 - 13.46	**0.030**

Key to Occupation grades: 1. Managers, directors and senior officials 2. Professional occupations 3. Associate professional and technical occupations 4. Administrative and secretarial occupations 5. Skilled trades 6. Caring, leisure and other services 7. Sales and customer service 8. Process, plant and machine operatives (empty) 9. Occupations requiring no specific training or skills. Students were categorized by their parents' occupation

Supplementary Appendices

Sources of bias

All observational studies are subject to bias. In addition to the sources of bias mentioned in the main text of the paper, we provide additional information about two specific sources of bias below.

Statement regarding potential bias associated with the source of cases and controls

An odds ratio estimate for an exposure can be free of bias due to differential attendance if both the following conditions apply: a) if the probability of referral or attendance among exposed cases (p1) is same as that in unexposed cases (p2), and b) if the same applies to controls, i.e. when p1=p2 & p3=p4. In practice, however, p1 may differ from p2 by k so that p1=kp2, in which case the selection of cases is biased by k. If much the same bias also applies to controls, i.e. if p3≈kp4 approximately, as they are largely from the same catchment population as cases, then there would be no serious bias from this source in the estimated odds ratio. The diversity of diagnoses in controls would help to ensure that no single eye condition associated with a particular push factor would dominate.

Potential bias due to differences in ethnicity amongst tertiary referrals with *Acanthamoeba* keratitis

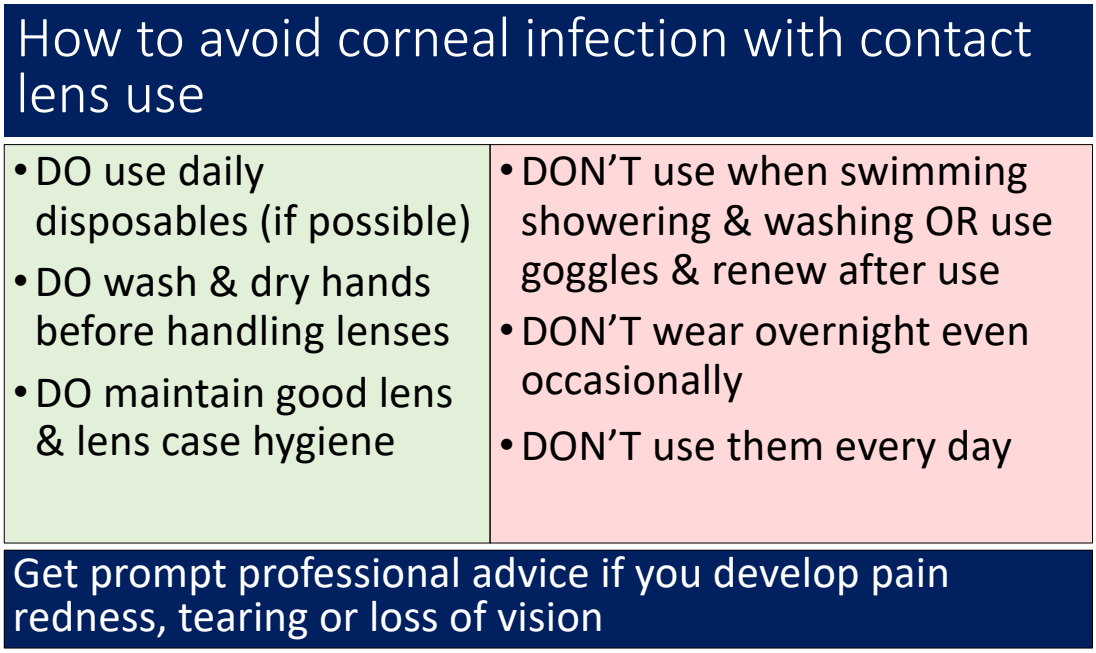
The unadjusted odds ratio (OR) we have given for British white DD users was 5.68 (CI: 1.51- 21.40) in Supp. Table 4b and was used for this analysis rather than the adjusted OR which is too complex to compare before and after the exclusions described here. There was a difference in the ethnicity of tertiary referral cases with a higher proportion of these being white British; this is a potential source of bias for the ethnicity findings. Amongst the 20 cases 11 were not tertiary referrals. Among the 66 controls none were assumed to be tertiary referrals. When the tertiary referral AK cases are excluded from the analysis the odds ratio for British white remained substantial at 4.26 (0.84 - 21.63), but less precise due to small numbers. As a result, we think we can still conclude that it's probable that the excess risk in British white subjects is present despite the potential bias of tertiary referral rates being higher in the British white subjects than for other ethnic categories but with loss of power due to small numbers.

Supplementary statement and graphic
An example of a statement recommended for inclusion with CL packaging

Risks and precautions:

- Corneal infections (causing corneal ulcers) in contact lens users are rare but can develop rapidly, causing permanent sight loss in some. Infections are caused by bacteria, fungi and *Acanthamoeba*.
- Other complications of CL use may be unpleasant, but do not cause vision loss.
- Infection risks are increased:
  - If contact lenses are worn overnight: either when using extended wear lenses, or when daily wear lenses are retained overnight.
- Risk of *Acanthamoeba* infection (and probably also severe bacterial infection) are increased:
  - In reusable (usually 1-4 weekly replacement) lens users compared to daily disposable lens users.
- Infection risks can be reduced by:
  - Using lenses and lens care products (solutions and cases) as recommended in the information leaflet
  - Avoiding contamination with water: clean and dry hands before insertion and removal, do not face wash, shower or swim in lenses - discard lenses used in these situations and replace with new lenses.
- Get prompt professional advice if you develop any unexpected pain, redness, tearing or loss of vision.

Graphic



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OTHER CONTRIBUTIONS: