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REVIEW ARTICLE

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Free-living amoebae in the water resources of Iran: a systematic review

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Abstract Free-living amoebae (FLA) are a group of protozoa with the capabilities of growth in the environment and invasion to the human body which have been isolated from different water sources. *Acanthamoeba*, *Naegleria*, and *Balamuthia* are the most important FLA. These cause a variety of severe complications of eye and central nervous system. Despite the fact that various studies have demonstrated the prevalence of FLA in different parts of Iran, there is no comprehensive evaluation and conclusion regarding the pollution of various water sources in Iran. This review was carried out to achieve the prevalence pattern of FLA in water resources across Iran to design appropriate health strategies. For this purpose, 8 online databases in English and Persian and also graduate thesis and national parasitology congresses were studied. The key words such as *"free living amoebae"*, *"Acanthamoeba"*, *"Naegleria"*, *"Hartmannella"*, *"Balamuthia"*, *"Sappinia"*, *"Vermamoebae"*, *"Valkampfia"*, "water resources", "water" and "Iran" were used to search articles between 1990 to 2017. From a total of 236 articles found, 38 reliable articles were included in the study. From the total number of investigated studies, the estimated prevalence was obtained as 36% among 2430 samples. Although *Acanthamoeba* prevalence has been considered as a priority, most kinds of free-living amoebae were found in all kinds of water resources. Due to the lack of free-living amoebae prevalence in more than a quarter of the Iranian provinces, more studies are recommended to achieve a better perspective to make comprehensive decisions to improve the hygiene of water resources.

Keywords Free living Amoeba · Water sources · Iran · Systematic review

Abbreviation

AK	Amoebic keratitis
PCR	Polymerase chain reaction
FLA	Free living amoeba
GAE	Granulomatous amoebic encephalitis
NNA	Non-nutrient agar

Introduction

Free-living amoebae (FLA) as amphizoic amoebae are a group of single cell organisms with the abilities of growth in natural environments such as water, soil, and dust. In appropriate conditions, they are pathogenic in humans and animals (Khan 2006). Although the FLA have been spread from pole to equator (Retana-Moreira et al. 2015; Lorenzo-Morales et al. 2007a; Yousuf et al. 2013; Winck et al. 2011; Onichandran et al. 2014; Kong 2009); their pathogenic cases are rarely reported which may be caused by lack of understanding from the part of the medical staff and misdiagnosis (Visvesvara et al. 2007). The FLA are generally found in two forms: trophozoite and cyst (as resistant

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form to physicochemical changes and disinfectants). Although their classification is still changing, we can say *Acanthamoeba*, *Naegleria*, *Balamuthia*, *Sappinia*, *Vermamoebae* and *Valkampfia* are the most important genera of FLA (Lorenzo-Morales et al. 2010).

Acanthamoeba genus is more prevalent compared to others and its infection have been reported frequently (Latifi et al. 2014; Niyyati et al. 2014; Ondriska et al. 2004; Stapleton et al. 2009; De Jonckheere 2003). *Acanthamoeba* in cases with impaired immune systems cause complications as skin ulcers, upper respiratory tract infection and granulomatous amoebic encephalitis (GAE) (Khan 2003). Nevertheless, there are many reports regarding the various cases of vision loss and blindness caused by amoebic keratitis (AK) in healthy people (Stapleton et al. 2009; Lorenzo-Morales et al. 2015). Of course, the same cases of AK caused by *Vermamoebae* and *Valkampfia* were reported (Abedkhojasteh et al. 2013). Based on the sequencing of stem 29-1 region of 18srRNA gene, *Acanthamoeba* are divided into 17 genotypes including T1–T17 and the T4, T3, and T5 genotypes are more prevalent and pathogenic (Kong 2009). A total of more than 30 known species of *Naegleria* genus, *N. fawleri* and *N. australiensis* are considered to be pathogenic by causing meningoencephalitis (Khan 2006). Usually parasites entrance via the central olfactory nervous systems is mediated by diving in contaminated water which causes poor prognosis of meningoencephalitis (Niyyati et al. 2010). *Vermamoeba vermiformis* genus which was previously called *Hartmannella vermiformis* or *Limax amoeba*, is considered to be the pathogen responsible for AK, currently (Niyyati et al. 2010; Lorenzo-Morales et al. 2007b). *Vannellidae* family as *V. persistens* have been isolated from various sources of water, but there is uncertainty about its pathogenicity (Nazar et al. 2012).

However, FLA have been isolated from different water sources such as water (Yousuf et al. 2013; Winck et al. 2011), water tanks (Lorenzo-Morales et al. 2006; Mosayebi et al. 2014), swimming pools (Dabirzadeh et al. 2015), water ponds and facades (Lorenzo-Morales et al. 2005a), rivers and lakes (Lorenzo-Morales et al. 2005b), fountains and aqueducts, wells and water channels (Armand et al. 2015), and even bottles of mineral water (Trabelsi et al. 2012). Soil, dust (Karamati et al. 2016; Niyyati et al. 2009a), air conditioning devices, hospital equipment (Lasjerdi et al. 2011a), dental units and ophthalmology equipment (Retana-Moreira et al. 2015; Lasjerdi et al. 2015), respiratory tract tube (Memari et al. 2015), animal waste (Lorenzo-Morales et al. 2007a), etc are the other sources for isolating FLA. The hygiene of water resources has always been of particular importance due to the different uses and constant exposure to humans. Moreover, today symbiosis of FLA has been proven with other microorganisms such as *Legionella*, *Toxoplasma*, *Campylobacter, Pseudomonas* and *Helicobacter* (Huang and Hsu 2010; Bonilla-Lemus et al. 2010). As mentioned above, the modes of entrance and the host immune system determined the severity and prognosis. Continuous or accidental exposure with contaminated water even in healthy people may lead to the disease (Lorenzo-Morales et al. 2005a, 2015). Therefore, due to strategic planning of water hygiene and the lack of a comprehensive study, this study was carried out to delineate the prevalence of FLA contamination in water sources.

Analysis method

This review has been conducted by searching English scientific databases such as PubMed, Science Direct, Web of Science, Scopus, Google Scholar and three national Persian databases (Magiran, SID, IranMedex) between 1990 and 2017. Search keywords included *"free-living amoebae", "Acanthamoeba", "Naegleria", "Hartmannella", "Balamuthia", "Sappinia", "Vermamoebae", "Valkampfia"*, "water resources", "water" and "Iran". Full papers and abstracts of the cross-sectional studies were selected in both Persian and English. Articles have survey the prevalence of FLA in water resources of Iran as well as studies of FLA species genotyping. Studies with other samples than water such as soil, equipment, humans or animals derivation were excluded. Articles were selected by inclusion and exclusion criteria, reliability of study design and validity of data and by two researchers separately.

Results

Of the 252 papers obtained from all of the databases, 38 articles were reliable and were scrutinized (Fig. 1). In the selected articles, 28 articles were scrutinized to assess the prevalence of merely *Acanthamoeba*. Amoeba culture was used as detection method in 12 studies while other studies used molecular methods (Fig. 2; Table 1).

FLA in drinking water (tank and tap water)

Despite all controversies surrounding the prevalence of FLA, they were seen in most studies on samples of drinking water (particularly *Acanthamoeba*). As an apparent contradiction, no contamination was found in Shiraz in 2015 (Armand et al. 2015) while 10% prevalence have been reported in previous studies with same sample (Ghadar-ghadr et al. 2012). This conflict was reported in Arak between 2010 and 2014 (Mosayebi et al. 2014;

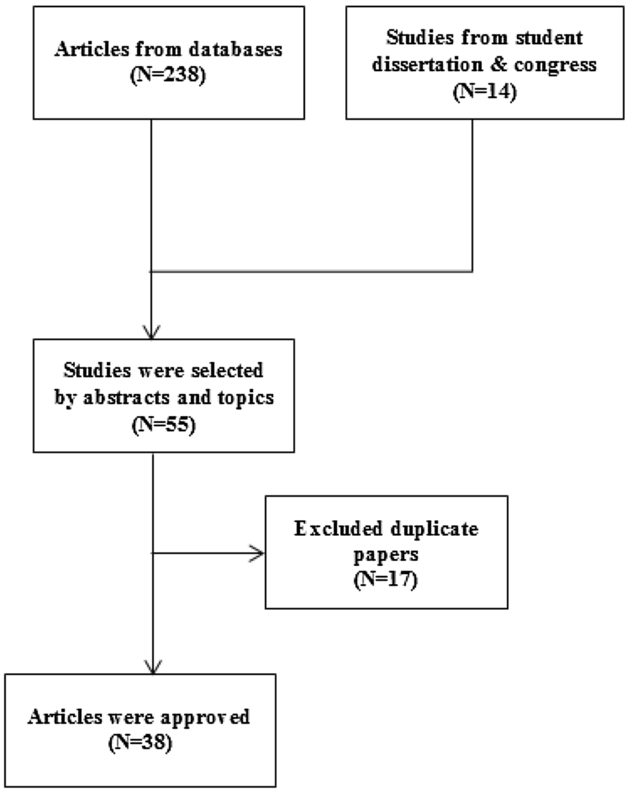


Fig. 1 Study diagram

Bagheri et al. 2010). Importantly, even one positive case has been reported in Ahvaz, Birjand and Semnan. Of course, in a comprehensive study on the tap water of 14 hospitals in the provincial capital, 12 positive cases were found (Bagheri et al. 2010). Although except *Acanthamoeba*, other FLA have been neglected in most studies, there are many reports of *Hartmannella*, *Naegleria*, *Thecamoeba*, and *Miniamoebae* (Niyyati et al. 2015a). Even though in many studies, genotype determination has not been conducted; in genotyping study of *Acanthamoeba*, T4 was considered as dominant genotype and T3, T5, T11 & T13 genotypes were the most common, respectively (Behniafar et al. 2015).

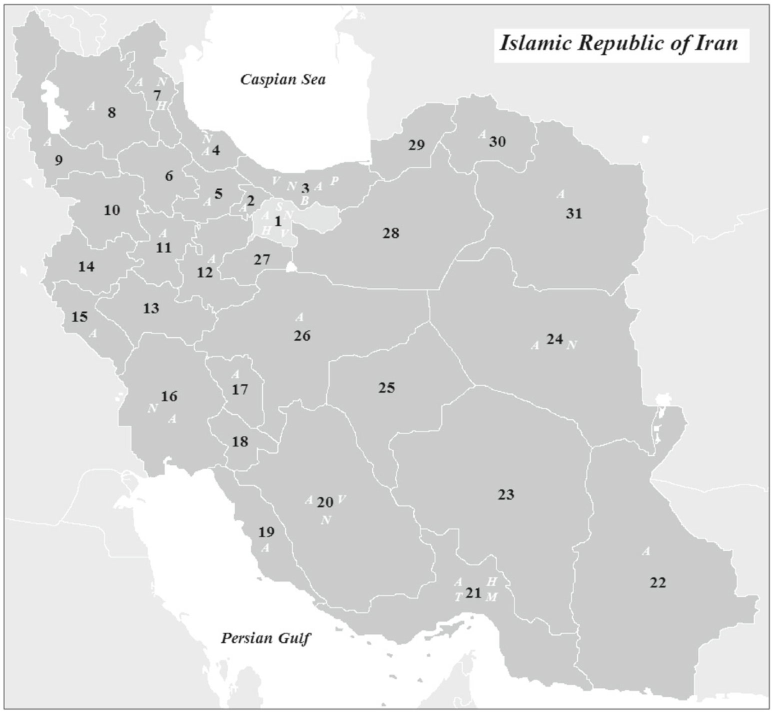
FLA in surface and groundwater

River water as the most popular surface water have been examined frequently. Rivers in Mazandaran and Gilan providences (northern Iran) as the rivers of Tehran province, have been studied more (Mahmoudi et al. 2012, 2015a, b). In other regions, more studies are required, even though few studies have been conducted in Bojnurd, Arak, Karaj, Kazeroun, Ahvaz, East and West Azarbaijan (Behniafar et al. 2015; Niyyati et al. 2012; Rahdar et al. 2012; Salehi 2014). The infestation rate of FLA was about 47%, among the total samples taken from various rivers. The most species were seen and *Acanthamoeba, Naegleria, V. vermiformis* and *Saccamoeba* had the highest prevalence, respectively. However, *Acanthamoeba* has still attracted the most attention, and this may be ignoring the other FLA (Mahmoudi et al. 2012; Rahdar et al. 2012; Salehi 2014). Other resources such as canals of agricultural water, wells, lakes and lagoons, aqueducts, dams and waterfalls have been studied to a limited extent. However, in some studies, the exact number of samples has not been mentioned, but through the mentioned cases, lakes (61.1%), wells (48.2%) and agricultural water channels (24.6%) were found as the most contaminated, respectively (Mahmoudi et al. 2012; Rahdar et al. 2012; Rezaian et al. 2003). Due to insignificant number of samples collected from aqueducts, dams and waterfalls, there is no reliable estimation of the prevalence (Mosayebi et al. 2014; Mahmoudi et al. 2015b). Similarly, *Acanthamoeba* was the main purpose of most studies, while other FLA such as *V. vermiformis, Saccamoeba, Naegleria* have also been detected (Mahmoudi et al. 2015a, b; Niyyati et al. 2012). Although in most cases, genotyping of *Acanthamoeba* have not been done, T4 genotype (89.4%) was considered as the most frequent and also T2, T3, T5 and T15 have been isolated (Behniafar et al. 2015; Mahmoudi et al. 2015b; Niyyati et al. 2012; Rahdar et al. 2012).

FLA in Recreational Water (pools, springs and sea)

Although there are limited studies on the prevalence of FLA in the Caspian Sea (North of Iran), most studies in recreational water have been done on springs and pools (Latifi et al. 2014; Behniafar et al. 2015; Badirzadeh et al. 2011; Solgi et al. 2012a, b). However, Acanthamoeba seen in most samples (80%) were collected from the Caspian Sea (Mahmoudi et al. 2015a, b). Moreover, various studies have indicated a prevalence of 27.2% on hot/cold springs in Ardebil, Mazandaran, Gilan, East Azerbaijan and Arak (Mosayebi et al. 2014; Behniafar et al. 2015; Badirzadeh et al. 2011; Solgi et al. 2012a, b). In addition to rare amoebae (such as *Vannella* and *Platyamoeba*), other FLA such as *Acanthamoeba, Vahlkamfidae, Naegleria, Hartmannella* and *Vermamoebae* have been isolated in springs (Mosayebi et al. 2014; Behniafar et al. 2015; Badirzadeh et al. 2011; Solgi et al. 2012a, b). Importantly, pathogenic amoebae *Balamuthia menderiallis* were isolated from hot springs (Latifi et al. 2016). Among the studies that performed sequencing, T4 and T3 are known as the most common genotypes of Acanthamoeba (Behniafar et al. 2015; Badirzadeh et al. 2011; Solgi et al. 2012a, b).

Surprisingly, only 5% of indoor swimming pool samples present FLA. The pools of Tehran, Kashan,



Province Name (no.)	Number of studies (no. of sample/Freq.%.)	Species (genotype*)
Tehran (1)	10 (395/32%)	*Acanthamoeba (T2, T4, T5, T6), Naegleria, S. limax, Vermamoeba vermiformis, Vannella persistens*
Alborz (2)	1 (4/50%)	*Acanthamoeba*
Mazandaran (3)	6 (295/43%)	*Vermamoebae, Vahlkampfiidae, Acanthamoeba (T2,T4,T5), Vannella, platyamoebae, Balamuthia mandrillaris, N. australiensis, N. americana, N.dobsoni, N. pagei, N. polaris*
Guilan (4)	5 (156/52%)	*Acanthamoeba (T4, T5), S. limax, Hartmannella, N. australiensis, N. pagei.*
Qazvin (5)	2 (40/80%)	*Acanthamoeba*
Zanjan (6)	-	--
Ardebil (7)	3 (58/52%)	*Acanthamoeba (T3, T4), Vahlkampfiidae, Naegleria (N. carteri and N. spp), V. vermiformis*
East Azerbaijan (8)	2 (73/27%)	*Acanthamoeba (T3, T4, T5, T13)*
West Azerbaijan (9)	2 (66/50%)	*Acanthamoeba (T4, T5), Hartmannella*
Kordestan (10)	-	--
Hamedan (11)	1 (N.D./75%)	*Acanthamoeba*
Markazi (12)	2 (60/55%)	*Acanthamoeba*
Lorestan (13)	-	--
Kermanshah (14)	-	--
Ilam (15)	1 (40/50%)	*Acanthamoeba (T2, T4)*
Khuzestan (16)	3 (420/13%)	*Acanthamoeba (T2, T4, T5), Naegleria*
ChaharMahal-o Bakhtiari (17)	1 (N.D./40%)	*Acanthamoeba*
Kohkiluyeh-o Boyer Ahmad (18)	-	--
Bushehr (19)	1 (N.D./66%)	*Acanthamoeba*
Fars (20)	3 (208/42%)	*Acanthamoeba (T4, T5, T15), V. vermiformis, Naegleria fowleri*
Hormozgan (21)	1 (55/38%)	*Hartmannellidae (V. vermiformis), Acanthamoeba (T3,T4), Miniamoebae, Tecamoebae*
Sistan-o Baluchestan (22)	1 (93/88%)	*Acanthamoeba (T3, T4, T5)*
Kerman (23)	-	--
South Khorasan (24)	1 (50/40%)	*Acanthamoeba, Vahlkamfiidae*
Yazd (25)	-	--
Esfahan (26)	3 (300/21%)	*Acanthamoeba*
Qom (27)	-	--
Semnan (28)	1 (N.D./0%)	--
Golestan (29)	-	--
North Khorasan (30)	1 (50/68%)	*Acanthamoeba (T4)*
Razavi Khorasan (31)	2 (67/16%)	*Acanthamoeba, Vahlkampfiidae*
Total	**2430 / 36%**	

Fig. 2 FLA distribution in Iran by province. (A): *Acanthamoeba* sp., (N): *Naegleria* sp., (B): *Balamuthia* sp., (V): *Vannella* sp., (H): *Hartmannella* or *V. vermiformis* sp., (P): *Platyamoebae* sp

Shiraz, Sari, Dehloran and Bojnoord (Rahdar et al. 2012; Salehi 2014; Rasti et al. 2011; Rezaeian et al. 2008) have been analyzed and *Acanthamoeba* on T4 genotype have been the predominant and T5, T3, and T6 genotype have also been reported(Armand et al. 2015; Niyyati et al. 2009b). Although there are reports of other FLA such as *Naegleria* and *Vermamoebae*, due to the lack of sufficient attention toward them, it's not reliable to report the decisive prevalence of FLA on Iran's pools (Armand et al. 2015).

Table 1 FLA prevalence in water source of Iran by article (M; multi city, C.M.; Culture medium)

Author(s)/Year (ref.)	Provenience (city)	Method	Sample type (Freq.%)	Genus (genotype)
Latifi et al. (2017)	Mazandaran (M.)	C.M. & PCR	Hot spring (54%)	*Vahlkamfiids (N. australiensis, N. dobsoni, N. americana, N. pagei, N. polaris and N. fultoni)*
Mafi et al. (2017)	Tehran (Tehran)	C.M.	Pool and park ponds (24%)	*Acanthamoeba, Hartmannella, Vahlkampfiids (Naegleria)*
Khezri et al. (2016)	West Azerbaijan (M.)	C.M. & PCR	River and tap water (45%)	*Acanthamoeba (T4, T2)*
Aghajani et al. (2016)	Sistan-o Baluchestan (M.)	C.M. & PCR	Pool and pond (41%)	*Acanthamoeba (T4, T5, T3)*
Aghajani et al. (2016)	Isfahan (Isfahan)	C.M.	Tap water and environmental water (45%)	*Acanthamoeba*
Shokri et al. (2016)	Mazandaran (Sari & suburbs)	C.M. & PCR	Lake, river, waterscape, sea, tap water, pool, waterhole, rice field and fishpond (55%)	*Acanthamoeba (T4, T2)*
Niyyati et al. (2016b)	Ilam (Dehloran)	C.M. & PCR	Recreational geothermal water; swimming pool, spring, river (50%)	*Acanthamoeba (T2, T4)*
Latifi et al. (2016)	Mazandaran (M.)	C.M. & PCR	Hot spring (3%)	*Balamuthia mandrillaris*
Niyyati et al. (2015c)	Guilan (Rasht)	C.M. & PCR	Recreational water; pond, pool, river (15%)	*Naegleria* spp. *(N. australiensis, N. pagei)*
Niyyati et al. (2015a)	Hormozgam (Kish)	C.M. & PCR	Tap water; household water resources (38%)	*Acanthamoeba (T3; A. griffini, T4; V. vermiformis, T5; A. lenticulata, T11), Hartmannella Vermiformis*
				Thecamoeba, miniamoebae
Mahmoudi et al. (2015a)	Guilan (M.)	C.M.	River, dam, lagoon (88%)	*Acanthamoeba*
Armand et al. (2015)	Fars (Shiraz)	C.M. & PCR	Pool, pond park and tap water (59%)	*Acanthamoeba (T4, T7, T5; A. lenticulata, T15; A. jacobsi), Vermamoebae*
Behniafar et al. (2015)	East Azerbaijan	C.M. & PCR	Tap water, river, hot/cold water springs, well (25%)	*Acanthamoeba (T4, T3, T13)*
Mahmoudi et al. (2015c)	Razavi Khorasan (Mashhad)	C.M.	Square fountain and ponds (10%)	*Acanthamoeba*
Mahmoudi et al. (2015b)	Guilan, Mazandaran, Alborz, Tehran.	C.M. & PCR	Tap water, hot spring, lake dam, river, sea, waterfall and pond (71%)	*Acanthamoeba (T4, T5), V. vermiformis, Saccamoeba limax*
Niyyati et al. (2015b)	Tehran (Tehran)	C.M. & PCR	Surface water (19%)	*Acanthamoeba (T4, T5)*
Dabirzadeh et al. (2015)	Sistan-o Baluchestan (Zahedan)	C.M. & PCR	Square fountain, ponds (88%)	*Acanthamoeba*
Behravan et al. (2015)	South Khorasan (Birjand)	C.M. & PCR	Surface water; square fountain and ponds water distribution stations (42%)	*Acanthamoeba, Vahlkamfiidae*
Salehi (2014)	North Khorasan (Bojnurd)	C.M. & PCR	Surface water; tap water, river, swimming pool, square fountain and creek (68%)	*Acanthamoeba (T4)*

Table 1 continued

Author(s)/Year (ref.)	Provenience (city)	Method	Sample type (Freq.%)	Genus (genotype)
Latifi et al. (2014)	Mazandaran (M.)	C.M.	Hot spring (26%)	*Acanthamoeba, Vahlkamfiidae, Vermamoebae, Vannella, Platyamoeba*
Mosayebi et al. (2014)	Markazi (M.)	C.M.	Drinking water; tap water, spring, aqueduct, well (61%)	–
Hooshyar et al. (2013)	Qazvin (Qazvin)	C.M. & PCR	Surface water (80%)	*Acanthamoeba (T2; A. palestinensis, T4; A. polyphaga)*
Ghadar-ghadr et al. (2012)	Fars (Shiraz)	C.M.	Tap water, well, water tank (35%)	*Acanthamoeba, Naegleria*
Hosseinbigi et al. (2012)	Qazvin (Qazvin)	C.M. & PCR	Square fountain and ponds (80%)	*Acanthamoeba*
Rahdar et al. (2012)	Khuzestan (ahvaz)	C.M. & PCR	Pool, river, tap water, pond, aqueduct (72%)	*Acanthamoeba (T4, T2)*
Mahmoudi et al. (2012)	Guilan (M.)	C.M.	Tap water, river, sea, waterfall and pond (70%)	*Acanthamoeba*
Nazar et al. (2012)	Tehran (Tehran)	C.M. & PCR	Recreational water environments; fountain and pond (54%)	*V. vermiformis, Vannella persistens*
Solgi et al. (2012a)	Ardebil (M.)	C.M. & PCR	Hot spring (27%)	*Hartmannella Vermiformis* *Naegleria*
Niyyati et al. (2012)	Tehran (suburbs of Tehran)	C.M. & PCR	Recreational surface water; river and pond (27%)	*Acanthamoeba (T4, T15; A. jacobsi)* *Naegleria (N. Fultoni, N. pagei, N. Clarki)*
Solgi et al. (2012a)	Ardebil (M.)	C.M. & PCR	Hot spring (33%)	*Thermotolerant Acanthamoeba (T4, T3;A. griffinii)*
Badirzadeh et al. (2011)	Ardebil (M.)	C.M. & PCR	Hot spring (78%)	*Acanthamoeba castellanii* *Vahlkamfiids*
Rasti et al. (2011)	Isfahan (Kashan)	C.M.	Swimming pool (0%)	–
Nazar et al. (2011)	Tehran (Tehran)	C.M. & PCR	Park pond and pool (32%)	*Acanthamoeba (T4, T5)*
Bagheri et al. (2010)	Iran (14 cap. of provincial)	C.M.	Tap water (48%)	*Acanthamoeba* spp.
Niyyati et al. (2009b)	Tehran (Tehran)	C.M. & PCR	Pool (1 case reported)	*Acanthamoeba (T2, T6)*
Eftekhar et al. (2009)	Tehran (Tehran)	C.M. & PCR	Square fountain and ponds (59%)	*Acanthamoeba*
Rezaeian et al. (2008)	Tehran (Tehran)	C.M.	Tap water, swimming pool and pond (33%)	*Acanthamoeba* spp.
Rezaian et al. (2003)	Khuzestan (Kazeroun)	C.M.	Surface water; spring, river, creek and pond (4%)	*Acanthamoeba, Naegleria*

FLA in stagnant water (ponds, fountains and streams)

Most studies have been conducted on fountains and ponds water in city squares and parks in Tehran, Shiraz, Qazvin, Mashhad, Birjand, Bojnoord, Sistan (Nazar et al. 2011, 2012; Dabirzadeh et al. 2015; Salehi 2014; Rezaeian et al. 2008; Behravan et al. 2015; Hosseinbigi et al. 2012; Niyyati et al. 2015b). *Acanthamoeba*, *Naegleria*, *Valkampfia*, *Hartmannella*, and *Vanella* were seen in 55% of samples, approximately. Due to the attention given to *Acanthamoeba* in many cases, other types of FLA have been neglected (Nazar et al. 2011, 2012; Dabirzadeh et al. 2015; Armand et al. 2015; Salehi 2014; Mahmoudi et al. 2015c; Rezaeian et al. 2008; Behravan et al. 2015; Hosseinbigi et al. 2012; Niyyati et al. 2015b). In few cases, genotyping were done and T4 genotype was identified as the dominant genotype, again. Other genotype such as T2, T3, T5 and T15 have been reported (Armand et al. 2015; Hooshyar et al. 2013).

Discussion and conclusion

In recent years, due to the importance of FLA, studies on FLA prevalence in Iranian water sources have increased. Although more than half of the studies have been conducted within the three provinces; Tehran, Mazandaran and Gilan, there is no epidemiological study in almost 30% of the provinces of Iran (Fig. 2). Due to suspicious reports of the contaminated cases in other countries and doubtful production process, the bottles of drinking mineral water are other points being neglected for investigation (Maschio et al. 2015; Rivera et al. 1981). Unfortunately, in many studies, *Acanthamoeba* were evaluated and other FLA were ignored (Fig. 2). Also, Iranian researchers have been concerned with genotyping, that's why today we cannot state dominant genotype, accurately (Fig. 3).

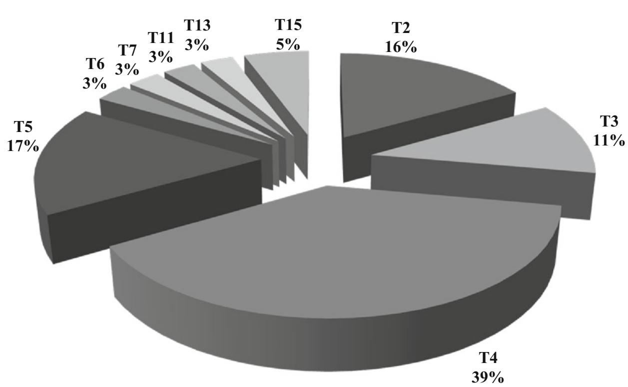


Fig. 3 *Acanthamoeba* genotype report by article (Freq.%)

However, due to the multiple uses of tap water, such as washing and bathing in addition to drinking in Iran, accurate and constant exploration is required. Overall, based on studies conducted in Iran on different water resources, FLA contamination is about 36%. It also seems that *Acanthamoeba* is the dominant species and T4 were estimated as the predominant genotypes of *Acanthamoeba* on Iran's water resources (Fig. 3). Although there are few comprehensive studies of epidemiological prevalence of FLA on water throughout the world, the prevalence of 36% seems close to reality. The prevalence of *Acanthamoeba* and *Naegleria* in drinking water on Pakistan (southeastern neighbor of Iran) have been estimated as 30 and 8%, respectively (Yousuf et al. 2013). In addition, antibodies against *Acanthamoeba* and *Naegleria* were found on the sera of whole local people. In addition, the lower sero-prevalence of antibody against *Balamuthia* demonstrates the significance of this rare and pathogenic amoeba. The presence of FLA in water resources in South Korea and US (Florida State) has been reported as 30-15% and 8.2%, respectively (Seal et al. 2006). These findings, confirmed the variety of effectiveness of water purification and distribution systems in different countries. It seems important to consider that *Acanthamoeba* was detected in tap water in one third of AK patients, in UK (Kilvington et al. 2004). This indicates the inefficiency of filtration methods of drinking water (i.e. ozone and activated carbon filtration) in replacing efficient methods (Thomas et al. 2008).

As expected, direct contact with the soil has been confirmed as the highest prevalence of FLA on aqueducts, wells, rivers, springs and agricultural canals. It should be noted that water resources are mentioned to have drinking, washing and entertainment usage in tourist areas, particularly (Mosayebi et al. 2014; Ghadar-ghadr et al. 2012; Salehi 2014; Rezaian et al. 2003). Due to constant population exposure, ponds, fountains and pools of squares and parks are considered as important resources of hand-made standing non-drinkable water. In addition to people who are directly in contact with them, they may be considered as a predisposing factor to people who inhale artificial water droplets containing FLA (especially *Naegleria)* produce by fountain (Nazar et al. 2011, 2012). Due to the patient's use of swimming pools and springs, appropriate notification should be given to patients with immune system disorders (Behniafar et al. 2015; Badirzadeh et al. 2011; Solgi et al. 2012a, b).

However, in most studies, it can be seen that FLA detection has been improved by observing cyst or trophozoite in non-nutrient agar (NNA) medium and have ignored verification by molecular methods. The morphological diagnostic methods as objective method depend on the technician skills and are accompanied by ignoring or misdiagnosis. However, PAGE as FLA diagnosis key can

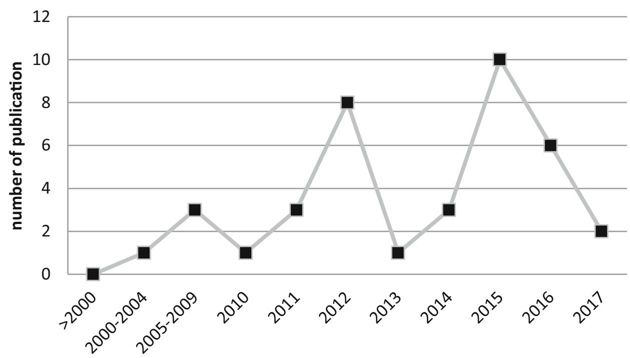


Fig. 4 Number of publications of FLA by Iranian scientist

be reliable when condensing methods and special staining (i.e. *Giemsa* and *Trichrome*) are used by experts, accurately (Garcia 2006; Page 1988). Another requirement to achieve reliable results is the accuracy of compounds present in *Page's* Amoeba saline solution. Negligence in any of the foregoing, may lead to some suspicious results (Rezaian et al. 2003; Rasti et al. 2011; Manesh et al. 2016).

Importantly, symbiosis with other microorganisms in FLA (particularly *Acanthamoeba*), except for a few cases is a neglected subject in Iranian scientific studies (Mahmoudi et al. 2015a; Niyyati et al. 2015b). Nevertheless, there are numerous evidences which admit the coexistence of a variety of microorganisms in FLA, i.e. *Legionella, Pseudomonas, Pasteurella, Aeromonas, mycobacteria* and *chlamydia* (Okude et al. 2012; Delafont et al. 2014; Zeybek and Binay 2014).

Recently, the reports of AK have increased in Iran (Hajialilo et al. 2015, 2016) (Fig. 4). On the other hand, comprehensive investigation have shown that FLA isolation is possible due to various sources in Iran such as; mouth or nose of human (Memari et al. 2016), soil (Niyyati et al. 2016a), animal feces (Niyyati et al. 2009b), hospital equipment (Lasjerdi et al. 2011b; Niyyati et al. 2015c). It could be helpful to make decisions for comprehensive health policy, detailed morphological and molecular examination of all resources, including water and soil to access the accurate prevalence of all species of FLA particularly *Acanthamoeba, Balamuthia, Naegleria, Hartmannella, Vanella, Vermamoebae, Saccamoeba, Miniamoebae, Sappinia* (and also less known amoebae i.e. *Mayorella, Korotnevella* and *Echinamoeba*) (Ramirez et al. 2014).

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Compliance with ethical standards

Conflict to interest All the authors declare that they have no conflict of interest.

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