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# Isolation and Genotyping of Acanthamoeba from Soil Samples in Markazi Province, Iran

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**Abstract**

**AIM:** A previous study confirmed the contamination of water sources with this parasite in Arak, Markazi Province, Iran. The current study investigated soil sources and determined the predominant genotype of Acanthamoeba in this region of Iran.

**MATERIAL AND METHODS:** Forty-eight soil samples, collected from different regions of Arak, Markazi province, Iran, were evaluated in this study. The samples were processed and identified by culturing on a specific medium, performing PCR assay, and sequencing the PCR products. Finally, using the NCBI database, the genotypes were determined.

**RESULTS:** Of 48 soil samples, 33.3% and 31.25% were contaminated with Acanthamoeba according to the culture and molecular assays, respectively. The majority of these isolates belonged to the T4, T5 and T6 genotypes of Acanthamoeba.

**CONCLUSION:** The genotypes of most isolates from soil samples in Arak similar to other regions of Iran belong to T4 genotype of this parasite. New sequence accession numbers include MG066681 and MG298785-MG298794.

## Introduction

Acanthamoeba is described as a free-living amoeba with a widespread distribution in the water and soil of various regions [1]. Host infection occurs through the entry of parasite cysts into the nose and eyes. Depending on the host conditions, this can lead to various diseases, such as granulomatous amebic encephalitis (GAE) or keratitis [2]. Water and soil are important reservoirs of this parasite, and to date, parasites have been isolated from different environmental sources in Iran [3]. For instance, 46.25% of environmental samples collected from Tehran were contaminated with this parasite; also, all soil samples were contaminated [4]. Also, 71.6% and 26% of water and soil samples from south of Iran were contaminated with Acanthamoeba, respectively [5].

A limited number of studies have examined water sources in different regions of Iran. In Isfahan, 45.16% of water sources were contaminated with this parasite, and a higher prevalence was found in environmental water sources in comparison with tap water [6]. Similar results have been reported from West Azerbaijan Province (Northwest of Iran) [7] and Shiraz [8]. Moreover, 70.3% of surface water samples

were contaminated with Acanthamoeba in North of Iran (Gilan Province) [9].

Morphological and molecular studies have revealed that the T4 strain is the predominant Acanthamoeba genotype in the environmental sources of Iran [3]. Considering the high prevalence of pathogenic Acanthamoeba strains in the environment, it is recognised as a dangerous organism. Also, it seems that fine airborne dust plays a role in the dissemination and transmission of this parasite [10]. The contamination of water sources with Acanthamoeba was confirmed by a previous study conducted in Markazi Province [11]; however, there is no information regarding the prevalence and isolates of this parasite in the soil of Markazi Province.

Therefore, this study aimed to assess the contamination status and genotypes of this parasite in soil samples from this region.

## Material and Methods

Forty-eight soil samples were collected from Arak, capital of Markazi Province, located on the crossroad of northern, eastern, southern, and western provinces of Iran (Arak, Iran; 34°00' N 49°40' E; Figure 1). Because of the presence of various industries, this city has many immigrants. In this study, the soil of parks and gardens were collected for analysis. For this purpose, approximately 50 g of soil was collected in sterile bags and transferred to the Parasitology and Mycology Laboratory, Arak University of Medical Sciences.


*Figure 1: Geographic Location of Arak city in Iran*

The soil samples were prepared using a 250-µm sieve and then a 0.45-µm nitrocellulose filter, as described in Figure 2. The nitrocellulose filter was transferred to a non-nutrient agar (NNA) plate, coated with E. coli (killed) at a temperature of 28°C. After monitoring the plates for four weeks, the surface of positive cultures was rinsed with sterile Page's saline. The parasites were centrifuged for 5 minutes at 1500 × g after collecting and washing them with phosphate buffer. These samples were used for molecular analysis.

According to the literature, the phenol-chloroform method was used for DNA extraction [12, 13]. For PCR amplification, genus-specific primers were used. To amplify a nearly 500-bp fragment of Acanthamoeba-specific 18S ribosomal DNA, reverse primer JDP2 (5′- TCTCACAAGCTGCTAGGGGAG TCA-3′) and forward primer JDP1 (5′-GGCCCAGATCGTTTACCGTGAA-3′) were used [14].

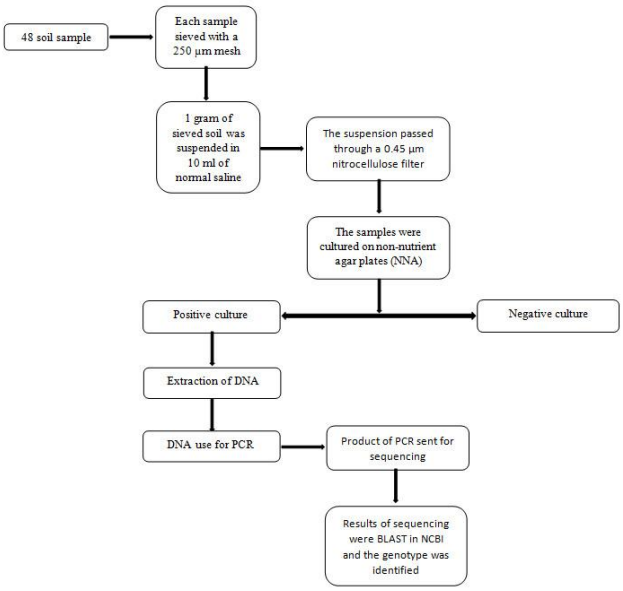


*Figure 2: Preparation and testing steps on soil samples*

A Master Mix Kit (CinnaGen Co.) was used to optimise the PCR reactions. A final volume of 50 µL was used for amplification in an Eppendorf thermocycler with incubation at 95°C for 3 minutes, followed by 35 cycles at 94°C for 45 seconds, at 55°C for 45 seconds, at 72°C for 45 seconds, and incubation at 72°C for 10 minutes. Using electrophoresis on 1.5% agarose gel, the products were assessed and then visualised under ultraviolet light. After sequencing the PCR products from positive samples, homology analysis was conducted through comparison of the sequences with Acanthamoeba DNA sequences available in the GenBank. The sequences generated in the current study were submitted to the GenBank database.

## Results

Sixteen out of 48 soil samples contained Acanthamoeba isolates according to the culturing method (16 samples, 33.3%). Molecular evaluation of the positive samples confirmed 15 (31.25%) isolates.

The eleven sequences (11 sequences) reported in the current study were submitted to the GenBank (accession numbers, MG066681 and MG298785-MG298794). Using the BLAST tool, homology of sequences was compared with the

sequences available in the GenBank. The specificity of the detected isolates is presented in Table 1.

**Table 1: Comparison of Acanthamoeba spp. isolates from Arak soil samples with available strains found in the GenBank database**

| Isolate sequence | Isolate Accession number | Name of Isolate | Genotype | Name of similar isolates in BLAST | Accession number of similar sequence (Genotype) | Region of origin | Reference |
|---|---|---|---|---|---|---|---|
| AGGTGAAATTCTTGGATTTATG AAAGATTAACTTCTGCGAAAGC ATCTGCCAAGGATGTTTTCATT AATCAAGAACGAAAGTTAGGG GATCGAAGACGATCAGATACC GTCGTAGTCTTAACCATAAACG ATGCCGACCAGCGATTAGGAG ACGTTGAATACAAAACACCACC ATGTATAATTCAGTATATGGCC AGTCAAATGGTTGTA TATTGTTTGTACATG | MG298785 | Acs2 | ND | Arc-T01 | MF470298.1 (ND) | United Kingdom | Asif, Unpublished |
| | | | | Arc-B08 | MF470259.1 (ND) | United Kingdom | Asif, Unpublished |
| | | | | Arc-NB03 | MF470254.1 (ND) | United Kingdom | Asif, Unpublished |
| | | | | BYB 2017-2 | MF113385.1 (T2) | USA | Martin-Perez et al.2017[15] |
| | | | | Arc-V13 | MF470280.1 (ND) | - | Asif, Unpublished |
| | | | | Arc-E08 | MF470243.1 (ND) | - | Asif, Unpublished |
| | | | | Arc-S07 | MF470242.1 (ND) | - | Asif, Unpublished |
| | | | | RG-HD186 | LC184519.1 (ND) | - | Rasti et al.2016, Unpublished |
| GGCCCAGATCGTTTACCGTGA AAAAATTAGAGTGTTCAAAGCA GGCAGATTCAATTTTCTGCCAC CGAATACATTAGCATGGGATAA TGGAATAGGACCCTGTCCTCCT ATCTTCAGTTGGTTAACTTGTA GAGGATCAGGGTAATGATTAAT AGGGATAGTTGGGGGCAT | MG066681 | AcAS4 | T4 | SSH40 | KU885380.1 (T4) | Spain | Reyes-Batlle et al. 2016[20] |
| CTGGGGCCCAGATCGTTTACC GTGAAAAAATTAGAGTGTTCAA AGCAGGCAGATTCAATTTTCTG CCACCGAATACATTAGCATGGG ATAATGGAATAGGACCCTGTCC TCCTTTTTTCAGTTGGTTAATAA CAGAGAGGATCAGGGTAATGA TTAATAGGGATAGTTG | MG298786 | Acs5 | T6 | CRIB-25 | EU273827.1 (T6) | France | Thomas V. (2008) [21] |
| GGGGTTGGCCCAGATCGTTTA CCGTGAAAAAATTAGAGTGTTC AAAGCAGGCAGATCCAATTTTC TGCCACCGAATACATTAGCATG GGATAATGGAATAGGACCCTGT CCTCCTATTTTCAGTTGGTTTTG GCAGCGCGAGGACTAGGGTAA TGATTAATAGGGATAGTTGGGG GCATTAATA | MG298787 | Acs7 | ND | EGM3 | EF050490.1 (ND) | India | Anand et al. Unpublished |
| AAAATTAGAGTGTTCAAAGCAG GCAGATTCAATTTTCTGCCACC GAATACATTAGCATGGGATAAT GGAATAGGACCCTGTCCTCCT CTTTTCAGTTGGTTAATTTACGT GTGAGGATCAGGGTAATGATTA ATAGGGATAGTTGGGGGCATT A | MG298788 | Acs9 | ND | Arc-SK07 | MF470308.1 (ND) | - | Asif, Unpublished |
| | | | | Arc-NB06 | MF470264.1 (ND) | - | Asif, Unpublished |
| | | | | T2/6C | JQ669661.2 (ND) | USA | Crary, Unpublished |
| | | | | T5-1 | EF378672.1 (T5) | - | Wildschutte et al. 2007 [23] |
| | | | | 250GILLE | GQ087290.1 (T2) | France | Year et al. 2007 [24] |
| ATTTTGGCCCAGATCGTTTACC GTGAAAAAATTAGAGTGTTCAA AGCGGGCAGATATTTTTCCTGC CACCGAATACATTAGCATGGGA TAATGGAATAGGACCCTGACCT CCTATTTTCAGTTGGTTTTGTTT ACAGCGAGGTTATATCAGGGTA ATGATTAATAGGGATAGTTGGG GGCATTAAT | MG298789 | Acs10 | T5 | FL1 | JQ418506.1 (T5) | Brazil | Otta et al. (2012) [25] |
| GGCCCAGATCGTTACCGTGAA AAAATTAGAGTGTTCAAAGCGG GCAGATATTTTTCCTGCCACCG AATACATTAGCATGGGATAATG GAATAGGACCCTGACCTCCTAT TTTCAGTTGGTTTTGTTACAGC GAGGTTATATCAGGGTAATGAT TAATAGGGATAGTTGGGGGCA TTA | MG298790 | Acs11 | ND | AG-2012 clone AR551 | JQ678613.1 (ND) | Spain | Garcia et al. (2013) [26] |
| TGAGATGGCCCAGATCGTTTAC CGTGAAAAAATTAGAGTGTTCA AAGCAGGCAGATCCAATTTTCT GCCACCGAATACATTAGCATGG GATAATGGAATAGGACCCTGTC CTCCTATTTTCAGTTGGTTTTG GCAGCGCGAGGACTAGGGTAA TGATTAATAGGGATAGTTGGGG GCATTAAT | MG298791 | Acs12 | T4 | JSS-2 | KM189416.1 (T4) | Jamaica | Todd CD. (2015) [27] |
| | | | | JWS-37 | KM189412.1 (T4) | Jamaica | Todd CD. (2015) [27] |
| | | | | JSS-24 | KM189408.1 (T4) | Jamaica | Todd CD. (2015) [27] |
| TTTTTGGCCCAGATCGTTTACC GTGAAAAAATTAGAGTGTTCAA AGCAGGCAGATCCAATTTTCTG CCACCGAATACATTAGCATGGG ATAATGGAATAGGACCCTGTCC TCCTATTTTCAGTTGGTTTTGG CAGCGCGAGGACTAGGGTAAT GATTAATAGGGATAGTTGGGG GCATTAATA | MG298792 | Acs13 | T4 | A29 | KT934544.1 (T4) | Venezuela | Wagner (2015) [28] |
| ATCGTTTACCGTGAAAAAATTA GAGTGTTCAAAGCGGGCAGAA ACTTTTTCCTGCCACCGAATAC ATTAGCATGGGATAATGGAATA GGACCCTGACCTCCTATTTTCA GTTGGTTTTGTTTACAGCGAGG TTCATCAGGGTAATGATTAATA GGGATAGTTGGGGGCATTAA | MG298793 | Acs14 | T5 | P7 | JQ268238.1 (T5) | Brazil | Alves Dde S. (2012) [29] |
| | | | | JWS-26 | KM189414.1(T5) | Jamaica | Todd CD. (2015) [27] |
| TGAAAAAAATTAGAGGGTTCAA AGCAGGCAGATTCAATTTTCTG CCACCGAATACATTAGCATGGG ATAATGGAATAGGACCCTGTCC TCCTATTTTCAGTTGGTTTTGG CAGCGCGAGGACTAGGGTAAT GATTAATAGGGATAGTTGGGG GCA | MG298794 | Acs16 | T4 | SSH40 | KU885380.1 (T4) | Spain | Reyes-Batlle M. (2016) [20] |

ND: not determined.

The genotyping study of these 11 positive specimens showed that 4 (36.4%), 2 (18.2%) and one (9.1%) sequence belonged to T4, T5 and T6 genotypes of Acanthamoeba, respectively. Phylogenic tree with the neighbor-joining method was shown in Figure 3.

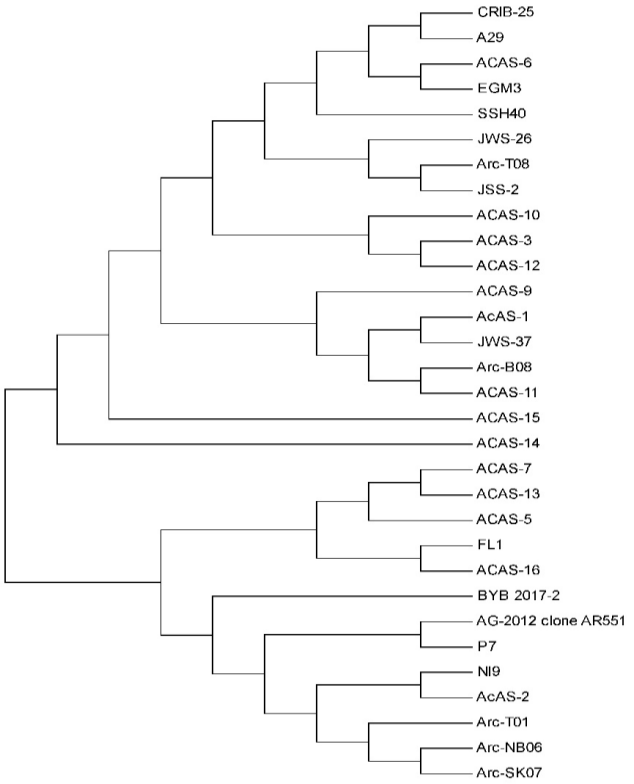


*Figure 3: Phylogenic tree constructed with the neighbour-joining tree using nucleotide sequences of DF3 region of the 18S rRNA gene by MEGA5 software*

## Discussion

Presence of Acanthamoeba spp. in natural resources of Iran has been confirmed in several studies. This organism is involved in dangerous infections of the nervous system and eyes [3]. The present study showed that 33.3% of soil samples from different parts of Arak were contaminated with free-living amoeba, and molecular examination confirmed that 31.25% of these contaminants were related to Acanthamoeba. Since previous studies have shown that water sources in Arak are contaminated with this parasite, the current results were not unexpected. However, the rate of soil contamination and the genotypes of the identified parasites should also be taken into consideration. In this study, seven out of eleven molecular-positive samples were contaminated with T4, T5 and T6 Acanthamoeba genotypes, while genotype of others cases was not determined.

The geographic location and climatic conditions of Markazi province have exposed it to the phenomenon of fine airborne dust. Airborne dust is a source of many microorganisms and has the potential to transfer Acanthamoeba. Therefore, identification of pathogenic microorganisms that can be transmitted by

soil and dust is important for proper planning and prevention of their spread. Strains of Acanthamoeba have been identified and reported in environmental sources from some regions in Iran. In the majority of these reports, the most prevalent Acanthamoeba genotype was T4 in water sources, comprising 62.96%, 91.7%, 83.3%, and 71.6% of water source samples from Shiraz [8], Tehran [30], Mazandaran [31], and Ahvaz [5], respectively. Also, the results of a systematic review showed that Acanthamoeba genotypes T4, T5, and T2 comprised 39%, 17%, and 16% of Acanthamoeba in water sources of Iran, respectively [32].

Some similar studies have examined soil and dust in Iran; T4 was the most common genotype of this parasite in soil and dust specimens in our country. Rezaeian et al., (2008) reported Acanthamoeba contamination in 100% of soil samples and 45.9% of dust samples; however, they did not investigate the parasite genotypes [4]. Niyyati and colleagues (2009) identified the first case of pathogenic genotype Acanthamoeba in dust samples collected from hospital wards of Iran. The isolated strains were related to genotypes T4, T5, and T11 (84.6%, 7.6%, and 7.6%, respectively) [10]. In another study conducted in South of Iran, three genotypes of Acanthamoeba, T2, T5, and T4, were isolated from soil, and T4 (86.6%) was the predominant genotype [5]. Another study demonstrated that 17.3% of soil samples were molecularly positive for T4 genotype Acanthamoeba [33]. Moreover, the soil samples in East Azerbaijan were contaminated with T3, T4, T5, and T11 genotypes of Acanthamoeba [34].

Comparison of genotypes obtained in the current study with other studies from Iran indicates that the genotypes of most isolates from soil samples belong to the unclassified group in Arak. All of these genotypes have been reported in environmental samples from other parts of the world, whereas T4 genotype was dominant in other regions of Iran. Future research should determine the causes of genetic differences between the isolates in our study and other research in Iran.

The results of this study confirmed that soil from parks and gardens has the potential for transgenic transmission of Acanthamoeba to humans. Therefore, individuals, especially children and immunocompromised people, are more likely to develop parasitic infections.

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