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Original Article

Infections with *acanthamoeba triangularis* in patients with renal failure

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Abstract

This study included 100 samples collected from patients with renal failure from the dialysis centers. NN-agar medium was used as culture, and one sample was infected with the *acanthamoeba*. It was diagnosed morphologically as *Acanthamoeba triangularis*. The diagnosis was confirmed by PCR technology, a genetic sequence analyzer and phylogenetic tree analysis based on the 18S ribosomal RNA gene. A total of 100 urine samples from patients with renal failure were collected from the dialysis center and microscopically examined; the result of direct examination of urine sediment was the presence of bacteria. The urine cultivation shows that one case of opportunistic amoeba infection was recorded. It was diagnosed as *Acanthamoeba triangularis*, and to ensure that the infection was real and not caused by contamination, another sample was taken from the urine of the same patient a month after the first collection and culture. The culture result was similar to the first sample, as the same parasite was isolated. The parasite was mostly triangular; the outer layer contained three open, while the trophozoite stage had many acanthopoda. This is the first record of *acanthamoeba triangularis* infection in renal failure patients.

Keywords: renal failure, *acanthamoeba triangularis,* amaranth, acanthopod, trophozoite.

Introduction

Acanthamoeba is a free-living opportunistic protozoan of an amoebae genus and is naturally widespread. It has been isolated from different soils, specks of dust, sands, swabs of nasal specimens, feces, stool, vegetables, reptiles, fish, birds, mammals, and different water samples involving freshwater, seawater, tapwater, bottled mineral water, swimming pool, aquarium and sewages. Trophozoites became small and oval to triangular shape in motility. It converts to smaller cystic form with a double layer in unsuitable conditions, like high temperature, high osmolarity and low pH [1]. In 1930, it was discovered and later put in the genus *acanthamoeba*. In 1960, authors described it as the causative agent of *Acanthamoeba* granulomatous encephalitis and in 1970, it was mentioned as the cause of keratitis [2]. The major sites of growth are the brain, cornea and lung. Also, they were found in the kidneys of cases with disseminated acanthamoebiasis. This depends on the host's immune status and the strain of amoebia [3]. They pose a threat to the life and health of humans. The infection rate has low incidence and relatively high mortality, like cerebral and extra-cerebral injuries in both immunocompetent and immunocompromised cases [4].

The identification of spp. was by morphological features, including the shape of ectocytes and endocysts and size. Biochemical and molecular biology techniques are helping in the identification [5]. There are 22 genotypes (T1-T22) that were determined by the 18S rRNA gene sequence analysis using the PCR [6].

A. castellani and *A. polyphagia* are the causative of severe eye infections. Other *Acanthamoeba spp.*, such as *A. Culbertson*, *A. Rhysodes*, *A. Lugdunensis*, *A. Hatchetti*, *A. Mauritaniensis*, *A. Griffini* of the T4 genotype, and also genotypes T1-3, T5, T6, and T11 have been recorded

Table 1: PCR primers for detection of *Acanthamoeba sp*. parasite based on amplification 18SrRNA gene.

Primers		Sequence 5'–3'	Product size
18SrRNA gene PCR primers	F	GGCCAGATCGTTTACCGTG	450–500 bp
	R	TCTCACAAGCTGCTAGGGAGTCA	

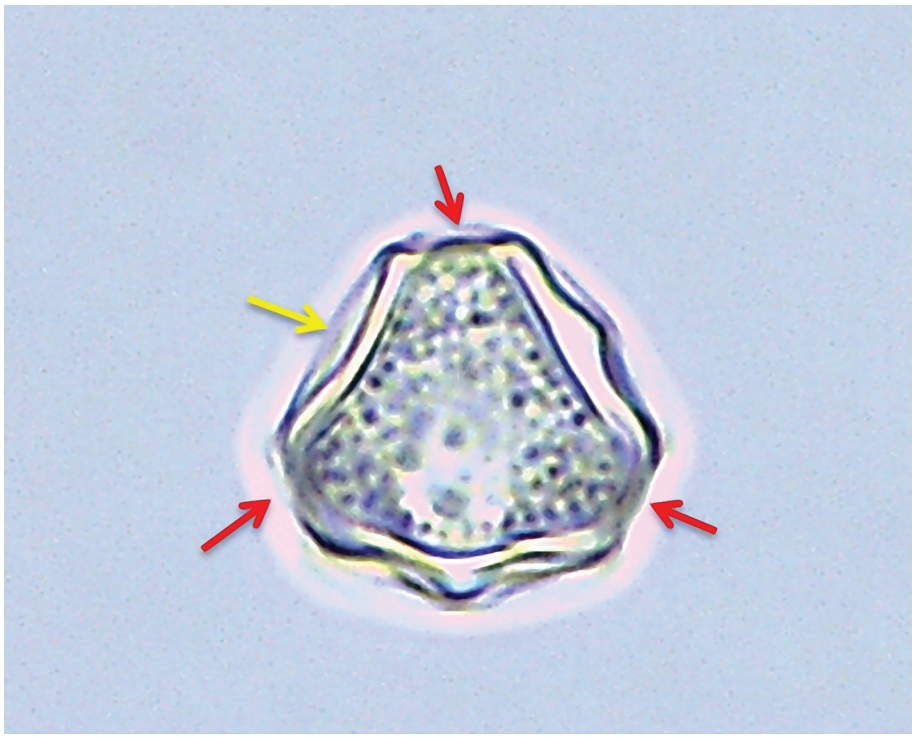

Figure 1: *Acanthamoeba triangularis* cyst stage outer wall yellow arrow ostiole red arrow original mag. 100X.

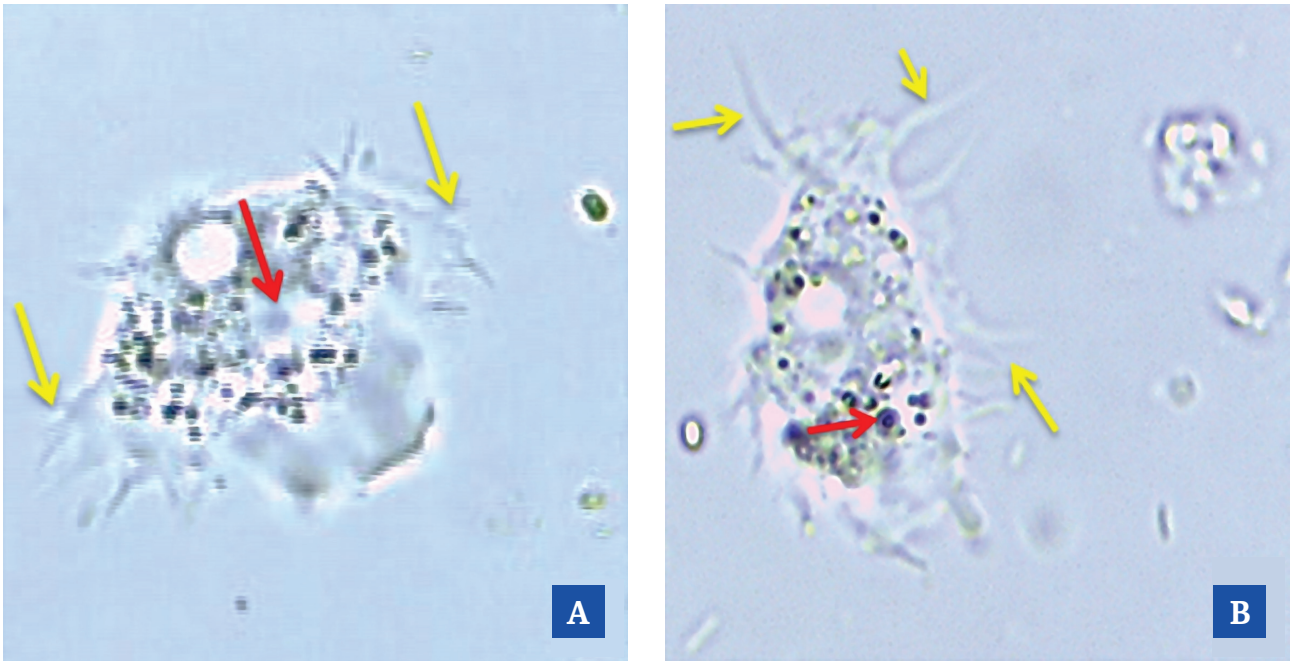

Figure 2 A, B: A. *triangularis* (Trophozoite) acanthopoda (yellow arrow); nucleus (red arrow), original mag. 100 X.

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to cause eye infections. Thus, all these cause devastating amoebic keratitis (AK) [7]. AK can be a challenge to ophthalmologists due to being misdiagnosed with bacterial and fungal infections [8].

Acanthamoeba spp. can be cultivated on an N-N agar plate covered* with *Enterobacter aerogenes* or *Escherichia coli* bacteria. The amebas ingest bacteria multiply, and completely cover the plate surface in a few days. Then, amebas are distinguished into cysts. Additionally, they can be cultured on mammalian cell cultures and in cell-free liquid medium [9].

Material and methods

One sample from 100 urine samples (15 ml) was collected from patients with renal failure from the dialysis centers, 72 males and 28 females, using sterile test tubes; the samples were precipitated using the centrifugation process (4000) cycles per minute for a period of five minutes. On glass slides and left to dry, then fixed with absolute methyl alcohol and left to dry. The remaining samples were planted on NN-agar medium; 10 ml of sterilized water was added and incubated at 37°C. The dishes were examined after four days of culture, and the examination continued for four weeks by making swabs for the cultured content. Using sterile swabs on glass slides and the slide cover using a Leica compound microscope, and after identifying the wave samples for parasitic infections, they were transferred to ethyl alcohol for PCR (Table 1).

Results

A total of 100 urine samples from patients with renal failure were collected from the dialysis center and microscopically examined. The result of direct examination of urine sediment was the presence of bacteria. The urine cultivation shows that one case of opportunistic amoeba infection was recorded. It was diagnosed as *Acanthamoeba triangularis*. To ensure that the infection is real and not caused by contamination, another sample was taken from the urine of the same patient A month after the first collection and cultured. The culture result was similar to the first sample, as the same parasite was isolated. The parasite was mostly triangular, and the outer layer contained three open

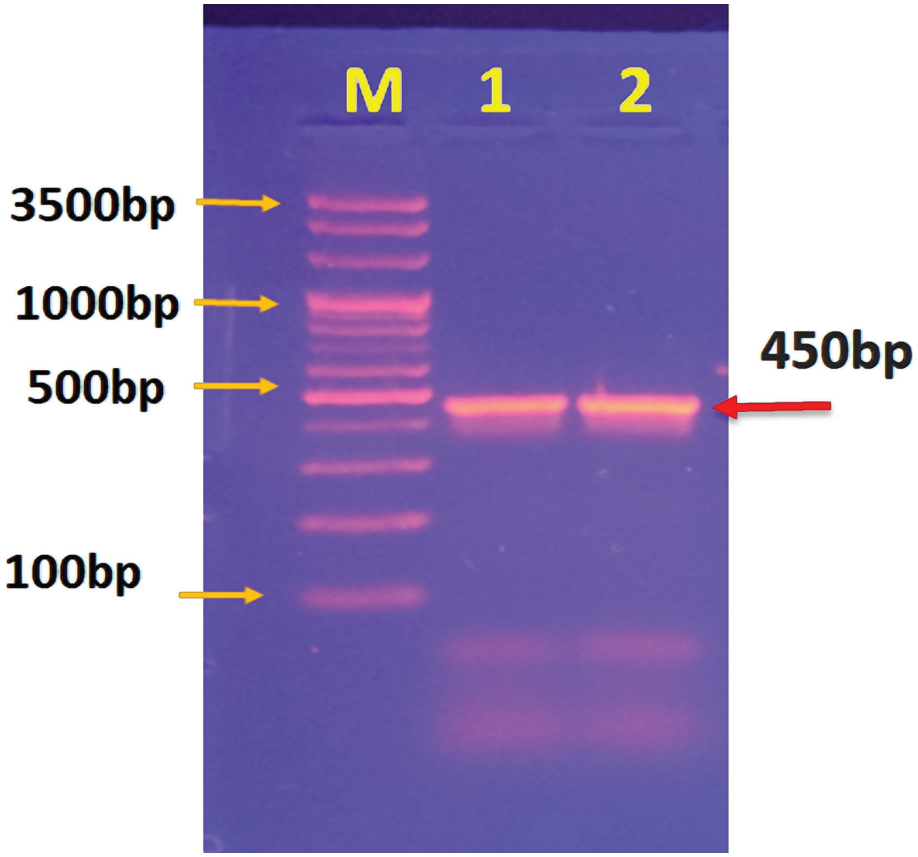

Figure 3: Agarose gel electrophoresis image that shows the analysis of PCR products of 18s ribosomal RNA gene from genomic DNA of *Acanthamoeba spp.* from urine samples.

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Local-IQ.isolate    AAAAAATTAGAGTGTTCAAAGCAGGCAGATCCAATTTTCTGCCACCGAATACATTAGCAT
MZ310461.1          AAAAAATTAGAGTGTTCAAAGCAGGCAGATCCAATTTTCTGCCACCGAATACATTAGCAT
KX018028.1          AAAAAATTAGAGTGTTCAAAGCAGGCAGATCCAATTTTCTGCCACCGAATACATTAGCAT
KJ094679.1          AAAAAATTAGAGTGTTCAAAGCAGGCAGATCCAATTTTCTGCCACCGAATACATTAGCAT
AB525819.1          AAAAAATTAGAGTGTTCAAAGCAGGCAGATCCAATTTTCTGCCACCGAATACATTAGCAT
DQ087296.1          AAAAAATTAGAGTGTTCAAAGCAGGCAGATCCAATTTTCTGCCACCGAATACATTAGCAT
AY703023.1          AAAAAATTAGAGTGTTCAAAGCAGGCAGATCCAATTTTCTGCCACCGAATACATTAGCAT
AF260719.1          AAAAAATTAGAGTGTTCAAAGCAGGCAGATCCAATTTTCTGCCACCGAATACATTAGCAT
KY072780.1          AAAAAATTAGAGTGTTCAAAGCAGGCAGATCCAATTTTCTGCCACCGAATACATTAGCAT
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Local-IQ.isolate    GGGATAATGGAATAGGACCCTGTCCTCCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACT
MZ310461.1          GGGATAATGGAATAGGACCCTGTCCTCCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACT
KX018028.1          GGGATAATGGAATAGGACCCTGTCCTCCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACT
KJ094679.1          GGGATAATGGAATAGGACCCTGTCCTCCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACT
AB525819.1          GGGATAATGGAATAGGACCCTGTCCTCCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACT
DQ087296.1          GGGATAATGGAATAGGACCCTGTCCTCCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACT
AY703023.1          GGGATAATGGAATAGGACCCTGTCCTCCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACT
AF260719.1          GGGATAATGGAATAGGACCCTGTCCTCCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACT
KY072780.1          GGGATAATGGAATAGGACCCTGTCCTCCTATTTTCAGTTGGTTTTGGCAGCGCGAGGACT
************************************************************

Local-IQ.isolate    AGGGTAATGATTAATAGGGATAGTTGGGGGCATTAATATTTAATTGTCAGAGGTGAAATT
MZ310461.1          AGGGTAATGATTAATAGGGATAGTTGGGGGCATTAATATTTAATTGTCAGAGGTGAAATT
KX018028.1          AGGGTAATGATTAATAGGGATAGTTGGGGGCATTAATATTTAATTGTCAGAGGTGAAATT
KJ094679.1          AGGGTAATGATTAATAGGGATAGTTGGGGGCATTAATATTTAATTGTCAGAGGTGAAATT
AB525819.1          AGGGTAATGATTAATAGGGATAGTTGGGGGCATTAATATTTAATTGTCAGAGGTGAAATT
DQ087296.1          AGGGTAATGATTAATAGGGATAGTTGGGGGCATTAATATTTAATTGTCAGAGGTGAAATT
AY703023.1          AGGGTAATGATTAATAGGGATAGTTGGGGGCATTAATATTTAATTGTCAGAGGTGAAATT
AF260719.1          AGGGTAATGATTAATAGGGATAGTTGGGGGCATTAATATTTAATTGTCAGAGGTGAAATT
KY072780.1          AGGGTAATGATTAATAGGGATAGTTGGGGGCATTAATATTTAATTGTCAGAGGTGAAATT
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Local-IQ.isolate    CTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATCTGCCAAGGATGTTTTCATTAACC
MZ310461.1          CTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATCTGCCAAGGATGTTTTCATTAACC
KX018028.1          CTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATCTGCCAAGGATGTTTTCATTAATC
KJ094679.1          CTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATCTGCCAAGGATGTTTTCATTAATC
AB525819.1          CTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATCTGCCAAGGATGTTTTCATTAATC
DQ087296.1          CTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATCTGCCAAGGATGTTTTCATTAATC
AY703023.1          CTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATCTGCCAAGGATGTTTTCATTAATC
AF260719.1          CTTGGATTTATGAAAGATTAACTTCTGCCAAAGCATCTGCCAAGGATGTTTTCATTAATC
KY072780.1          CTTGGATTTATGAAAGATTAACTTCTGCGAAAGCATCTGCCAAGGATGTTTTCATTAATC
* ***************************** *************************

Local-IQ.isolate    AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
MZ310461.1          AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
KX018028.1          AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
KJ094679.1          AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
AB525819.1          AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
DQ087296.1          AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
AY703023.1          AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
AF260719.1          AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
KY072780.1          AAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACG
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Local-IQ.isolate    ATGCCGACCAGCGATTAGGTGACGTTGAATACAAAACACCACCATCGGCGCGGTCGTCCT
MZ310461.1          ATGCCGACCAGCGATTAGGAGACGTTGAATACAAAACACCACCATCGGCGCGGTCGTCCT
KX018028.1          ATGCCGACCAGCGATTAGGAGACGTTGAATACAAAACACCACCATCGGCGCGGTCGTCCT
KJ094679.1          ATGCCGACCAGCGATTAGGAGACGTTGAATACAAAACACCACCATCGGCGCGGTCGTCCT
AB525819.1          ATGCCGACCAGCGATTAGGAGACGTTGAATACAAAACACCACCATCGGCGCGGTCGTCCT
DQ087296.1          ATGCCGACCAGCGATTAGGAGACGTTGAATACAAAACACCACCATCGGCGCGGTCGTCCT
AY703023.1          ATGCCGACCAGCGATTAGGAGACGTTGAATACAAAACACCACCATCGGTGCGGTCGTCCT
AF260719.1          ATGCCGACCAGCGATTAGGAGACGTTGAATACAAAACACCACCATCGGTGCGGTCGTCCT
KY072780.1          ATGCCGACCAGCGATTAGGAGACGTTGAATACAAAACACCACCATCGGTGCGGTCGTCCT
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Local-IQ.isolate    TGGCGTCTCGGTCCTTCACGGGGCCGGGGCGCGGGGGCGGCTTAGCCCGGTGGCACCGGT
MZ310461.1          TGGCGTCTCGGTCCTTCACGGGGCCGGGGCGCGGGGGCGGCTTAGCCCGGTGGCACCGGT
KX018028.1          TGGCGTCTCGGTCCTTCACGGGGCCGGGGCGCGGGGGCGGCTTAGCCCGGTGGCACCGGT
KJ094679.1          TGGCGTCTCGGTCCTTCACGGGGCCGGGGCGCGGGGGCGGCTTAGCCCGGTGGCACCGGT
AB525819.1          TGGCGTCTCGGTCCTTCACGGGGCCGGGGCGCGGGGGCGGCTTAGCCCGGTGGCACCGGT
DQ087296.1          TGGCGTCTCGGTCCTTCACGGGGCCGGGGCGCGGGGGCGGCTTAGCCCGGTGGCACCGGT
AY703023.1          TGGCGTCTCGGTCCTTCACGGGGCCGGGGCGCGGGGGCGGCTTAGCCCGGTGGCACCGGT
AF260719.1          TGGCGTCTCGGTCCTTCACGGGGCCGGGGCGCGGGGGTGGCTTAGCCCGGTGGCATCGGT
KY072780.1          TGGCGTCTCGGTCCTTCACGGGGCCGGGGCGCGGGGGTGGCTTAGCCCGGTGGCACCG-T
* ** ********************* ************************************
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Figure 4: Multiple sequence alignment of 18S ribosomal RNA gene analysis in local *Acanthamoeba sp*. urine isolates and NCBI-Gen-bank associated *Acanthamoeba spp*. isolates. This was constructed using (ClustalW alignment tool. Online). This showed the nucleotide alignment similarity as (*) and substitution mutations in the 18S ribosomal RNA gene among isolates.

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(Figure 1), while the trophozoite stage had many acanthopoda (Figure 2 A, B).

DNA Sequence results

The DNA sequencing method was carried out for genetic species typing of 18S ribosomal RNA gene analysis of *Acanthamoeba spp.* in urine isolates and NCBI-Gen-bank related *Acanthamoeba spp.* isolates, (Figure 3). The phylogenetic tree genetic correlation analysis was revealed that *Acanthamoeba spp.* in urine isolates were have closely associated with NCBI-BLAST *Acanthamoeba triangularis* isolate (MZ310461.1) at total genetic changes (0.0080–0.0020%) (Figure 4).

The homology sequence identity between *acanthamoeba spp.* urine isolates and NCBI-Gen-bank related *Acanthamoeba triangularis* isolate (MZ310461.1) was showed genetic homology sequence identity ranged from (99.76%) (Figure 5).

Finally, *Acanthamoeba sp.* urine isolates were submitted to NCBI Genbank and identified by accession numbers (OP315258.1) (Table 2).

Discussion

Acanthamoeba is one of the free-living amoebae with high-risk factors for direct contact infection with protozoa. It is widespread naturally and in artificial environments [10]. This parasite can cause many sight-threatening conditions, such as AK and fatal granulomatous amoebic encephalitis (GAE) [11]. *Acanthamoeba* detection is upon morphological appearances and molecular techniques using PCR and DNA sequencing for clinico-epidemiological purposes [11].

In our case, *Acanthamoeba spp.* strains were diagnosed from clinical source samples in Misan province using morphological features and PCR methods.

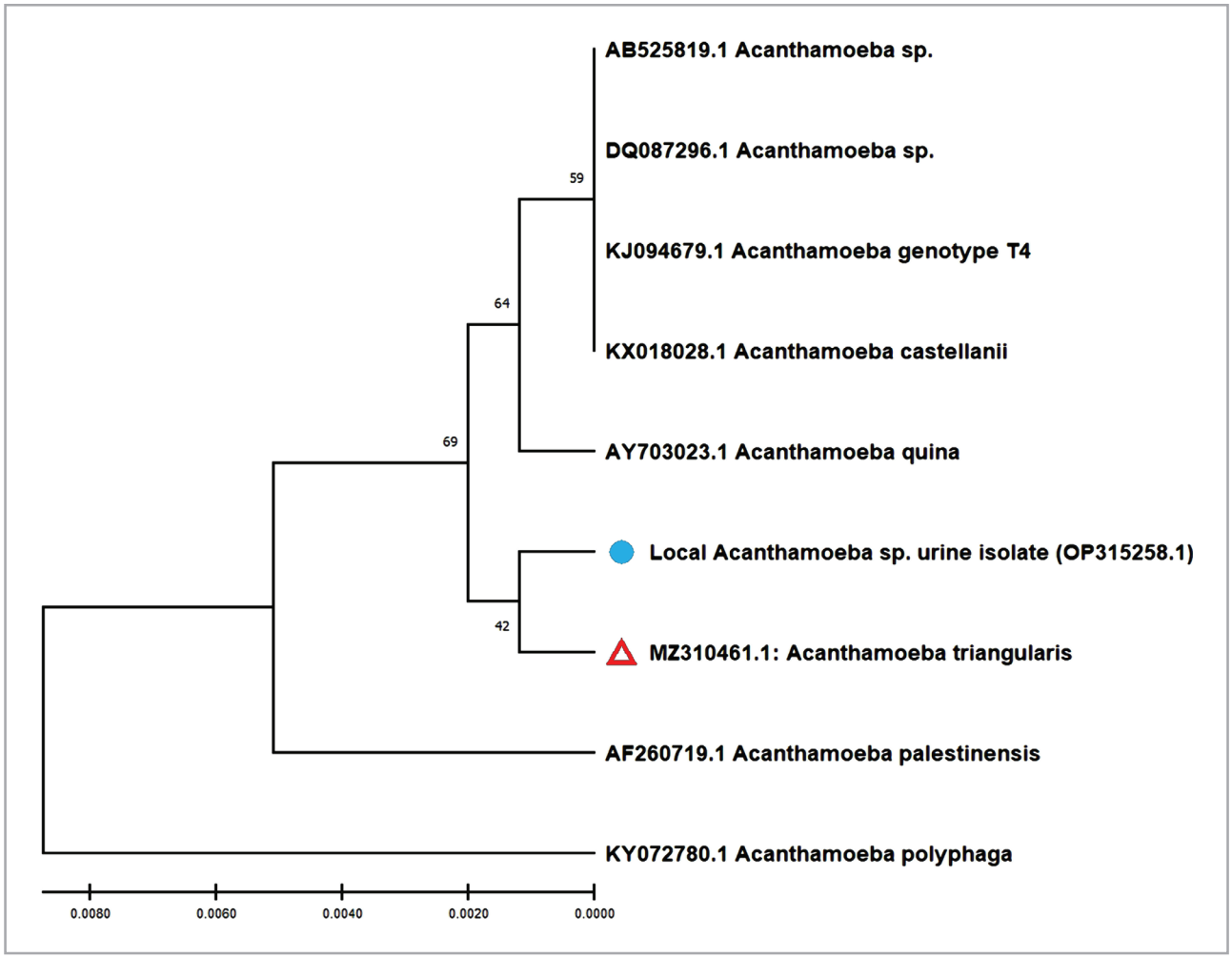

Figure 5: Analysis of phylogenetic tree-based 18S ribosomal RNA gene (partial sequence) in local *Acanthamoeba spp.* urine isolates. The tree was constructed using Un-weighted Pair Group methods with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The *Acanthamoeba spp.* urine isolate was closed related to NCBI-BLAST *Acanthamoeba triangularis* isolate (MZ310461.1) at total genetic changes (0.008–0.0020%).

Table 2: The NCBI-BLAST Homology Sequence identity percentage between local *Acanthamoeba sp*. Urine isolates and NCBI-BLAST closed genetic-related *Acanthamoeba* species isolate,

***Acanthamoeba sp.* Isolate**	**Accession number**	**Homology sequence identity (%)**		
		Identical Coccidia	**Accession number**	**Identity (%)**
***Acanthamoeba sp.* urine isolate**	OP315258.1	*Acanthamoeba triangularis*	MZ310461.1	99.76%

It was found that one species belonged to the genus *Acanthamoeba spp*. It is *Acanthamoeba triangularis* for the first time in Iraq. *Acanthamoeba* was isolated from the urine of a 45-year-old man with renal failure. The report showed that *Acanthamoeba triangularis* was observed in one sample from 100 samples by PCR.

Acanthamoeba triangualaris is cultivated on NN-agar. This report shows other organisms like *Microsporidia* spp. in one case (1%) and Yeasts in four cases (4%) under microscopic examination.

A. triangularis is an opportunistic parasite belonging to a group of parasites that cause brain, skin and eye infections. A pore-forming parasite called A. triangularis has three prongs that end in openings, which is why this name is given to this parasite in Iraq [11].

According to Kot *et al*. [12], the presence of the parasite in the urine may be due to the ascent of the parasite through the urethra and the passage towards the bladder. This is a weak possibility, but the bladder's content is not suitable for the parasite and is coming from the kidney as a result of injury. Also, continuous urination in this way leads to cleaning the urinary tract so that the infection may come from areas beyond the urinary tract [12].

Usually, it is determined in the lungs after the patient's death, and the disease varies on the host's immunity and the parasite. Pulmonary failure is considered the second leading reason for mortality in this group of patients who are susceptible to blood transfusions and lung infections and who are undergoing pulmonary dialysis [13].

Acanthamoeba spp. is the most opportunistic parasite, is endemic naturally and has been isolated from different environments, including soils, air, sewage, seawater, swimming pools, household tapwater, bottledwater, dental hygiene units, hospital rooms, air conditioning filters, contact lens and also have been isolated from skin, oral cavity and mouth [14].

Conclusion

Acanthamoeba spp. strains were diagnosed from clinical source samples in Misan province using morphological features and PCR methods. *Acanthamoeba sp*. urine isolates were submitted to NCBI Genbank and identified by accession numbers (OP315258.1). This is the first record of *acanthamoeba triangularis* infection in renal failure patients.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the University of Basrah (approval ID: #4 of 3/5/293 at 21/10/2021).

Consent to participate

Written informed consent was obtained from the patient for publication of the detail of his medical case and any accompanying images.

References

1. Kot K, Łanocha-Arendarczyk NA, Kosik-Bogacka DI. Amoebas from the genus *acanthamoeba* and their pathogenic properties. Ann. Parasitol. 2018;64:299–308. doi: 10.17420/ap6404.164.
2. Khan NA. *Acanthamoeba*: biology and increasing importance in human health. FEMS Microbiol Rev. 2006, 30 (4): 564-95.
3. Kot K, Kosik-Bogacka D, Wojtkowiak-Giera A, Kolasa-Wołosiuk A, Łanocha-Arendarczyk N. The expression of TLR2 and TLR4 in the kidneys and heart of mice infected with *acanthamoeba spp*. Parasit. Vectors. 2020;13:480. doi: 10.1186/s13071-020-04351-4.
4. Łanocha-Arendarczyk N, Kosik-Bogacka D, Galant K, Zaorski W, Kot K, Łanocha A. Pełzaki wolno żyjące o właściwościach patogenicznych dla człowieka [Pathogenic free-living amoeba]. Postępy Mikrobiologii. 2017; 56: 106-112
5. Derda M, Wojtkowiak-Giera A, Kolasa-Wołosiuk A, Kosik-Bogacka D, Hadaś E, Jagodziński P.P., Wandurska-Nowak E. 2016. *Acanthamoeba* infection in lungs of mice expressed by toll-like receptors (TLR2 and TLR4). Experimental Parasitology 165: 30-34. doi:10.1016/j.exppara.2016.02.012

https://doi.org/10.46389/rjd-2023-1358

6. 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. Journal of Infection and Public Health. 2018; 11: 377-383. doi:10.1016/j.jiph.2017.09.013
7. Maycock, N.J.R.; Jayaswal, R. Update on *Acanthamoeba* keratitis: Diagnosis, treatment, and outcomes. Cornea 2016, 35, 713–720.
8. Hong, J.; Ji, J.; Xu, J.; Cao, W.; Liu, Z.; Sun, X. An unusual case of *Acanthamoeba* polyphaga and Pseudomonas aeruginosa keratitis. Diagn. Pathol. 2014, 9, 105.
9. GOVINDA S. VISVESVARA, JAMES H. MAGUIRE. In: Tropical Infectious Diseases (Second Edition), 2006.
10. Hanady MM. (2017). Isolation and experimental infection of some opportunistic amoebas from clinical and environmental in Basra. Ph thesis, College of science, University of Basra.
11. Bruno –da Rocha –Azevedo, Herberto T, Francine M. Diagnosis of infections caused by pathogenic free-living amoebae. Interdisciplinary perspectives on infectious disease. 2009;2009:251-406.
12. Kot, K.; Kosik-Bogacka, D.; Wojtkowiak-Giera, A.; Kolasa-Wołosiuk, A. and Łanocha-Arendarczyk, N. (2020).The expression of TLR2 and TLR4 in the kidneys and heart of mice infected with *acanthamoeba spp*. Parasit. Vectors, 13, 480.
13. Niyyati M, Arab-Mazar Z, Lasjerdi Z, Lorenzo Morales J, Espotin A, Yadegarynia D. (2017). Molecular characterization of *Acanthamoeba* strains isolated from the oral cavity of hemodialysis patients in Iran. Parasitol Res;116(11):2965-9. doi: 10.1007/s00436-017-5605-9.
14. Marciano-Carbal F, Carbal GA. *Acanthamoeba spp*. as agents of disease in human.Clinical Microbiology Reviews. 2003;16(2):127-307.