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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].

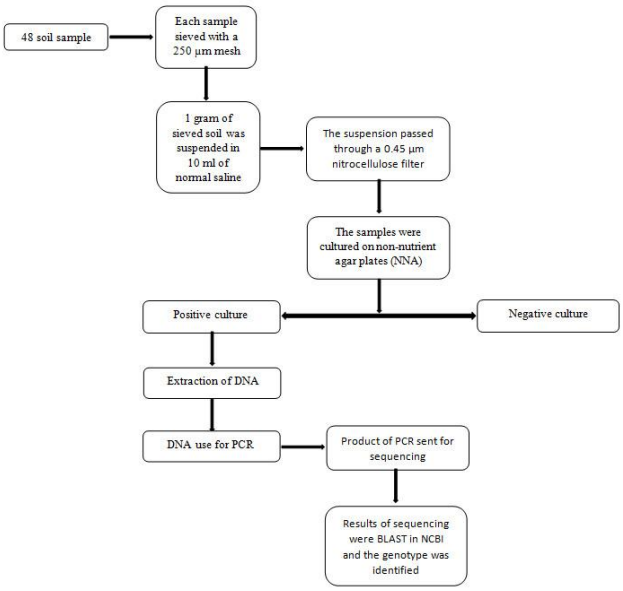

Figure 2: Preparation and testing steps on soil samples

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.

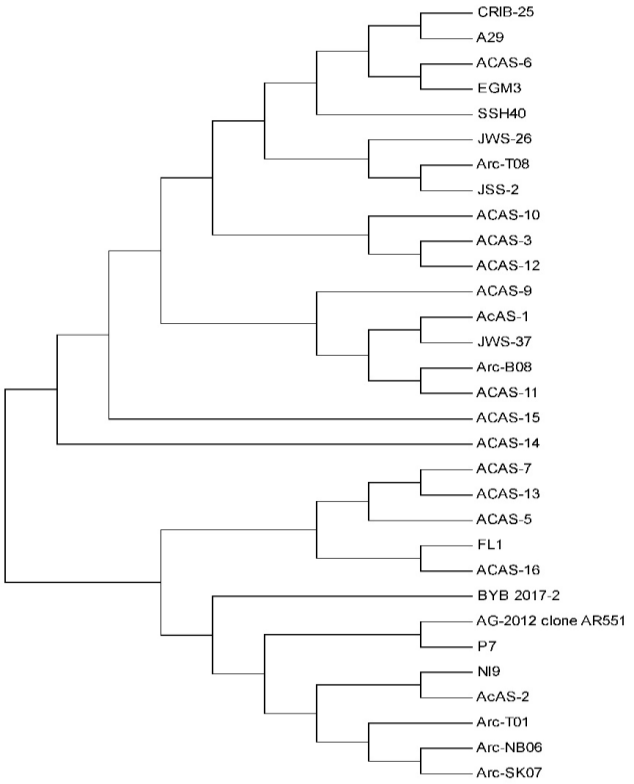

Figure 3: Phylogenic tree constructed with the neighbour-joining tree using nucleotide sequences of DF3 region of the 18S rRNA gene by MEGA5 software

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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References

1. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri, and Sappinia diploidea. FEMS Immunol Med Microbiol. 2007; 50(1):1-26. https://doi.org/10.1111/j.1574-695X.2007.00232.x PMid:17428307

2. Ahmed Khan N. Pathogenesis of Acanthamoeba infections. Microb Pathog. 2003; 34(6):277-285. https://doi.org/10.1016/S0882-4010(03)00061-5

3. Niyyati M, Rezaeian M. Current Status of Acanthamoeba in Iran: A Narrative Review Article. Iran J Parasitol. 2015; 10(2):157-163. PMid:26246812 PMCid:PMC4522290

4. Rezaeian M, Niyyati M, Farnia S, Haghi AM. Isolation of Acanthamoeba spp. from different environmental sources. Iran J Parasitol. 2008; 3(1):44-47.

5. Rahdar M, Niyyati M, Salehi M, Feghhi M, Makvandi M, Pourmehdi M, et al. Isolation and Genotyping of Acanthamoeba Strains from Environmental Sources in Ahvaz City, Khuzestan Province, Southern Iran. Iran J Parasitol. 2012; 7(4):22-26. PMid:23323088 PMCid:PMC3537466

6. Mohammadi Manesh R, Niyyati M, Yousefi HA, Eskandarian AA. Isolation of Acanthamoeba spp. from different water sources in Isfahan, central Iran, 2014. J Parasit Dis. 2016; 40(4):1483-1486. https://doi.org/10.1007/s12639-015-0716-7 PMid:27876971 PMCid:PMC5118342

7. Khezri A, Fallah E, Mostafazadeh M, Spotin A, Shahbazi A, Mahami-Oskouei M, et al. Molecular and Morphometric Characterization of Acanthamoeba spp. from Different Water Sources of Northwest Iran as a Neglected Focus, Co-Bordered With the Country of Iraq. Jundishapur J Microbiol. 2016; 9(11):e38481. https://doi.org/10.5812/jjm.38481 PMid:28138374 PMCid:PMC5240160

8. Armand B, Motazedian MH, Asgari Q. Isolation and identification of pathogenic free-living amoeba from surface and tap water of Shiraz City using morphological and molecular methods. Parasitol Res. 2016; 115(1):63-68. https://doi.org/10.1007/s00436-015-4721-7 PMid:26412057

9. Mahmoudi MR, Taghipour N, Eftekhar M, Haghighi A, Karanis P. Isolation of Acanthamoeba species in surface waters of Gilan province-north of Iran Parasitol Res. 2012; 110(1):473-477.

10. Niyyati M, Lorenzo-Morales J, Rahimi F, Motevalli-Haghi A, Martin-Navarro CM, Farnia S, et al. Isolation and genotyping of potentially pathogenic Acanthamoeba strains from dust sources in Iran. Trans R Soc Trop Med Hyg. 2009; 103(4):425-427. https://doi.org/10.1016/j.trstmh.2008.12.007 PMid:19185896

11. Mosayebi M, Ghorbanzadeh B, Eslamirad Z, Ejtehadifar M, Rastad B. The Isolation and Detection of Acanthamoeba in Rural Water Sources of Arak, Iran. Medical Laboratory Journal. 2014; 7(4):66-71.

12. Eslamirad Z, Ghaffarifar F, Shojapour M, Khansarinejad B, Sadraei J. A preliminary Study: Expression of Rhoptry Protein 1 (ROP1) Toxoplasma gondii in Prokaryote System. Jundishapur J Microbiol. 2013; 6(6):e10089. https://doi.org/10.5812/jjm.10089

13. Ghaffarifar F, Dalimi A, Eslamirad Z, Sharifi Z. Cloning rhoptry protein 1 (ROP1) gene of Toxoplasma gondii (RH) in expression vector. Archives of Razi Institute. 2008; 63(2):11-17.

14. Schroeder JM, Booton GC, Hay J, Niszl IA, Seal DV, Markus MB, et al. Use of subgenic 18S ribosomal DNA PCR and sequencing for genus and genotype identification of

Acanthamoebae from humans with keratitis and from sewage sludge. J Clin Microbiol. 2001; 39(5):1903-1911. https://doi.org/10.1128/JCM.39.5.1903-1911.2001 PMid:11326011 PMCid:PMC88046

15. Martin-Perez T, Criado-Fornelio A, Avila-Blanco M, Perez-Serrano J. New Advances in the Biology of Acanthamoeba spp. (Protozoa: Amoebozoa): An Opportunistic Pathogen Found in Contact Lenses In: Arno F, Rein E, editors. Recent Progress in Eye Research. USA: Nova Science Publishers, 2017:1-89.

16. Geisen S, Fiore-Donno AM, Walochnik J, Bonkowski M. Acanthamoeba everywhere: high diversity of Acanthamoeba in soils. Parasitol Res. 2014; 113(9):3151-3158. https://doi.org/10.1007/s00436-014-3976-8 PMid:24951165

17. Lorenzo-Morales J, Ortega-Rivas A, Martinez E, Khoubbane M, Artigas P, Periago MV, et al. Acanthamoeba isolates belonging to T1, T2, T3, T4 and T7 genotypes from environmental freshwater samples in the Nile Delta region, Egypt. Acta Trop. 2006; 100(1-2):63-69. https://doi.org/10.1016/j.actatropica.2006.09.008 PMid:17078918

18. Stothard DR, Schroeder-Diedrich JM, Awwad MH, Gast RJ, Ledee DR, Rodriguez-Zaragoza S, et al. The evolutionary history of the genus Acanthamoeba and the identification of eight new 18S rRNA gene sequence types. J Eukaryot Microbiol. 1998; 45(1):45-54. https://doi.org/10.1111/j.1550-7408.1998.tb05068.x PMid:9495032

19. Gast RJ, Ledee DR, Fuerst PA, Byers TJ. Subgenus systematics of Acanthamoeba: four nuclear 18S rDNA sequence types. J Eukaryot Microbiol. 1996; 43. https://doi.org/10.1111/j.1550-7408.1996.tb04510.x

20. Reyes-Batlle M, Zamora-Herrera J, Vargas-Mesa A, Valeron-Tejera MA, Wagner C, Martin-Navarro CM, et al. Acanthamoeba genotypes T2, T4, and T11 in soil sources from El Hierro island, Canary Islands, Spain. Parasitol Res. 2016; 115(8):2953-2956. https://doi.org/10.1007/s00436-016-5048-8 PMid:27075307

21. Thomas V, Loret JF, Jousset M, Greub G. Biodiversity of amoebae and amoebae-resisting bacteria in a drinking water treatment plant. Environ microbiol. 2008; 10(10):2728-2745. https://doi.org/10.1111/j.1462-2920.2008.01693.x PMid:18637950

22. Reyes-Batlle M, Todd CD, Martin-Navarro CM, Lopez-Arencibia A, Cabello-Vilchez AM, Gonzalez AC, et al. Isolation and characterization of Acanthamoeba strains from soil samples in Gran Canaria, Canary Islands, Spain. Parasitol Res. 2014; 113(4):1383-1388. https://doi.org/10.1007/s00436-014-3778-z PMid:24449449

23. Wildschutte H, Lawrence JG. Differential Salmonella survival against communities of intestinal amoebae. Microbiology. 2007; 153(Pt 6):1781-1789. https://doi.org/10.1099/mic.0.2006/003616-0 PMid:17526835

24. Yera H, Zamfir O, Bourcier T, Ancelle T, Batellier L, Dupouy-Camet J, et al. Comparison of PCR, microscopic examination and culture for the early diagnosis and characterization of Acanthamoeba isolates from ocular infections. Eur J Clin Microbiol Infect. Dis. 2007; 26(3):221-224. https://doi.org/10.1007/s10096-007-0268-6 PMid:17393203

25. Otta DA, Rott MB, Carlesso AM, da Silva OS. Prevalence of Acanthamoeba spp. (Sarcomastigophora: Acanthamoebidae) in wild populations of Aedes aegypti (Diptera: Culicidae). Parasitol Res. 2012; 111(5):2017-2022. https://doi.org/10.1007/s00436-012-3050-3 PMid:22828934

26. Garcia A, Goni P, Cieloszyk J, Fernandez MT, Calvo-Begueria L, Rubio E, et al. Identification of free-living amoebae and amoeba-associated bacteria from reservoirs and water treatment plants by molecular techniques. Environ Sci Technol. 2013; 47(7):3132-4310. https://doi.org/10.1021/es400160k PMid:23444840

27. Todd CD, Reyes-Batlle M, Martin-Navarro CM, Dorta-Gorrin A, Lopez-Arencibia A, Martinez-Carretero E, et al. Isolation and genotyping of Acanthamoeba strains from soil sources from Jamaica, West Indies. J Eukaryot Microbiol. 2015; 62(3):416-421. https://doi.org/10.1111/jeu.12197 PMid:25393552

28. Wagner C, Reyes-Batlle M, Ysea MA, Perez MV, de Rondon CG, Paduani AJ, et al. Genotyping of clinical isolates of Acanthamoeba genus in Venezuela. Acta parasitol. 2016; 61(4):796-801. https://doi.org/10.1515/ap-2016-0110 PMid:27787218

29. Alves Dde S, Moraes AS, Nitz N, de Oliveira MG, Hecht MM, Gurgel-Goncalves R, et al. Occurrence and characterization of Acanthamoeba similar to genotypes T4, T5, and T2/T6 isolated from environmental sources in Brasilia, Federal District, Brazil. Exp Parasitol. 2012; 131(2):239-244. https://doi.org/10.1016/j.exppara.2012.04.011 PMid:22546341

30. Niyyati M, Lasjerdi Z, Nazar M, Haghighi A, Nazemalhosseini Mojarad E. Screening of recreational areas of rivers for potentially pathogenic free-living amoebae in the suburbs of Tehran, Iran. J Water Health. 2012; 10(1):140-146. https://doi.org/10.2166/wh.2011.068 PMid:22361709

31. Shokri A, Sarvi S, Daryani A, Sharif M. Isolation and Genotyping of Acanthamoeba spp. as Neglected Parasites in North of Iran. Korean J Parasitol. 2016; 54(4):447-453. https://doi.org/10.3347/kjp.2016.54.4.447 PMid:27658596 PMCid:PMC5040085

32. Saburi E, Rajaii T, Behdari A, Kohansal MH, Vazini H. Free-living amoebae in the water resources of Iran: a systematic review. J Parasit Dis. 2017; 41(4):919-928. https://doi.org/10.1007/s12639-017-0950-2 PMid:29114120 PMCid:PMC5660050

33. Niyyati M, Ebrahimi M, Haghighi A, Haydari S. Isolation and genotyping of Acanthamoeba spp. from recreational soil of parks in Tehran, Iran. Armaghane danesh. 2013; 18(7):530-538.

34. Karamati SA, Niyyati M, Lorenzo-Morales J, Lasjerdi Z. Isolation and molecular characterization of Acanthamoeba genotypes isolated from soil sources of public and recreational areas in Iran. Acta parasitol. 2016; 61(4):784-789. https://doi.org/10.1515/ap-2016-0108 PMid:27787217