Detection of potentially pathogenic free-living amoebae from the Caspian Sea and hospital ward dust of teaching hospitals in Guilan, Iran

Mohammad Reza Mahmoudi, Nozhat Zebardast, Frederick R. Masangkay and Panagiotis Karanis

ABSTRACT

Free-living amoebae (FLA) thrive in diverse environmental conditions. The present study aimed to define the FLA distribution from the Caspian Sea as well as from hospital ward dust from Guilan, Iran. Seawater (20) and hospital ward dust samples (100) were collected from May to June 2018. Seawater samples were vacuum filtered through a 0.45 μm pore-size membrane. Dust was collected using sterile gauze, washed with sterile distilled water, with washings collected thereafter. Washings were similarly filtered as seawater samples. FLA from the filtered material was cultivated in non-nutrient agar. Molecular analysis was performed by PCR and sequencing using specific primers for *Acanthamoeba*, *Naegleria*, and *Vermamoeba*/*Hartmanella*. Culture and PCR returned 50 and 65% positivity, respectively, for seawater samples where sequencing revealed *Acanthamoeba* T2, T5 and T6 genotypes and *A. palestinensis* and *A. lenticulata*, as well as *N. dobsoni* and *N. clarki*. In addition, 30% amoebic growth and 16% PCR detection were observed from hospital ward dust samples where sequencing revealed *Acanthamoeba* T2, T4 and T11 genotypes and *A. castellanii*, *A. palestinensis* and *A. stevensoni* as well as *N. clarki*. For both seawater and dust samples, *Acanthamoeba* was the dominant isolate. The detection of potentially pathogenic FLA from seawater may pose a threat to the public, while the presence of the same in dust spells threats to both hospital staff and patients, in particular, immunocompromised individuals. Public education, awareness, improved sanitation and hygiene, and the crafting of diagnostic strategies for the early detection of FLA in humans are necessary for the mitigation and management of potential human infection cases.

Key words | *Acanthamoeba*, dust, genotype, *Naegleria*, polymerase chain reaction, seawater

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HIGHLIGHTS

- Free-living amoebae (FLA) detected in environmental and infrastructure-derived samples.
- Pathogenic genotypes of FLA detected in the Caspian Sea.
- Pathogenic FLA detected in hospital wards for immunocompromised patients.
- Pathogenic genotypes of FLA detected in hospital ward dust.
- Hygiene and policies are necessary to protect humans against FLA infections.

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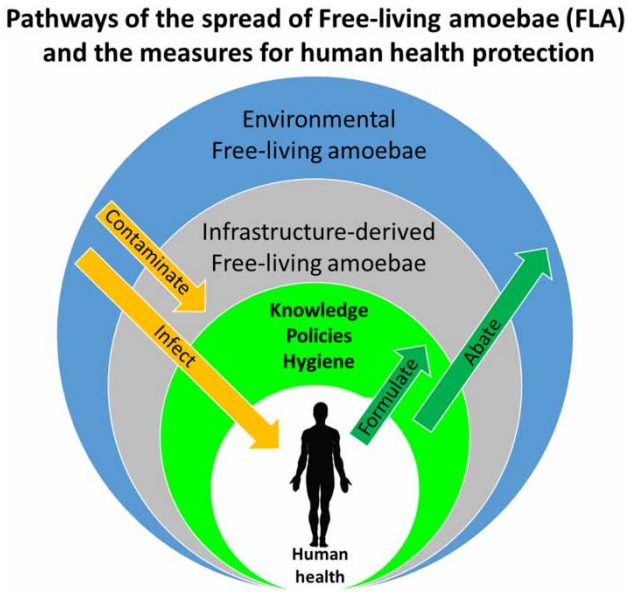
GRAPHICAL ABSTRACT



INTRODUCTION

Free-living amoebae (FLA) including the genera *Acanthamoeba*, *Naegleria*, and *Vermamoeba/Hartmannella* are distributed worldwide. Some species belonging to FLA are potentially pathogenic to humans and thrive in diverse environmental habitats including freshwater and marine systems (Abdul Majid *et al.* 2017). FLA is known to cause epidermal, neurological, and ocular infections. The ocular infection of *Acanthamoeba* keratitis (AK) is more prevalent than epidermal and neurological infections and is commonly reported from individuals with a history of swimming, diving, wearing contact lenses, and those with poor contact lens hygiene (Todd *et al.* 2015). To date, 20 distinct genotypes (T1 to T20) of *Acanthamoeba* have been reported based on rRNA sequences. Notably, genotype T4 is the most frequently isolated from clinical cases. Other isolated genotypes associated with human infections include T2, T3, T5, T6, T11, and T15 (Castro-Artavia *et al.* 2017). Although 40 species of *Naegleria* have been identified to date, only *N. fowleri* is found to cause lethal brain infections even in healthy individuals (Abdul Majid *et al.* 2017). In addition, FLA such as an *Acanthamoeba* can serve as hosts for some pathogenic microorganisms (Scheid 2018). Similarly, *Vermamoeba/Hartmanella* can host endosymbionts of bacteria, fungi, and viruses (Masangkay *et al.* 2018). This amoeba-endosymbionts relationship may further lead to the development of virulence and antibiotic resistance. Contamination with FLA commonly occurs through water and dust contact, thereby necessitating its detection from aquatic and dust sources to further define and understand the transmission dynamics from the environment to humans.

The presence of FLA in water sources have been reported in Iran and other parts of the globe (Lorenzo-Morales *et al.* 2005; Edagawa *et al.* 2009; Kao *et al.* 2012; Mahmoudi *et al.* 2012; Abdul Majid *et al.* 2017; Al-Herrawy *et al.* 2017; Milanez *et al.* 2020). However, there were only a few studies on the detection of FLA from seawater (Sawyer *et al.* 1977; Bayer & Grouyps 2004; Lorenzo-Morales *et al.* 2005; Liu *et al.* 2006) and from hospital ward dust samples (Silva & Rosa 2003; Carlesso *et al.* 2007, 2010; Lasjerdi *et al.* 2015; Golestani *et al.* 2018). In as much as FLA, contamination of environmental water

sources are risk factors for human transmission, the presence of the same in infrastructure-derived vectors such as dust from hospital wards, places a large number of high-risk individuals such as cancer, haemodialysis, transplantation, and eye trauma patients, as well as other patients under an immunocompromised or immunosuppressed status to morbid and possibly mortal consequences.

Guilan province is one of the northernmost provinces of Iran that extends along the Caspian Sea. The Caspian Sea is the largest lake in the world, is also a fully-fledged Sea, and does not have any way out to any ocean. Because of the Caspian Sea, northern Iran is one of the most touristic parts of the country where every year, millions of people frequent the region and can enjoy the beautiful sea and engage in a plethora of water-sports and recreational activities.

The present study aimed to define the distribution of FLA from environmental and infrastructure-derived samples. Seawater samples collected from the coastal areas of the Caspian Sea, as well as dust samples from teaching hospital wards, provided vital information in FLA diversity in the province and potential sources of FLA infection in Guilan, Iran.

MATERIALS AND METHODS

Study site

This cross-sectional descriptive study was conducted in Guilan province, northern Iran from May to June 2018. Guilan province (37.2809°N, 49.5924°E) is located alongside the Caspian Sea. This territory in Iran has a humid subtropical climate with the heaviest rainfall records averaging as much as 190 cm on the south western coast. The Map of Guilan province and the sampling points along with the coastal areas of the Caspian Sea as well as the location of the hospital study sites in Rasht city are shown in Figure 1.

Collection of seawater and hospital ward dust samples

A total of 20 seawater samples, 500 ml each were randomly collected from four swimming areas of the Caspian Sea. These sampling points were located in public places,

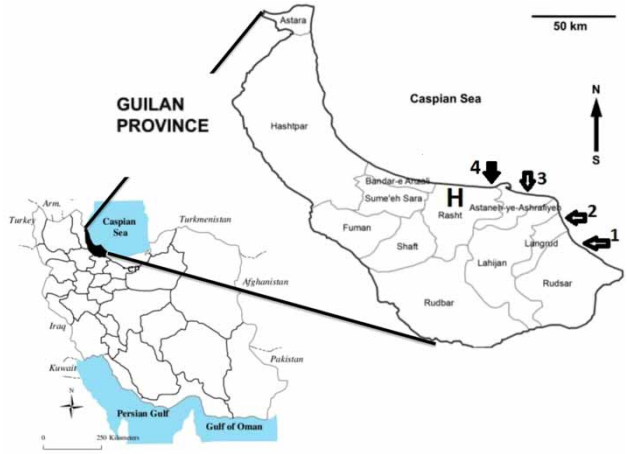


Figure 1 | Map of Guilan Province and sampling points. **1** Roudsar, **2** Chamkhaleh, **3** Kiyashahr, and **4** Zibakenar Caspian Sea coastal areas seawater sampling points; **H**, hospitals study sites in Rasht.

which are frequented for water-sports and recreational activities. Five samples from each sampling point were taken from a distance of 20 to 100 cm from the shoreline at a depth of approximately 10 to 30 cm. A total of 100 hospital ward dust samples were collected using 2×2 cm sterile gauze by wiping windows, air conditioners, and floors of patients' rooms in two teaching hospitals that included the ophthalmology and otorhinolaryngology, dialysis, transplantation, and chemotherapy wards in Rasht-Guilan province. The gauze/dust samples were placed inside 50 ml falcon tubes and immediately transported to the laboratory for processing.

Processing of samples and FLA culture

Seawater samples were filtered through 0.45 μm Nitrocellulose-Millipore™ membrane filters using a vacuum pump. Dust samples were harvested from sterile gauze by washing with 250 ml sterile distilled water with the washings filtered similarly as mentioned above. The membrane filters were inverted onto non-nutrient agar plates (NNA) lawned with heat-killed *Escherichia coli*. The plates were incubated at 37 °C and examined daily for the presence of cysts and trophozoites for 14 days using an inverted light microscope (Mahmoudi *et al.* 2015a, 2015b). Microscopic detection and identification of FLA were performed based on the criteria by Pussard & Pons (1977). Subcultures were performed to

obtain homogenous growth by cutting 5 mm portions of NNA positive for amoebic growth and placed culture-side down on a new NNA plate.

DNA extraction and molecular analysis

DNA was harvested from homogenous culture-positive NNA plates by moistening the surface of the agar with 3 ml PBS (pH 7.4), gently scraped with L shape rod, and the cysts/trophozoites suspension transferred to sterile test tubes and centrifuged at 2,000 rpm for 5 min. DNA was extracted using a tissue DNA extraction kit (FAVORGEN Biotech Corporation, founded in Taiwan) following the manufacturer's protocols with some modifications. Amoebic cysts/trophozoites suspensions were subjected to 10 freeze–thaw cycles of 5 min freezing using liquid nitrogen (−196 °C) and boiling in a water bath for 5 min (Mahmoudi *et al.* 2015c). Primary PCR was performed using three primer sets (Table 1). The first primer set was an *Acanthamoeba* specific primer set (JDP1 and JDP2) used to amplify approximately 500 bp of 18S rDNA gene called Diagnostic Fragment 3 (DF3; Schroeder *et al.* 2001; Edagawa *et al.* 2009). The second primer set (NA1 and NA2) was used to amplify approximately 900 bp of the small subunit of rRNA was used to target other genera of FLA like *Vermamoeba/Hartmanella* (Niyyati *et al.* 2012), while the third primer set (ITS1 and ITS2) was used to amplify approximately 470 bp on the 18S rRNA gene adjacent to the ITS1 region and the 23S rRNA gene almost 70 nucleotides from the ITS region to detect Vahlkampfids such as *Naegleria* (Pelandakis *et al.* 2000). Amplicons were visualized on 1.5% agarose gel stained with ethidium bromide solution under UV illumination. A 100-bp DNA ladder was used as a DNA size marker. PCR amplicons were sequenced at Codon Genetic Group (Iran). Nucleotide sequencing was performed on amplicons and compared with reference sequences from GenBank.

RESULTS

Culture and molecular detection of FLA from the Caspian Sea

Amoebic growth was observed in 50% (10/20) of Caspian Sea shoreline seawater samples, while PCR provided 65% (13/20) positivity for *Acanthamoeba* and *Naegleria* species. It is important to emphasize that all sampling points were positive for FLA with Kiyashahr and Zibakenar area having the highest FLA detection for culture at 80% (4/5) and 60% (3/5), respectively, while PCR detection of *Acanthamoeba* spp. at 45% (9/20) was higher compared with *Naegleria* spp. at 20% (4/20) (Tables 2 and 4). Sequencing of *Acanthamoeba* isolates revealed five T2 (55%), three T5 (33%), and one T6 (11%) genotype. The sequences of the isolates demonstrated similarities to *A. palestinensis* and *A. lenticulata*. In addition, sequences amplified using *Naegleria* primer sets demonstrated similarities to *N. clarki* and *N. dobsoni*. Derived sequences were deposited in GenBank under accession numbers MT981400, MT981208, MT973990, MT893135 to MT893140, MT893142, MT893144, and MT893146 (Table 4). BLAST search/similarity was performed with most sequences demonstrating more than 96% similarity.

Table 1 | Primer sets and thermal cycling conditions used in the present study

FLA	Primer sequence	Thermal cycling conditions
Acanthamoeba spp.	JDP1: 5-GGCCCAGATCGTTTACCGTGAA-3′ JDP2: 5′-TCTCACAAGCTGCTAGGGAGTCA-3′	94 °C for 3 min; 35 cycles of 94 °C for 35 s, 56 °C for 45 s, 72 °C for 45 s; followed by a final extension at 72 °C for 5 min
Vermamoeba/Hartmanella spp.	NA1 5′-GCT CCA ATA GCG TAT ATT AA-3′ NA2 5′-AGA AAG AGC TAT CAATCT GT-3	94 °C for 1 min, 35 cycles of 94 °C for 35 s; 50 °C for 45 s, 72 °C for 1 min. The extension time was prolonged for 5 min at 72 °C
Naegleria spp.	ITS1 5-GAACCTGCGTAGGGATCATTT-3 ITS2 5-TTTCTTTTCCTCCCCTT ATTA-3	94 °C for 1 min, 35 cycles of 94 °C for 35 s, 55 °C for 45 s, 72 °C for 1 min. The extension time was prolonged for 5 min at 72 °C

Table 2 | Frequency of FLA and genotypes detected from Caspian Sea shoreline seawater samples

			PCR positive		Species/Genotypes	
Sampling points	**Total samples**	**Culture positive**	***Acanthamoeba* spp.**	***Naegleria* spp.**	***Acanthamoeba* spp./Genotype**	***Naegleria* spp.**
Roudsar	5	2 (40%)	2	1	*A. palestinensis*/T2 ($n = 1$) *A. palestinensis*/T6 ($n = 1$)	*N. dobsoni* ($n = 1$)
Chamkhaleh	5	1 (20%)	1	1	*A. palestinensis*/T2 ($n = 1$)	Negative
Kiyashahr	5	4 (80%)	3	1	*A. palestinensis*/T2 ($n = 2$) *A. lenticulata*/T5 ($n = 1$)	*N. clarki* ($n = 1$)
Zibakenar	5	3 (60%)	3	1	*A. palestinensis*/T2 ($n = 1$) *A. lenticulata*/T5 ($n = 2$)	*N. dobsoni* ($n = 1$)
Total	20	10 (50%)	9 (45%)	4 (20%)	9 sequences	3 sequences

Culture and molecular detection of FLA from hospital ward dust samples

Amoebic growth was observed in 30% (30/100) of hospital ward dust samples, while PCR provided 16% (16/100) positivity for *Acanthamoeba* and *Naegleria* species. It is important to emphasize that all hospital wards were positive for FLA in both culture and PCR methods (Tables 3 and 4). The rates of culture detection per ward were highest at the transplantation ward at 38% (6/16) and lowest at the dialysis ward at 22% (6/27). The rate of PCR detection was higher for *Acanthamoeba* spp. at 14% (14/100) compared with *Naegleria* spp. at 2% (2/100). Sequencing revealed the presence of *Acanthamoeba* genotypes T2 at 50% (7/14), T4 at 43% (6/14), and T11 at 7% (1/14) which were composed of *A. palestinensis*, *A. stevensoni*, *A. castellanii*, and *A. polyphaga* (Accession numbers: MT893147 to MT893156, MT893134, MT893141, MT893143, and MT893145). In addition, *Naegleria* (*N. clarki*; Accession number: MT973989) was detected from the Ophthalmology/Otorhinolaryngology ward only. BLAST search/similarity was performed with most sequences demonstrating more than 96% similarity.

DISCUSSION

FLA in environmental water samples

To the best of our knowledge, this study submits the first comprehensive report on the presence of potentially pathogenic FLA in the Caspian Sea and hospital ward dust samples from Guilan, Iran. With the increasing number of FLA cases, detection efforts should be increased, especially from environmental waters due to the FLA's ability to cause life-threatening infections to humans (Abdul Majid *et al.* 2017). There are only a few reports in the detection of FLA from seawater (Sawyer *et al.* 1977; Lorenzo-Morales *et al.* 2005; Liu *et al.* 2006; Munson & Paget 2006; De Jonckheere 2007) and hospital ward dust worldwide (Carlesso *et al.* 2007, 2010; Costa *et al.* 2010).

Table 3 | Frequency of FLA and genotypes detected from hospital ward dust samples

			PCR positive		Species/Genotypes	
Hospital wards	**Total samples**	**Culture positive**	***Acanthamoeba* spp.**	***Naegleria* spp.**	***Acanthamoeba* spp./Genotype**	***Naegleria* spp.**
Opthalmology Otorhinolarngology	46	14 (30%)	7	2	*A. palestinensis*/T2 ($n = 7$)	*N. clarki*
Dialysis	27	6 (22%)	1	Negative	*A. castellanii*/T4 ($n = 1$)	Negative
Transplantation	16	6 (38%)	3	Negative	*A. castellanii*/T4 ($n = 3$)	Negative
Chemotherapy	11	4 (36%)	3	Negative	*A. polyphaga*/T4 ($n = 1$) *A.* genotype/T4 ($n = 1$) *A. stevensoni*/T11 ($n = 1$)	Negative
Total	100	30 (30%)	14 (14%)	2 (2%)	14 sequences	1 sequence

Table 4 | Point sources of FLA and accession numbers of sequences deposited in GenBank

Point source		Species/genotype	GenBank accession no.
Caspian Sea shoreline seawater samples	Roudsar	*A. palestinensis*/T2	MT893136
		A. palestinensis/T6	MT893140
		N. dobsoni	MT981208
	Chamkhaleh	*A. palestinensis*/T2	MT893135
	Kiyashahr	*A. palestinensis*/T2	MT893138, MT893139
		A. lenticulata/T5	MT893144
		N. clarki	MT973990
	Zibakenar	*A. palestinensis*/T2	MT893142
		A. lenticulata/T5	MT893137, MT893146
		N. dobsoni	MT981400
Hospital ward dust samples	Ophthalmology Otorhinolaryngology	*A. palestinensis*/T2, *N. clarki*	MT893141, MT893143, MT893145, MT893147, MT893148, MT893149, MT893150, MT973989
	Dialysis	*A. castellanii*/T4	MT893154
	Transplantation	*A. castellanii*/T4	MT893156, MT893153, MT893134
	Chemotherapy	*A. polyphaga*/T4	MT893155
		A. genotype/T4	MT893151
		A. stevensoni/T11	MT893152

In Guilan, previous reports on FLA, in particular, *Acanthamoeba* have been submitted (Mahmoudi *et al.* 2012, 2015b). However, there remains a scarcity of data on the prevalence of pathogenic Acanthamoeba isolates from seawater and hospital ward dust in this region. In the present study, *Acanthamoeba* was detected from 50% of the Caspian Sea water samples. Similarly, previous studies reported *Acanthamoeba* from seawater in Jamaica (49.6%) (Lorenzo-Morales *et al.* 2005), North Sea coastal sediments (82.9%) (Munson & Paget 2006), coastal sands of Granary Island (Pussard & Pons 1977), Canyon Lake in Arizona (De Jonckheere 2007), and sea and ocean sediments (Bayer & Grouyps 2004; Liu *et al.* 2006; Castro-Artavia *et al.* 2017).

Interestingly, in the present study, *Acanthamoeba* genotypes have been isolated from seawater, here it is revealed that *Acanthamoeba* species and genotypes detected in the Caspian Sea can tolerate saline environment and may contribute to its pathogenic potential against humans and animals (Khan 2009).

In addition, increasing global and water temperatures contributing to increased salt concentrations in freshwater systems may influence adaptive patterns of osmo-tolerance and thermo-tolerance in amoebic species. Relative to these, great curiosity lies in the osmo-thermo-adaptive capacity of FLA, in particular the pathogenic genotypes, and how these hypothesized evolutionary or adaptive changes may influence the burden and gravity of amoebic diseases at present and in the future (Milanez *et al.* 2020).

With the use of more sensitive and specific molecular detection methods, such as PCR, information on waterborne protozoan parasites has increased in the last years (Mahmoudi *et al.* 2015c). According to PCR and sequencing results in the present study, *Acanthamoebae* isolated from seawater samples belonged to genotypes T2 (55%), T5 (33%), and T6 (11%) which were in line with a study by Maghsood *et al.* (2005), who reported *Acanthamoeba* T2 genotype as the predominant genotype in water sources of Iran. Genotypes T2 and T6 are environmental isolates that are phylogenetically close to one another and have been reported to cause *Acanthamoeba* keratitis (Maghsood *et al.* 2005; Kao *et al.* 2012). In a study by Booton *et al.* (2004), *Acanthamoebae* were isolated from beach sand in southern Florida and nearly all isolates were T4 genotypes. In the current study, 33% of seawater isolates demonstrated sequence similarities with genotype T5. T5 genotype is found in the environment and has been reported to cause keratitis and fatal disseminated infection in immunocompromised heart transplant patients (Spanakos *et al.* 2006; Barete *et al.* 2007). In addition, *N. clarki* and *N. dobsoni* were

isolated from three seawater samples from the shorelines of the Caspian Sea used by humans for water-sports and recreational activities. *Naegleria* species have been isolated from river sediments from lakes in the USA (De Jonckheere 2007). Of the 40 species of *Naegleria*, only *N. fowleri* has been reported to cause meningoencephalitis in humans (Abdul Majid *et al.* 2017). The present study revealed that *Acanthamoeba* spp. was more prevalent in the study sites compared with *Naegleria* species. Perhaps the detection of the *Acanthamoeba* more than *Naegleria* is due to the much shorter life of *Naegleria* cysts in the environment (Al-Herrawy *et al.* 2017).

FLA in infrastructure-derived dust samples

There are only a few studies on the detection of FLA from infrastructure-derived sources such as hospital ward dust and was usually investigated in terms of only a single ward or a single hospital (Silva & Rosa 2003; Carlesso *et al.* 2007, 2010; Lasjerdi *et al.* 2015; Golestani *et al.* 2018). The present study conducted a comprehensive survey of dust samples obtained from various wards in two teaching hospitals catering for immunodeficiency and eye injury patients in Guilan, Iran. In the present study, *Acanthamoeba* and *Naegleria* species were detected in 14 and 2% of the hospital ward dust samples, respectively. In another study, *Acanthamoeba* was identified in 52.9 and 42.86% of dust samples in hospital wards in Tehran (Lasjerdi *et al.* 2011, 2015), and hospital wards in Kashan (Golestani *et al.* 2018), respectively. In addition, the isolation rate of *Acanthamoeba* from dust collected from different hospitals in Brazil was 34% (Silva & Rosa 2003), 23% (Carlesso *et al.* 2007), and 35% (Carlesso *et al.* 2010). Since viable potentially pathogenic FLA was revealed to be present in hospitals wards caring for immunocompromised or eye trauma patients, opportunistic infection cases are inevitable unless appropriate interventions are set into motion. Initiatives to address the presence of potentially pathogenic FLA inside healthcare institutions are necessary to abate risks to the health of clients, staff, and patients with faulty immune systems.

In the present study, PCR and sequencing results identified, T2, T4, and T11 genotypes of *Acanthamoeba* from hospital ward dust samples (Tables 3 and 4). A study from Brazil in a hospital environment revealed that *Acanthamoeba* T4 was the predominant genotype from dust samples (Carlesso *et al.* 2010). Moreover, T4, T2, and T11 genotypes were isolated from the hospital dust from previous studies in Iran (Niyyati *et al.* 2009; Golestani *et al.* 2018). In addition, genotype T11 was reported in keratitis patients in Iran, which was previously isolated from environmental samples (Hajialilo *et al.* 2016). This defines the path of the spread of environmental FLA into infrastructural spaces and places humans at risk of contracting potentially pathogenic FLA even within grey structures and utilities. In the present study, the *Naegleria* spp. isolated from three hospital ward dust samples and the isolation rate of *Naegleria*, in general, was lower than *Acanthamoeba*. This may be due to its lower prevalence in the environment and its susceptibility to chlorine-based disinfectants applied in the hospitals (Visvesvara *et al.* 2007). The *Naegleria* findings in the present study are in agreement with results from Silva & Rosa (2003) and Teixeira *et al.* (2009). In addition, the fact that FLA can act as natural vectors for pathogenic bacteria has a significant implication on the sector of public health (Zhao *et al.* 2010; Balczun & Scheid 2017; Scheid 2018). Our samples were collected from wards caring for immunocompromised and eye trauma patients, thereby posing a significant risk for contracting not only nosocomial amoebic infections but bacterial, fungal, and viral infections as well (Pagnier *et al.* 2008; Zhao *et al.* 2010; Mahmoudi *et al.* 2015a).

Guilan province in northern Iran annually attracts a multitude of tourists and all the water sources included in the present study are utilized by humans for various water-sports and recreational activities. Public health organizations should craft initiatives to disseminate the necessary information to all stakeholders, in particular, high-risk individuals, including contact lens wearers on the necessary precautions to prevent amoebic infections caused by FLA. Although some FLA isolates in the present study are not considered pathogenic, the same can still act as hosts for endosymbionts of pathogenic bacteria, fungi, viruses, and virophages (Cateau *et al.* 2008; Masangkay *et al.* 2018). This relationship protects the endosymbionts from external stresses and acts as a dispersal mechanism across various habitats and biological hosts. Mimiviruses, Pandoraviruses, and Pithoviruses are a few examples of viral endosymbionts detected within FLA (Cateau *et al.* 2008; Balczun & Scheid

2017; Scheid 2018). Since FLA is found in ubiquity from a variety of sources such as water, soil, and dust, pathogenic airborne and waterborne endosymbionts within the FLA should be of interest to Public Health Authorities as well.

CONCLUSION

The present study describes the first expanded evidence on the molecular and epidemiologic distribution of *Acanthamoeba* and *Naegleria* species from the largest lake in the world, the Caspian Sea. Also, the pathway of the spread of FLA from the environment to infrastructures has been preliminarily defined through the detection of FLA in hospital ward dust samples in Guilan, Iran. The detection of pathogenic genotypes provides evidence of the inevitability of human infectious cases if no initiatives are enacted for protection from environmental and infrastructural contamination. This evidence of environmental and infrastructure-derived FLA poses risks for human health, in particular, the immunocompromised population and can be more complicated because of the potential presence of pathogenic endosymbionts as well. All this should be taken into consideration by the health authorities and all stakeholders in crafting policies to prevent, detect, diagnose, and manage human FLA infections.

ETHICAL APPROVAL

Ethical approval was obtained from the Ethical Committee Board of the Guilan University of Medical Sciences (Ref. No. approved this study protocol IR.GUMS.REC.1399.267).

FUNDING

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

M.R.M. acquisition of data; M.R.M. and N.Z. analysis and interpretation of data; M.R.M. drafted the manuscript, evaluated the results, and provided administrative, technical, and material supports; F.R.M. performed critical revision of the manuscript and provided administrative support; P.K. drafted the manuscript and performed critical revision of the manuscript, evaluated the results, and provided administrative and editorial support.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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