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Review

The global epidemiology and clinical diagnosis of *Acanthamoeba* keratitis

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ABSTRACT

Acanthamoeba keratitis is a rare parasitic infection of the cornea that can lead to permanent blindness if not diagnosed and treated promptly. We collected data on the incidences of *Acanthamoeba* keratitis from 20 countries and calculated an annual incidence of 23,561 cases, with the lowest rates in Tunisia and Belgium, and the highest in India. We analyzed 3755 Acanthamoeba sequences from the GenBank database across Asia, Europe, North America, South America, and Oceania and genotyped them into T1, T2, T3, T4, T5, T10, T11, T12, and T15. Many genotypes possess different characteristics, yet T4 is the most prevalent genotype. As efficient treatment against *Acanthamoeba* remains lacking, prevention from early diagnosis via staining, PCR, or in vivo confocal microscopy (IVCM) becomes significant for the condition's prognosis. IVCM is the most recommended approach for the early detection of *Acanthamoeba*. If IVCM is unavailable, PCR should be used as an alternative.
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Introduction

Acanthamoeba keratitis (AK) is a rare but potentially vision-threatening corneal infection [1,2]. It has been reported worldwide and presented an increased number of cases year after year, but its total global incidence remains unclear. The pathogenesis of AK is

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studied, as the Acanthamoeba has two distinct stages in its life cycle: active growing form (trophozoite) and resilient form (cyst). Currently, there is no single efficient agent to target AK [3], thus the early diagnosis of AK is crucial to prevent the disease from progressing to an advanced stage [4]. However, making a presumptive diagnosis based on clinical features is challenging because initial symptoms and signs are non-specific and mild. Combined multiple methods (clinical manifestations, microbiological examinations, confocal imaging, and molecular techniques) to diagnose were always recommended, but the diagnostic interpretation of these parameters deserves attention.

In 1974 and 1992, Naginton et al. and Jin et al. reported the first case of AK in the world and China, respectively [5,6]. Since then, contact lenses and trauma have been considered the major risk factors for AK [7–10]. In developed countries, contact lens hygiene [2,11–14], especially soft contact lenses [15], and water quality [16] become the most concerning extrinsic risk factors for AK. For example, a significant rise in AK was seen in the 1980 s in the United States, when contact lens was first introduced [17], and the increased incidence in Iowa in 1994 was most likely caused by water contamination due to regional flooding [18]. In contrast, AK is more commonly caused by ocular trauma in developing countries with either stone, dust, mud, or vegetative materials and water contamination [19–21]. For example, less than 5% of AK cases were contact lens users in India [22,23] and 53.1% of patients were associated with trauma, and more than half (50.8%) of them were farmers from our study in China [24]. Updating global AK incidence to identify potential risk factors could significantly impact its early management and prevention. Yet, little epidemiological research has been conducted to estimate its global burden.

Various types of species can cause corneal infection of *Acanthamoeba*. Previously, according to the morphology of endocyst and ectocyst: prominent star-shaped cysts, polygonal cysts with cyst arms, or round cysts without cyst arms, over 24 *Acanthamoeba* species were grouped as I, II, and III since 1977 [25]. Group II was widely isolated, including *A. castellanii, A. polyphaga*, *A. hatchetti, A. rhysodes*, *A. lugdunensis*, *A. quina*, and *A. griffini* [26]. This classification is simple but superficial, which has limited benefit for clinical decisions making. Meanwhile, the outer appearances of *Acanthamoeba* spp. may change accordingly with the culture media's ionic strength, thus it is challenging to keep the morphology consistency of the same cyst in different studies [26,27]. Now, with 18 S ribosomal RNA typing and phylogenetic analysis, 23 different genotypes (T1 to T23) have been designated [27,28], of which the most frequent disease-causing genotype in all continents is grouped as T4, accounting for over 85% of AK. Different genotypes possess different characteristics, among which T4 strains were considered the most virulent due to their firmer attachment to host epithelial cells [27,29]. Non-T4 genotypes, though relatively rare [30,31], have been reported to have delayed diagnosis, increased resistance to medicines, and poorer prognosis [31,32]. Although genotypes of *Acanthamoeba* spp. associated with keratitis (T2, T3, T4, T5, T7, T8, T9, T10, T11, T12, T13, and T15) have been reported [27], unpublished *Acanthamoeba* spp. sequences from the NCBI nucleotide database and samples derived from ocular surface and contact lens apparatus need to be analyzed furtherly.

In clinical practice, patients with typical presentations, including radial keratoneuritis, crude salt-like granular dense infiltration, and corneal ring infiltration, are easy to diagnose, while patients with atypical lesions or corneal infections at the early stages could be misdiagnosed as herpes simplex keratitis, fungal or bacterial keratitis [33,34]. Therefore, a comprehensive analysis and careful assessment based on the clinical manifestation are required. Laboratory tests become confirmatory for the diagnosis, of which conventional approaches, namely, the corneal smear for *Acanthamoeba* culture, remain the gold standard but have low sensitivity [35,36]. Polymerase chain reaction (PCR) and in vivo confocal microscopy (IVCM) are becoming increasingly important in clinical practices due to their high sensitivity and specificity [37]. Overall, this review aims to provide an updated estimate of the global incidence rate of AK with associated risk factors and a comprehensive analysis of the *Acanthamoeba* genotypes distribution with the existing literature and NCBI GenBank database to guide and facilitate clinicians' decision-making.

Epidemiology

Method

The systematic literature on the epidemiology of AK was reviewed using PubMed, MEDLINE, Embase, CINAHL, and Web of Science from July 1975 to Dec 2021. The following search terms were applied alone or simultaneously to perform the queries: acanthamoeba keratitis, amoeba keratitis, parasitic eye infections, acanthamoeba infection of the cornea, infectious keratitis, microbial keratitis, incidence, prevalence, or epidemiology. Only articles with the diagnostic criteria, including clinical manifestations and one positive laboratory index to collect AK cases, could be included. The quality of these articles presenting the incidence of AK within a defined population was evaluated with the GRADE scoring system (Supplementary Table 1) based on diagnostic accuracy, sample size, and reporting time [38]. Articles with scores equal to or greater than two were deemed acceptable. Mixed infectious keratitis (e.g., bacteria and *Acanthamoeba* cultured positively or seen on the smear) was included and taken as one case of AK. The estimated incidence rate for each country/region was calculated as follows: for example, 20 AK patients were diagnosed in Sydney, Australia, between January 1997 and December 2002 [12], a period of 5.9 years; thus, the annualized AK cases were 3.3. The population was extrapolated from online databases and, in this case, from the Australian Bureau of Statistics, the population of New South Wales was 6.7 million in December 2002 (access from: https://www.abs.gov.au/), and the estimated incidence was recorded as 0.5 per 1 million for this period. The same calculation strategy was applied to the rest articles. A weighted mean was calculated via the Arcsine and Square Root Transformations if multiple incidence rates were available for a country. After obtaining the incidences of each country, the global incidence can be estimated using the same weighted strategy.

Based on the diagnostic fragment 3 (DF3) region of *Acanthamoeba* within the nuclear 18 S ribosomal RNA gene [39], sequences associated with ocular infections were retrieved for genotyping (Supplementary Figure 2) with the following screening processes: (1) *Acanthamoeba* sequences were reviewed in the NCBI GenBank database using the search word "*Acanthamoeba*" and "18 S" before March 2022. (2) Sequences isolated from samples of non-AK sources were excluded, including from the environment, non-ocular sites, immunosuppressed patients, plants, and animals, etc. (3) Sequences without DF3 region were discarded. All the included sequences were aligned with MAFFT (version 7.490), and the aligned sequences were visualized with AliView (version 1.28) [40]. DF3 region was trimmed from the alignment, and poorly aligned regions were edited manually and automatically with BMGE (version 1.12) [41]. Reference sequences of existing genotypes (T1-T23) of *Acanthamoeba* were retrieved from the website developed by The Ohio State University (https://u.osu.edu/ acanthamoeba/) [42]. Genotype T4 was sub-genotyped into T4A, T4B, T4C, T4D, T4E, T4F, and T4Neff. The phylogenetic tree was constructed with an approximate maximum likelihood tree method using FastTree (version 2.1.10) with the -gtr and -nt options [43] and visualized using the R package ggtree (version 2.2.4) [44].

Due to its difficulty of obtaining an accurate and timely diagnosis, adjuvant imaging examination and molecular diagnostic techniques

Table 1
Estimated annual incidence of *Acanthamoeba* keratitis by countries and regions.

Continent	Country /Region	Duration	Study type	Total of AK cases	Annualized AK cases	Population (million)	Incidence (Per million)	Reference
North America	*Canada*						*1.4*	
	British Colombia	2003–2007	Case control study	42	8.4	4.2	2.0	Fraser et al.[53]*
	Toronto	1999–2006	Retrospective study	41	5.1	2.6	2.0	McAllum et al.[56]
	USA						*1.0*	
	USA	2007–2009	Multicenter study	348	139.2	169.3	0.8	Yoder et al.[52]
	USA	2005–2007	Case control study	158	63.2	169.3	0.4	Verani et al.[51]
	Philadelphia	1995–2005	Retrospective study	31	2.8	1.5	1.9	Thebpatiphat[50]
	Houston	1997–2007	Retrospective study	45	4.3	1.9	2.2	Wilhelmus et al.[54]
	Chicago- Kenosha	2003–2005	Population-based study	40	16.0	12.1	1.3	Joslin et al.[34]* *
Europe	*Sweden*						*0.4*	
	Orebro County	2004–2014	Retrospective study	1	0.1	0.3	0.4	Sagerfors et al.[66]
	UK						*4.3*	
	Bristol	1990–1995	Retrospective study	21	4.2	0.5	7.92	Illingworth et al.[4]
	England	1992–1996	Retrospective study	243	60.8	41.0	1.4	Radford et al.[57]*
	West Scotland	1995–1995	Prospective cohort study	14	21.0	3.0	7	Seal et al.[58]* *
	England & Wales	1998–1999	Multicenter study	48	48.0	42.4	1.1	Radford et al.[59]*
	London	2011–2016	Retrospective study	NR	50.3	8.6	5.8	Carnt et al.[11]
	London	2000–2011	Retrospective case series	178	16.2	7.6	2.1	Iovieno et al.[124]
	Coventry	2017–2018	Retrospective study	9	9.0	0.4	22.1	Hassan et al.[60]
	Denmark						*0.9*	
	Aarhus	1994–2018	Case control study	65	2.7	3.0	0.9	Nielsen et al.[45]
	Netherlands						*1.9*	
	Netherlands	2009–2015	Retrospective case series	224	32.0	16.9	1.9	Randag et al.[46]
	Belgium						*0.2*	
	Belgium	1997–2003	Retrospective study	17	2.4	11.3	0.2	Verhelst et al.[47]
	Portugal						*4.5*	
	Porto	2009–2018	Retrospective study	58	5.8	1.3	4.5	Oliveira et al.[8]
	Spain						*1.7*	
	Madrid	2010–2011	Retrospective case series	7	5.6	3.3	1.7	Arnalich-Montiel[31]
	Italy						*1.7*	
	Sassari	2008–2015	Prospective case series	4	0.6	0.3	1.7	Pinna et al.
Oceania	*Australia*						*1.7*	
	New South Wales	1997–2002	Retrospective case series	20	3.3	6.7	0.5	Butler et al.[12]
	Melbourne	2010–2016	Retrospective review	NR	5.0	3.9	1.3	Lee et al.[33]
	Brisbane	2003–2007	Retrospective case series	13	3.1	1.8	1.7	Ku et al.[49]
	New Zealand						*5.2*	
	Auckland	2009–2016	Retrospective case series	52	8.3	1.0	5.5	McKelvie et al.[48]
Asia	*China*						*0.6*	
	Beijing	1991–2013	Retrospective review	259	11.8	16.0	0.7	Jiang et al.[24]
	Hongkong	1997–1998	Case control study	6	4.2	7.3	0.6	Lam et al.[21]
	Singapore						*1.1*	
	Singapore	2000–2007	Retrospective cohort study	42	6.0	5.6	1.1	Por et al.[63]
	India						*15.2*	
	Madurai	1988–2009	Retrospective study	372	17.0	1.3	13.1	Lalitha et al.[62]
	Coimbatore	2017–2019	Prospective study	40	28.2	1.6	17.6	Raghavan et al.[22]
	Israel						*0.6*	
	Israel	2008–2012	Multicenter studies	23	4.6	7.7	0.6	Graffi et al.[64]*
Africa	*Egypt*						*5.0*	
	Mansoura	2013–2015	Retrospective study	5	2.5	0.5	5.0	Badawi et al.
	Tunisia						*0.2*	
	Tunisia	2011–2016	Retrospective study	14	2.3	11.0	0.2	Zbiba et al.[67]
Latin America	*Brazil*						*0.8*	
	Sao Paulo	1987–2006	Retrospective review	185	9.3	16.3	0.6	Carvalho et al.[9]
	Sao Paulo	2002–2007	Retrospective study	95	15.8	18.2	0.9	Moriyama et al.[13]
	Argentina						*0.8*	
	Cordoba city	2000–2016	Cohort study	20	1.3	1.5	0.8	Casero et al.[14]
Global							**2.9**	

Note: * These articles presented incidence directly. * * These articles presented cases of *Acanthamoeba* keratitis within a defined population. NR: not reported.

have been applied. In this regard, a hierarchical retrieval strategy was used in MEDLINE to investigate the diagnostic technology of AK. This section reviews and analyzes the diagnostic performances, advantages, and limitations of these diagnostic modalities including clinical manifestations, microbiological investigations, IVCM examination, and PCR.

Global and national AK incidences

In this study, 3998 articles were identified for annual incidence evaluation, and 130 records were used for full-text assessment after the title and abstract screening. Among them, 94 full-text articles were excluded either because no AK cases were reported or the research period still needs to be provided. Only 36 studies from 20 countries were left to be further analyzed, including four articles presenting incidence directly, two articles showing AK cases within a defined population, and 30 other studies providing sufficient information to calculate annualized national incidence through statistics (Supplementary Figure 1).

Based on our data on AK incidences collected worldwide (Table 1), the global annual incidence of AK is calculated to be 23,561 cases, which is equivalent to 2.9 cases per million people. The

highest estimated incidence occurs in India (15.2 cases per million people), while the lowest is in Tunisia and Belgium (0.2 cases per million people) (Fig. 1). Since its discovery [5], AK incidence fluctuated with an upward trend [45–50], and outbreaks tend to be confined to a specific region. Following the worldwide popularization of contact lenses in the 1980 s, the incidence of AK began to rise globally.

In the USA, the incidence of AK continuously rose from 1999 to 2007 [51,52], increasing from 0.4 to 0.8 cases per million people (Fig. 2). Although Advanced Medical Optics Complete Moisture Plus (AMOCMP) was recalled in May 2007, the incidence of AK still elevated in the following years [51]. Both USA and Canada have witnessed a rise in the incidence of AK since 2003 [53,54]. In the Chicago area, from 2003 to 2005, outbreaks of AK occurred, and the incidence was estimated to be 1.3 cases per million people with uneven distribution [55], in which decreased disinfection by-products by Environmental Protection Agency (EPA) regulations may play a role. Meanwhile, a remarkably increased incidence of AK in Philadelphia and Houston was also noticed in 2004, reaching approximately 1.9 and 2.2 cases per million people [50,54], respectively. Elevated incidence since 2004 in multiple regions in the USA may not be a coincidence. In British Columbia, Canada, the incidence of AK rose from 0.3 to 2.0 cases per million people from 1995 to 2005 [54], which coincided with the outbreak of AK in Toronto in 2003 when its incidence ascended to 2.0 cases per million people [56]. The incidence trend in Canada was in parallel with that in the USA, both of which have a rapid rise of AK incidence as of 2003 compared with previous years.

In Europe, the United Kingdom had the highest incidence of AK, and its growth was probably associated with the prevalence of contact lenses since 1992 [4]. In Bristol, the incidence of AK seemed to rise after 1990; since then it has increased to approximately 7.92 cases per million people [4]. Fortunately, there was a decline in AK between 1995 and 1996 in England, where its incidence dropped to 1.4 cases per million people [57]. In West Scotland, the incidence of AK was extremely high, with approximately 7.0 cases per million people [58]. In Wales, the incidence of AK fluctuated from 1.3 cases to 1.1 cases per million people from 1997 to 1999 [59]. From then onwards, outbreaks of AK appeared to be absent until 2012–2014, when its incidence was estimated to be as high as 5.8 cases per million people.[11] Moreover, Coventry in England had a dramatically increased incidence of AK from 2017 to 2018 (Fig. 2) [60].

In Oceania, the incidence of AK in New South Wales, Australia, was approximately 0.5 cases per million people between 1997 and 2002 [12]. In Melbourne, although spikes were seen in 2002, 2005, and 2007, the overall trend of AK declined from 1998 to 2016, with an average incidence rate of 3.1 and 1.3 cases per million people during 2004–2009 and 2010–2016 [61], respectively. The incidence of AK in Brisbane was approximately 1.7 cases per million people [49], similar to that of Melbourne. In Asia, the incidence of AK was remarkably high in Coimbatore, India, reaching 17.6 cases per million people from 2017 to 2019 [22], among which some patients were coinfected with fungi and bacteria. The incidence of AK rose in 1993 and 2002 in Madurai, India, where it was estimated to be 13.1 cases per million people [62]. China, Singapore, and Israel had similar incidence rates, at 0.6, 1.1, and 0.6 cases per million people [21,24,63,64], respectively. The incidences of other countries are illustrated in Table 1 and Fig. 2.

Risk factors

Risk factors for AK vary by country. In developing countries, trauma remains the leading risk factor for AK and is estimated to be associated with 27% of cases (Table 2) [23,62]. A retrospective review in India between 1999 and 2002 found that all 33 patients with AK were agricultural workers without contact lenses wearing, and all of them experienced ocular trauma (Table 2) [23]. A study in China between 1997 and 2003 showed that 50.8% of patients (96/189) were farmers, followed by students (23.8%), suggesting the vulnerable population for AK [24]. In developed countries, contact lens wearing for refractive and cosmetic purposes is the leading risk for AK, estimated to be associated with 86% of cases (Table 2) [54,58,63–67]. In Australia, declined AK incidence in Melbourne was probably the result of its cleaner water supplies [61], while in the UK, the rising incidence started in 1992 when chlorine disinfectant systems may be responsible as it was resisted by *Acanthamoeba* cysts, and a declined incidence from 1995 to 1996 was probably due to improved lens care and disinfection system[4]. Secondly, insufficient disinfection of lens solutions may also be a threat. In America, the increased incidence since 2004 and the outbreak in 2007 was probably due to the insufficient efficacy of the solution against *Acanthamoeba*, namely AMOCMP [51,52,68]. In British Columbia, Canada, a lens solution containing taurine and propylene glycol was likely a contributing factor for its increased AK incidence

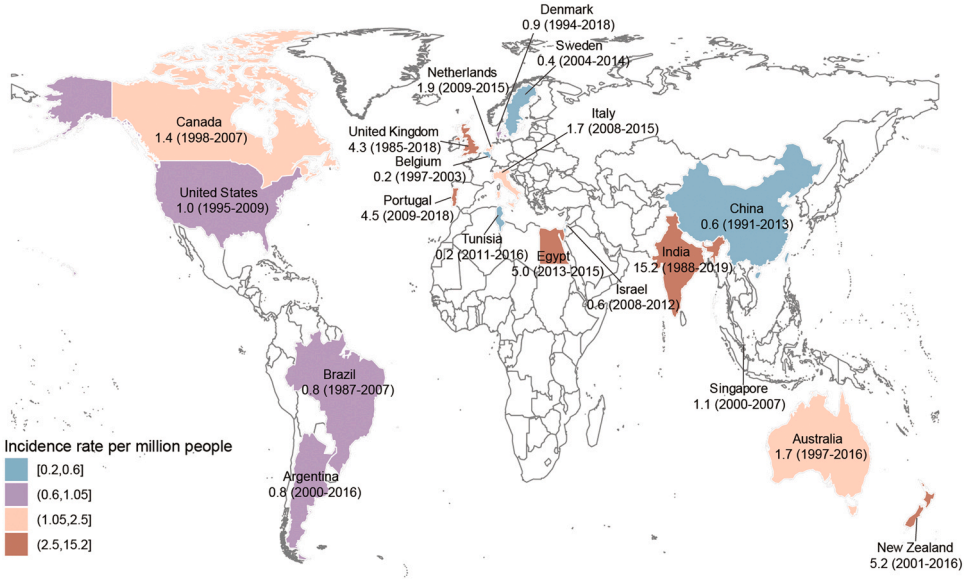


Fig. 1. The estimated annual incidence of *Acanthamoeba* keratitis by regions (as defined by the UN). The figure used with permission from co-author Xu.

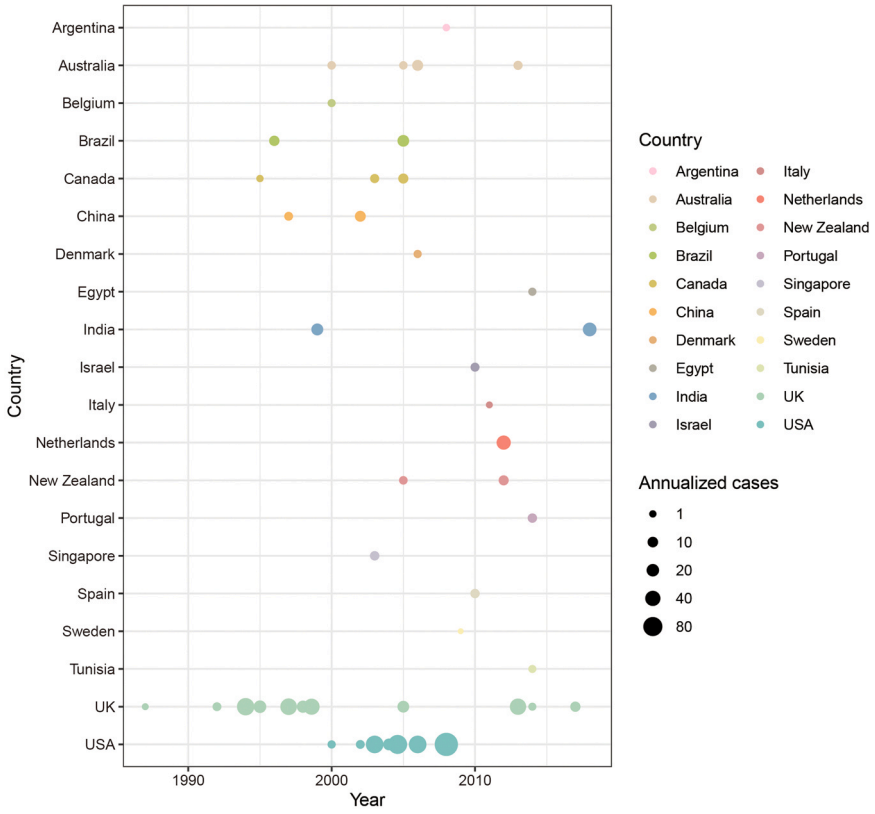


Fig. 2. Temporal dot plot showing the distribution of annualized cases of *Acanthamoeba* keratitis patients across different countries. The color of the dot corresponds to the countries of *Acanthamoeba* keratitis patients. The size of the dot indicates the number of *Acanthamoeba* keratitis patients in that country for that year.

from 2003 to 2007 (2 cases per million people per year), as the recall of this solution saw a lower AK incidence thereafter (0.5 cases per million people per year) [53]. Lastly, long-term contact lens wearing, especially for orthokeratology purposes as seen in rigid contact lens wearers, increases the risks of lens-induced microtrauma and hypoxia of the corneal epithelium, which undermine users' ocular barrier against *Acanthamoeba* [69].

Furthermore, there have been more cases of AK reported between June to August in England, Hong Kong, and Canada, indicating a seasonal trend [56,57,70]. This is likely because amoeba may prefer warmer environments and people are more likely to be exposed to water, through activities such as swimming and bathing during the summer [71]. Water hardness may also play a role in the geographical variation of AK in the UK[59], where citizens supplied with hard water (200 calcium carbonate mg/l or more) had a threefold increased risk of AK compared to those supplied with soft water (0–99 calcium carbonate mg/l) [59,72]. Other minor risk factors of AK include steroid use [73], topical anaesthetic use [74], coinfections with microbes [75], complications with systemic diseases [76], and cosmetics wearing [77].

Genotyping identification

From 1994–2021, 3755 relevant isolates were identified in the GeneBank database. A total of 940 *Acanthamoeba* isolates that met the inclusion standard were genotyped, and nine different genotypes (T1, T2, T3, T4, T5, T10, T11, T12, and T15) were identified. The earliest *Acanthamoeba* isolates in our collection were from 1994, and a peak in the number of collected sequences appeared in 2014 when a rise in T3 and T15 could be noticed (Fig. 3A). The prevalence of *Acanthamoeba* genotypes in different continents was presented in Supplementary Table 2.

Among 940 isolates, 823 (823/940, 87.6%) isolates were designated to genotype T4 with a preponderance of T4A (315/823, 38.3%), followed by T4B (163/823, 19.8%) and T4D (109/823, 13.2%) (Supplementary Figure 3). Asia (425/940, 45.2%) and Europe (267/940, 28.4%) had the most reported cases, followed by South America (127/940, 13.5%), North America (111/940, 11.8%), Africa (9/940, 1.0%), and Oceania (1/940, 0.1%) (Fig. 3B). Considering the sub-genotypes, T4A was the predominant one in Asia, Europe, South America, and North America, along with T3 being the second most common except for South America, where no T3 strains have been reported. T15 accounted for a large proportion in Europe (16/267, 5.9%) compared with that in Asia (5/425, 1.2%), North America (0/111, 0%), South America (0/127, 0%), and Oceania (0/1, 0%).

Diagnosis

Early clinical suspicion of AK is an important issue. If a contact lens-related eye infection is not responding well to typical antimicrobial (bacteria, fungus, or viruses) agents, AK should be considered as a potential cause [20,78,79]. Additionally, a detailed medical history, including corneal trauma with exposure to contaminated water or soil, corticosteroid therapy, and ocular surface disease should be considered [25,78,80–82]. Laboratory examinations, such as cytological smear, the culture of amoebae, PCR, etc., should be performed to confirm the diagnosis. Many factors can impact the diagnostic methods' specificity and sensitivity [83]. To achieve accurate diagnosis, multiple methods have been used in various regions around the world [79].

Clinical manifestation

Clinical signs are vital in advanced cases, and treatment should be initiated as soon as possible. Within the first two weeks of AK,

Table 2
Risk factors for *Acanthamoeba* keratitis at different regions and duration.

Country/Region	Duration	Number of cases	Predisposing factors								Reference
			CL	Trauma	OSD	Swim with CL	Tap water	Shower with CL	Extended CL use	others	
Brazil/Sao Paulo	1987–2006	185	82.7%	17.2%	NR	NR	NR	NR	NR	NR	Carvalho et al., 2009[9]
India/Tirunelveli	1999–2002	33	0%	100%	0%	NR	NR	NR	NR	Corneal injury with mud 84.9%	Bharathi et al., 2007[23]
China/Beijing	1991–2013	259	29.8%	53.1%	4.5%	NR	NR	NR	NR	NR	Jiang et al., 2015[24]]
UK/England	1992–1996	243	92.6%	10.3%	2.1%	19.3%	5.8%	NR	90.9%	Non-sterile CL rinsing 4.5%	Radford et al., 1998[57]
Denmark/Aarhus	1994–2018	65	89.2%	NR	NR	NR	NR	NR	NR	Corticosteroids 49.2%	Nielsen et al., 2019[45]
USA/Philadelphia	1995–2005	20	100.0%	NR	NR	25.0%	NR	NR	25.0%	Well water exposure 40.0%	Thebpatiphat et al., 2007[50]
UK/England and Wales	1997–1998	106	87.7%	14.2%	6.5%	31.7%	NR	NR	NR	Irregular disinfection 59.7%	Radford et al., 2002[59]
USA/Houston	1997–2007	45	100.0%	NR	NR	NR	NR	NR	NR	NR	Wilhelmus et al., 2008[54]
Canada/Toronto	1999–2006	41	92.9%	NR	NR	2.4%	NR	NR	2.4%	Saline solution 12.5%	McAllum et al., 2009[56]
Singapore	2000–2007	42	100.0%	NR	NR	43.3%	NR	50.0%	36.7%	Expired lenses 60.0%, Napping with CL 73.3%	Por et al., 2009[63]
Brazil/Sao Paulo	2002–2007	95	100.0%	NR	NR	12.6%	7.4%	8.4%	1.1%	Saline solution 24.2%	Moriyama et al., 2008[13]
Australia/Melbourne	2004–2009	34	86.1%	NR	NR	19.3%	22.6%	NR	38.7%	Omitted disinfection 22.6%	Lee et al., 2018[61]
USA	2005–2007	105	88.6%	NR	NR	NR	NR	NR	NR	Multipurpose solution 74.3%	Verani et al., 2009[51]
New Zealand/Auckland	2009–2016	52	96.0%	2.0%	NR	19.0%	4.0%	12.0%	25.0%	Expired CL solution 6.0%	McKelvie et al., 2018[48]
Netherlands	2009–2015	224	95.1%	NR	NR	NR	NR	NR	NR	NR	Randag et al., 2019[46]
India/Coimbatore	2017–2019	40	5.0%	NR	NR	NR	NR	NR	NR	NR	Raghavan et al., 2019[22]
Overall			86%	27%	4%	22%					

Note: CL, contact lens; OSD, ocular surface disease; NR, not reported.

patients are likely to present epithelial lesions (Fig. 4A) and multifocal stromal infiltrates with micro erosions [84], which may cause severe ocular pain despite the mild clinical signs [85]. Within the first month, complement activation, ring infiltrates composed of leukocytes, and perineural infiltrates stemming from the limbus may occur [84]. Notably, corneal ring infiltrates and radial neuritis are highly associated with AK (Fig. 4B, C) [82], especially when they coexist with salt-like dense infiltrate dots and/or groove-shaped corneal melting [24]. After months of infection, non-specific conditions can be noticed, such as sterile uveitis, secondary glaucoma, cataract, chorioretinitis, and retinal vasculitis [78,84].

In vivo confocal microscopy

IVCM, as a non-invasive "in vivo corneal biopsy", can penetrate the corneal layers and provide real-time images of corneal structures and pathologies at the cellular and subcellular levels with high magnification and resolution [86,87]. Under IVCM, *Acanthamoeba* cysts are observed mainly in the epithelium and anterior stroma [82,88]. Typical cysts were defined as highly reflective structures (approximately 10–15 μm), with double-walled structure, a bright spot, or signet ring appearance (Fig. 4D, E, F). The specific arrangement of clustered and string-like structures presents another characteristic for cyst identification. In contrast, *Acanthamoeba* trophozoites are more difficult to identify because of their irregular shapes and their similarity to corneal stromal cells [26]. The diameter of *Acanthamoeba* trophozoites is approximately 25–40 μm and its reflective density is medium [89]. The nucleus of trophozoites presents a circular dark area, the center of which is dense and highly reflective [90]. Compared to smear and culture, the sensitivity of IVCM for AK is up to 88–100% and its specificity is above 84–91% (Table 3) [91,92]. Therefore, it has been considered the primary method for diagnosing AK when available, or at least it serves as a confirmatory test [93–95]. IVCM can also be used to evaluate the progression of AK. The density and infiltration depth of the cysts increased significantly with AK severity. A higher density and deeper infiltration of cysts were associated with larger corneal ulcers in AK [96].

Smear and culture

As in conventional laboratory approaches, the effectiveness of smear and culture in diagnosing AK should never be underestimated. In clinical practice, identification of *Acanthamoeba* via microscopy has become an important method for diagnosing AK (Table 3). The quality of smears depends on the sample collection, specimen processing, smear production and staining. Before sample collection, the glass slide and Kimura platinum spatula were sterilized using an alcohol lamp flame, the cornea was anesthetized, and lesions were exposed via a lid speculum. Then the edge and base of the ulcer were scraped using a Kimura platinum spatula under the slit-lamp microscope or surgical operation microscope when necessary to prevent corneal perforation [82]. The obtained sample was smeared as a monolayer on glass slides to avoid cysts overlapping, it was then fixed with 95% methanol, and submitted for laboratory examination. Giemsa [25], calcofluor white [97], lactophenol-cotton blue (LPCB) [98], and acridine orange [99] stains are often used to identify pathogens easily. Among these stains, Giemsa can be used to observe the different stages of *Acanthamoeba,* including trophozoites, mature cysts, pre-cysts, and empty cysts. Given that trophozoites are fragile and easily broken during the smear procedure, they are not often detected and cysts are the more common structure found in corneal scraping (Fig. 4G, H). A pre-cyst is a transitional structure that is slightly larger and has a single-wall structure. Mature cysts are round or polygonal double-walled structures with a diameter of 10–20 μm (Fig. 4I). Under Giemsa

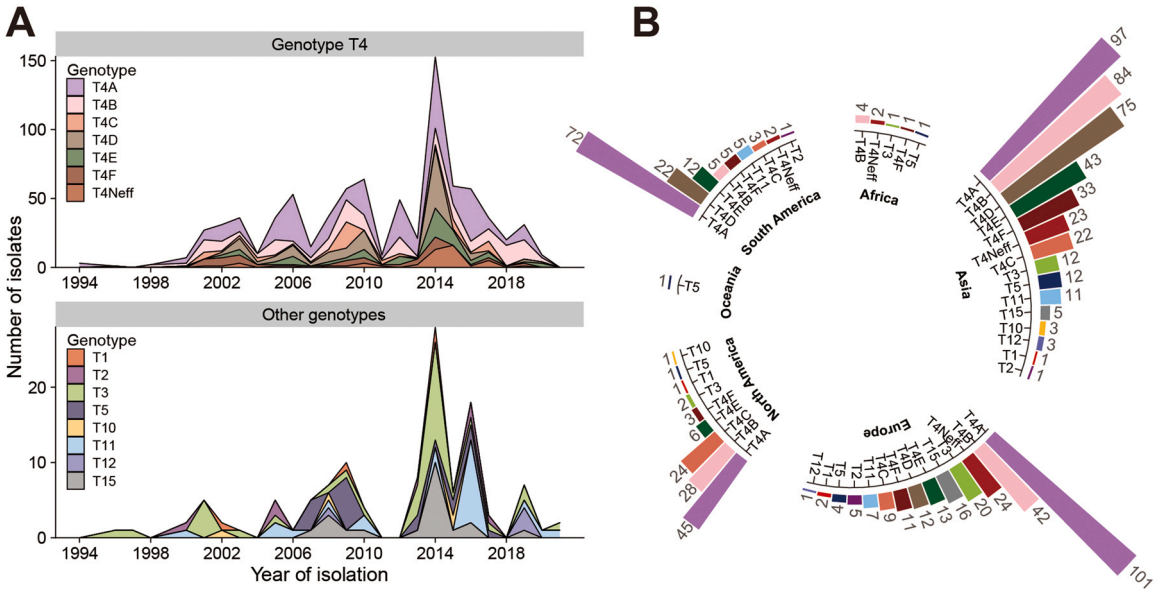


Fig. 3. Genotype distributions of 940 *Acanthamoeba* isolates in this study. (A) Temporal distribution of the *Acanthamoeba* genotypes included in the study. The top and bottom panels display the temporal distribution of the T4 genotype and the remaining genotypes, respectively. (B) Prevalence of *Acanthamoeba* genotypes in different continents.

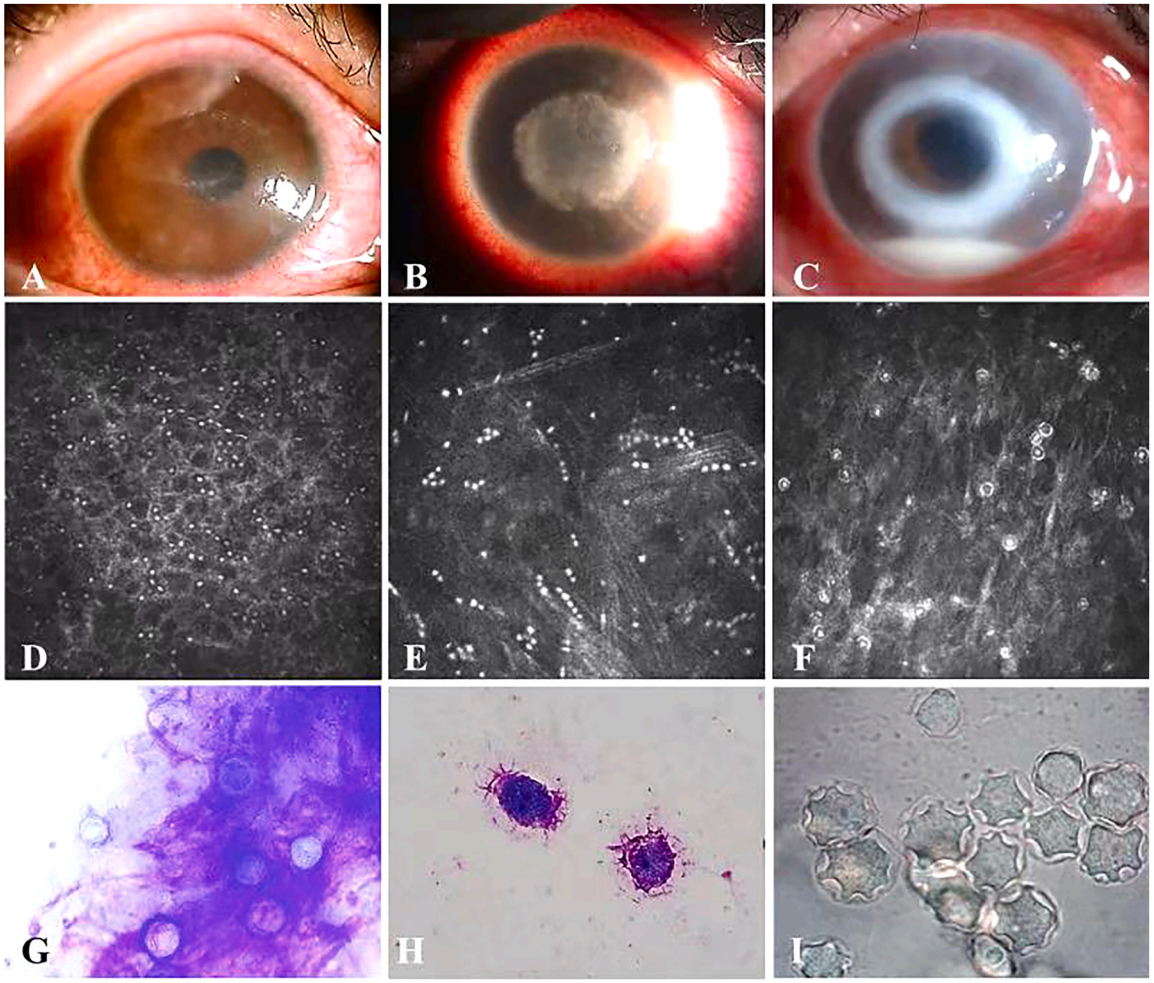


Fig. 4. Clinical manifestations and etiological diagnosis of *Acanthamoeba* keratitis. Slit lamp photographs of *Acanthamoeba* keratitis (A~C). (A) Epitheliitis with pseudodendrite. (B) Epitheliitis with radial neuritis demonstrating an inflamed, nodular corneal nerve. (C) Ring infiltrate and hypopyon. Confocal microscopy of *Acanthamoeba* keratitis (D~F). Note the multiple bright white opacities of cysts distributed in clusters throughout the scan. (D) The bright spot. (E) High reflection spots arranged in clumps or in line. (F) Double-walled structure. Smear and culture examination of *Acanthamoeba* keratitis (G~I). (G) *Acanthamoeba* cyst with double-walled structure from a superficial corneal scraping (Giemsa Stain, ×1000). (H) *Acanthamoeba* trophozoite presented oval or quasi-circle in shape with spine or pseudopodia (Giemsa Stain, ×1000). (I) Circle cysts with thick double wall seen in culture (×1000).

Table 3
Comparison between different methods in the diagnosis of *Acanthamoeba* keratitis.

Diagnostic method	Study	No. of Sample	Reference method	Sensitivity	Specificity	Advantages	Limitations
Smear							
Giemsa	Boggild et al.[103]	107	Standard criteria[a]	55%	100%	1. Giemsa, Gram, and Calcofluor white are easy and fast procedures.	1. Difficulty in distinguishing cysts from host macrophages and epithelial cells.
Calcofluor white	Megha et al.[104]	300	PRS[a]	64%	100%	2. KOH can be used to identify fungi, Nocardia and *Acanthamoeba*.	2. Giemsa, Gram were difficult to differentiate fungi from acanthamoeba.
Gram	Bharathi et al.[23]	3298	Culture	60%	100%		3. KOH is corrosive chemicals.
KOH	Bharathi et al.[23]	3298	Culture	91%	100%		1. 24 h to 3 weeks for culture.
Culture	Yera et al.[36]	1217	LCA[a]	67%	100%	1. Gold standard for AK diagnosis.	2. Discomfort after sampling.
	Boggild et al.[103]	107	Standard criteria[a]	74%	100%	2. Easy to take.	3. Affected by the quality of the specimen.
	Megha et al.[104]	300	PRS[a]	82%	100%		1. Only cysts can be recognized
In vivo confocal microscopy	Kanavi et al.[91]	133	Smear and culture	100%	84%	1. High sensitivity and specificity.	2. Need proficient expertise
	Vaddavalli et al.[92]	148		88%	91%	2. Noninvasive images evaluation.	3. Expensive and not widely available technique.
PCR	Goh et al.[37]	25	Culture	75%	70%	1. Obtain results within 60 min.	1. Genomes from dead *Acanthamoeba* can give positive results.
	Yera et al.[36]	1217	LCA[a]	73%	99%	2. High sensitivity and specificity.	2. Previous treatment may give false-negative results.
	Boggild et al.[103]	107	Standard criteria[a]	65%	100%		
	Boggild et al.[103]	107	Standard criteria[a]	90%	91%		
NGS assay	Holmgaard et al.[110]	200	Real-time PCR	88%	100%	1. Provide genotype info	1. Species need further analyses
						2. Identify relevant pathogens	2. Requires much labor and cost

[a] LCA (latent class analysis), refers to the reference standard which comprised results from PCR with/without culture; PRS (proposed reference standard), refers to that the sample was regarded as positive if the sample was positive in any two of the four tests (culture, smear, cPCR, qPCR); Standard criteria refers to that the sample was regarded as positive if the sample was positive in any two of the four tests (culture, smear, PCR using Nelson primers, PCR using JDP primers)

staining, ectocysts and endocysts are stained blue, cytoplasm is light pink, and the dense granules in the cytoplasm are dark blue [100]. If the contents of the cysts (cytoplasm, nucleus, and nucleolus) are released, empty cysts with a light blue stain will be left behind. Although the methods of Giemsa, Gram, and Calcofluor white are simple and fast, and have high specificity (100%) for diagnosis, their low sensitivity (55–60%) is a concern. After a comparative study of 3298 cases of infective keratitis, KOH staining was found to have high sensitivity (91%) and specificity (100%) for AK diagnosis [83]. Clinically, it has often been used to identify *Acanthamoeba,* fungi, and *Nocardia* (Table 3) [83,101].

Despite its low sensitivity and positivity rate, *Acanthamoeba* culture remains the current gold standard for AK diagnosis in clinical practice and can be carried out simply and successfully when IVCM and PCR are not available [102]. Cultures demonstrate 100% specificity, and their sensitivity can be as high as 67% and even up to 82% (Table 3) [36,103,104]. Similar to the corneal smear, the cornea is first anesthetized, and the lesions are exposed via a lid speculum. Sterilized transport swabs were then placed on the lesions for 5 s to collect the debrided samples. The culture technique included the direct inoculation of the suspected samples onto 1.5% Page's non-nutrient agar plates or in test tubes with live *Escherichia coli* or *Enterobacter aerogenes* at 28–30 °C for 7–10 days [25,78,105]. In cases of prior antibiotics use with amoebicidal action, the incubation time should be prolonged. Secondarily, samples in liquid > 200 µL should be well mixed, centrifuged, and resuspended in 200 µL of sterile saline for culture [25]. Agar culture plates should be examined via inverted phase contrast microscopy every day for up to 10 days to screen for this pathogen [25]. In addition, tracks left by the migration of trophozoites should be observed around the inoculation site with low magnification of the microscope after one or two days of incubation [71]. To ensure that the pathogen's structure is detected, sliced agar cuttings from the surface of non-nutrient agar plates are usually recommended for examination with a high magnification of the light microscope.

Molecular biology

PCR can provide rapid and effective identification of the *Acanthamoeba* [60]. Previous studies have found that microscopic examination and culture are significantly less sensitive than PCR assays for detecting *Acanthamoeba* in ocular samples [106–108]. When performed using a limited sample size, PCR has a sensitivity ranging from 65% to 90% and a specificity of 96–100% [36,37,103] (Table 3). Yera et al. found that combining different PCR assays increased diagnostic sensitivity (73.3% by one assay vs. 93.3% by combining two or three assays) [36]. As some cases of AK may be coinfected with bacteria, fungi, viruses, and chlamydia, polymicrobial co-infection could be identified and analyzed by Next Generation Sequencing (NGS) of the ribosomal genes in corneal scrapings [109]. Based on an NGS assay, Holmgaard et al. detected the presence of *Acanthamoeba*-specific sequences with a specificity of 100% and sensitivity of 88% [110].

Discussion and conclusions

Even though *Acanthamoeba* is the most common cause of protozoal infections among free-living amoeba, AK is a rare disease worldwide [111]. The currently reported studies mainly consist of case series, with less research on its epidemiology at the national scale. Understanding the global incidence of AK, identifying its risk factors and clinical features, and weighing the pros and cons of laboratory tests and IVCM may help ophthalmologists make optimal clinical decisions.

According to our estimation, India has the highest AK incidence, at 15.2 cases per million per year, followed by New Zealand, Egypt,

Table 4
Genotypes of *Acanthamoeba* and their biological characteristics.

Genotype	Biological characteristics	Reference
T1	Higher proteolytic activity	Costa et al., 2021[122]
T2	1. Probably contain pathogenic and non-pathogenic clusters 2. Abundant in Iran environmental samples	Maghsood et al., 2005[121]
T3	Resistant to multipurpose contact lens cleaning solutions	Ledee et al., 2009[123]
T4	1. Higher proteolytic activity 2. Firm attachment to host cells	Costa et al., 2021[122] Ng et al., 2017
T5	1. Resistant to multipurpose contact lens cleaning solutions 2. Resistant to PHMB and chlorhexidine	Ledee et al., 2009[123] Iovieno et al., 2010[124]
T10	Rarely found in keratitis and encephalitis	Booton et al., 2005
T11	Poor osmo-tolerance	Duarte et al., 2013
T12	Use serine protease to degrade collagen, fibronectin, and fibrinogen	Khan and Naveed, 2006
T15	Thermotolerant at 37–42℃	Corsaro et al., 2017[32]

Portugal, and the UK (5.2, 5.0, 4.5, and 4.3 cases per million per year, respectively). AK incidence was previously estimated to range from 1 to 5 and 1–85 in the USA, Europe, Australia, and the UK, respectively [57,58,112,113]. This variation in AK incidence in developed countries may be due to water hardness, the changing number of contact lens wearers, the withdrawal of problematic multipurpose contact lens solution, and the persistence of Acanthamoeba in domestic water [34,51,111]. The high association between CL wearing and AK in developed countries suggests that poor contact lens hygiene increases *Acanthamoeba* infection, and long-term wearing impairs the ocular surface, which reduces host defense against this protozoan. The limescale formed within the water distribution system provides a habitat for *Acanthamoeba* [114]. Therefore, any involvement in the usage of tap water or diluted disinfectants, such as rinsing or storing lenses with tap water [69], topping off lens solution rather than using fresh one [69], bathing or swimming with contact lens [69,113], substituting commercial saline for homemade saline [69,115], etc., increases the risk of *Acanthamoeba* infection and should be avoided. Unfortunately, epidemiological data from Oceania, Asia, Latin America, and Africa were scant, and certain included studies focused on patients with severe infective keratitis. As such, underdiagnosis in the whole population should be considered. Some studies only provide the number of eyes infected by *Acanthamoeba* or the number of corneal scrapes rather than the direct patient number; therefore, the number of new cases of AK may not be accurate.

It is important to note that an increasing trend in the incidence of AK occurred on all continents which reminds individuals to pay attention to lens hygiene and ophthalmologists to consider AK when similar signs and symptoms present [79,116]. The pseudo-dendriform lesion in AK is smooth, while dendrites in herpetic keratitis are distorted [84]. In contrast to mycotic and bacterial keratitis whose stromal infiltrates are monofocal, infiltrations of AK tend to be multifocal, partially transparent, and have dot-like appearances. Multifocal lesions in AK should be differentiated from those in fungal keratitis which has coarse and raised lesions, along with obscure boundaries, satellite lesions, and hypopyon [84]. Though corneal smear and culture are widely available, it is recommended to use IVCM and PCR for the early detection of *Acanthamoeba* if possible. Combined laboratory testing may help to ensure diagnosis, which has already been conducted in Africa, the Middle East, Australasia, Europe, North America, South America, and Asia [117]. Gene analysis may play a role in understanding the pathophysiology of AK. In this study, nine different *Acanthamoeba* genotypes related to ocular infections were identified, and T4 became the predominant and most virulent genotype in every continent. Clinically, *Acanthamoeba* genotypes may help understand AK's pathophysiology (Table 4) [118]. The adhesion of *Acanthamoeba* onto the cornea probably initiates its infection [119,120]. The mannose-binding protein in T4 strains was found to bind host corneal epithelial cells more firmly [120,121], which may explain the prevalence and virulence of this genotype in AK [29]. T4 and T1 strains tend to possess higher proteolytic activities [122], leading to early immune response and opacification of the cornea. T3 and the fourth one, T5, were more resistant to multipurpose contact lens cleaning solutions than T4 strains [123]. T5 is mainly isolated from the environment [124], the infection of which possibly indicates a history of ocular contamination.

Overall, we estimated that the global incidence of AK is 2.9 cases per million people, and the incidence trend in some regions is rising, reminding ophthalmologists to differentiate AK to prevent misdiagnosis. Further research is needed to improve this situation.

Author contributions

YHZ did the paper review, collected primary data, created figures and tables, and wrote original draft. XZX assisted in *Acanthamoeba* genotype data analysis and data interpretation. ZYW conceived the study design, reviewed epidemiology data, and revised and commented on the text. KC assisted in epidemiology data analysis. ZJZ did the *Acanthamoeba* keratitis literature search. QFL reviewed key papers, contributed much of the diagnosis part, revised and finalized the writing.

Declaration of Interests

We declare no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2023.03.020.

References

[1] Varacalli G, Di Zazzo A, Mori T, Dohlman TH, Spelta S, Coassin M, et al. Challenges in Acanthamoeba keratitis: a review. J Clin Med 2021;10(5). PubMed PMID: 33804353. eng. https://pubmed.ncbi.nlm.nih.gov/33804353.
[2] Moore MB, McCulley JP, Newton C, Cobo LM, Foulks GN, O'Day DM, et al. Acanthamoeba keratitis. A growing problem in soft and hard contact lens wearers. Ophthalmology 1987;94(12):1654–61. PubMed PMID: 3431835. eng. https://pubmed.ncbi.nlm.nih.gov/3431835.
[3] Lorenzo-Morales J, Martín-Navarro CM, López-Arencibia A, Arnalich-Montiel F, Piñero JE, Valladares B. Acanthamoeba keratitis: an emerging disease gathering

importance worldwide? Trends Parasitol 2013;29(4):181–7. PubMed PMID: 23433689. Epub 2013/02/26. eng.
[4] Illingworth CD, Cook SD, Karabatsas CH, Easty DL. Acanthamoeba keratitis: risk factors and outcome. Br J Ophthalmol 1995;79(12):1078–82. PubMed PMID: 8562539. Pubmed Central PMCID: PMC505344. Epub 1995/12/01. eng.
[5] Naginton J, Watson PG, Playfair TJ, McGill J, Jones BR, Steele AD. Amoebic infection of the eye. Lancet 1974;2(7896):1537–40. PubMed PMID: 4140981. eng. https://pubmed.ncbi.nlm.nih.gov/ 4140981.
[6] Jin XY, Lo SY, Zhang WH. Diagnosis, treatment and prevention of Acanthamoeba keratitis. [Ophthalmol China] Chin J Ophthalmol 1992;1:67–71.
[7] Ibrahim YW, Boase DL, Cree IA. Factors affecting the epidemiology of Acanthamoeba keratitis. Ophthalmic Epidemiol 2007;14(2):53–60. PubMed PMID: 17464851. eng. https://pubmed.ncbi. nlm.nih.gov/17464851.
[8] Oliveira-Ferreira C, Leuzinger-Dias M, Tavares-Ferreira J, Torrão L, Falcão-Reis F. Microbiological profile of infectious keratitis in a Portuguese Tertiary Centre. J Ophthalmol 2019;2019:6328058. PubMed PMID: 31772767. Pubmed Central PMCID: PMC6854955 publication of this paper. Epub 2019/11/28. eng.
[9] Carvalho FR, Foronda AS, Mannis MJ, Höfling-Lima AL, Belfort R, Jr de, Freitas D. Twenty years of acanthamoeba keratitis. Cornea 2009;28(5):516–9. PubMed PMID: 19421047. Epub 2009/05/08. eng.
[10] de Lacerda A.G., Lira M. Acanthamoeba keratitis: a review of biology, pathophysiology and epidemiology. Ophthalmic Physiol Opt. 2021;41(1):116–135. PubMed PMID: 33119189. eng. https://pubmed.ncbi.nlm.nih.gov/33119189.
[11] Carnt N, Hoffman JM, Verma S, Hau S, Radford CF, Minassian DC, et al. Acanthamoeba keratitis: confirmation of the UK outbreak and a prospective case-control study identifying contributing risk factors. Br J Ophthalmol 2018;102(12):1621–8. PubMed PMID: 30232172. Epub 2018/09/21. eng.
[12] Butler TK, Males JJ, Robinson LP, Wechsler AW, Sutton GL, Cheng J, et al. Six-year review of Acanthamoeba keratitis in New South Wales, Australia: 1997-2002. Clin Exp Ophthalmol 2005;33(1):41–6. PubMed PMID: 15670077. Epub 2005/01/27. eng.
[13] Moriyama AS, Hofling-Lima AL. Contact lens-associated microbial keratitis. Arq Bras Oftalmol 2008;71(6 Suppl):32–6. PubMed PMID: 19274408. Epub 2009/03/11. eng.
[14] Casero RD, Mongi F, Laconte L, Rivero F, Sastre D, Teherán A, et al. Molecular and morphological characterization of Acanthamoeba isolated from corneal scrapes and contact lens wearers in Argentina. Infect Genet Evol 2017;54:170–5. PubMed PMID: 28676340. Epub 2017/07/06. eng.
[15] Page MA, Mathers WD. Acanthamoeba keratitis: a 12-year experience covering a wide spectrum of presentations, diagnoses, and outcomes. J Ophthalmol 2013;2013:670242. PubMed PMID: 23840938. eng. https://pubmed.ncbi.nlm.nih.gov/23840938.
[16] Chynn EW, Lopez MA, Pavan-Langston D, Talamo JH. Acanthamoeba keratitis. Contact lens and noncontact lens characteristics. Ophthalmology 1995;102(9):1369–73. PubMed PMID: 9097775. eng. https://pubmed.ncbi.nlm.nih.gov/9097775.
[17] Stehr-Green JK, Bailey TM, Brandt FH, Carr JH, Bond WW, Visvesvara GS. Acanthamoeba keratitis in soft contact lens wearers. A case-control study. Jama 1987;258(1):57–60.
[18] Meier PA, Mathers WD, Sutphin JE, Folberg R, Hwang T, Wenzel RP. An epidemic of presumed Acanthamoeba keratitis that followed regional flooding. Results of a case-control investigation. Arch Ophthalmol 1998;116(8):1090–4. PubMed PMID: 9715690. Epub 1998/08/26. eng.
[19] Stehr-Green JK, Bailey TM, Visvesvara GS. The epidemiology of Acanthamoeba keratitis in the United States. Am J Ophthalmol 1989;107(4):331–6. PubMed PMID: 2929702. eng. https://pubmed.ncbi.nlm.nih.gov/2929702.
[20] Sharma S, Garg P, Rao GN. Patient characteristics, diagnosis, and treatment of non-contact lens related Acanthamoeba keratitis. Br J Ophthalmol 2000;84(10):1103–8. PubMed PMID: 11004092. eng. https://pubmed.ncbi.nlm.nih.gov/11004092.
[21] Lam DS, Houang E, Fan DS, Lyon D, Seal D, Wong E. Incidence and risk factors for microbial keratitis in Hong Kong: comparison with Europe and North America. Eye 2002;16(5):608–18. PubMed PMID: 12194077. Epub 2002/08/24. eng.
[22] Raghavan A, Baidwal S, Venkatapathy N, Rammohan R. The Acanthamoeba-fungal keratitis study. Am J Ophthalmol 2019;201:31–6. PubMed PMID: 30721687. Epub 2019/02/06. eng.
[23] Bharathi JM, Srinivasan M, Ramakrishnan R, Meenakshi R, Padmavathy S, Lalitha PN. A study of the spectrum of Acanthamoeba keratitis: a three-year study at a tertiary eye care referral center in South India. Indian J Ophthalmol 2007;55(1):37–42. PubMed PMID: 17189885. Epub 2006/12/27. eng.
[24] Jiang C, Sun X, Wang Z, Zhang Y. Acanthamoeba keratitis: clinical characteristics and management. Ocul Surf 2015;13(2):164–8. PubMed PMID: 25881999. eng. https://pubmed.ncbi.nlm.nih.gov/ 25881999.
[25] Lorenzo-Morales J, Khan NA, Walochnik J. An update on Acanthamoeba keratitis: diagnosis, pathogenesis and treatment. Parasite 2015;22:10. PubMed PMID: 25687209. Pubmed Central PMCID: PMC4330640. Epub 2015/02/18. https://www.ncbi.nlm.nih.gov/pubmed/25687209.
[26] Khan NA. Acanthamoeba: biology and increasing importance in human health. FEMS Microbiol Rev 2006;30(4):564–95. PubMed PMID: 16774587. eng. https://pubmed.ncbi.nlm.nih.gov/16774587.
[27] Diehl MLN, Paes J, Rott MB. Genotype distribution of Acanthamoeba in keratitis: a systematic review. Parasitol Res 2021;120(9):3051–63. PubMed PMID: 34351492. eng. https://pubmed.ncbi.nlm. nih.gov/34351492.
[28] Putaporntip C, Kuamsab N, Nuprasert W, Rojrung R, Pattanawong U, Tia T, et al. Analysis of Acanthamoeba genotypes from public freshwater sources in Thailand reveals a new genotype, T23 Acanthamoeba bangkokensis sp. nov. Sci Rep 2021;11(1):17290. PubMed PMID: 34453084. eng. https://pubmed.ncbi.nlm.nih.gov/34453084.
[29] Garate M, Marchant J, Cubillos I, Cao Z, Khan NA, Panjwani N. In vitro pathogenicity of Acanthamoeba is associated with the expression of the mannose-binding protein. Invest Ophthalmol Vis Sci 2006;47(3):1056–62. PubMed PMID: 16505041. eng. https://pubmed.ncbi.nlm.nih.gov/16505041.
[30] Maycock NJR, Jayaswal R. Update on Acanthamoeba keratitis: diagnosis, treatment, and outcomes. Cornea 2016;35(5):713–20. PubMed PMID: 26989955. eng. https://pubmed.ncbi.nlm.nih. gov/26989955.
[31] Arnalich-Montiel F, Lumbreras-Fernández B, Martín-Navarro CM, Valladares B, Lopez-Velez R, Morcillo-Laiz R, et al. Influence of Acanthamoeba genotype on clinical course and outcomes for patients with Acanthamoeba keratitis in Spain. J Clin Microbiol 2014;52(4):1213–6. PubMed PMID: 24430449. eng. https://pubmed.ncbi.nlm.nih.gov/24430449.
[32] Corsaro D, Köhsler M, Montalbano Di Filippo M, Venditti D, Monno R, Di Cave D, et al. Update on Acanthamoeba jacobsi genotype T15, including full-length 18S rDNA molecular phylogeny. Parasitol Res 2017;116(4):1273–84. PubMed PMID: 28190156. eng. https://pubmed.ncbi.nlm.nih.gov/28190156.
[33] Lee WB, Gotay A. Bilateral Acanthamoeba keratitis in Synergeyes contact lens wear: clinical and confocal microscopy findings. Eye Contact Lens 2010;36(3):164–9. PubMed PMID: 20386314. eng. https://pubmed.ncbi.nlm.nih.gov/20386314.
[34] Joslin CE, Tu EY, Shoff ME, Booton GC, Fuerst PA, McMahon TT, et al. The association of contact lens solution use and Acanthamoeba keratitis. Am J Ophthalmol 2007;144(2):169–80. PubMed PMID: 17588524. eng. https://pubmed.ncbi.nlm.nih.gov/17588524.
[35] Fanselow N, Sirajuddin N, Yin XT, Huang AJW, Stuart PM. Acanthamoeba Keratitis, pathology, diagnosis and treatment. Pathogens 2021;10(3). PubMed PMID: 33801905. Pubmed Central PMCID: PMC7998329. Epub 2021/04/04. https://www.ncbi.nlm.nih.gov/pubmed/33801905.
[36] Yera H, Ok V, Lee Koy Kuet F, Dahane N, Ariey F, Hasseine L, et al. PCR and culture for diagnosis of keratitis. Br J Ophthalmol 2021;105(9):1302–6. PubMed PMID: 33099504. eng. https://pubmed.ncbi.nlm.nih.gov/33099504.
[37] Goh JWY, Harrison R, Hau S, Alexander CL, Tole DM, Avadhanam VS. Comparison of in vivo confocal microscopy, PCR and culture of corneal scrapes in the diagnosis of acanthamoeba keratitis. Cornea 2018;37(4):480–5. PubMed PMID: 29256983. eng. https://pubmed.ncbi.nlm.nih.gov/29256983.
[38] Brown L, Leck AK, Gichangi M, Burton MJ, Denning DW. The global incidence and diagnosis of fungal keratitis. Lancet Infect Dis 2021;21(3):e49–57. PubMed PMID: 33645500. Epub 2021/03/02. eng.
[39] Fuerst P.A., Booton G.C., Crary M. Phylogenetic Analysis and the Evolution of the 18S rRNA Gene Typing System of Acanthamoeba. J Eukaryot Microbiol. 2015 Jan-Feb;62(1):69–84. PubMed PMID: WOS:000348718000010. English. < Go to ISI > ://WOS:000348718000010.
[40] Larsson A. AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 2014;30(22):3276–8. PubMed PMID: 25095880. Pubmed Central PMCID: PMC4221126. Epub 2014/08/07. https://www.ncbi.nlm.nih.gov/pubmed/25095880.
[41] Criscuolo A, Gribaldo S. BMGE (Block mapping and gathering with entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. BMC Evol Biol 2010;10(210). PubMed PMID: 20626897. Pubmed Central PMCID: PMC3017758. Epub 2010/07/16. https://www.ncbi.nlm.nih.gov/pubmed/20626897.
[42] Fuerst PA, Booton GC. Species, sequence types and alleles: dissecting genetic variation in Acanthamoeba. Pathogens 2020;9(7). PubMed PMID: 32630775. Pubmed Central PMCID: PMC7400246. Epub 2020/07/08. https://www.ncbi.nlm.nih.gov/pubmed/32630775.
[43] Price MN, Dehal PS, Arkin AP. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One 2010;5(3):e9490. PubMed PMID: 20224823. Pubmed Central PMCID: PMC2835736. Epub 2010/03/13. https://www.ncbi.nlm.nih.gov/pubmed/20224823.
[44] Yu G.C., Smith D.K., Zhu H.C., Guan Y., Lam T.T.Y. GGTREE: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol. 2017 Jan;8(1):28–36. PubMed PMID: WOS:000393305300004. English. < Go to ISI > ://WOS: 000393305300004.
[45] Nielsen SE, Ivarsen A, Hjortdal J. Increasing incidence of Acanthamoeba keratitis in a large tertiary ophthalmology department from year 1994 to 2018. Acta Ophthalmol 2019. PubMed PMID: 31885189. Epub 2019/12/31. eng.
[46] Randag AC, van Rooij J, van Goor AT, Verkerk S, Wisse RPL, Saelens IEY, et al. The rising incidence of Acanthamoeba keratitis: a 7-year nationwide survey and clinical assessment of risk factors and functional outcomes. PLoS One 2019;14(9):e0222092. PubMed PMID: 31491000. Pubmed Central PMCID: PMC6731013. Epub 2019/09/07. eng.
[47] Verhelst D, Koppen C, Van Looveren J, Meheus A, Tassignon MJ. Contact lens-related corneal ulcers requiring hospitalization: a 7-year retrospective study in Belgium. Acta Ophthalmol Scand 2006;84(4):522–6. PubMed PMID: 16879575. Epub 2006/08/02. eng.
[48] McKelvie J., Alshiakhi M., Ziaei M., Patel D.V., McGhee C.N. The rising tide of Acanthamoeba keratitis in Auckland, New Zealand: a 7-year review of presentation, diagnosis and outcomes (2009–2016). Clin Exp Ophthalmol. 2018 Aug;46(6):600–7. PubMed PMID: 29412494. Epub 2018/02/08. eng.
[49] Ku JY, Chan FM, Beckingsale P. Acanthamoeba keratitis cluster: an increase in Acanthamoeba keratitis in Australia. Clin Exp Ophthalmol 2009;37(2):181–90. PubMed PMID: 19723126. Epub 2009/09/03. eng.
[50] Thebpatiphat N, Hammersmith KM, Rocha FN, Rapuano CJ, Ayres BD, Laibson PR, et al. Acanthamoeba keratitis: a parasite on the rise. Cornea 2007;26(6):701–6. PubMed PMID: 17592320. Epub 2007/06/27. eng.

[51] Verani JR, Lorick SA, Yoder JS, Beach MJ, Braden CR, Roberts JM, et al. National outbreak of Acanthamoeba keratitis associated with use of a contact lens solution, United States. Emerg Infect Dis 2009;15(8):1236–42. PubMed PMID: 19751585. Pubmed Central PMCID: PMC2815976. Epub 2009/09/16. eng.
[52] Yoder JS, Verani J, Heidman N, Hoppe-Bauer J, Alfonso EC, Miller D, et al. Acanthamoeba keratitis: the persistence of cases following a multistate outbreak. Ophthalmic Epidemiol 2012;19(4):221–5. PubMed PMID: 22775278. Epub 2012/07/11. eng.
[53] Fraser MN, Wong Q, Shah L, Holland SP, Morshed M, Isaac-Renton J, et al. Characteristics of an Acanthamoeba keratitis outbreak in British Columbia between 2003 and 2007. Ophthalmology 2012;119(6):1120–5. PubMed PMID: 22365067. Epub 2012/03/01. eng.
[54] Wilhelmus KR, Jones DB, Matoba AY, Hamill MB, Pflugfelder SC, Weikert MP. Bilateral acanthamoeba keratitis. Am J Ophthalmol 2008;145(2):193–7. PubMed PMID: 18222188. Epub 2008/01/29. eng.
[55] Joslin CE, Tu EY, McMahon TT, Passaro DJ, Stayner LT, Sugar J. Epidemiological characteristics of a Chicago-area Acanthamoeba keratitis outbreak. Am J Ophthalmol 2006;142(2):212–7. PubMed PMID: 16876498. Epub 2006/08/01. eng. https://www.ajo.com/article/S0002-9394(06)00507-1/fulltext.
[56] McAllum P, Bahar I, Kaiserman I, Srinivasan S, Slomovic A, Rootman D. Temporal and seasonal trends in Acanthamoeba keratitis. Cornea 2009;28(1):7–10. PubMed PMID: 19092396. Epub 2008/12/19. eng.
[57] Radford CF, Lehmann OJ, Dart JK. Acanthamoeba keratitis: multicentre survey in England 1992-6. National Acanthamoeba Keratitis Study Group. Br J Ophthalmol 1998;82(12):1387–92. PubMed PMID: 9930269. Pubmed Central PMCID: PMC1722455. Epub 1999/02/04. eng.
[58] Seal DV, Kirkness CM, Bennett HG, Peterson M. Population-based cohort study of microbial keratitis in Scotland: incidence and features. Cont Lens Anterior Eye 1999;22(2):49–57. PubMed PMID: 16303406. Epub 2005/11/24. eng.
[59] Radford CF, Minassian DC, Dart JK. Acanthamoeba keratitis in England and Wales: incidence, outcome, and risk factors. Br J Ophthalmol 2002;86(5):536–42. PubMed PMID: 11973250. Pubmed Central PMCID: PMC1771120. Epub 2002/04/26. eng. https://www.ncbi.nlm.nih.gov/pmc/ articles/PMC1771120/pdf/bjo08600536.pdf.
[60] Hassan F, Bhatti A, Desai R, Barua A. Analysis from a year of increased cases of Acanthamoeba Keratitis in a large teaching hospital in the UK. Cont Lens Anterior Eye 2019;42(5):506–11. PubMed PMID: 31018907. Epub 2019/04/26. eng.
[61] Lee MH, Abell RG, Mitra B, Ferdinands M, Vajpayee RB. Risk factors, demographics and clinical profile of Acanthamoeba keratitis in Melbourne: an 18-year retrospective study. Br J Ophthalmol 2018;102(5):687–91. PubMed PMID: 28844988. Epub 2017/08/29. eng.
[62] Lalitha P, Lin CC, Srinivasan M, Mascarenhas J, Prajna NV, Keenan JD, et al. Acanthamoeba Keratitis in South India: a longitudinal analysis of epidemics. Ophthalmic Epidemiol 2012;19(2):111–5. English.https://www.embase.com/search/results?subaction=viewrecord&id=L364520926&from=export http://dx.doi.org/10.3109/09286586.2011.645990.
[63] Por YM, Mehta JS, Chua JL, Koh TH, Khor WB, Fong AC, et al. Acanthamoeba keratitis associated with contact lens wear in Singapore. Am J Ophthalmol 2009;148(1):7–12. .e2. PubMed PMID: 19403109. Epub 2009/05/01. eng.
[64] Graffi S, Peretz A, Jabaly H, Koiefman A, Naftali M. Acanthamoeba keratitis: study of the 5-year incidence in Israel. Br J Ophthalmol 2013;97(11):1382–3. (Nov).
[65] McKelvie J, Patel D, McGhee C. Cosmetic contact lens-related Acanthamoeba keratitis. Clin. Exp. Ophthalmol. 2009:419–20.
[66] Sagerfors S, Ejdervik-Lindblad B, Söderquist B. Infectious keratitis: isolated microbes and their antibiotic susceptibility pattern during 2004-2014 in Region Örebro County, Sweden. Acta Ophthalmol 2020;98(3):255–60. (May).
[67] Zbiba W, Abdesslem NB. Acanthamoeba keratitis: An emerging disease among microbial keratitis in the Cap Bon region of Tunisia. Exp Parasitol 2018;192:42–5. PubMed PMID: 29859227. Epub 2018/06/03. eng.
[68] Tu EY, Joslin CE. Recent outbreaks of atypical contact lens-related keratitis: what have we learned? Am J Ophthalmol 2010;150(5). 602-8.e2. PubMed PMID: 21036209. Pubmed Central PMCID: PMC2991148. Epub 2010/11/03. eng.
[69] Cope JR, Collier SA, Schein OD, Brown AC, Verani JR, Gallen R, et al. Acanthamoeba keratitis among rigid gas permeable contact lens wearers in the United States, 2005 through 2011. Ophthalmology 2016;123(7):1435–41. PubMed PMID: 27117780. Pubmed Central PMCID: PMC4921294. Epub 2016/04/28. eng.
[70] Houang E, Lam D, Fan D, Seal D. Microbial keratitis in Hong Kong: relationship to climate, environment and contact-lens disinfection. Trans R Soc Trop Med Hyg 2001;95(4):361–7. PubMed PMID: 11579873. Epub 2001/10/03. eng.
[71] Sun X. Acanthamoeba keratitis: Diagnosis and Treatment. Springer; 2018.
[72] Voyatzis G, McElvanney A. Bilateral Acanthamoeba keratitis in an experienced two-weekly disposable contact lens wearer. Eye Contact Lens 2007;33(4):201–2. PubMed PMID: 17630630. Epub 2007/07/17. eng.
[73] Markowitz SM, Sobieski T, Martinez AJ, Duma RJ. Experimental Acanthamoeba infections in mice pretreated with methylprednisolone or tetracycline. Am J Pathol 1978;92(3):733–44. PubMed PMID: 686155. eng. https://pubmed.ncbi.nlm.nih.gov/686155.
[74] Kurna SA, Sengor T, Aki S, Agirman Y. Ring keratitis due to topical anaesthetic abuse in a contact lens wearer. Clin Exp Optom 2012;95(4):457–9. PubMed PMID: 22443111. eng. https://pubmed.ncbi. nlm.nih.gov/22443111.
[75] Matsumoto Y, Kodama A, Goto E, Kawakita T, Dogru M, Tsubota K. The relation of ocular surface irregularity and visual disturbance in early stage Acanthamoeba keratitis. Eye Contact Lens 2017;43(1):51–6. PubMed PMID: 26783979. eng. https://pubmed.ncbi.nlm.nih.gov/26783979.
[76] Stapleton F, Carnt N. Contact lens-related microbial keratitis: how have epidemiology and genetics helped us with pathogenesis and prophylaxis. Eye 2012;26(2):185–93. PubMed PMID: 22134592. eng. https://pubmed.ncbi.nlm.nih.gov/22134592.
[77] Taher EE, Méabed EMH, Abdallah I, Abdel Wahed WY. Acanthamoeba keratitis in noncompliant soft contact lenses users: genotyping and risk factors, a study from Cairo, Egypt. J Infect Public Health 2018;11(3):377–83. PubMed PMID: 28965795. Epub 2017/10/03. eng.
[78] Dart JKG, Saw VPJ, Kilvington S. Acanthamoeba keratitis: diagnosis and treatment update 2009. Am J Ophthalmol 2009;148(4). PubMed PMID: 19660733. eng. https://pubmed.ncbi.nlm.nih.gov/ 19660733.
[79] Hammersmith KM. Diagnosis and management of Acanthamoeba keratitis. Curr Opin Ophthalmol 2006;17(4):327–31. PubMed PMID: 16900022. eng. https://pubmed.ncbi.nlm.nih.gov/16900022.
[80] Marciano-Cabral F, Cabral G. Acanthamoeba spp. as agents of disease in humans. Clin Microbiol Rev 2003;16(2):273–307. PubMed PMID: 12692099. eng. https://pubmed.ncbi.nlm.nih.gov/12692099.
[81] McClellan K, Howard K, Niederkorn JY, Alizadeh H. Effect of steroids on Acanthamoeba cysts and trophozoites. Invest Ophthalmol Vis Sci 2001;42(12):2885–93. PubMed PMID: 11687533. Epub 2001/11/01. eng.
[82] Wang Y, Feng X, Jiang L. Current advances in diagnostic methods of Acanthamoeba keratitis. Chin Med J (Engl) 2014;127(17):3165–70. PubMed PMID: 25189964. eng. https://pubmed.ncbi.nlm.nih.gov/ 25189964.
[83] Bharathi MJ, Ramakrishnan R, Meenakshi R, Mittal S, Shivakumar C, Srinivasan M. Microbiological diagnosis of infective keratitis: comparative evaluation of direct microscopy and culture results. Br J Ophthalmol 2006;90(10):1271–6. PubMed PMID: 16837544. eng. https://pubmed.ncbi.nlm.nih.gov/16837544.
[84] Szentmary N, Daas L, Shi L, Laurik KL, Lepper S, Milioti G, et al. Acanthamoeba keratitis - Clinical signs, differential diagnosis and treatment. J Curr Ophthalmol 2019;31(1):16–23. PubMed PMID: 30899841. Pubmed Central PMCID: PMC6407156. Epub 2019/03/23. https://www.ncbi.nlm.nih. gov/pubmed/30899841.
[85] Chin J, Young AL, Hui M, Jhanji V. Acanthamoeba keratitis: 10-year study at a tertiary eye care center in Hong Kong. Contact Lens Anterior Eye: J Br Contact Lens Assoc 2015;38(2). PubMed PMID: 25496910. eng. https://pubmed.ncbi.nlm.nih.gov/25496910.
[86] Sun X, Zhang Y, Li R, Wang Z, Luo S, Gao M, et al. Acanthamoeba keratitis: clinical characteristics and management. Ophthalmology 2006;113(3):412–6. PubMed PMID: 16427699. eng. https://pubmed. ncbi.nlm.nih.gov/16427699.
[87] Labbé A, Khammari C, Dupas B, Gabison E, Brasnu E, Labetoulle M, et al. Contribution of in vivo confocal microscopy to the diagnosis and management of infectious keratitis. Ocul Surf 2009;7(1):41–52. PubMed PMID: 19214351. eng. https://pubmed.ncbi.nlm.nih.gov/19214351.
[88] Winchester K, Mathers WD, Sutphin JE, Daley TE. Diagnosis of Acanthamoeba keratitis in vivo with confocal microscopy. Cornea 1995;14(1):10–7. PubMed PMID: 7712728. eng. https://pubmed.ncbi.nlm.nih.gov/7712728.
[89] Chopra R, Mulholland PJ, Hau SC. In vivo confocal microscopy morphologic features and cyst density in Acanthamoeba keratitis. Am J Ophthalmol 2020;217:38–48. PubMed PMID: 32278770. eng. https://pubmed.ncbi.nlm.nih.gov/32278770.
[90] Rezaei Kanavi M, Naghshgar N, Javadi MA, Sadat Hashemi M. Various confocal scan features of cysts and trophozoites in cases with Acanthamoeba keratitis. Eur J Ophthalmol 2012;22. Suppl 7:S46-S50. PubMed PMID: 22427148. eng. https://pubmed.ncbi.nlm.nih.gov/22427148.
[91] Kanavi MR, Javadi M, Yazdani S, Mirdehghanm S. Sensitivity and specificity of confocal scan in the diagnosis of infectious keratitis. Cornea 2007;26(7):782–6. PubMed PMID: 17667609. eng. https://pubmed.ncbi.nlm.nih.gov/17667609.
[92] Vaddavalli PK, Garg P, Sharma S, Sangwan VS, Rao GN, Thomas R. Role of confocal microscopy in the diagnosis of fungal and acanthamoeba keratitis. Ophthalmology 2011;118(1):29–35. PubMed PMID: 20801515. eng. https://pubmed.ncbi.nlm.nih.gov/20801515.
[93] Füst Á, Tóth J, Simon G, Imre L, Nagy ZZ. Specificity of in vivo confocal cornea microscopy in Acanthamoeba keratitis. Eur J Ophthalmol 2017;27(1):10–5. PubMed PMID: 27312210. Epub 2016/10/30. eng.
[94] Kobayashi A, Yokogawa H, Yamazaki N, Ishibashi Y, Oikawa Y, Tokoro M, et al. In vivo laser confocal microscopy findings of radial keratoneuritis in patients with early stage Acanthamoeba keratitis. Ophthalmology 2013;120(7):1348–53. PubMed PMID: 23499062. eng. https://pubmed.ncbi.nlm.nih. gov/23499062.
[95] Kaufman SC, Musch DC, Belin MW, Cohen EJ, Meisler DM, Reinhart WJ, et al. Confocal microscopy: a report by the American Academy of Ophthalmology. Ophthalmology 2004;111(2):396–406. PubMed PMID: 15019397. eng. https://pubmed.ncbi.nlm.nih.gov/15019397.
[96] Wei Z, Cao K, Wang L, Baudouin C, Labbé A, Liang Q. Corneal changes in Acanthamoeba keratitis at various levels of severity: an in vivo confocal microscopic study. Transl Vis Sci Technol 2021;10(7):10. PubMed PMID: 34110388. eng. https://pubmed.ncbi.nlm.nih.gov/34110388.
[97] Wilhelmus KR, Osato MS, Font RL, Robinson NM, Jones DB. Rapid diagnosis of Acanthamoeba keratitis using calcofluor white. Arch Ophthalmol 1986;104(9):1309–12. PubMed PMID: 2428343. eng. https://pubmed.ncbi.nlm.nih.gov/2428343.
[98] Thomas PA, Kuriakose T. Rapid detection of Acanthamoeba cysts in corneal scrapings by lactophenol cotton blue staining. Arch Ophthalmol

1990;108(2):168. PubMed PMID: 2302096. eng. https://pubmed.ncbi.nlm.nih.gov/2302096.
[99] Hahn TW, O'Brien TP, Sah WJ, Kim JH. Acridine orange staining for rapid diagnosis of Acanthamoeba keratitis. Jpn J Ophthalmol 1998;42(2):108–14. PubMed PMID: 9587842. eng. https://pubmed.ncbi.nlm.nih.gov/9587842.
[100] El-Sayed NM, Hikal WM. Several staining techniques to enhance the visibility of Acanthamoeba cysts. Parasitol Res 2015;114(3):823–30. PubMed PMID: 25346196. eng. https://pubmed.ncbi.nlm.nih. gov/25346196.
[101] Sharma S, Silverberg M, Mehta P, Gopinathan U, Agrawal V, Naduvilath TJ. Early diagnosis of mycotic keratitis: predictive value of potassium hydroxide preparation. Indian J Ophthalmol 1998;46(1):31–5. PubMed PMID: 9707845. eng. https://pubmed.ncbi.nlm.nih. gov/9707845.
[102] Tu EY, Joslin CE, Sugar J, Booton GC, Shoff ME, Fuerst PA. The relative value of confocal microscopy and superficial corneal scrapings in the diagnosis of Acanthamoeba keratitis. Cornea 2008;27(7):764–72. PubMed PMID: 18650660. eng. https://pubmed.ncbi.nlm.nih.gov/18650660.
[103] Boggild AK, Martin DS, Lee TY, Yu B, Low DE. Laboratory diagnosis of amoebic keratitis: comparison of four diagnostic methods for different types of clinical specimens. J Clin Microbiol 2009;47(5):1314–8. PubMed PMID: 19321727. eng. https://pubmed.ncbi.nlm.nih.gov/19321727.
[104] Megha K, Sharma M, Gupta A, Sehgal R, Khurana S. Microbiological diagnosis of Acanthamoebic keratitis: experience from tertiary care center of North India. Diagn Microbiol Infect Dis 2021;100(2):115339. PubMed PMID: 33618203. eng. https://pubmed.ncbi.nlm.nih.gov/33618203.
[105] da Rocha-Azevedo B, Tanowitz HB, Marciano-Cabral F. Diagnosis of infections caused by pathogenic free-living amoebae. Inter Perspect Infect Dis 2009;2009:251406. PubMed PMID: 19657454. eng. https://pubmed.ncbi.nlm.nih.gov/19657454.
[106] Lehmann OJ, Green SM, Morlet N, Kilvington S, Keys MF, Matheson MM, et al. Polymerase chain reaction analysis of corneal epithelial and tear samples in the diagnosis of Acanthamoeba keratitis. Invest Ophthalmol Vis Sci 1998:1261–5.
[107] Vodkin MH, Howe DK, Visvesvara GS, McLaughlin GL. Identification of Acanthamoeba at the generic and specific levels using the polymerase chain reaction. J Protozool 1992;39(3):378–85. PubMed PMID: 1640385. eng. https://pubmed.ncbi.nlm.nih.gov/1640385.
[108] Yera H, Zamfir O, Bourcier T, Ancelle T, Batellier L, Dupouy-Camet J, et al. Comparison of PCR, microscopic examination and culture for the early diagnosis and characterization of Acanthamoeba isolates from ocular infections. Eur J Clin Microbiol Infect Dis 2007;26(3):221–4. PubMed PMID: 17393203. eng. https://pubmed.ncbi.nlm.nih.gov/17393203.
[109] Li Z, Breitwieser FP, Lu J, Jun AS, Asnaghi L, Salzberg SL, et al. Identifying corneal infections in formalin-fixed specimens using next generation sequencing. Invest Ophthalmol Vis Sci 2018;59(1):280–8. PubMed PMID: 29340642. eng. https://pubmed.ncbi.nlm.nih.gov/29340642.
[110] Holmgaard DB, Barnadas C, Mirbarati SH, O'Brien Andersen L, Nielsen HV, Stensvold CR. Detection and identification of and other nonviral causes of infectious keratitis in corneal scrapings by real-time PCR and next-generation sequencing-based ^{16}S–^{18}S gene analysis. J Clin Microbiol 2021;59(2). PubMed PMID: 33239372. eng. https://pubmed.ncbi.nlm.nih.gov/33239372.
[111] Stapleton F. The epidemiology of infectious keratitis. Ocul Surf 2021. PubMed PMID: 34419639. eng. https://pubmed.ncbi.nlm.nih.gov/34419639.
[112] Cheng KH, Leung SL, Hoekman HW, Beekhuis WH, Mulder PG, Geerards AJ, et al. Incidence of contact-lens-associated microbial keratitis and its related morbidity. Lancet 1999;354(9174):181–5. PubMed PMID: 10421298. eng. https://pubmed.ncbi.nlm.nih.gov/10421298.
[113] Schaumberg DA, Snow KK, Dana MR. The epidemic of Acanthamoeba keratitis: where do we stand? Cornea 1998;17(1):3–10. PubMed PMID: 9436873. Epub 1998/01/22. eng.
[114] Seal D, Stapleton F, Dart J. Possible environmental sources of Acanthamoeba spp in contact lens wearers. Br J Ophthalmol 1992;76(7):424–7. PubMed PMID: 1627513. eng. https://pubmed.ncbi.nlm.nih.gov/1627513.
[115] Mathers WD, Sutphin JE, Folberg R, Meier PA, Wenzel RP, Elgin RG. Outbreak of keratitis presumed to be caused by Acanthamoeba. Am J Ophthalmol 1996;121(2):129–42. PubMed PMID: 8623882. Epub 1996/02/01. eng.
[116] Visvesvara GS. Infections with free-living amebae. Handb Clin Neurol 2013;114:153–68. PubMed PMID: 23829906. eng. https://pubmed.ncbi.nlm.nih.gov/23829906.
[117] Bouten M, Elsheikha HM. Diagnosis and management of Acanthamoeba keratitis: a continental approach. Parasitologia 2022;2(3):167–97.
[118] Possamai CO, Loss AC, Costa AO, Falqueto A, Furst C. Acanthamoeba of three morphological groups and distinct genotypes exhibit variable and weakly interrelated physiological properties. Parasitol Res 2018;117(5):1389–400. PubMed PMID: 29532218. Epub 2018/03/14. eng.
[119] Clarke DW, Niederkorn JY. The pathophysiology of Acanthamoeba keratitis. Trends Parasitol 2006;22(4):175–80. PubMed PMID: 16500148. eng. https://pubmed.ncbi.nlm.nih.gov/ 16500148.
[120] Hurt M, Neelam S, Niederkorn J, Alizadeh H. Pathogenic Acanthamoeba spp secrete a mannose-induced cytolytic protein that correlates with the ability to cause disease. Infect Immun 2003;71(11):6243–55. PubMed PMID: 14573643. eng. https://pubmed.ncbi.nlm.nih.gov/14573643.
[121] Maghsood AH, Sissons J, Rezaian M, Nolder D, Warhurst D, Khan NA. Acanthamoeba genotype T4 from the UK and Iran and isolation of the T2 genotype from clinical isolates. J Med Microbiol 2005;54(Pt 8):755–9. PubMed PMID: 16014429. eng. https://pubmed.ncbi.nlm.nih.gov/16014429.
[122] Costa AO, Chagas IAR, de Menezes-Neto A, Rêgo FD, Nogueira PM, Torrecilhas AC, et al. Distinct immunomodulatory properties of extracellular vesicles released by different strains of Acanthamoeba. Cell Biol Int 2021;45(5):1060–71. PubMed PMID: 33448518. eng. https://pubmed.ncbi. nlm.nih.gov/33448518.
[123] Ledee DR, Iovieno A, Miller D, Mandal N, Diaz M, Fell J, et al. Molecular identification of t4 and t5 genotypes in isolates from acanthamoeba keratitis patients. J Clin Microbiol 2009;47(5):1458–62. PubMed PMID: 19321730. Pubmed Central PMCID: PMC2681865. Epub 2009/03/27. eng.
[124] Iovieno A, Oechsler RA, Ledee DR, Miller D, Alfonso EC. Drug-resistant severe Acanthamoeba keratitis caused by rare T5 Acanthamoeba genotype. Eye Contact Lens 2010;36(3):183–4. PubMed PMID: 20461886. eng. https://pubmed.ncbi.nlm.nih.gov/20461886.