

## Case report

# Can *Acanthamoeba* keratitis be properly diagnosed without culture in the real-world clinical microbiology laboratory?: a case report

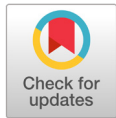
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## Abstract

*Acanthamoeba* species are ubiquitous, free-living organisms found in the environment. They can cause a sight-threatening cornea disease, termed *Acanthamoeba* keratitis, and are often misdiagnosed, causing delayed administration of the correct treatment. Herein, we report a case of *Acanthamoeba* keratitis diagnosed without culture. A 12-year-old girl with a history of wearing contact lenses presented with complaints of pain, irritation, and hyperemia in the left eye. Corneal scraping-smear slide, and liquids with contact lenses were submitted to the clinical microbiology laboratory. Cultures of *Acanthamoeba* spp. were not available; thus, they were stained with calcofluor white. The isolation of *Acanthamoeba* from the corneal scraping allowed the detection of trophozoites and cysts based on their morphological characteristics. PCR targeting the 18S rRNA gene and subsequent sequencing revealed 99% identity with the *Acanthamoeba* spp. Although it is challenging to find real-world evidence of *Acanthamoeba* in clinical microbiology without using culture methods, this case underscores the need for clinical microbiology laboratories to maintain their inspection capabilities.

**Keywords:** *Acanthamoeba* keratitis, cyst, trophozoite, morphology



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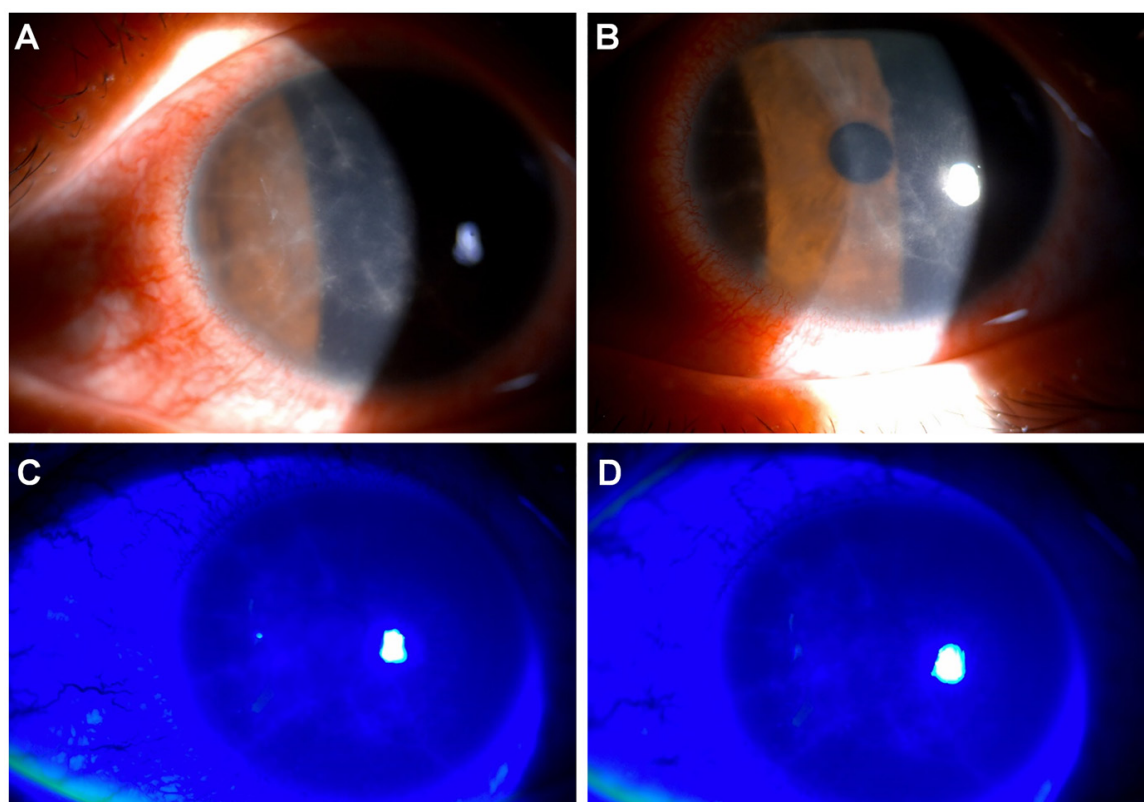
## Introduction

*Acanthamoeba* species are ubiquitous, free-living organisms found in the environment. They can cause a sight-threatening cornea disease, termed *Acanthamoeba* keratitis, and are often misdiagnosed, causing delayed treatment. Although the gold standard diagnosis is amoeba isolation from corneal scraping samples, it is challenging to find evidence of *Acanthamoeba* in real-world clinical microbiology practice. Herein, we report a case of *Acanthamoeba* keratitis diagnosed without culture, and describe several diagnostic methods for *Acanthamoeba* that can be useful in actual clinical microbiology laboratories.

## Case report

**Patient information:** A 12 year-old girl with a history of wearing contact lenses presented with pain, irritation, and hyperemia in the left eye visited the ophthalmology clinic at Asan Medical Center, South Korea.

**Clinical findings:** During slit-lamp microscopic examination of the patient's left eye, radial keratoneuritis was observed in the central corneal stroma, whereas the vitreous and retina showed no abnormalities (Fig. 1).



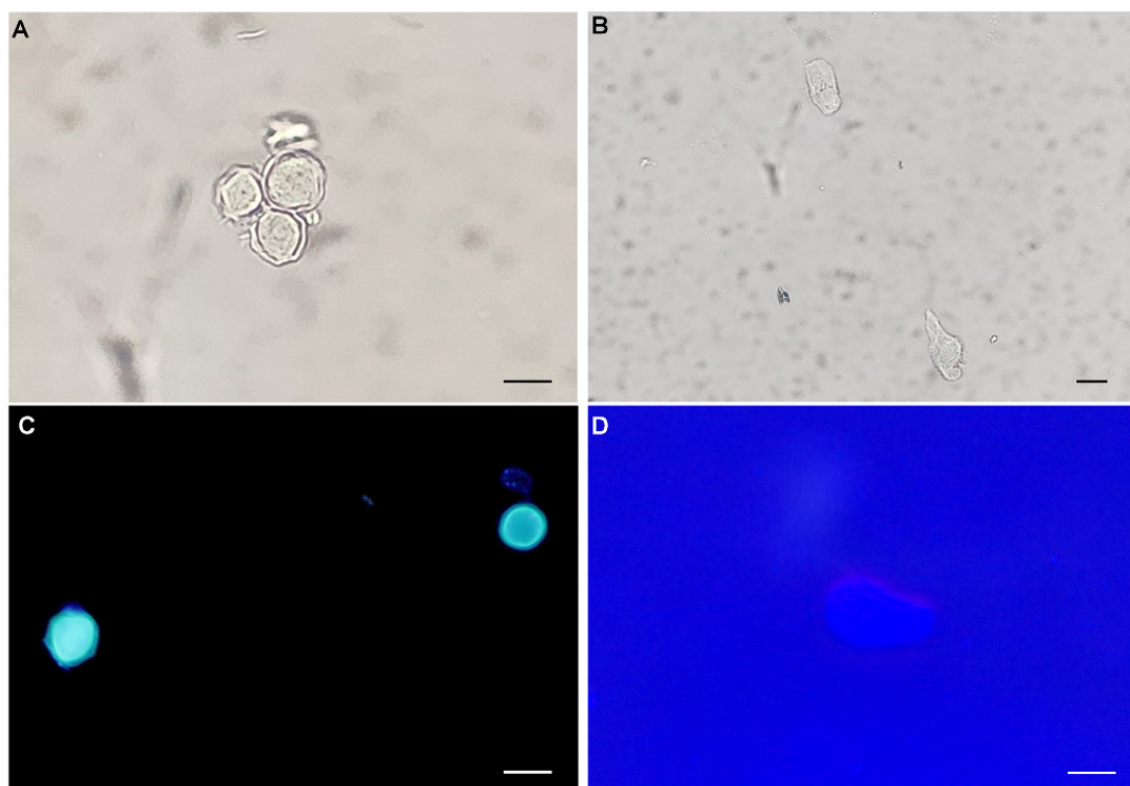
**Fig. 1.** Anterior segment photographs of the patient in this case study. The top two anterior segment images were taken under slit-lamp microscope (A, B) and the bottom two images were under cobalt blue light after fluorescein dye staining (C, D). During the slit-lamp microscopy examination of the patient's left eye, radial keratoneuritis was observed in the central corneal stroma, while vitreous and retina showed no abnormalities.

**Diagnostic assessment:** Corneal scraping was performed, and a direct smear slide was prepared. The slide and liquids with contact lenses were submitted to a clinical microbiology laboratory. Cultures for *Acanthamoeba* spp. were not available; thus, they were stained with calcofluor white as previously described [1]. Isolation of *Acanthamoeba* spp. from the corneal scraping and liquids allowed the detection of trophozoites and cysts under both light and fluorescence microscopes (Fig. 2). The *Acanthamoeba* cysts were approximately 10–15  $\mu\text{m}$  in diameter, round, with a plica-like surface (Fig. 2A, 2C). The viable cysts were round or oval with double cyst walls, that is, ectocyst and endocyst walls. The ectocysts contracted into wrinkles and were separated from the thin and smooth endocysts. The trophozoites (about 20–25  $\mu\text{m}$  in diameter) were pleomorphic and exhibited large lamellipodium of varying size and shape. A single large nucleus and numerous vacuoles with diverse contents in trophozoites were clearly observed by light microscopy but were unremarkable by fluorescent microscopy (Fig. 2B, 2D). The microscopic morphology was consistent with *Acanthamoeba* spp. reported to the ophthalmology department. To molecularly identify the microorganisms, a segment of the 18 small subunit ribosomal ribonucleic acid (rRNA) gene was

amplified. Genomic DNA was extracted from the samples using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Subsequently, polymerase chain reaction (PCR) was conducted employing the primer set JDP1 (5'-GGCCCAGATCGTTTACCGTGAA-3') and JDP2 (5'-TCTCACAAGCTGCTAGGGAGTCA-3'), as documented in the literature [2]. Briefly, the PCR process involved an initial incubation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and elongation at 72°C for 1 min, with a final elongation step at 72°C for 10 min to complete the amplification process. The PCR product, 436 base pairs in length, was sent to Cosmo Genetech for direct sequencing. The chromatograms of sequences were trimmed manually and assembled using the SeqMan software (DNASTAR). Basic local alignment search tool searches of the assembled sequences revealed 99% identity with *Acanthamoeba* spp. (GenBank accession no. MG825460.1) and conclusively identified the microorganism as *Acanthamoeba* spp.

**Therapeutic intervention:** Based on the morphological characteristics observed under the microscope, the patient was prescribed 0.02% polyhexamethylene biguanide eyedrops as a treatment on the day of the visit to our hospital.

**Follow-up and outcomes:** The patient underwent five months of pharmacological treatment, and the initially observed radial keratoneuritis showed significant improvement.



**Fig. 2.** Morphological analysis of *Acanthamoeba* spp. using calcofluor-white staining. (A) *Acanthamoeba* cysts in a group under light microscopy (400×, scale bar = 10 μm). (B) *Acanthamoeba* trophozoites with a single large nucleus and numerous vacuoles were conspicuous under light microscopy (200×, scale bar = 10 μm). (C) Remarkable *Acanthamoeba* cysts with double cyst walls under fluorescence microscopy (400×, scale bar = 10 μm). (D) *Acanthamoeba* trophozoites were relatively unremarkable under fluorescence microscopy (400×, scale bar = 10 μm).

## Discussion

Since the first report of *Acanthamoeba* keratitis in 1975, its incidence has increased, particularly among contact lens users [3]. In Korea, most published reports on the clinical patterns and treatments of *Acanthamoeba* keratitis have been case reports [4,5]; however, the incidence is perceived to be low, and the exact prevalence remains unclear [6]. The gold standard for diagnosing *Acanthamoeba* keratitis involves scraping the corneal epithelium, particularly from areas with severe epithelial damage, and culturing on *Escherichia coli*-seeded non-nutrient agar plates. This method allows the observation of *Acanthamoeba* predation paths on *E. coli*, demonstrating a detection rate of 60%–76% [7]. Several additional tests have been suggested to ascertain the growth rate, temperature tolerance, or pathogenicity of isolated amoebae, and axenization using proteose peptone-yeast extract-glucose medium is required [8]. Nonetheless, this culture method is time-consuming, taking a 1 week or more, which delays diagnosis and treatment. Although culture has been recognized as the gold standard for *Acanthamoeba* laboratory diagnosis [1], it has not been well-established in clinical laboratories. Instead of culture, several staining methods, such as lactophenol-cotton blue, acridine orange, calcofluor white, silver, and hematoxylin and eosin, can be useful for the identification of *Acanthamoeba* spp. from clinical specimens [1,9]. In this study, we found that *Acanthamoeba* cysts appeared light green on staining with calcofluor white. Direct microscopy of the original sample can detect characteristic cysts and trophozoites [1]. In this case, the causative agent was diagnosed through observation of *Acanthamoeba* morphology via a direct smear of the original sample without performing an axenic culture. However, relying solely on morphology for *Acanthamoeba* keratitis diagnosis through microscopic examination can lead to a misdiagnosis without experienced inspectors. This necessitates quality control in clinical laboratories, and continuous education regarding this uncommon pathogen. In addition to direct observation, we performed PCR using JDP1 and JDP2 primer sets, as previously described. The PCR method is well-established for detecting the 18S rRNA gene, facilitating genotyping in most cases through sequencing [2]. In addition to sequencing, real-time PCR has been preferred in clinical laboratories because there is no need for postamplification handling, leading to faster analysis and reduced risk of amplicon contamination [10]. Several real-time PCR-based assays have been developed and evaluated [11], but no commercial real-time PCR kits for the diagnosis of *Acanthamoeba* keratitis are available in Korea. Financial and procedural hurdles due to the non-reimbursable status of axenic cultures and PCR in certain diagnostic frameworks should be further overcome. Although it is challenging to find real-world evidence of *Acanthamoeba* in clinical microbiology without using culture methods, this case underscores the need for clinical microbiology laboratories to maintain their inspection capabilities. Furthermore, this study highlights the need for insights into *Acanthamoeba* diagnosis in clinical laboratories using various diagnostic tools.

## Ethics statement

The study was approved by the Institutional Review Board of Asan Medical Center (No. 2024-0519) and the requirement for informed consent was waived because of the retrospective nature of the study.

## Conflicts of interest

Eun Jeong Won has been an associate editor of the *Annals of Clinical Microbiology* since January 2024. However, she was not involved in the review process of this article. No other potential conflict of interest relevant to this article was reported.

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