Diagnostic Microbiology & Infectious Disease 114 (2026) 117247

Contents lists available at ScienceDirect

Diagnostic Microbiology & Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Retrospective study of Acanthamoeba keratitis: Three-year experience with an integrated clinical and diagnostic workflow

Margherita Ortalli [a,b,#], Antonio Moramarco [c,d,#], Piera Versura [c,d], Giovanna Liguori [a,b], Giuseppe Russello [e], Simone Ambretti [a,b,*], Simone Baiocchi [b], Giacomo Nigrisoli [a], Michele Potenza [c], Luigi Fontana [c,d,1], Tiziana Lazzarotto [a,b,1]

[a] Department of Medical and Surgical Sciences, Alma Mater Studiorum, University of Bologna, Bologna 40138, Italy
[b] Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna 40138, Italy
[c] Ophthalmology Unit, DIMEC, Alma Mater Studiorum, Università di Bologna, Bologna 40126, Italy
[d] IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna 40138, Italy
[e] Microbiology Unit, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia 42122, Italy

ARTICLE INFO

Keywords:
Acanthamoeba keratitis
Clinical evaluation
Integrated diagnostic workflow
Therapy

ABSTRACT

Acanthamoeba keratitis (AK) is a rare but sight-threatening corneal infection, caused by free-living protozoa of the genus *Acanthamoeba*. Although cases are rising in high-income countries, the nonspecific presentation and overlapping features with other keratitis frequently result in misdiagnosis of AK, with poorer outcomes and impaired quality of life. A delayed diagnosis can allow the infection to progress to deeper corneal layers, increasing the risk of severe structural damage and requiring emergency keratoplasty to preserve ocular integrity and regain vision. This retrospective study describes a three-year experience at a Northern Italy tertiary center using an integrated diagnostic workflow combining clinical evaluation, in vivo confocal microscopy, culture, and -since 2024- real-time PCR. Among 39 patients with suspected AK, 11 cases were confirmed. Culture showed 92 % sensitivity in first diagnoses but decreased to 77 % when relapses were included. PCR, introduced as a routine tool in 2024, showed 100 % sensitivity in first diagnoses and proved crucial in detecting low-burden or culture-negative relapses. Confocal microscopy reached a sensitivity and specificity of 82 %. Early targeted anti-amoebic therapy, guided by this integrated approach, contributed to favorable outcomes in most cases, limiting the need for surgical intervention. These findings support the utility of combining methods for timely and accurate diagnosis of AK. Despite ongoing challenges in the clinical management of AK, our findings reinforce the critical value of adopting molecular diagnostics to enable earlier, more accurate identification and targeted treatment.

1. Introduction

Infectious keratitis is a sight-threatening corneal disease that may lead to ulceration and opacification, with severe cases potentially leading to permanent vision loss [1]. Infectious keratitis can be broadly categorized into two main types: microbial keratitis (MK), caused by bacteria, fungi, or parasites, and viral keratitis, most commonly associated with herpesviruses [2]. The clinical course of the infection heavily depends on the timeliness and accuracy of diagnosis, as well as the prompt initiation of appropriate treatment [3,4].

Among the various causes of MK, *Acanthamoeba* keratitis (AK) stands out as a rare but often misdiagnosed corneal infection. It is caused by amoebae of the genus *Acanthamoeba*, which are ubiquitous free-living protists found in different environmental reservoirs, including tap water, swimming pools, hot tubs, and soil [5]. First described in 1974 in England [6], AK has since been linked to several risk factors, including corneal trauma, swimming in contaminated water, contact lens use during showers, poor lens hygiene, and exposure to contaminated lens solutions [7].

Although *Acanthamoeba* is historically considered a rare cause of MK, the incidence of AK has been steadily rising in recent decades, marking it as an emerging pathogen of significant concern [3].

* Corresponding author.
E-mail address: simone.ambretti3@unibo.it (S. Ambretti).
# These authors contributed equally to this work.
[1] These authors shared senior authorship.

https://doi.org/10.1016/j.diagmicrobio.2025.117247
Received 10 October 2025; Received in revised form 24 December 2025; Accepted 29 December 2025
Available online 7 January 2026

This trend has been particularly evident in high-income countries, where increased use of contact lenses, coupled with suboptimal hygiene practices, has been identified as a major contributing factor. In fact, studies in Europe have reported a growing number of AK cases, with incidence rates reaching approximately 1-5 cases per million contact lens users annually, with cases mainly linked to urban environments and tap water exposure [8,9]. Similarly, reports from the USA indicate an increasing burden of AK, particularly in regions with high contact lens use and limited public awareness about preventative measures [9].

The rising incidence of AK necessitates heightened clinical awareness and improved diagnostic strategies. Timely diagnosis and intervention are critical to preserving vision, as AK's nonspecific presentation often mimics other keratitis forms, leading to dangerous diagnostic delays.

The diagnostic workflow for AK currently relies on a combination of clinical suspicion, corneal imaging and microbiological investigations. While culture remains the gold standard for detecting *Acanthamoeba*, advancements in diagnostic technology have introduced highly sensitive and rapid methods such as polymerase chain reaction (PCR) and *in vivo* confocal microscopy (IVCM) [9,10]. These innovations have significantly enhanced diagnostic accuracy and speed, enabling earlier treatment and better patient outcomes [11].

In this study, we describe the standardized diagnostic approach implemented at the IRCCS AOU Policlinico di Sant'Orsola in northern Italy for the diagnosis and management of AK. Our strategy aimed to streamline the diagnostic workflow, improve the case detection rate, and optimize patient care in a tertiary referral center of the country.

2. Methods

2.1. Ethics statement

The study was conducted in accordance with the declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Area Vasta Emilia Centro (study number: CE AVEC: 901/2022/Oss/AOUBo). All participants gave their written, informed consent.

2.2. Settings and samples

This is a retrospective observational study performed at the IRCCS AOU Policlinico di Sant'Orsola of Bologna, northern Italy during a 36-month period (from January 2022 to December 2024).

All cases of suspected AK referred to the Ophthalmology Unit of the IRCCS AOU Policlinico di Sant'Orsola were included in this study. Clinical suspicion was primarily based, according to the most up-to-date data available in the literature, on keratitis with known risk factors (e.g., positive history of contact lens use and/or exposure to non-sterile water while wearing lenses) and signs indicative of *Acanthamoeba* infection, including severe ocular pain and photophobia out of proportion, ring-shaped stromal infiltrate (ring infiltrate), rapidly progressing stromal disease and persistent corneal ulceration unresponsive to standard antibiotic therapy [12,13]. In each case, corneal scraping was performed at the Ophthalmology Unit; AK cases were defined based on clinical signs, IVCM together with diagnostic confirmation, in our setting achieved through culture and/or PCR.

2.3. Diagnostic workflow

An integrated diagnostic strategy was applied: standardized clinical evidence was combined with frontline IVCM evaluation supported by traditional culture. Corneal scraping was performed using 21-gauge needles or sterile Kimura platinum spatulas at the base and the infiltrate's leading edge. The corneal scrapings were transferred on the solid media in multiple “C” shaped streaks, directly in the operating theater, in cultures plates previously provided by the Microbiology Unit of the same hospital, in order to simplify the workflow and reduce the risk of sample contamination. From January 2024, a real-time PCR assay was also incorporated in the diagnostic work-flow as a complementary test alongside culture examination; in order to perform the molecular test, part of the scraping sample was transferred to an Eswab directly in the operating theater and sent to the Microbiology Unit along with the culture sample.

2.4. In vivo confocal microscopy

IVCM is a highly useful diagnostic tool for AK, in fact it provides a non-invasive, high-resolution view of corneal structures at the subcellular level [14,15]. This examination was performed utilizing an HRT3 RCM (Heidelberg Engineering, Heidelberg, Germany), enabling the direct identification of trophozoites and cysts, which appear as hyperreflective, well-defined structures (Fig. 1). Within our case series, IVCM was systematically implemented in all patients exhibiting clinical signs indicative of AK.

2.5. Acanthamoeba culture

The *Acanthamoeba* culture medium was specifically formulated and custom-prepared by Vacutest Kima (Padova, Italy) based on a standardized protocol provided by our laboratory [16], ensuring the production of a high-quality Non-Nutrient Agar (NNA) optimized for *Acanthamoeba* isolation and growth. NNA plates were inoculated with corneal scrapings directly in the operating theater as abovementioned.

Subsequently, upon arrival at the laboratory, 1 mL of Page’s amoeba saline solution, enriched with a fresh *Escherichia coli* suspension adjusted to a 2 McFarland turbidity standard, was inoculated onto the NNA plate. The culture plate was incubated at 30°C in aerobic atmosphere for 7 days and monitored daily under a low-power (100x) microscope for the appearance of *Acanthamoeba* trophozoites and cysts, *Acanthamoeba* trophozoites and cysts were identified based on the size and morphological characteristics, as the presence of finger-like tapering pseudopodia in trophozoites, double walled cysts with the inner wall that is retracted to give a polygonal- or star-shaped appearance (Fig. 2).

2.6. Molecular test for Acanthamoeba detection

A commercial PCR kit, ParoReal Kit Acanthamoeba T4 CE IVD marked (LyonDX, Pordenone, Italy), was used to detect *Acanthamoeba* DNA. The DNA extraction from clinical samples was performed by using the Maxwell® CSC Pathogen Total Nucleic Acid Kit (Promega, Wisconsin, USA), following the manufacturer's recommendations and employing the semiautomated platform Maxwell® CSC Intrument (Promega, Wisconsin, USA). The assay amplifies a segment of the 18S ribosomal (r) RNA gene of *Acanthamoeba* genotype T4 species [17]. PCR runs were performed employing the CFX Real Time PCR detection System (Bio--Rad, California, USA). The total processing time for the sample was around 4 hours.

2.7. Treatment of AK patients

Treatment of AK consisted of a prolonged topical regimen involving a combination of biguanides and aromatic diamidines. The biguanides employed included polyhexamethylene biguanide (PHMB) and chlorhexidine, with the former administered at a concentration of 0.08 % and the latter at 0.06 %. The aromatic diamidine hexamidine was administered at a concentration of 0.1 %, occasionally in combination with biguanides [18,19].

According to the empirical treatment strategy described by Dart et al [12], used at Moorfields Eye Hospital of London: PHMB 0.08 % was administered, every hour for 5 days, then every two hours for 7 days, six times daily for 7 days, and four times until complete clinical resolution [20]. When hexamidine was used in combination, it was administered at a concentration of 0.1 % with the same frequency as PHMB. Chlorhexidine 0.06 % was introduced in combination with, not as a replacement

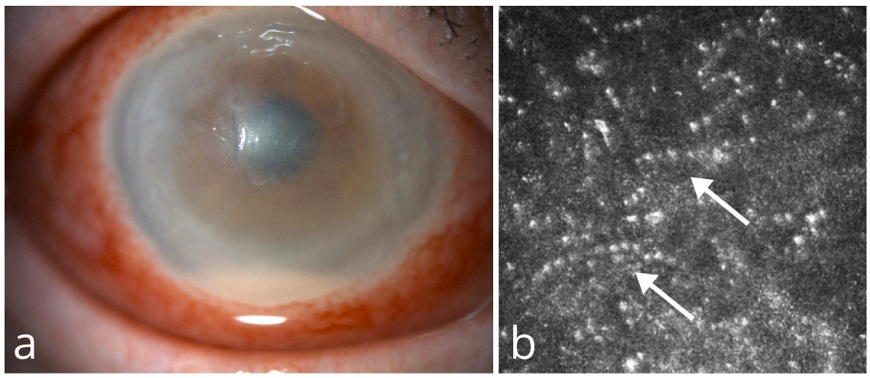

Fig. 1. a) Wide epithelial defect with stromal infiltration and a 360° annular appearance involving the mid-peripheral cornea; presence of an inferior hypopyon measuring approximately 1 mm. b) IVCM scan of the same eye at a depth of 200 μm, showing, indicated by the arrows, small polygonal structures measuring 10-15 μm in diameter, consistent with *Acanthamoeba* spp. cysts arranged in string-like formations.

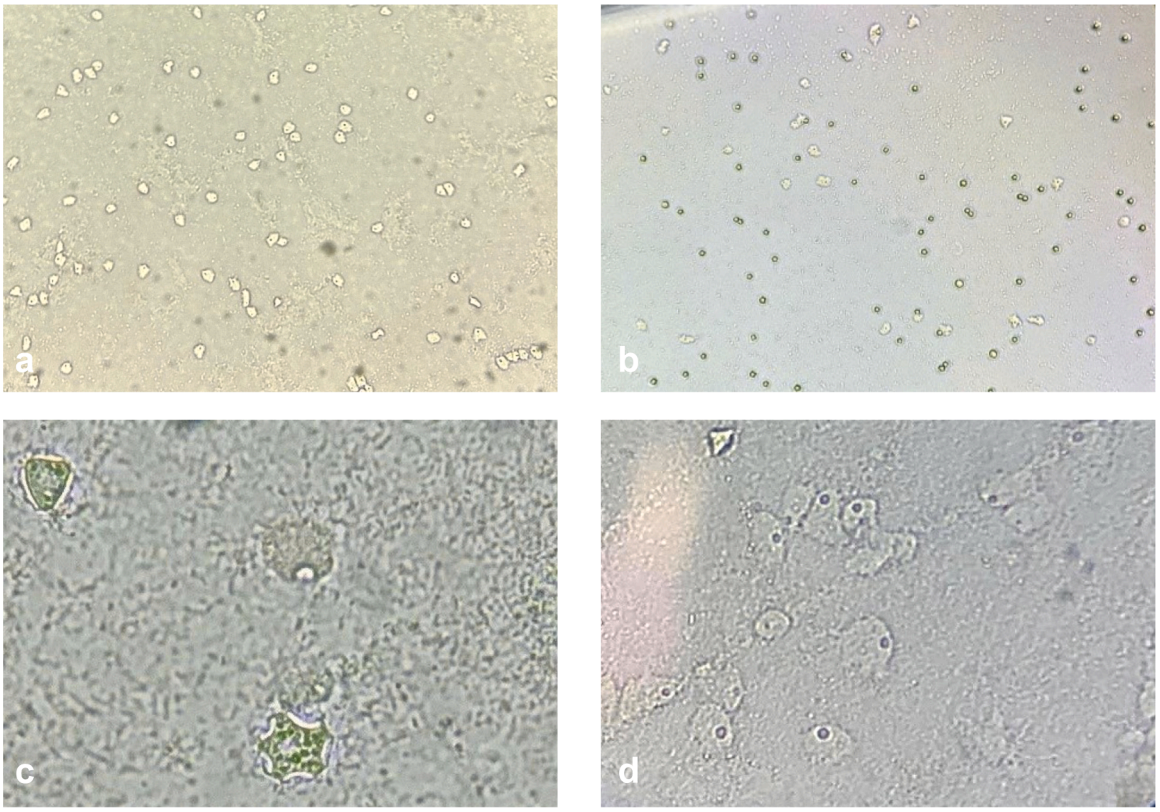

Fig. 2. a) Trophozoites of *Acanthamoeba* spp. grown on NNA, 100x magnification. b) Trophozoites and cysts of *Acanthamoeba* spp. grown on NNA, 100x magnification. In the upper section of the image, the imprint of the corneal scraping on the culture medium can be observed. c) Cysts of *Acanthamoeba* spp. with polygonal and star-shaped appearance, 200x magnification. d) Trophozoites of *Acanthamoeba* spp., 200x magnification.

for, PHMB in cases with suboptimal initial response, following a similar tapering schedule [12].

3. Results

During the study period, 105 patients were admitted to the Ophthalmology Unit of the IRCCS AOU Policlinico di Sant'Orsola with clinical signs suggestive of MK, among whom 39 showed symptoms indicative of AK (Fig. 3).

These 39 patients presented with severe ocular pain and marked photophobia, that were out of proportion to the clinical findings. Common clinical features included the presence of a ring infiltrate, a persistent corneal ulcer unresponsive to standard antibiotic therapy and rapidly progressing stromal disease. These signs occurred in patients with known risk factors for AK, such as a history of contact lens use (n=32) and/or exposure to non-sterile water while using or cleaning them (n=7). Eighteen patients were male and twenty-one were female, with an age range between 12 and 81 years.

All suspected AK cases underwent microbiological diagnostic testing, which included culture of corneal scraping for all analyzed patients. From January 2024, corneal scraping samples obtained from 11 patients were analyzed using both culture and PCR. Among the AK suspected cases, 11 out of 39 (28 %) were confirmed as positive for *Acanthamoeba* by culture and/or PCR (Fig. 3).

Culture positivity was confirmed in 10 out of 11 cases, demonstrating a sensitivity of 92 % with a maximum incubation period of 72 hours

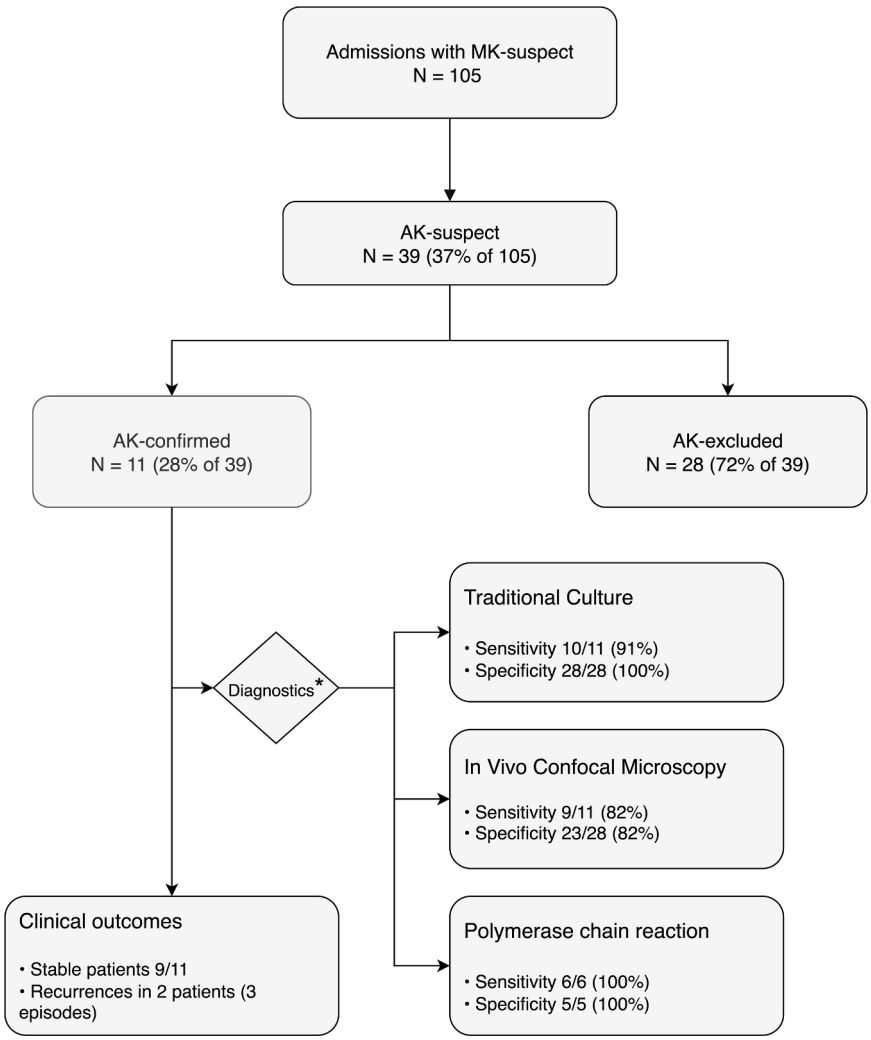

Fig. 3. Flow chart of patients with suspected MK; diagnostic performance and clinical outcomes are shown. *All reported sensitivity and specificity values refer exclusively to first episodes of *Acanthamoeba* keratitis; recurrences were not included.

(range: 30-72 hours), with characteristic *Acanthamoeba* trophozoites and cysts detecTable upon microscopic examination (Fig. 2).

Since the integration of the real-time PCR kit into the diagnostic routine, 11 suspected samples were tested using the molecular method, with 6 yielding positive results. The diagnosis of AK was excluded in the 5 negative samples, demonstrating a 100 % sensitivity for real-time PCR in the context of first diagnosis of AK. The five PCR-negative samples were excluded from AK diagnosis because culture and IVCM findings were concordantly negative, and all patients showed a favorable clinical response to non-amoebic therapy, further supporting the absence of *Acanthamoeba* infection.

IVCM yielded positive results in 9 out of 11 confirmed cases of AK, thus exhibiting a sensitivity of 82 %. Among 28 patients without AK, 23 tested negative by IVCM, resulting in a specificity of 82 % (Fig. 3).

Among the 11 confirmed cases, two patients (case#1 and case#2) suffered from recurrent AK, both testing positive by PCR and negative by culture. Furthermore, case#2 experienced clinical signs of a second AK relapse, but his corneal scraping tested negative by both PCR and culture. IVCM performed during the first relapse of both case#1 and case#2, demonstrated nonspecific inflammatory signs consistent with general corneal inflammation but did not reveal any distinctive features indicative of *Acanthamoeba* infection.

All patients with clinical suspicion of AK were initially treated empirically with a fourth-generation fluoroquinolone (moxifloxacin), followed by a targeted anti-amoeba therapy when AK diagnosis was confirmed. Among the 11 patients diagnosed with AK, three received a combination therapy of PHMB and hexamidine, four were treated with PHMB and chlorhexidine, and the remaining four underwent PHMB monotherapy. The choice between monotherapy and combination regimens was guided by the clinical severity at the time of diagnosis. Topical corticosteroids were introduced only after confirming the effectiveness of antiamoebic therapy and observing clear signs of regression in corneal infiltration. Notably, anti-inflammatory treatment was initiated at least 15 days after the onset of antiamoebic therapy. The average duration of topical therapy across all cases was approximately 250 days.

The first recurrence occurred in a 46-year-old patient (case#1) who had received PHMB therapy for six months, combined with hexamidine for one month. Ninety days after discontinuing all topical medications, she presented with a new stromal infiltration. Although culture results were negative, a four-month treatment regimen was initiated, consisting of PHMB for the entire duration and chlorhexidine for two months. PCR testing, subsequently performed, confirmed the recurrence.

The second recurrence involved a 40-year-old patient (case#2), a monthly contact lenses wearer, who had stopped PHMB therapy after four months due to apparent clinical improvement. Approximately one week later, he returned with subjective worsening and the reappearance of a corneal ring infiltrate. PCR testing confirmed the recurrence of AK, leading to the start of a PHMB and chlorhexidine-based regimen, PHMB was stopped after 4 month, while chlorhexidine was interrupted after 3

month from the start of the recurrence.

Furthermore, case#2 experienced clinical signs of a second AK relapse, but his corneal scraping tested negative by both PCR and culture. The case was therefore misdiagnosed as fungal keratitis and treated accordingly. Due to the lack of clinical improvement, the patient underwent penetrating keratoplasty (PK) approximately 40 days after symptom onset. The diagnosis of the second relapse in case#2 was therefore only clinical, while both culture and PCR failed to identify this episode and IVCM was not performed.

4. Discussion

Acanthamoeba keratitis is a challenging ocular infection often causing severe visual impairment if not promptly diagnosed and treated. AK remains underdiagnosed despite diagnostic advancements, primarily due to its nonspecific clinical presentation and the lack of standardized, widely accessible diagnostic protocols [21]. Traditionally, culture-based methods from corneal scrapings have been considered the gold standard for AK detection, offering high specificity but prolonged turnaround times. Recently, IVCM and molecular diagnostics, particularly PCR-based assays, have emerged as valuable tools for improving sensitivity and reducing diagnostic delays. However, as many neglected diseases, AK suffers from a lack of standardized diagnostic protocols and limited investment in research and development aimed at improving diagnostic methodologies and commercial kits, resulting in variations in laboratory protocols across different centers.

Several studies have highlighted the high sensitivity (77 %-100 %) and specificity (84 %-100 %) of *in vivo* confocal microscopy in detecting the infection, as well as its ability to identify possible coinfections (e.g., fungal) [14,22]. However, interpreting confocal images requires significant expertise to distinguish trophozoites from leukocytes or other corneal cells, and deep stromal infiltrates or marked inflammation may hinder diagnostic accuracy. Furthermore, the high cost and limited availability of this equipment is a barrier to routine use. It is also worth emphasizing that the presence of 5 false positives detected in our series by IVCM, underscores its limitation when used as a standalone diagnostic tool. As a result, it is considered a valuable diagnostic tool only when integrated with microbiological methods, rather than as a stand-alone method [15].

The sensitivity of the culture method for diagnosing AK, as reported in the literature, varies between 33 % and 67 %. Through the optimization of our workflow, i.e. direct transfer of corneal scrapings onto culture plates in the operating theater, we achieved a noTable improvement in accuracy, with culture sensitivity reaching 92 % in the context of first diagnosis of AK. Among the 11 cases diagnosed as AK positive, the undetected case by culture was attributed to fungal contamination of the culture medium, likely due to the Page's solution, which interfered with the growth of *Acanthamoeba*. Furthermore, when considering the two patients who experienced a relapse, the culture results were negative, thus leading to a decrease of the sensitivity to 77 %. This finding aligns with established literature demonstrating the progressive decline in culture method sensitivity in relapsed cases and in patients undergoing active treatment [23,24]. In case#1, culture yielded a negative result, and at the time of diagnosis PCR was not integrated into the diagnostic routine. However, given the presence of characteristic clinical signs previously described, the clinician opted to proceed with the targeted AK treatment, which resulted in the complete resolution of AK signs. To further support this diagnosis, a frozen sample of case#1 was retrospectively analyzed by PCR, leading to the detection of *Acanthamoeba* DNA, thus confirming that the initial culture result was a false negative result. In case#2, culture was inconclusive due to fungal contamination, but PCR identified *Acanthamoeba* DNA, although at low levels, which was consistent with a relapse of infection. Unfortunately, during the second relapse, PCR failed to detect *Acanthamoeba* DNA, which may be attributed to a low parasitic load or suboptimal sampling conditions, which may have compromised the sensitivity of molecular detection.

Timely AK diagnosis is critical; PCR provides results within 6-24 hours, significantly reducing diagnostic turnaround time compared to culture. We observed that the incorporation of PCR significantly improved our diagnostic workflow by reducing turnaround time and offering superior sensitivity, as also other studies have shown [25]. In fact, the molecular test allowed the detection of *Acanthamoeba* infection in a patient whose culture was contaminated by fungi (a crucial finding supported by the high DNA concentration detected -low Ct value- and confirmed by characteristic clinical presentation) and enabled the identification of 2 relapses that tested negative by conventional culture methods. These findings underscore the importance of integrating molecular diagnostics with culture to enhance sensitivity, especially in challenging cases where culture limitations could lead to delayed or missed diagnoses.

Moreover, a highly relevant issue is that the early-stage AK is often mistaken for bacterial, herpetic or fungal keratitis, leading to delays in proper treatment. Given these challenges, incorporating PCR as an early diagnostic tool in cases of keratitis unresponsive to antibiotics could greatly reduce misdiagnosis and improve clinical decision-making.

Corneal biopsy is a valuable diagnostic option when microbiological/molecular tests are inconclusive [25]. Studies suggest histopathology is often more reliable than culture-particularly post-therapy-due to its ability to detect resistant stromal cysts. However, we excluded biopsy from our protocol since the combination of microbiological, molecular, IVCM consistently yielded a definitive diagnosis. Additionally, the single remaining negative case was structurally unsuitable for safe biopsy [26].

Regarding treatment outcomes, 9 patients out of 11 were tapered off anti-amoebic therapy, after an average of 36 weeks, with no current signs of recurrence. Both recurrent cases in our series received anti-amoebic therapy for a shorter duration than the mean treatment length. Although limited, this observation supports the literature's consistent emphasis on the necessity of prolonged therapy -often exceeding 12 months- as a single surviving amoeba can re-establish infection [12,24].

At the time of initial evaluation, all patients had extremely poor visual acuity: only one patient had a decimal VA of logMAR 1.0, whereas the remaining 10 could perceive only hand motions. Upon discontinuation of topical therapy, 2 out of 11 patients still had hand-motion vision, 7 out of 11 had counting-fingers vision, and 2 out of 11 had VA of logMAR 1.0. The poor visual outcome was attributable to post-keratitis leucoma involving the optical axis in all cases. No patient in our series underwent corneal cross-linking (CXL). While some isolated reports suggest potential benefits of CXL in resistant infectious keratitis, its precise role remains uncertain due to heterogeneous protocols and inconsistent outcomes in observational studies [27]. CXL may be considered only in exceptional, non-responsive cases or as a temporary measure before urgent keratoplasty, but it was not deemed suitable for our cohort. Except for the single patient who underwent therapeutic penetrating keratoplasty, early treatment avoided in all patients the need for urgent "hot" surgery, which is often associated with poor visual outcome, and prevented extensive full-thickness corneal damage that would have otherwise required a penetrating keratoplasty. This suggest that the overall favorable prognosis can be largely attributed to the timely microbiological diagnosis, which enabled early and targeted treatment. Importantly, once a condition of clinical stability is achieved, patients can be considered candidates for corneal transplantation with optical purposes, aimed at visual rehabilitation.

Not least, although the introduction of PCR involves higher costs compared with traditional culture, the overall workflow should be seen as cost-effective, since timely and accurate diagnosis reduces the risk of misdiagnosis, prolonged therapy, and costly invasive procedures such as emergency keratoplasty. By integrating culture, IVCM, and PCR in a standardized pathway, our approach balances diagnostic performance with economic sustainability and can be practically implemented in other referral centres.

This study has some limitations, including its retrospective nature and the small sample size. Nevertheless, our experience provides valuable insights into the real-world application of an integrated diagnostic strategy and highlights the critical role of molecular techniques and combined methods in addressing the challenges associated with AK detection.

5. Conclusion

Our study underscores the necessity of integrating multiple diagnostic methods within a standardized AK pathway and highlights the need for clear referral system to specialized centers. Broader implementation of PCR -particularly in tertiary settings and suspected antibiotic-resistant keratitis- could significantly reduce time to diagnosis, thus improving patient outcomes and mitigating long-term complications.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Margherita Ortalli: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Antonio Moramarco:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Piera Versura:** Writing – review & editing, Visualization, Validation, Supervision, Data curation. **Giovanna Liguori:** Writing – review & editing, Visualization, Methodology, Conceptualization. **Giuseppe Russello:** Writing – review & editing, Visualization, Investigation. **Simone Ambretti:** Writing – review & editing, Visualization, Validation, Supervision. **Simone Baiocchi:** Writing – review & editing, Formal analysis, Data curation. **Giacomo Nigrisoli:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Michele Potenza:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Luigi Fontana:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Conceptualization. **Tiziana Lazzarotto:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Cabrera-Aguas M, Khoo P, Watson SL. Infectious keratitis: A review. Clin Experiment Ophthalmol 2022;50:543–62. https://doi.org/10.1111/ceo.14113.
[2] Durand ML, Barshak MB, Chodosh J. Infectious keratitis in 2021. JAMA 2021;326:1319–20. https://doi.org/10.1001/jama.2021.0424.
[3] Russello G, Moramarco A, Vizzini L, Farina C, Fontana L, Carretto E. Diagnostic approach and epidemiology of Microbial keratitis: findings from an Italian Tertiary Care center. Diagn Microbiol Infect Dis 2021;101:115470. https://doi.org/10.1016/j.diagmicrobio.2021.115470.
[4] Truong DT, Bui M-T, Memon P, Cavanagh HD. Microbial keratitis at an urban public hospital: A 10-year update. J Clin Exp Ophthalmol 2015;6:498. https://doi.org/10.4172/2155-9570.1000498.
[5] Somani SN, Ronquillo Y, Moshirfar M. Acanthamoeba keratitis. Statpearls. Treasure Island (FL): StatPearls Publishing; 2025.
[6] Azzopardi M, Chong YJ, Ng B, Recchioni A, Logeswaran A, Ting DSJ. Diagnosis of acanthamoeba keratitis: past, present and future. Diagn Basel Switz 2023;13:2655. https://doi.org/10.3390/diagnostics13162655.
[7] Bao M, Bao H, Wang S, Zhou H. The pathogenesis, risk factors, diagnosis and treatment of acanthamoeba keratitis. Front Med 2025;12. https://doi.org/10.3389/fmed.2025.1559224.
[8] Stellwagen A, MacGregor C, Kung R, Konstantopoulos A, Hossain P. Personal hygiene risk factors for contact lens-related microbial keratitis. BMJ Open Ophthalmol 2020;5. https://doi.org/10.1136/bmjophth-2020-000476.
[9] Zhang Y, Xu X, Wei Z, Cao K, Zhang Z, Liang Q. The global epidemiology and clinical diagnosis of *acanthamoeba* keratitis. J Infect Public Health 2023;16:841–52. https://doi.org/10.1016/j.jiph.2023.03.020.
[10] Hoffman JJ, Dart JKG, De SK, Carnt N, Cleary G, Hau S. Comparison of culture, confocal microscopy and PCR in routine hospital use for microbial keratitis diagnosis. Eye 2022;36:2172–8. https://doi.org/10.1038/s41433-021-01812-7.
[11] Fanselow N, Sirajuddin N, Yin X-T, Huang AJW, Stuart PM. Acanthamoeba Keratitis, pathology. Diagnosis Treatment. Pathogens 2021;10:323. https://doi.org/10.3390/pathogens10030323.
[12] Dart JKG, Saw VPJ, Kilvington S. Acanthamoeba keratitis: diagnosis and treatment update 2009. Am J Ophthalmol 2009;148:487–99. https://doi.org/10.1016/j.ajo.2009.06.009. e2.
[13] Raghavan A, Bhosale N, Baidwal S, Bellamkonda P, N K, Vijayaraghavan P, et al. Acanthamoeba keratitis - is there another perspective: extrapolations from the Acanthamoeba - fungal keratitis study. Arch Clin Exp Ophthalmol 2020;2:17–21. https://doi.org/10.46439/ophthalmology.2.010.
[14] Chidambaram JD, Prajna NV, Larke NL, Palepu S, Lanjewar S, Shah M, et al. Prospective study of the diagnostic accuracy of the In vivo laser scanning confocal microscope for severe microbial keratitis. Ophthalmology 2016;123:2285–93. https://doi.org/10.1016/j.ophtha.2016.07.009.
[15] Villani E, Baudouin Christophe, Efron Nathan, Hamrah Pedram, Kojima Takashi, Patel Sanjay V, et al. Vivo confocal microscopy of the ocular surface: from bench to bedside. Curr Eye Res 2014;39:213–31. https://doi.org/10.3109/02713683.2013.842592.
[16] Manuale illustrato di diagnostica parassitologica - Zanichelli n.d. https://www.zanichelli.it/ricerca/prodotti/manuale-illustrato-di-diagnostica-parassitologica (accessed July 2, 2025).
[17] Corsaro D, Venditti D. Molecular evidence for a new lineage within the acanthamoeba T4 genotype. Parasitol Res 2023;122:1445–50. https://doi.org/10.1007/s00436-023-07844-0.
[18] Papa V, der Meulen I van, Rottey S, Sallet G, Overweel J, Asero N, et al. Safety and tolerability of topical polyhexamethylene biguanide: a randomised clinical trial in healthy adult volunteers. Br J Ophthalmol 2022;106:190–6. https://doi.org/10.1136/bjophthalmol-2020-317848.
[19] Oldenburg CE, Acharya NR, Tu EY, Zegans ME, Mannis MJ, Gaynor BD, et al. Practice patterns and opinions in the treatment of acanthamoeba keratitis. Cornea 2011;30:1363. https://doi.org/10.1097/ICO.0b013e31820f7763.
[20] Dart JKG, Papa V, Rama P, Knutsson KA, Ahmad S, Hau S, et al. The Orphan drug for Acanthamoeba Keratitis (ODAK) Trial: PHMB 0.08 % (Polihexanide) and Placebo versus PHMB 0.02 % and propamidine 0.1. Ophthalmology 2024;131:277–87. https://doi.org/10.1016/j.ophtha.2023.09.031.
[21] Garg D, Daigavane S. A Comprehensive Review on Acanthamoeba keratitis: an overview of epidemiology, risk factors, and therapeutic strategies. Cureus n.d.;16:e67803. https://doi.org/10.7759/cureus.67803.
[22] 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;37:480. https://doi.org/10.1097/ICO.0000000000001497.
[23] Walochnik J, Scheikl U, Haller-Schober E-M. Twenty years of acanthamoeba diagnostics in Austria. J Eukaryot Microbiol 2015;62:3–11. https://doi.org/10.1111/jeu.12149.
[24] Lorenzo-Morales J, Khan NA, Walochnik J. An update on Acanthamoeba keratitis: diagnosis, pathogenesis and treatment. Parasite Paris Fr 2015;22:10. https://doi.org/10.1051/parasite/2015010.
[25] Roth M, Balasiu A, Daas L, Holtmann C, Servera A, Walckling M, et al. Impact of implementation of polymerase chain reaction on diagnosis, treatment, and clinical course of acanthamoeba keratitis. Graefes Arch Clin Exp Ophthalmol 2023;261:1951–9. https://doi.org/10.1007/s00417-023-05993-7.
[26] Hudson J, Al-Khersan H, Carletti P, Miller D, Dubovy SR, Amescua G. Role of corneal biopsy in the management of infectious keratitis. Curr Opin Ophthalmol 2022;33:290–5. https://doi.org/10.1097/ICU.0000000000000852.
[27] Chan TCY, Lau TWS, Lee JWY, Wong IYH, Jhanji V, Wong RLM. Corneal collagen cross-linking for infectious keratitis: an update of clinical studies. Acta Ophthalmol (Copenh) 2015;93:689–96. https://doi.org/10.1111/aos.12754.