

Review articles

Acanthamoeba spp. from water and soil sources in Iran: a systematic review and meta-analysis

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ABSTRACT. *Acanthamoeba*, an opportunistic protozoan parasite, is distributed in the environment globally, causes serious human infections, including a fatal encephalitis and keratitis. The purpose of this study was to evaluate the prevalence and genotyping of *Acanthamoeba* spp. from different water and soil sources in Iran. Six English language and four Persian language databases were searched to identify studies published from March 2005 to October 2016. Random effect model and Stats Direct statistical software was used for the meta-analysis. This review resulted in a total 34 articles meeting the including criteria. The overall prevalence rate of *Acanthamoeba* spp. among the 1850 water and soil samples in Iran with using random-effect model was estimated 42.7%. The genotyping results of the *Acanthamoeba* isolates showed that T4 (81.2%) was a predominant strain in Iran. In addition, other genotypes, T2, T3, T4, T5, T6, T11, T13, T15, mixed T3/T4 and mixed T2/T6, were obtained in the environmental samples of Iran. This study may be the first systematic review and meta-analysis of *Acanthamoeba* prevalence and genotyping in different environmental sources of Iran. Knowledge about wide distribution of *Acanthamoeba* T4 in the environmental sources may help physicians to manage the patients and susceptible persons in Iran.

Key words: *Acanthamoeba*, prevalence, systematic review, soil, water, Iran

Introduction

Free-living amoebas (FLA) from the genus *Acanthamoeba*, are widely distributed in many parts of the world. *Acanthamoeba* has been found in soil, dust, plants, vegetables, contact lens solutions, water supplies, tap water, fresh water, sea water, aquariums, bottled mineral water, swage, sludge and even in animal feces, human tissues and cavities [1–3].

In some condition, they can enter human bodies from various sources, and afterwards live as opportunistic parasites causing pathogenic consequences. Thus, environmental sources can provide an important potential risks for human infection [4]. These amoebae can cause granulomatous amoebic encephalitis (GAE), amoebic keratitis (AK), cutaneous lesions, pulmonary and kidney infections. *Acanthamoeba* is

also responsible for life threatening infections in immunodeficiency patients [5,6]. Moreover, *Acanthamoeba* strains have been described as reservoir of pathogenic microorganisms such *Vibrio cholerae*, *Mycobacterium tuberculosis*, *Yersinia enterocolitica*, *Escherichia coli*, *Enterobacter cloacae*, *Listeria monocytogenes*, *Helicobacter pylori*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Salmonella enterica*, *Shigella dysenteriae*, *Staphylococcus aureus* and viruses, which can then cause host infection [7,8]. Due to their potential effect on human health, early detection of *Acanthamoeba* infection is crucial.

Acanthamoeba species can be divided into three morphological groups, based on cyst measure and structure. However, molecular analysis of the 18S

nuclear small-subunit rRNA gene and the sequencing of diagnostic fragment 3 (DF3) of 18S rRNA gene obtained *via* PCR have classified *Acanthamoeba* isolates into 21 genotypes (T1-T21) [9–11].

Recently, infections related to *Acanthamoeba* strains have been increased in many countries, including Iran [12,13]. Spotin et al. [4] reviewed the genetic variability of *Acanthamoeba* spp., among the Iranian clinical isolates and environmental sources in different regions of Iran [14]. Knowledge about prevalence of *Acanthamoeba* spp. in different environments could be helpful for control and prevention of infection in human. Despite increasing health concerns about *Acanthamoeba* strains, there is no comprehensive data about the status of *Acanthamoeba* in environmental sources of Iran. This systematic review and meta-analysis study was performed to evaluate the prevalence of *Acanthamoeba* spp. and their genotypes in water and soil sources of Iran.

Search strategy

Six English language (PubMed, Web of Science, Ebsco, Science Direct, Google Scholar and Scopus) and four Persian language databases (SID, IranMedex, Magiran and Irandoc) were searched from March 2005 to October 2016. In addition, dissertations and national conference proceedings were surveyed carefully. In order to avoid missing any articles, whole references of papers were checked carefully. The search terms which used alone or combined were “Free Living Amoeba”, “*Acanthamoeba*”, “Soil”, “Water”, “Genotype”, “Prevalence” and “Iran”. The search was restricted to English and Persian language articles (Fig.1).

Studies selection

Studies were identified by two reviewers independently and confirmed by a third reviewer. All Persian and English studies that estimated the prevalence and genotypes of *Acanthamoeba* spp. in environmental sources of Iran were included in this review. In addition to duplications, studies with the following features were excluded: 1) the studies that did not separately represent the sample size of soil and water, 2) articles that surveyed the contamination percentage of other free-living amoebae, other than *Acanthamoeba* spp., 3) studies that have been carried out qualitatively.

Data extraction

The quality of the meta-analysis was evaluated using the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist [15], which included several items that we considered essential for good reporting of observational studies. All required data such as first author, year of the study, type of sample, study methodology, study area, number of examined samples, and number of positive samples, prevalence and genotypes were extracted from each of the eligible articles and entered to Microsoft Excel software.

Data Analysis

Stats Direct statistical software (Cheshire, UK) was used for data analysis. For each study, standard deviation of the prevalence was calculated using a binomial distribution formula. The heterogeneity index between the results was estimated based on Q test and I square indicator. In addition, the degree of heterogeneity and random effect model were applied for point estimation of studies. To assess factors affecting heterogeneity, a meta-regression method was used. Point estimation of the prevalence of contamination with 95% confidence interval (CI) is shown in forest plots. The size of each square demonstrated the weight of every study, while the crossed lines indicate the confidence intervals. A protocol of this systematic review and meta-analysis is available [16].

Results

From the ten databases, 34 articles (prevalence of *Acanthamoeba* spp. 15; just genotyping of *Acanthamoeba* spp. 1; and the prevalence and genotyping of it in 18 articles) met the eligibility criteria and were selected for inclusion in this systematic review and meta-analysis [3, 13,17–45]. The results of literature searches are shown in Table 1 and 2. Two different types of diagnostic tests were used to evaluate prevalence of *Acanthamoeba* spp. in water and soil specimens of Iran as following: morphological examination and molecular method. The most used diagnostic method was morphological examination (20 studies), followed by both morphological and molecular methods (11 studies) and just molecular method (3 studies).

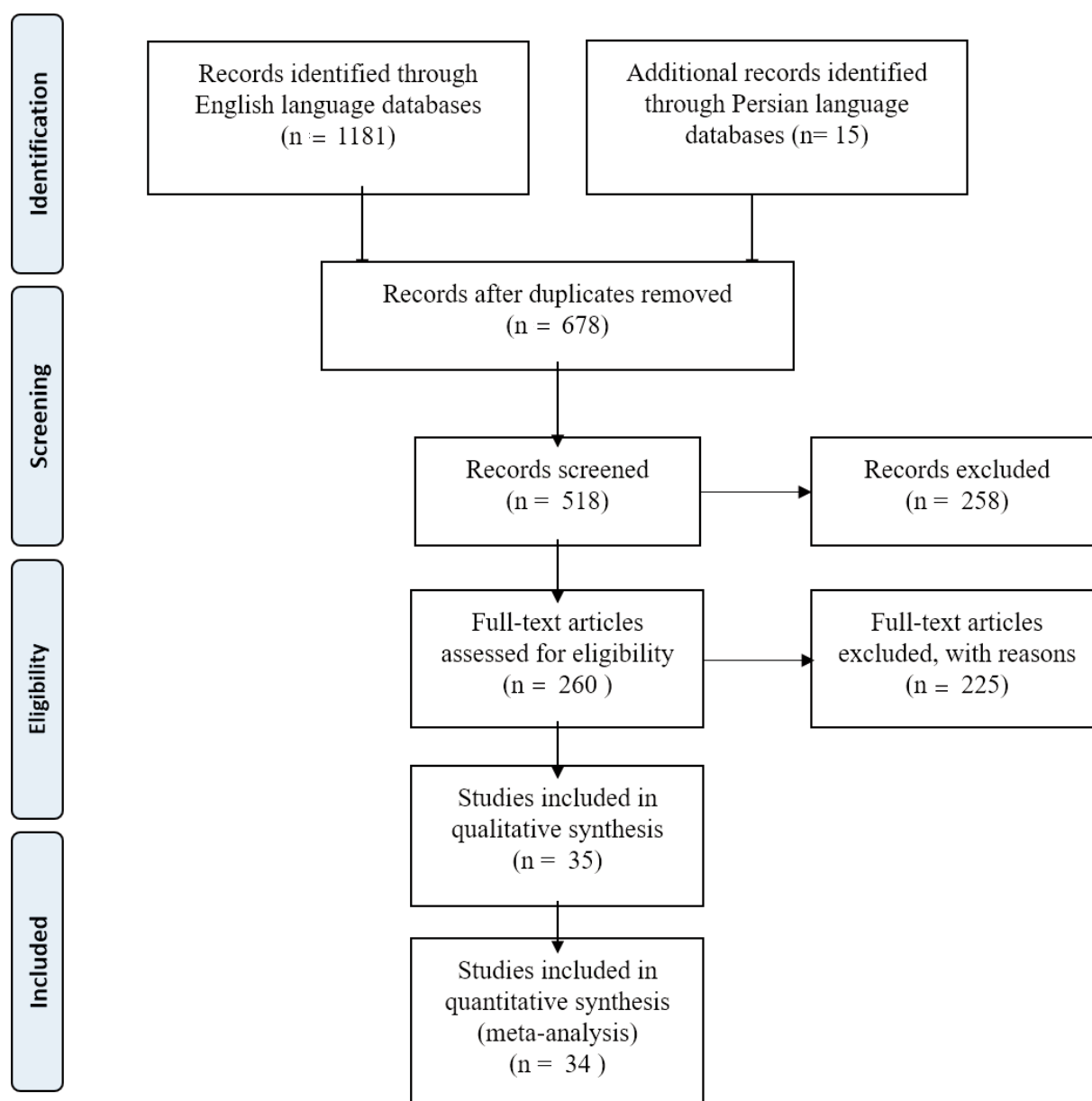


Fig. 1. Flowchart describing the study design process

Descriptive analysis of *Acanthamoeba* spp. prevalence in water and soil sources of Iran

Of 1850 water and soil samples, 757 cases contaminated to *Acanthamoeba* and were included in the meta-analysis. Results of heterogeneity tests showed a wide variation between the prevalence estimates among in different studies, and the Q statistic was also very large ($Q=268.3$, $P<0.0001$; $I^2=88.1$). Overall prevalence of *Acanthamoeba* spp. among environmental samples using random-effects model were 42.7% (95% CI, 36.2%–49.4%). The forest plot diagram of this review is illustrated in Fig. 2.

From the twenty seven publications, 1532 water samples were included in the current systematic review and meta-analysis. The Q statistic and the pooled prevalence were 245/2 ($P<0.0001$) and 42.6%, (95% CI, 35.04%–50.34%), respectively. Figure 3 shows the forest plot diagram.

A total number of 318 samples from six studies were examined for the prevalence of *Acanthamoeba* spp. among soil specimens. In this group, the Q statistic was 22.6 ($P=0.0004$) with inconsistency ($I^2=78\%$), and the pooled estimation of *Acanthamoeba* species prevalence was 42.1% (95% CI, 30.2%–54.4%) (Fig. 4).

Prevalence of *Acanthamoeba* genotypes in water and soil sources of Iran

From 238 publications of the literature searches based on *Acanthamoeba* genotypes in environmental

samples, 19 records were potentially appropriate for inclusion in this systematic review (Fig. 1). The baseline characteristics of the studies are described in Table 3.

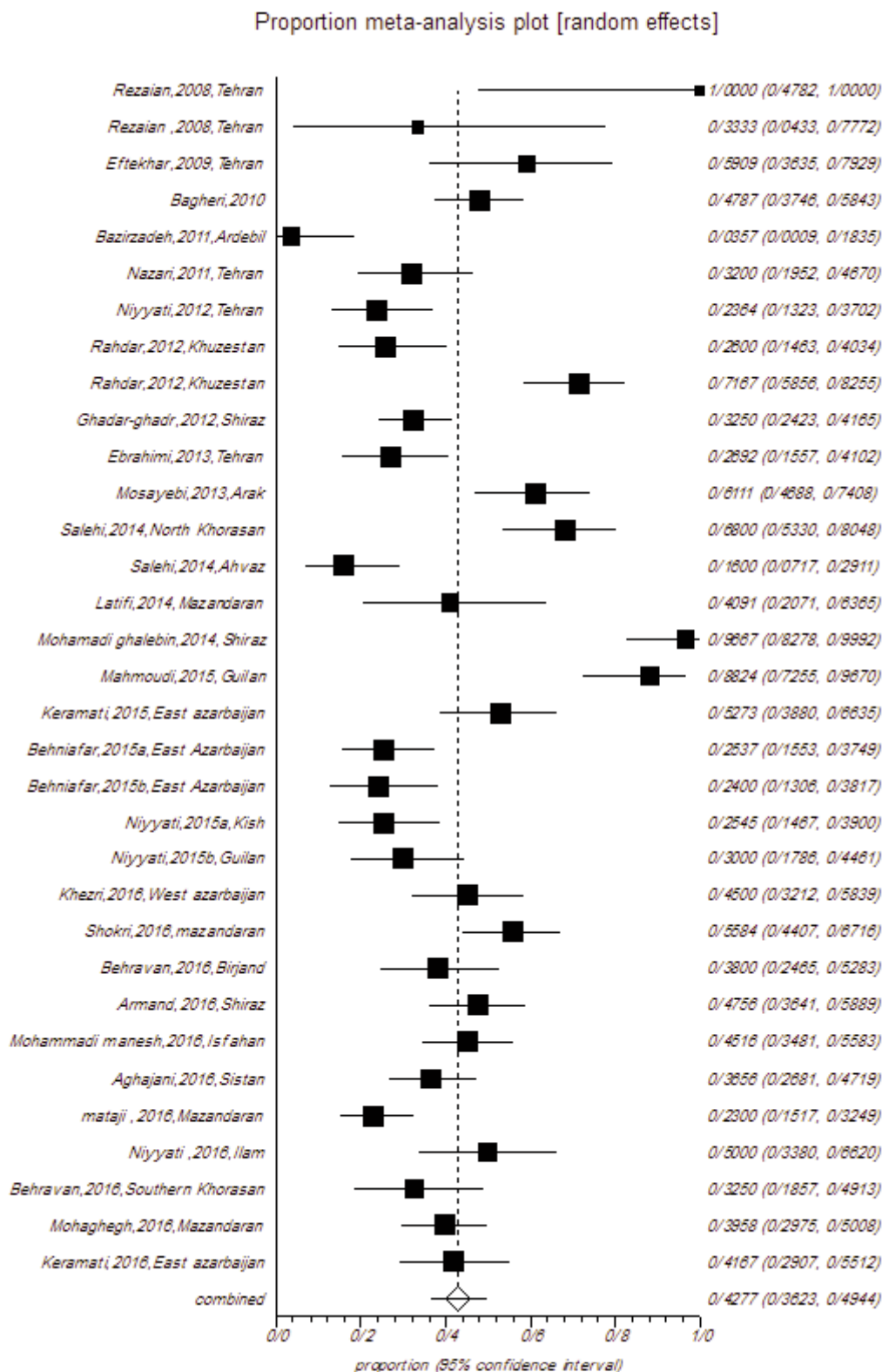


Fig. 2. Forest plot diagram of studies showing prevalence (%) of *Acanthamoeba* spp. in environmental specimens in Iran

Table 1. Baseline characteristics of included studies about *Acanthamoeba* spp. prevalence in water sources of Iran

Study area	Sample	Method	No. examined samples	No. positive samples	Prevalence (%)	References
Tehran	Tap water, swimming pool	Morphological	6	2	33.3	[17]
Tehran	Surface water	Morphological and molecular	22	13	59	[18]
13 cities	Tap water	Morphological	94	45	48	[19]
Ardebil	Hot spring	Morphological	28	3.6	1	[20]
Tehran	Recreational water	Morphological and molecular	50	16	32	[21]
Tehran	River sources	Morphological and molecular	55	13	80	[22]
Khuzestan	Surface water	Morphological and molecular	60	43	71.6	[23]
Shiraz	Water resources	Morphological	120	39	32.5	[24]
Arak	Surface water	Morphological	54	33	61.11	[25]
Bojnurd	Water resources	Morphological and molecular	50	34	68	[26]
Ahvaz	Tap water	Morphological	50	8	16	[41]
Mazandaran	Hot spring	Morphological	22	9	40.9	[27]
Shiraz	Surface water	Morphological	30	29	99.6	[39]
East Azerbaijan	Water resources	Microscopic and molecular	67	17	25.4	[45]
East Azerbaijan	Water resources	Microscopic	50	12	60	[28]
Kish Island	Tap water	Morphological	55	14	66.7	[42]
Guilan	Water resources	Morphological	50	15	30	[30]
West Azerbaijan	Water resources	Morphological and molecular	60	27	45	[31]
Sari/Mazandaran	Water	Morphological and molecular	77	43	55.8	[3]
Birjand	Surface water	Morphological	50	19	38	[29]
Shiraz	Surface water	Morphological and molecular	82	39	47.56	[33]
Isfahan	Water resources	Morphological	93	42	45.16	[40]
Guilan	River water	Molecular	34	30	88	[13]
Sistan	Pools water	Molecular	93	38	46.34	[34]
Tonekabon/ Mazandaran	Rivers	Morphological	100	23	23	[35]
Ilam	Geothermal rivers	Morphological	40	20	50	[36]
Southern Khorasan	Surface waters	Morphological	40	13	32.5	[29]

Genetic characterization of the *Acanthamoeba* samples, using PCR and 18S rRNA gene sequencing demonstrated that genotypes T2, T3, T4, T5, T6, T11, T13, T15, mixed T3/T4 and mixed T2/T6 were identified in Iran. Results displayed that, genotypes T4 and T5 had the highest frequency and their prevalence in the environmental samples were 81.2% (95% CI, 73.0%–88.3%) and 16.04% (95% CI, 9.4%–23.9%), respectively (Table 4).

From 241 water samples searched in fifteen studies, the prevalence of genotypes T4 and T5 were 79.4% (95% CI, 69.7%–87.6%) and 16.8% (95% CI, 9.1%–26.3%), respectively (Table 4).

Because other genotypes were investigated in few studies, it wasn't possible to carry out the meta-analysis. Therefore, we entered the average numbers of the other genotypes in Table 5.

Discussion

The presence of *Acanthamoeba* as an amphizoic opportunistic protozoan parasite in environmental sources can be a health concern especially for high risk persons such as contact lens wearers and immunosuppressed patients [46]. In addition, the amoebae may also act as reservoirs of other

Table 2. Baseline characteristics of included studies about *Acanthamoeba* spp. prevalence in soil sources of Iran

Study area	Sample	Method	No examined samples	No positive samples	Prevalence(%)	References
Tehran	Soil	Morphological	5	5	100	[17]
Khuzestan	Soil	Morphological and molecular	50	13	26	[23]
Tehran	Soil	Morphological	52	14	26.9	[37]
East Azerbaijan	Soil	Morphological and molecular	55	29	52.7	[44]
Sari	Soil	Morphological	96	38	39.6	[38]
East Azerbaijan	Soil	Morphological	60	25	41.6	[43]

Table 3. Baseline characteristics of included studies about *Acanthamoeba* spp. genotypes of water and soil sources in Iran

Study area	Sample	Number examined	Genotype (%)	References
Hamedan, Tehran, Ramsar, Tonekabon	Water	12	T2 (58.3) T4 (33.3) Not determined (8.3)	[62]
Tehran	Water	1	T2/T6 (100)	[61]
Tehran	Water	13	T4 (92.3) T15 (7.7)	[22]
Ardebil	Water	1	T4 (100)	[20]
Khuzestan	Water	9	T2 (6.6) T4 (53.3)	[23]
Qazvin	Water	14	T4 (78.6) T2 (21.4)	[68]
Tehran	Water	16	T4 (87.5) T5 (12.5)	[21]
Bojnurd	Water	10	T4 (100)	[26]
East Azerbaijan	Water	17	T3 (23.52) T4 (58.82) T5 (5.88) T13 (5.88) Mixed T3/T4 (5.88)	[45]
Guilan	Water	13	T4 (100)	[30]
Kish Island	Water	14	T4 (57.1) T3 (14.2) T5 (21.42) T11 (7.1)	[42]
Guilan	Water	12	T4 (61.11) T5 (5.55)	[13]
Shiraz	Water	48	T4 (62.96) T5 (33.33) T15 (3.71)	[33]
Mazandaran	Water	18	T4 (83.3) T2 (16.7)	[3]
Sistan	Water	38	T4 (89.47) T5 (7.9) T3 (2.63)	[34]
Tehran	Soil	7	T4 (100)	[61]
Khuzestan	Soil	6	T5 (16.66) T4 (83.33)	[23]
Tehran	Soil	9	T4 (100)	[37]
East Azerbaijan	Soil	17	T3 (11.76) T4 (76.47) T5 (5.88) T11 (5.88)	[43]

Table 4. The prevalence of *Acanthamoeba* T4 and T5 in environmental sources of Iran (results of meta-analysis based of random effect model)

Sample	Genotypes	Prevalence (%)	Number of examined samples	Number of identified isolates	I ² (inconsistency)	Cochran Q	P-V ₀
Water and soil	T4	81.2	280	220	63.2%	48.9	0.0001
Water and soil	T5	16.04	174	29	45.7%	13.2	0.1
Water	T4	79.4	241	186	66.6%	41.9	0.0001
Water	T5	16.8	151	27	49.0%	11.7	0.06

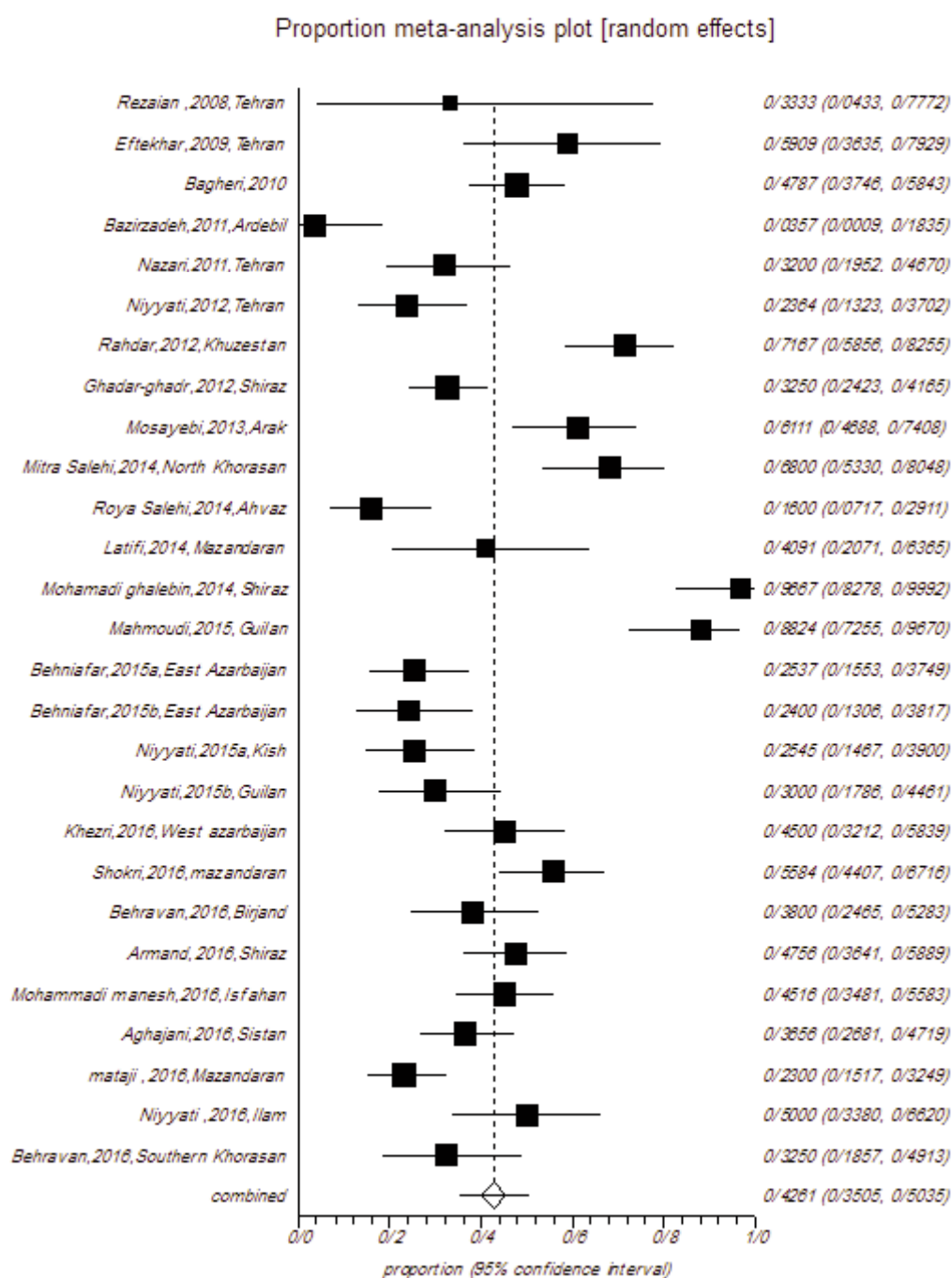


Fig. 3. Forest plot diagram of studies showing prevalence (%) of *Acanthamoeba* spp. in water samples of Iran

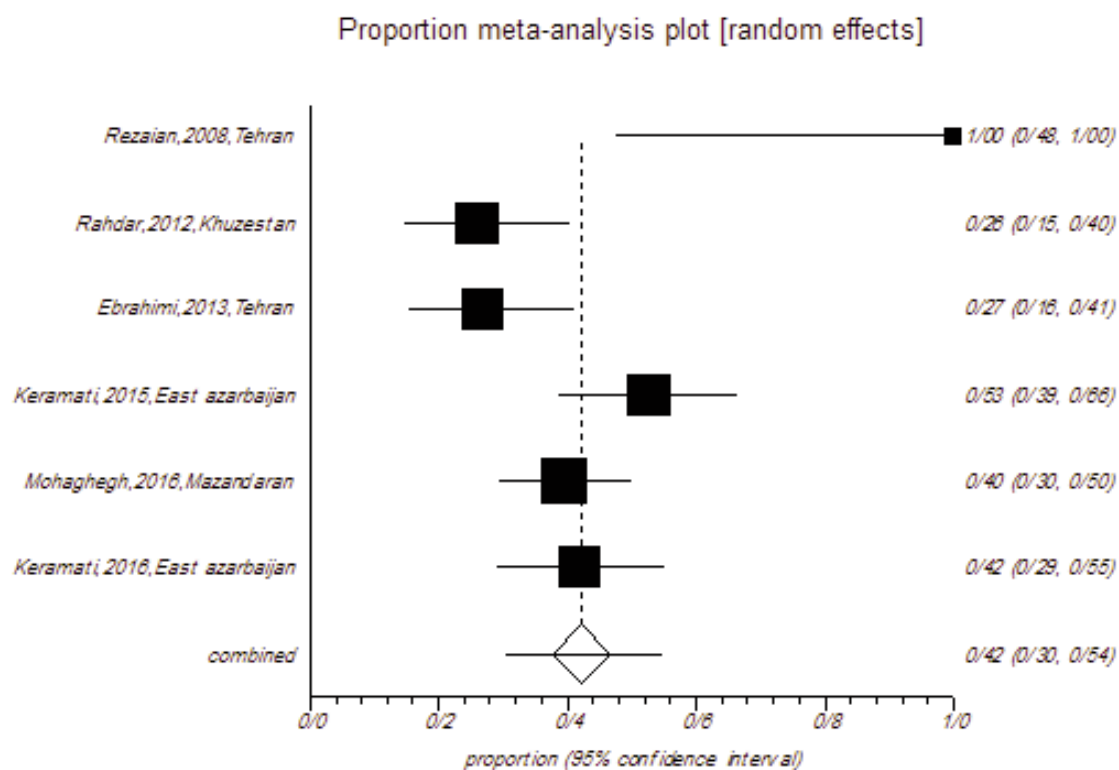


Fig. 4. Forest plot diagram of studies showing prevalence (%) of *Acanthamoeba* spp. in soil samples of Iran

organisms pathogenic for humans: protozoan, fungal, bacterial and viral organisms which can survive and even multiply within the amoeba cells [7]. Investigations on the distribution of the amoebic species in various environmental samples such as water and soil have been conducted around the world. Potentially pathogenic strains are recognized in environmental samples and reported all over the world [47–50].

Findings of this review showed that the overall

prevalence of *Acanthamoeba* spp. in environmental sources in Iran was 42.7%. Percentage of *Acanthamoeba* genus was higher in water samples (42.6%) than in soil samples (42.1%). In Turkey and Pakistan, the estimation of *Acanthamoeba* in environmental sources was found to be 60% and 70%, respectively [51,52]. Similarly to our results, *Acanthamoeba* have been observed in 39.1% of environmental water samples in Italy and 43.24% of water samples in Egypt [8,53].

Table 5. Average number of *Acanthamoeba* genotypes in water and soil samples of Iran

Sample	Genotypes	Number of study	Number of examined water samples	Number of identified genotypes
Water	T2	4	53	7
Water	T3	3	69	7
Water	T11	1	14	1
Water	T13	1	17	1
Water	T15	2	61	3
Water	Mixed T3/T4	1	17	1
Water	Mixed T2/T6	1	1	1
Soil	T3	1	17	2
Soil	T4	4	39	34
Soil	T5	2	23	2
Soil	T11	1	17	1

Results showed that *Acanthamoeba* genus have been isolated from different water sources of Iran including sea water, swimming pools, lakes, ponds, rivers, hot spring, tap water, agricultural canals and other resources. The presence of *Acanthamoeba* spp. in tap water can be a risk for those who drink and use this water in their life. Iran has several tourist attractions due to the presence of recreational areas, including hot springs, rivers and natural warm pools. Swimming in waters contaminated with *Acanthamoeba* spp. can threaten the health of people at risk.

In general, the presence of *Acanthamoeba* spp. in surface waters is significant, because they are available sources of recreational and potable water. It increases the hazard of keratitis and other health problems caused by *Acanthamoeba* genus in population. In the present study, genetic characterization among *Acanthamoeba* isolates using molecular methods displayed T2, T3, T4, T5, T6, T11, T13, T15, mixed T3/T4 and mixed T2/T6 as frequent genotypes in Iran. According to studies conducted in Turkey, *Acanthamoeba* T2, T3, T4, T7 and T9 genotypes were identified in environmental samples [51,54]. In addition, the results of water samples in Pakistan showed that there are seven different pathogenic and non-pathogenic genotypes, including T2-T10, T4, T5, T7, T15, T16, and T17 [55].

High variation observed in the prevalence of *Acanthamoeba* in worldwide can be related to several factors, such as water temperature or soil composition in distinct geographical regions, various climatic conditions, and diagnostic methods (microscopic or molecular) [56]. Studies by some researchers were limited to morphological methods [17,20]. Although *Acanthamoeba* spp. can be identified by the morphological features of cysts, the morphological characteristics of cysts can change with cultivation conditions and be variable within the similar strain. The most proposed method for identification of *Acanthamoeba* is molecular analysis [12].

Also, different environmental and climatic factors may lead to distribution of diverse genotypes [57], and further study is expected to clarify this.

The T4 (81.2%) and T5 (16.04%) were the most common genotypes of *Acanthamoeba* in environmental samples in Iran. Due to insufficient number of articles, other genotypes of *Acanthamoeba* in water and soil samples were not checked by meta-analysis. Edagawa et al. [47] in Japan, Huang

and Hsu [58] in Taiwan demonstrated that T3 and T15 genotypes are predominant in water samples in these regions. The results of the current study are in agreement with findings of Stothard, who reported that T4 is the most common genotype isolated from environmental sources and patients with GAE and AK infections in USA [59]. Several studies have shown that T4 genotype is the main etiological agent of *Acanthamoeba*-related infection such as GAE, AK and cutaneous infections in Iran and worldwide [55,60,61]. Moreover, researchers reported an enhanced rate of keratitis due to *Acanthamoeba* spp. with T4 genotype [61,62]. This genotype has special characteristic that make them to be more virulent with higher binding and remarkable cytotoxicity on host cells [53,62]. Also, this genotype has become an increasingly important because of wide distribution in environmental sources and the resistance of cysts to disinfectant [1,63]. *Acanthamoeba* with T4 genotype likely to be infected by *Legionella* spp. and *Neochlamydia* spp., thus, this parasite can help growth and transportation of waterborne pathogens. Coskun et al. [64] reported that the prevalence of free-living amoebae infected to pathogenic microorganisms in drinking water system was 16%. Therefore, the presence of T4 genotype in water is a hygienic risk for immunosuppressed patients including HIV positive patients, graft patients, patients undergoing corticosteroid and chemotherapy, pregnant women, diabetes, cirrhosis, lupus, patients with corneal trauma and contact lens wearers engaged in water-related recreation [22,65].

In our study, the second prevalent genotype of *Acanthamoeba* identified from environmental samples was the T5 genotype. For the first time, Spanakos et al. [66] confirmed that *Acanthamoeba* T5 in clinical sample could be pathogenic. Further, Iovieno et al. [67] determined a drug-resistant of T5 genotype in one keratitis patient. However, the reason for the fewer prevalence of T5 genotype in clinical samples, compared to environmental ones, is not clear and is worth further research.

In present study *Acanthamoeba* can be found in different environmental sources in Iran with high frequency. Thus, implication of alarming signs in recreational areas and education to high risk people such as contact lens wearers is of special importance. Moreover, to decrease the infections of *Acanthamoeba*, health authorities must monitor and disinfect tap waters with appropriate disinfectants. Knowledge about prevalence of this protozoan in

the environmental sources may help physicians to manage the patients and susceptible persons. The authors suggest that an investigation of the relationship between environment and the patient's infections is important. It is also recommended that studies be conducted using molecular diagnostic techniques.

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