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## Original Article

# *Acanthamoeba* Spp. Detection in Contact Lens Wearers and Non-Wearers in Iraq

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***Abstract***

***Background:*** *Acanthamoeba* spp. are free-living amoebae found in a broad range of environments. *Acanthamoeba* spp. are responsible for about 20% of keratitis infections in contact lens wearers. We aimed to detect *Acanthamoeba* spp. and determined the prevalence of *Acanthamoeba* spp. in contact lens wearers to increase health awareness of the risks of spreading it.

***Methods:*** The current study included an investigation of free-living opportunistic amoeba *Acanthamoeba* spp. in eye swab samples among students in the College of Sciences at the University of Thi-Qar/Iraq. Eighty-eight samples were collected from the eyes of both sexes and for both lens wearers and non-lens wearers from January to April 2024.

***Results:*** The current study results showed that the percentage of *Acanthamoeba* was 4.5% (4/88). All positive cases were in females who were lens wearers only, and no cases were recorded in males or females who had no contact lens. *Acanthamoeba* spp. were identified morphologically based on characteristics of active trophozoites and cyst forms, and molecular identified by conventional PCR using *Acanthamoeba* primers JPD1/ JPD2. Additionally, sequencing analysis was performed on positive samples and their alignment sequences were analyzed using BLAST. The results showed a homologous identity (100%) for four samples with the reference isolate *Acanthamoeba* spp. *18S ribosomal RNA* gene (MK390853.1). It was registered as one isolate of *Acanthamoeba* spp. in gene bank with accession number (PQ661179.1).

***Conclusion:*** the current findings indicate the prevalence of *Acanthamoeba* spp. in contact lens wearers, which is a health risk, especially in females.

## Introduction

*Acanthamoeba* is a widespread amoeba that is frequently isolated from environmental samples. It can be found in various habitats, such as air, soil, water, hospital water supplies, and dental water units (1). *Acanthamoeba* spp. can tolerate a broad range of extreme environmental conditions, are thermo-tolerant, and cause infection in human (2). *Acanthamoeba* life cycle includes two stages (trophozoites and cysts). Extremes in temperature, dryness, and pH are among the harshest conditions that dormant cysts with low metabolic activity may withstand (3).

It is even commonly found in man-made environments, such as public pools, surgerical tools, and contact lens solutions. Certain *Acanthamoeba* species can result in *Acanthamoeba* keratitis (AK), an infection that may be visually dangerous (4,5). Acanthamoebic keratitis, a disease of the cornea, and granulomatous amoebic encephalitis (GAE), an infection of the central nervous system (CNS) in both human and animals, are the two main pathologies caused by *Acanthamoeba* spp. pathogens. Moreover, *Acanthamoeba* frequently causes skin infections linked to GAE (6). Infectious keratitis (IK) can be caused by *Acanthamoeba* spp., which, if unchecked, can result in blindness (7). Contact lens wearers frequently develop keratitis linked to *Acanthamoeba* spp. (8).

Further, reports of infection in the skin and *Acanthamoeba* pneumonia have been made. In addition, the carcinogenic characteristic for *Acanthamoeba* spp. to be as a "Trojan Horse," can assist the organism in introducing a host inside, which causes bacterial or mixed infections, and is a secondary reason for protozoan infection (9).

The classification depends on morphological characteristics and cyst size; 25 species were diagnosed and registered in *Acanthamoeba* genus. Group I included 4 species that are *Astronyxis, Astrium tubiashi, Comandoni,* and *Echinulata* with a diameter less than 18 µm and large cysts and trophozoites. While Group II included 11 species that are in natural and man-made habitats,they are also more prevalent .Cysts with a mediumsize, and a diameter of less than 18 µm. Further,the endocyst is ovoid, polygonal, or triangle-shaped, while the exocyst is rough and undulant. There are differences in the area between the outer and inner walls. Group III included 5 species of small cysts that are the least common, with a diameter less than 18 µm. The endocyst is globose or ovoid, while it is not polygonal and stellate. The exocyst is extremely thin and invisible during the existence of the creases (10).

Recently, genetic detection for the classification of the *Acanthamoeba* genus has been dependent on the sequence of 18S ribosomal RNA and has reported twenty-two genotypes, including T1-T21 (11). The most common distribution of *Acanthamoeba* genotypes is T4, which causes infection in human (12).

Therefore, we aimed to detect *Acanthamoeba* spp. and determined the prevalence of *Acanthamoeba* spp. in contact lens wearers to increase health awareness of the risks of spreading it.

## Materials and Methods

### *Eye sample collection*

Between January and April of 2024, samples were taken from the students' eyes at the Sciences College /University of Thi-Qar /Iraq, aged 18 to 24 years. The samples were taken using a sterile cotton swab dampened with Page Amoebic Saline (PAS). The lower conjunctiva in the eyes was then swabbed in a rolling motion from the lateral canthus toward the medial canthus (13).

### *Ethical approval*

Oral consent from student participants to collect eye swabs was obtained. Besides, ethical approval of sample collection was obtained

by the Ethical Committee in University of Thi-Qar /Iraq (Ref: 52360, dated 20/ 12/2023)

### *Samples culture*

Non-nutrient agar (NNA) consists of dissolving 15 g agar in 1 Liter saline solution, including NaCl (0.12 g), $MgSO_4.7H_2O$ (0.004 gm), $CaCl_2.2H_2O$(0.004g), $Na_2HPO_4$(0.142g), $KH_2PO_4$ (0.136 g), then dissolving all materials in 1 liter distilled water. After sterilizing NNA media, it was put in the sterile plate. Some heat-killed *Escherichia coli* were added 10 drops on each NNA plate, and spread. The sample swabs were transferred to NNA plates, then these plates were incubated at 28 °C. The plates were examined daily for 14 days by the light microscope to detect trophozoites and cysts (14). Morphological diagnosis depended on the shape of the cyst according to (10).

### *Genetic analysis*

Following morphological characterization according to (10). Cells ($1\times10^7$) from the NNA plate to 1.5 (ml Eppendorf tube were transferred after washing with PBS solution, then centrifuged for 5 min. at (300 xg). The supernatant was discarded then cells were re-suspended in PBS solution (200 µl) by pipette to extract DNA for *Acanthamoeba* spp. by the Geneaid kit, Korea. Conventional PCR was applied to the identity of *Acanthamoeba* spp. Two primers from Alpha DNA company were used according to Schroeder et al. (15) forward JPD1 (5'GGCCAGATCGTTTACCGTG 3') and reverse JPD2 (5' TCTCACAAGC TGCTAGGGAGTCA 3'). PCR product yield from the 18S rRNA genes in *Acanthamoeba* spp. was 450–500 bp. PCR condition included initial denaturation (95 °C/10 min ), second denaturation (35 sec / 95 °C) for 35 cycles, annealing (35 sec / 56° C/ 35cycles) and extension (40 sec / 72° C/35 cycles) and 10 min for final extension / 72 °C. PCR product were electrophoresed on (1.5%) agarose gel staining with Ethidium bromide to visualize via UV. The sequencing analysis was performed for positive samples of PCR amplicon by sending them to Macrogen company /Korea. After that the sequences were analyzed by the Basic Local Alignment Search Tool in NCBI.

## Results

### *Microscopic detection of Acanthamoeba spp.*

The percentage of presence *Acanthamoeba* was 4.5% (4/88). All positive cases were in females who were lens wearers only, and no cases were recorded in males or females who had no lens wearers' contact lens (Table 1).

**Table 1:** Occurrence of *Acanthamoeba* spp. in eye swabs

| Groups | Total samples | *Acanthamoeba* spp. positive | |
|---|---|---|---|
| | | N | % |
| **Female** | 44 | 4 | 9.09 |
| **Male** | 44 | 0 | 0 |
| **Total** | **88** | **4** | **4.5** |
| **Lens wearers** | 25 | 4 | 16 |
| **Non- Lens wearers** | 63 | 0 | 0 |
| **Total** | **88** | **4** | **4.5** |

*Acanthamoeba* spp. were identified morphologically based on characteristics of active trophozoites and cyst forms. After four days of cultivation, the trophozoite stage appeared. The trophozoites characterized in all isolates had irregular forms, measuring between 29 and 34 µm. The shape of *Acanthamoeba* trophozoites was characterized by a conspicuous contractile vacuole and the presence of acanthopodia, that is, projections of pseudopodia like a needle. Trophozoites also moved freely (Fig. 1).

The cyst phase is characterized with a wrinkled surface, an irregular form, and a prominent double wall, measuring around 15-17 µm (Fig. 2).

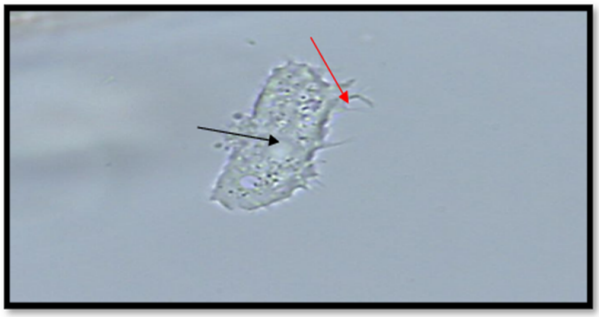

**Fig. 1:** *Acanthamoeba* trophozoite unstained from eye swab and cultured on NN-agar, acanthapodia (red arrow), and contractile vacuole (black arrow) unstained (100X)

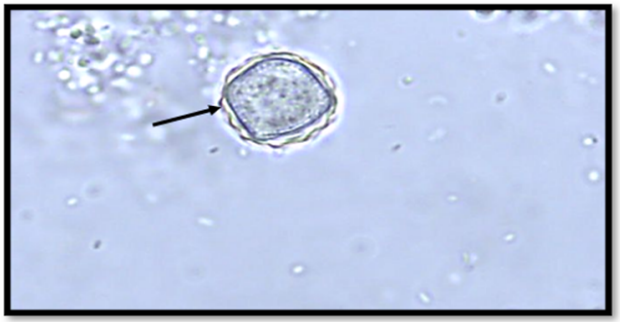

**Fig. 2:** *Acanthamoeba* spp. cyst unstained from eyes swab and cultured on NN-agar, wrinkled surface (black arrow) (100X)

### *Genetic detection and sequencing analysis of Acanthamoeba spp.*

This study includes an examination of all samples that were microscopically positive for *Acanthamoeba* spp. by conventional PCR using the *Acanthamoeba* primer JPD1/JPD2. All of the positive samples (Four samples) were shown to belong to *Acanthamoeba* spp. based on the electrophoresis of the 500 bp PCR products (Fig. 3).

After sequencing analysis for PCR products, one isolate registered in the gene bank with accession number(PQ661179.1) genotype 4 (Fig. 4) and alignment sequences by BLAST the results showed a homologous identity (100%) for four samples with reference isolate *Acanthamoeba* spp. (Genotype 4) *18S ribosomal RNA* gene (MK390853.1) in NCBI (Fig. 5).

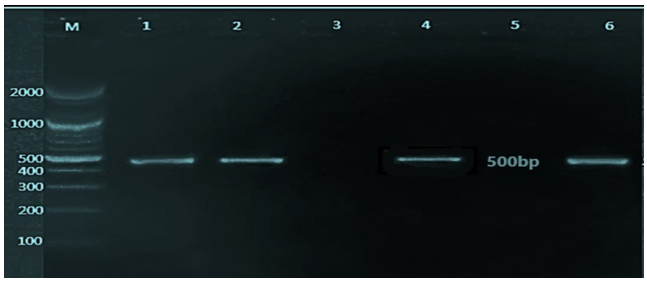


**Fig. 3:** PCR amplicon *Acanthamoeba* spp. (500 bp) for *18S rDNA* gene from eye swabs samples. M: DNA Marker (100-2000 bp) lanes (1,2,4,6) positive samples and lanes (3,5) negative control

**Acanthamoeba sp. isolate Ac1Eye small subunit ribosomal RNA gene, partial sequence**

GenBank: PQ661179.1

GenBank Graphics

>PQ661179.1 Acanthamoeba sp. isolate Ac1Eye small subunit ribosomal RNA gene, partial sequence
GCCACCGAATACATTAGCATGGGATAATGGAATAGGACCCTGTCCTCCTATTTTCAGTTGGTTTTGGCAG
CGCGAGGACTAGGTAATGATTAATATGGATAGTTGGGGGCATTAATATGTAATTGTCAGAGGTGAAATTC
TTGGATTTATGAAAGATTAACTTCTGCGAAAGCATCTGCCAAGGATGTTTTCATTAATCAAGAACGAAAG
TTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACGATGCCGACCAGCGATTAGGAG
ACGTTGAATACAAAACACCACCATCGGCGCGGTCGCCCTTGGCGTTCGTGTTCACGCACGGGCGCGAGGG
CGGCTTAGCCCG

**Fig. 4:** *18rDNA* gene sequence of one isolate for *Acanthamoeba* spp. in the current study registered in gene bank with accession number (PQ661179.1) genotype 4

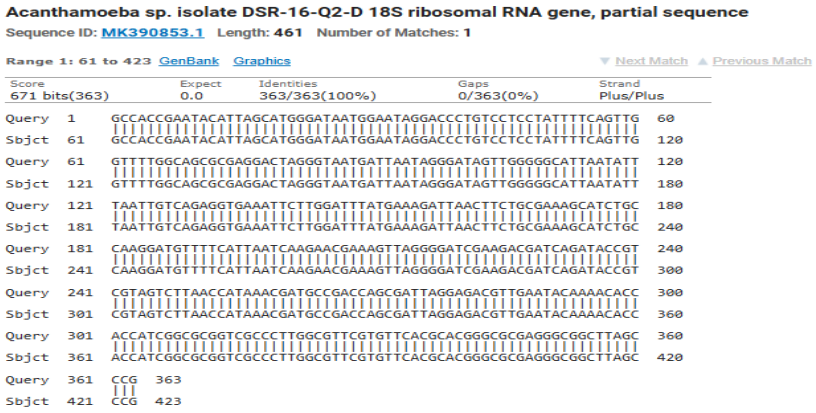


**Fig. 5:** Sequence alignment of *Acanthamoeba* spp. with reference isolate MK390853.1

## Discussion

One of the most often isolated amoebas in environmental samples is the free-living protozoan *Acanthamoeba*. It is widely distributed and found in many different environments, such as home water supplies, dental offices, hospitals, the air, and soil (16,17). *Acanthamoeba* strains are responsible for up to 20% of infectious keratitis in CL wearers (18). The present study is the first in Thi–Qar Province – Iraq, that included an investigation of opportunistic free amoebae, *Acanthamoeba* spp., in eye swab samples among students at College of Sciences, University of Thi-Qar especially with the few previous studies in this field in Iraq.

The current results showed that the percentage of presence of *Acanthamoeba* was 4.5% (4/88). *Acanthamoeba* were identified morphologically based on characteristics of active trophozoites and cyst forms according to (19), and molecular identified by conventional PCR using the *Acanthamoeba* primer JPD1 / JPD2. Another study indicated that PCR is a more sensitive methodology than direct microscopy of culture; nonetheless, to obtain more comprehensive results of the true presence of free-living amoeba in ambient water samples, the employment of both PCR and culture methods is recommended (20). All positive cases were in females who were lens wearers only, and no cases were recorded in males or females who had no lens wearers' contact lenses. The results of the current study are consistent with other studies. Aldin et al. (21) observed a total of 96 samples from corneal scrapings and contact lens scrapings gathered from corneal patients; three patients had *Acanthamoeba* spp. Infections in Baghdad. Cosmetic lenses and disinfectant solutions are a major transmissible mode for infection with *Acanthamoeba* spp. (22).

Also, this result agrees with another study (23), who found that the incidence of *Acanthamoeba* in the eyes was high among lens wearers. This is biologically plausible because *in vitro* studies demonstrate that *Acanthamoeba* species are largely resistant to contact lens solutions (24,25).

Also, one study indicated in North Africa, and Tunisia T4 genotype was used to diagnose *Acanthamoeba* Keratitis cases in five patients from corneal abscess (26). Another study diagnosed *Acanthamoeba* Keratitis in 14 cases out of 230 corneal scraped specimens. In most cases ages were between 19 – 27 yr and had a history using contact lens (27). Many studies in several countries recorded *Acanthamoeba* Keratitis in wearers of contact lenses, such as United Kingdom (28), Austria (29) Netherlands (30), this outbreak reasons due to contact lens misuse, does not cleaning the lenses. These outcomes agree with the current study.

The present study is consistent with another study that showed five genotypes of *Acanthamoeba* spp. in 138 patients; these genotypes included T2, T3, T4, T9, and T11) in 138 patients and reported T4 genotype was common and more prevalent in contact lenses and corneal scrape samples (31).

Another study observed corneal patients infected with *Acanthamoeba* spp. according to sequence analysis of amoebic keratitis isolates belonged to the T4 genotype with 94.1%, while one sample (5.8%) belonged to T11 genotype (32).

One of study indicated *Acanthamoeba* keratitis (*Acanthamoeba castellani*) by detection of genotype T2 using PCR and sequencing analysis in two cases of infection, one of the cases had a history of wearing contact lenses, and the other had no wearing lenses and no history of trauma. So, the significant risk factors for *Acanthamoeba* keratitis are wearing contact lenses, trauma, and contaminated water (33).

## Conclusion

The current study showed the prevalence of *Acanthamoeba* spp. in contact lens wearers, which is a health risk, especially in females. Besides, the sequencing analysis for positive samples indicated the homologous identity 100% with the reference isolate in NCBI with accession number MK390853.1. Therefore, one isolate of *Acanthamoeba* spp. was registered in the gene bank with an accession number (PQ661179.1).

## Conflicts interest

There are no conflicts of interest

## Acknowledgements

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