



Seasonal patterns of incidence, demographic factors and microbiological profiles of infectious keratitis: the Nottingham Infectious Keratitis Study

Darren Shu Jeng Ting ^{1,2} · Charlotte Shan Ho ² · Jessica Cairns³ · Bhavesh P. Gopal² · Ahmad Elshah^{1,2} · Mouhamed Al-Aqaba^{1,2} · Tim Boswell⁴ · Dalia G. Said^{1,2} · Harminder S. Dua ^{1,2}

Received: 13 May 2020 / Revised: 22 October 2020 / Accepted: 28 October 2020 / Published online: 12 November 2020

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Abstract

Purpose The purpose of this study is to examine the seasonal patterns of incidence, demographic factors and microbiological profiles of infectious keratitis (IK) in Nottingham, UK.

Methods A retrospective study of all patients who were diagnosed with IK and underwent corneal scraping during 2008–2019 at a UK tertiary referral centre. Seasonal patterns of incidence (in per 100,000 population-year), demographic factors, culture positivity rate and microbiological profiles of IK were analysed.

Results A total of 1272 IK cases were included. The overall incidence of IK was highest during summer (37.7, 95% confidence interval (CI): 31.3–44.1), followed by autumn (36.7, 95% CI: 31.0–42.4), winter (36.4, 95% CI: 32.1–40.8) and spring (30.6, 95% CI: 26.8–34.3), though not statistically significant ($p = 0.14$). The incidence of IK during summer increased significantly over the 12 years of study ($r = 0.58$, $p = 0.049$), but the incidence of IK in other seasons remained relatively stable throughout the study period. Significant seasonal variations were observed in patients' age (younger age in summer) and causative organisms, including *Pseudomonas aeruginosa* (32.9% in summer vs. 14.8% in winter; $p < 0.001$) and gram-positive bacilli (16.1% in summer vs. 4.7% in winter; $p = 0.014$).

Conclusion The incidence of IK in Nottingham was similar among four seasons. No temporal trend in the annual incidence of IK was observed, as reported previously, but there was a significant yearly increase in the incidence of IK during summer in Nottingham over the past decade. The association of younger age, *P. aeruginosa* and gram-positive bacilli infection with summer was likely attributed to contact lens wear, increased outdoor/water activity and warmer temperature conducive for microbial growth.

Supplementary information The online version of this article (<https://doi.org/10.1038/s41433-020-01272-5>) contains supplementary material, which is available to authorised users.

✉ Harminder S. Dua
profdua@gmail.com

¹ Academic Ophthalmology, Division of Clinical Neuroscience, School of Medicine, University of Nottingham, Nottingham, UK

² Department of Ophthalmology, Queen's Medical Centre, Nottingham, UK

³ School of Medicine, University of Nottingham, Nottingham, UK

⁴ Department of Microbiology, Nottingham University Hospital, Nottingham, UK

Introduction

Infectious keratitis (IK) is a common ophthalmic emergency characterised by a variety of manifestations, including corneal ulceration, stromal infiltrates and varying degree of anterior chamber reaction. It is responsible for ~2 million monocular blindness per year, with higher rates reported in developing countries [1]. A wide range of microorganisms, including bacteria, fungi, viruses and parasites, particularly *Acanthamoeba*, have been implicated in IK [2, 3]. In view of the diverse causative microorganisms, broad-spectrum topical antimicrobial treatment is often commenced initially and supplemented by adjuvant therapies when required [4–6].

IK is primarily diagnosed on clinical grounds with the support of microbiological investigations, commonly in the form of corneal scraping for microscopy, culture and

Table 1 Summary of the seasonal trends in the rate and microbiological profiles of infectious keratitis in the literature, in the order of chronology.

Year	Authors	Study period	Sample size ^a	Location	Overall seasonal rate	Microbiological profiles ^b
2008	Green et al. [10]	1999–2004	253	Brisbane, Australia	Not examined	<i>P. aeruginosa</i> (in summer); <i>S. pneumoniae</i> (in winter)
2009	Ibrahim et al. [11]	1997–2003	1786	Portsmouth, UK	Summer > winter > autumn > spring	Not examined
2012	Lin et al. [12]	2006–2009	6967	Southeast India	Summer > winter > spring/autumn	Fungi (in summer); <i>P. aeruginosa</i> (in July–December)
2013	Otri et al. [13]	2007–2010	129	Nottingham, UK	Summer > spring > winter > autumn	Not examined
2015	Ni et al. [14]	2009–2012	313	Philadelphia, US	Spring > autumn > summer > winter	Bacteria (in spring)
2016	Gorski et al. [15]	2008–2013	155	New York, US	Summer > winter > spring > autumn	<i>P. aeruginosa</i> (in summer)
2018 ^c	Walkden et al. [16]	2004–2015	4229	Manchester, UK	Winter > autumn > spring > summer	<i>P. aeruginosa</i> (in summer); CoNS (in autumn); <i>Candida</i> (in summer)
2020	Ting et al. (current study)	2008–2019	1272	Nottingham, UK	Summer > autumn > winter > spring	<i>P. aeruginosa</i> (in summer); Gram-positive bacilli (in summer)

^aNumber of cases of infectious keratitis.

^bCausative microorganisms that demonstrated significant seasonal predilection.

^cThe reported seasonal rate refers to the culture positivity rate of infectious keratitis but not the overall rate of infectious keratitis.

sensitivity testing. However, this current diagnostic approach is challenged by several issues, including the variably low culture yield, the slow turnaround time for positive results (usually 24–48 h from the corneal samples being taken), contamination and the possibility of polymicrobial infection [1, 7, 8]. As the specific cause of IK is often indistinguishable from the clinical features, gaining knowledge about the patterns of microbiological profiles of IK in a particular region may provide additional guidance to the clinicians on the antimicrobial therapy.

Geographical and temporal variations of IK have been well reported in the literature, with bacteria and fungi being shown as the most common microorganisms responsible for IK in developed and developing countries, respectively [2, 3, 9]. However, examination of the seasonal trends in the incidence and causative microorganisms of IK remains limited (Table 1) [10–16]. So far, there are only three studies in the literature that examined the seasonal variations in the rate of IK in the UK [11, 13, 16]. Otri et al. [13] previously reported a higher proportion of IK during the summer season in Nottingham between 2007 and 2010; however, only 129 cases of sight-threatening IK were included in the study. In addition, only one UK study, conducted in Manchester, examined the seasonal variations in the causative microorganisms of IK [16].

We recently reported the incidence, causative microorganisms and in vitro antibiotic susceptibility of IK in Nottingham, UK, over the past decade [17]. We observed a relatively stable trend of incidence (estimated at 34.7 per 100,000 population-year) and *Pseudomonas aeruginosa* was found to be the most common organism for IK. However, the seasonal patterns of these aspects have not been elucidated. In view of the paucity of literature, this study aimed to provide an up-to-date and comprehensive examination of the seasonal variations in the incidence, demographic factors, culture positivity rate, microbiological profiles and antibiotic susceptibility of IK in Nottingham.

Materials and methods

This was a retrospective study of all patients who were diagnosed with IK and underwent corneal scraping between January 2008 and December 2019 (a 12-year period) at the Queen's Medical Centre (QMC), Nottingham, UK. The study method used was similar to the previous study but with a different objective and a slightly different study period [17]. Cases were identified through the local microbiology electronic database. QMC was the only tertiary ophthalmic referral centre in the city of Nottingham with an embedded eye casualty that was open 24 h a day throughout the year to manage patients with emergency and urgent ophthalmic conditions, including IK. There were two

other nearby hospitals in the East Midlands region, including Kings Mill Hospital and Derby Royal Hospital, which covered a different subset of the population outside Nottingham and were not included in our local database.

Based on the departmental guideline for IK, all patients presenting with sight-threatening corneal ulcers—defined as size > 1 mm diameter, central location, associated melting or hypopyon or atypical presentation—were subjected to microbiological investigation such as corneal scraping for microscopy (with gram staining), microbial culture and sensitivity testing [17]. Corneal scrapes were inoculated on chocolate agar (for fastidious organisms), blood agar (for bacteria) and Sabouraud dextrose agar (for fungi). For suspected cases of Acanthamoeba keratitis, non-nutrient *Escherichia coli*-enriched agar plate was used for inoculation. All cultures were incubated for at least 1 week (and up to 3 weeks for suspected Acanthamoeba keratitis cases). The identity of the microorganisms was confirmed through standard culture and bacteriology tests. Corneal scraping was repeated in the same eye when the patient was unresponsive to treatment regardless of positive or negative outcome of the first culture. These cases were only counted as one clinical episode.

For descriptive and analytic purposes, the causative microorganisms were categorised into gram-positive and gram-negative bacteria, fungi and Acanthamoeba. Seasons were divided into winter (22 December to 21 March), spring (22 March to 21 June), summer (22 June to 21 September) and autumn (22 September to 21 December), as defined by the internationally recognised astronomical seasons and previous studies [14, 15, 18]. The population in Nottingham was estimated at the range between 300,000 and 328,000 people during the 12-year study period (<https://www.ukpopulation.org/nottingham-population/>). The number of populations used to estimate the yearly incidence of IK in Nottingham, UK, is provided in Supplementary Table 1.

The study was conducted in accordance with the tenets of Declaration of Helsinki and was approved by the Nottingham University Hospitals NHS Trust as a service evaluation study (reference number: 19–265C).

Statistical analysis

Statistical analysis was performed using SPSS version 26.0 (IBM SPSS Statistics for Windows, Armonk, NY, USA). Chi-square test or one-way analysis of variance (ANOVA) was performed, where appropriate, to analyse the seasonal patterns of incidence, demographic factors and microbiological profiles of IK among the four seasons. All continuous data were presented as mean \pm standard deviation and/or 95% confidence interval (CI). Pearson's correlation coefficient (r) analysis was performed to examine the

incidence of IK in each season over time and was interpreted as weak ($r = 0.00$ – 0.40), moderate ($r = 0.41$ – 0.69) or strong ($r = 0.70$ – 1.00), with negative values being interpreted in the same way [19]. p value of ≤ 0.05 was considered statistically significant. When multiple subgroups were analysed in chi-square test, crude Bonferroni-type adjustment was used to keep the overall false positive rate or alpha level at 0.05 (e.g., if comparison of five subgroups was performed, the adjusted p value of ≤ 0.01 (based on $0.05/5$) was considered significant) [20].

Results

Overall description

During the 12-year study period, a total of 1272 cases of IK were included. The mean patient's age was 50.0 ± 22.2 years and 50.2% were male. Of all cases, 468 (36.8%) cases were culture positive with 549 microorganisms being identified (Table 2).

Seasonal pattern in incidence of IK

The overall incidence of IK (in per 100,000 population-year) was highest during summer (37.7, 95% CI: 31.3–44.1), followed by autumn (36.7, 95% CI: 31.0–42.4), winter (36.4, 95% CI: 32.1–40.8) and spring (30.6, 95% CI: 26.8–34.3), though the overall difference was not statistically significant ($p = 0.14$; Fig. 1). Over the 12-year study period, there was a significant yearly increase in the incidence of IK during summer ($r = 0.58$, $p = 0.049$), but the incidence of IK in other seasons remained stable over time (Fig. 2).

Seasonal patterns of demographic factors and microbiological profiles of IK

A total of 549 causative microorganisms were identified during the study period. There was a small but significant difference in the patient's age among the four seasons ($p = 0.044$), with a younger group of patients (48.3 ± 22.2 years) presenting during the summer and older group of patients (52.4 ± 22.6 years) presenting during the winter (Table 2). In addition, seasonal predilection was observed in some causative organisms such as *P. aeruginosa* (32.9% in summer vs. 14.8% in winter; $p < 0.001$) and gram-positive bacilli (16.1% in summer vs. 4.7% in winter; $p = 0.014$), which included *Propionibacterium* spp., *Corynebacterium* spp. and *Bacillus* spp. (Table 2). There were no seasonal variations in gender, culture positivity rate and antibiotic susceptibility of IK demonstrated among the four seasons.

Table 2 Summary of the seasonal patterns in demographic factors, culture positivity rate and microbiological profiles of infectious keratitis in Nottingham, UK, between January 2008 and December 2019.

	Winter <i>n</i> (%)	Spring <i>n</i> (%)	Summer <i>n</i> (%)	Autumn <i>n</i> (%)	<i>p</i> value*
Age, years	52.4 ± 22.6	51.1 ± 22.5	48.3 ± 22.2	48.4 ± 21.5	<u>0.044</u>
Gender					0.88
Female	163 (49.7)	138 (50.2)	174 (51.3)	159 (48.2)	
Male	165 (50.3)	137 (49.8)	165 (48.7)	171 (51.8)	
Culture result					0.69
Positive	114 (34.8)	101 (36.7)	133 (39.2)	120 (36.4)	
Negative	214 (65.2)	174 (63.3)	206 (60.8)	210 (63.6)	
Organisms ^a					
Gram-positive	70 (54.7)	73 (60.3)	77 (49.7)	78 (53.8)	0.37
<i>Staphylococci</i>	46 (35.9)	38 (31.4)	35 (22.5)	40 (27.6)	0.055
Streptococci ^c	18 (14.1)	17 (14.0)	17 (11.0)	24 (16.6)	0.50
Bacilli	6 (4.7)	18 (14.9)	25 (16.1)	14 (9.7)	<u>0.014</u>
Gram-negative	48 (37.5)	38 (31.4)	70 (45.2)	55 (37.9)	0.14
<i>P. aeruginosa</i> (PA)	19 (14.8)	17 (14.0)	51 (32.9)	38 (26.2)	<u><0.001</u>
Non-PA	29 (22.7)	21 (17.4)	19 (12.2)	17 (11.7)	0.036
Fungi	4 (3.1)	4 (3.3)	4 (2.6)	5 (3.4)	0.98
Acanthamoeba	6 (4.7)	6 (5.0)	4 (2.6)	7 (4.8)	0.70
Antibiotics, % (Y/N) ^b					
Cephalosporin	81.8 (27/6)	90.9 (20/2)	85.2 (23/4)	90.0 (18/2)	0.75
Aminoglycoside	97.5 (79/2)	92.6 (63/5)	97.2 (104/3)	97.7 (86/2)	0.28
Fluoroquinolone	92.4 (97/8)	93.2 (82/6)	96.6 (115/4)	97.2 (104/3)	0.27

Significant *p*-values are underlined.

Continuous values are presented in mean ± standard deviation.

*Comparison was made among the four seasons using chi-square test or ANOVA test, where appropriate. *p* value of ≤0.05 was considered statistically significant. Adjusted *p* value, using crude Bonferroni-type adjustment, was used when multiple pair-wise comparisons were performed. This adjustment was performed for analysis of organisms (at first and second levels separately) and antibiotics.

^aIncluded all culture positive cases only and some cases cultured more than one organism. Comparison of organisms among four seasons was performed; (1) first level examining the four main groups, namely, gram-positive and gram-negative bacteria, fungi and Acanthamoeba; and (2) second level examining only the difference in the five bacterial subgroups.

^bRefers to antibiotic susceptibility, presented in % of susceptibility (Y = susceptible/N = resistant). The total number may vary as not all organisms were tested against all three classes of antibiotics.

^cIncluded two cases of *Enterococcus faecalis* (one in spring and one in summer).

Discussion

Seasonal cyclicity is a common feature of infectious diseases in general [21]. Depending on the causative pathogens, geographical and temporal factors and host susceptibility, certain diseases are more common in particular seasons [21, 22]. For instance, influenza and rotavirus-related gastroenteritis were shown to be more common during the winter season (in temperate zones) [21, 23], whereas tuberculosis peaked during summer in some countries such as the UK [23, 24]. Understanding of the seasonal patterns of infectious diseases, including IK, could have important implications on the public health, disease control and biology [21].

To the best of our knowledge, this represents the most up-to-date and largest study examining the seasonal

variations in incidence, demographic factors and microbiological profiles of IK in Nottingham, UK. We observed that IK was most prevalent during summer (37.7 per 100,000 population-year), accompanied by a significant increase over the past decade. This was similar to other studies conducted in the UK [11, 13] and in other parts of the world such as India [12] and the US [15], which also reported a higher rate of IK during summer. Gaining knowledge on the seasonal rate or incidence of IK helps increase the vigilance for IK among clinicians, including ophthalmologists and non-ophthalmologists who work at the front-line service such as accident and emergency department and primary care setting, during the prevalent season.

Plausible explanations for this seasonal phenomenon include raised temperature, which may help the

microorganisms to flourish, increased outdoor activities, contact with water and use of contact lenses during the summer period, which could increase the risk of corneal injury and infection [15]. However, further studies examining the seasonal variations of the risk factors are required to elucidate the findings observed in our study.

Interestingly, Walkden et al. [16] reported that the culture positivity rate of IK was highest during winter and lowest during summer but it is uncertain whether the overall seasonal incidence of IK in their region could be inferred from these findings.

In addition, we observed significant seasonal variations in *P. aeruginosa* and gram-positive bacilli during the past decade. *P. aeruginosa* infection was most commonly observed during summer and was responsible for 33% of all IK. Similarly, a higher rate of *P. aeruginosa* infection in summer has been reported in other studies [10, 12, 15, 16], which was attributed to warmer temperature and use of contact lens. We also observed a significantly higher proportion of gram-positive bacilli infection during summer when compared to winter. Gram-positive bacilli, including *Propionibacterium spp.* and *Corynebacterium spp.*, are common ocular surface commensals [25, 26] and the growth has been shown to be most active or optimal at the temperature between 30 and 37 °C [27], which may account for the higher rate of these infections during summer. Furthermore, Lin et al. [12] have also demonstrated a significantly higher rate of fungal infection during summer in Southeast India. The number of fungal or Acanthamoeba infection was very low (<5%) in our study and any seasonal variation was observed.

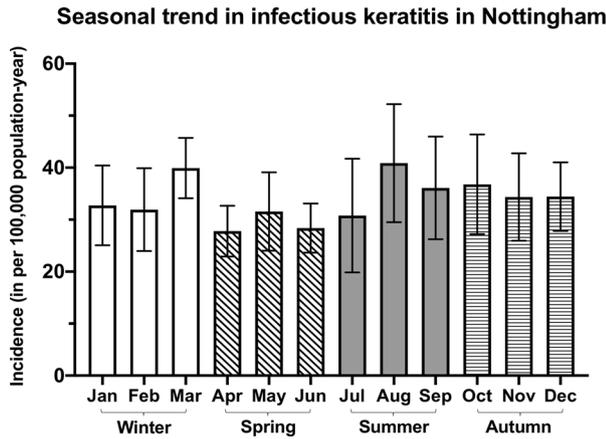


Fig. 1 Seasonal patterns in the incidence of infectious keratitis in Nottingham, UK, between January 2008 and December 2019. The monthly incidence is presented as mean with 95% confidence interval (depicted by the error bars). For better graphical presentation purpose, “22 Dec–21 Jan” was referred to as month “January”, “22 Jan–21 Feb” was referred to as month “February”, and so on.

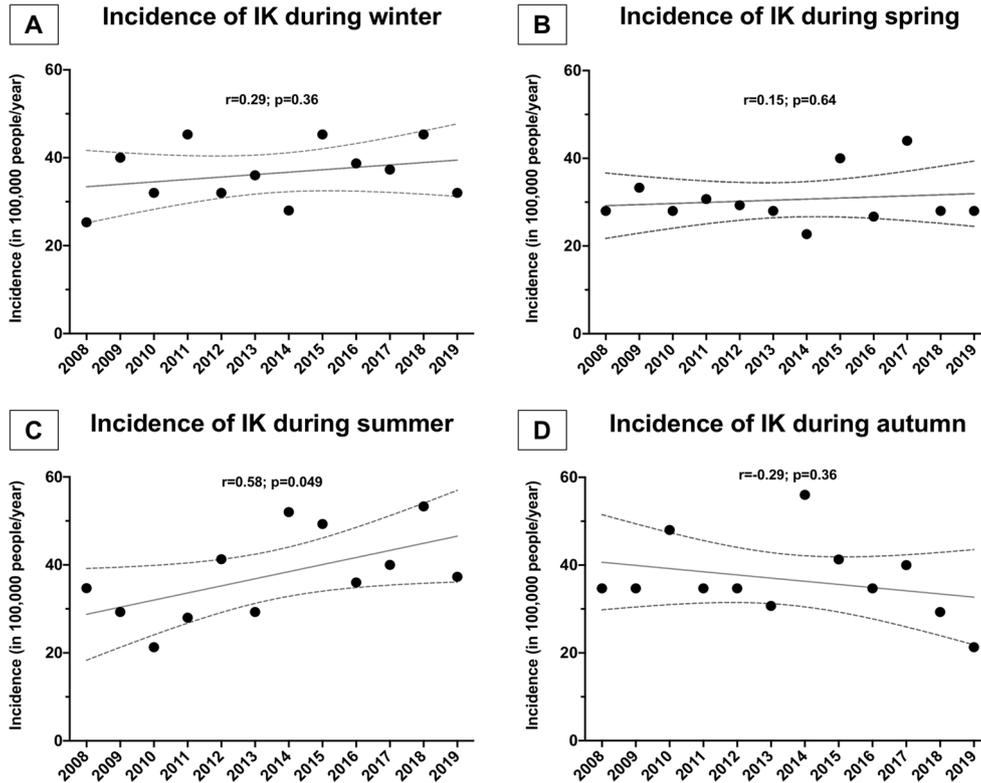


Fig. 2 A summary of the temporal changes of the annual incidence of infectious keratitis of each season. Temporal changes of the incidence of infectious keratitis in Nottingham, UK, during: **A** winter; **B** spring; **C** summer; and **D** autumn.

Interestingly, studies have also shown that postoperative infection may be higher during summer. For instance, Anthony et al. [28] demonstrated that surgical site infections following knee and hip arthroplasty were most common in summer, with increased re-admission for treatment of post-surgical infection during the same season. It would be interesting to examine whether this observation can be generalised to IK following ocular surface and/or refractive surgeries, particularly our study found that there was a significant higher rate of infection related to ocular surface commensals (i.e., *Propionibacterium* spp. and *Cornebacterium* spp.) during the summer season.

One of the limitations of our study is that we only included IK cases that had undergone corneal scraping; therefore, the overall incidence of IK in our region is likely to be underestimated. Nevertheless, there was no seasonal disparity in the practice pattern (e.g., culture method or threshold for performing corneal scraping) in our unit, suggesting that the findings related to the seasonal variations of IK observed in our study should not be affected. Cases referred from elsewhere usually have scrapes performed and antibiotic treatment initiated at the referring hospital. Culture results from these patients would not be captured in our microbiology database. Another limitation is that the full representation of the causative microorganisms in this study was hindered by the relatively low positive culture rate, which is a common issue in many IK studies [1]. Emerging investigative techniques such as in vivo confocal microscopy [29, 30], MALDI-TOF mass spectrometry [31, 32], polymerase chain reaction and/or next generation sequencing [33] and artificial intelligence-assisted systems [34] could potentially enhance the diagnostic yield of IK in the future.

In conclusion, there has been a significant increase in IK during summer in Nottingham, UK, over the past decade. Increased awareness of IK during this season should be raised among the general public and the healthcare service. Gram-positive bacilli and *P. aeruginosa* infections are significantly more common in summer and these observations may provide additional guidance on the antimicrobial therapy used in our region. Further studies investigating the correlations between these observations and the predisposing factors of IK will be beneficial.

Summary

What was known before

- Infectious keratitis is a common yet potentially sight-threatening ocular condition.
- Seasonal variation in the rate/incidence of infectious keratitis has been previously demonstrated; however,

influence on the causative microorganisms is not well known.

What this study adds

- This represents the most up-to-date and comprehensive study examining the seasonal pattern of infectious keratitis in Nottingham, UK.
- There has been a significant increase in the incidence of infectious keratitis during summer in Nottingham.
- Infections caused by *Pseudomonas aeruginosa* and gram-positive bacilli are more commonly observed during the summer season.

Acknowledgements

Funding DSJT acknowledges support from the Medical Research Council/Fight for Sight Clinical Research Fellowship (MR/T001674/1) and the Fight for Sight/John Lee, Royal College of Ophthalmologists Primer Fellowship (24CO4).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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