RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.1583675
İst Tıp Fak Derg 2025 / J Ist Faculty Med 2025

INVESTIGATION OF *ACANTHAMOEBA* SPP. WITH CULTURE AND MOLECULAR METHODS IN THE ENVIRONMENTAL WATER SAMPLES

ÇEVRESEL SU ÖRNEKLERİNDE *ACANTHAMOEBA* SPP.'NİN KÜLTÜR VE MOLEKÜLER YÖNTEMLER İLE ARAŞTIRILMASI

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Cite this article as: Efe Ş, Boral Ö, İşsever H. Investigation of Acanthamoeba spp. with culture and molecular methods in the environmental water samples. J Ist Faculty Med 2025;88(2):146-154. doi: 10.26650/IUITFD.1583675

ABSTRACT

Objective: *Acanthamoeba* spp. are free-living amoebae found in a variety of environments, including seawater, lakes, rivers, stagnant waters, swimming pools, bottled waters, ventilation ducts, air conditioning units, sewage systems, soil, and in-hospital dialysis and eye wash units. Although infections caused by *Acanthamoeba* spp. are infrequent, they are characterised by high mortality and can lead to serious clinical problems. *Acanthamoeba* causes *Acanthamoeba* keratitis (AK) in healthy individuals and contact lens users. In immunocompromised individuals, it can lead to granulomatous amoebic encephalitis (GAE) and lung and skin infections. In this study, we investigated the presence of *Acanthamoeba* species in environmental water samples collected from various cities across Türkiye. We employed a range of culture and molecular methods for this analysis.

Material and Methods: A total of 100 samples were collected from different cities and water sources in Türkiye. Water samples were filtered through a 0.45 µm diameter cellulose nitrate membrane filter using a vacuum device, allowing approximately 100 ml to pass in about 30 s. The filtered water samples were cultured on Non-Nutrient Agar *E.coli* (NNA-E.coli) and Buffered Charcoal Yeast Extract Agar media. Samples that were considered culture-positive were stored at -20 °C for DNA isolation.

Results: Among the 100 samples, 27 (27%) *Acanthamoeba* spp. were detected without differentiation by the media. In the NNA-E. coli medium, 22 (22%) *Acanthamoeba* spp. were produced, while 19 (19%) were produced in the BCYE medium. No significant difference was found between the two media. A total of 25 *Acanthamoeba* spp. were detected using RT-PCR (25%). The compatibility of the media with RT-PCR was found to be statistically significant ($p<0.005$) and bidirectional, as calculated by the kappa coefficient.

ÖZET

Amaç: *Acanthamoeba* spp. serbest yaşayan amiplerden olup çevrede deniz sularında, göllerde, nehirlerde, durgun sularda, yüzme havuzlarında, şişelenmiş sularda, havalandırma kanallarında, klima ünitelerinde, lağım sularında, toprakta, hastanelerin diyaliz üniteleri ve göz yıkama üniteleri gibi çok çeşitli yerlerde bulunabilmektedir. *Acanthamoeba* spp.'ye bağlı enfeksiyonlara az rastlanmasına rağmen yüksek ölüm ile karakterize olup ciddi klinik problemler oluşturmaktadır. *Acanthamoeba* sağlıklı bireylerde ya da kontakt lens kullanıcılarında *Acanthamoeba* keratitine (AK) neden olmaktadır. Bağışıklık sistemi baskılanmış kişilerde granülomatöz amebik ensefalit (GAE), akciğer ve deri enfeksiyonlarına sebep olmaktadır. Çalışmamızda Türkiye'nin çeşitli şehirlerinden toplanan çevresel su örneklerinde, *Acanthamoeba* spp. varlığının farklı kültür ve moleküler yöntem ile araştırılması amaçlanmıştır.

Gereç ve Yöntem: Türkiye'nin farklı şehirlerinden ve su kaynaklarından toplam 100 örnek toplanmıştır. Su örnekleri kültür yöntemi ve DNA izolasyonu için 0.45 µm çapında selüloz nitrat membran filtreden yaklaşık 30 saniyede 100 ml geçecek şekilde vakum cihazı ile filtrelenmiştir. Filtrelenen su örnekleri NNA-*E.coli* ve Tamponlu Kömür Maya Özütü besiyerlerine kültüre edilmiştir. Kültür sonucu pozitif olarak kabul edilen örnekler DNA izolasyonu için -20 °C dondurucuya kaldırılmıştır.

Bulgular: Toplanan 100 örneğin kültür sonuçları besiyeri ayrımı yapılmaksızın 27 (%27) *Acanthamoeba* spp. tespit edilmiştir. NNA-E. coli besiyerinde 22 (%22) BCYE besiyerinde 19 (%19) *Acanthamoeba* spp. üretilmiştir. İki besiyeri arasında anlamlı bir farklılık bulunamamıştır. RT-PCR yöntemi ile toplam 25 *Acanthamoeba* spp. saptanmıştır (%25). Besiyerlerinin RT-PCR ile uyumu kappa katsayısı

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Submitted/Başvuru: 12.11.2024 • **Revision Requested/Revizyon Talebi:** 03.02.2025 •
Last Revision Received/Son Revizyon: 17.03.2025 • **Accepted/Kabul:** 18.03.2025 • **Published Online/Online Yayın:** 17.04.2025

Samples in which *Acanthamoeba* spp. cysts were detected were evaluated based on morphological criteria. Based on the size and shape of the cysts, it was observed that the majority of the detected samples belonged to groups II and III.

Conclusion: There are few studies comparing *Acanthamoeba* growth in different culture media. There is a need to discover newer and more accessible culture media for this amoeba.

Keywords: *Acanthamoeba* spp., environment water samples, real time PCR, culture

hesaplanarak istatiksel olarak (p<0,005) anlamlı ve iki yönlü olarak kabul edilebilir bulunmuştur. *Acanthamoeba* spp. kisti saptanan örnekler morfolojik kriterlere göre değerlendirilmiştir. Kistlerin büyüklük ve şekilleri baz alınarak yapılan sınıflamada ağırlıklı olarak tespit edilen örneklerin grup II ve grup III'e ait oldukları görülmüştür.

Sonuç: *Acanthamoeba* büyümesini farklı kültür ortamlarında karşılaştıran az sayıda çalışma yapılmıştır. Bu amip için daha yeni ve daha erişilebilir kültür ortamları keşfedilmesine ihtiyaç vardır.

Anahtar kelimeler: *Acanthamoeba* spp., çevresel su örnekleri, real time PCR, kültür

INTRODUCTION

Free-living amoebae (FLA) are widely found in nature, soil, and water. Although these amoebae have a cosmopolitan distribution, they can survive in difficult conditions. Some species and genotypes of the FLA of *Acanthamoeba, Naegleria, Balamuthia* and *Sappinia* cause infections in humans (1, 2). Among FLAs, the most common are *Acanthamoeba* species, which cause infections, especially in immunocompromised individuals. The predisposing conditions include diabetes, acquired immune deficiency syndrome (AIDS), cirrhosis, human immunodeficiency virus (HIV) infection, chronic renal failure, systemic lupus erythematosus, malignancy, chemotherapy, and organ transplants, including stem cell transplantation (3, 4). *Acanthamoeba* can be found in water (tap water, well, pool, spa, dam, lake, sea), air, and soil (beach sands, potting soil). The transmission routes include inhalation of wind-borne cysts, direct skin contact, and haematogenous spread (2). It has also been reported to cause keratitis in people who use contact lenses. To date, 23 genotypes (T1-T23) of *Acanthamoeba* have been identified (5). *Acanthamoeba* spp. causes a severe brain infection known as fatal granulomatous amoebic encephalitis (GAE). This condition typically begins quietly and progresses slowly over time. The incubation period for GAE can range from several weeks to several months. Patients with GAE often present to the hospital with symptoms such as fever, headache, vomiting, seizures, changes in sensory perception, and loss of consciousness. As the disease progresses, intracranial pressure, seizures, loss of consciousness, coma, and death occur (6). Another *Acanthamoeba* infection is *Acanthamoeba* keratitis (AK), which is a rare infection of the cornea. The use of contact lenses is the leading risk factor for Acanthamoeba keratitis. Serological studies have shown that 90%-100% of individuals with no history of AK had antibodies specific for *Acanthamoeba* antigens. This indicates that exposure is common, but the disease is extremely rare (6).

The aim of the present study was to investigate the presence of *Acanthamoeba* spp. in environmental water samples collected from various cities in Türkiye using different culture and molecular methods.

MATERIALS AND METHODS

Within the scope of the study, 100 samples were collected from different sources, mostly sea water, pool water, tap water, hot springs, baths, rivers, lakes, dams and tank water, from the cities marked in Figure 1 between August 2021 and March 2023 (Figure 1). Water samples were collected in 500 ml sterile plastic bottles. The date and places

Figure 1: Cities where the samples were collected

where the samples were taken were noted on each sample. The collected samples were placed in a thermal bag inbrought to the laboratory of the Department of Parasitology in İstanbul University, İstanbul Faculty of Medicine.

Filtering of the water samples

The water samples that were brought to the Parasitology Laboratory of İstanbul University, İstanbul Faculty of Medicine in sterilised plastic bottles were filtered with a vacuumed device, passing 100 ml approximately in 30 s through a 0.45 µm diameter cellulose nitrate membrane filter (AISIMO, England) for the culturing method and DNA isolation. In order to prevent the drying of the surface of the filter, the filtering process continued until 3-5 ml of water remained on the surface. The surface of the filter membrane was divided into equal parts with a sterile scalpel, and one part was preserved at -20 °C for DNA isolation. The remaining part was divided into two equal parts and one half was placed on Non-Nutrient Agar (NNA) and the other half was placed on Buffered Charcoal Yeast Extract Agar (BCYE) and covered upside down. The edges of the Petri dishes were covered with parafilm to prevent drying. Then, the NNA-*E. coli* was incubated at 30 °C and BCYE was incubated at 37 °C. Images of melting or opening on the surface in areas covered with bacteria were considered suspicious, and the surface was scraped with a sterile scalpel, suspended in approximately 2 ml of Page Saline, vortexed and centrifuged, and then microscopically examined. The samples considered positive were stored at -20 °C for DNA isolation.

Culturing method

Three different media were used in this study. Brain heart infusion agar (BHI) was used to grow *Escherichia coli*, and NNA and Buffered charcoal - yeast extract agar (BCYE) were used to culture the environmental water samples.

Brain heart infusion agar (BHIA) (LABM – United Kingdom)

49 g of powdered BHI was added to 1000 ml of distilled water and mixed well. After adjusting the pH to 7.4±0.2, the prepared mixture was sterilised in an autoclave at 121 °C for 15 min. It was cooled to 47°C, poured into Petri dishes and allowed to dry. It was then covered with parafilm and placed at 4°C.

NNA

Page's saline

A solution was prepared by dissolving 0.12 g NaCl, 0.004 MgSO4.7H2O, 0.004 g CaCl2.2H2O, 0.142 g Na2HPO4, and 0.136 g KH2PO4 in 1000 ml distilled water, with the pH adjusted to 6.8±0.1.

NNA

The mixture was prepared by adding 15.0 g agar and 1000 ml of Page's saline, and the pH was adjusted to 7.0±0.2. The prepared mixture was sterilised in an autoclave at 121 °C for 15 min. It was then poured into Petri dishes and stored at 4 °C. When the sample was to be planted, 18-24 hours old *E.coli* (ATCC 25922) previously grown on BHIA was suspended densely in 5 ml page saline solution, then autoclaved at 121 °C for 15 min and the suspension was collected with a sterile pipette. 1 ml was taken and spread on the NNA surface with the help of a swab. It was then placed in the oven at 30 °C for 24 h to dry.

Buffered charcoal–yeast extract agar (BCYE) (BD BBL, France)

To prepare BCYE, 2.4 grammes of KOH was added to 1000 ml of distilled water and stirred to dissolve. Then, 38.3 grammes of BCYE was added and mixing continued. Heating and mixing were carried out to completely dissolve the powder. The pH was adjusted to 6.8±0.1 and the prepared mixture was sterilised in an autoclave at 121 °C for 15 min. Then, it was cooled to 45-50 °C and 4 ml of 10% filter-sterilised L-Cysteine HCl solution was added. Then, by mixing, the pH was adjusted to 6.8±0.2. Finally, it was poured into Petri dishes and left to dry. It was then covered with parafilm and placed at 4 °C.

DNA isolation of the culture samples

Isolation of amoebae cultured on NNA medium and BCYE medium was performed according to the procedure steps specified in the QIAamp® DNA Minikit QIAGEN.

Real-time polymerase chain reaction

The real-time polymerase chain reaction (RT-PCR) was performed using the *Acanthamoeba* spp. 18S ribosomal RNA (18S) gene Genesig® Standard Kit.

RESULTS

Water samples brought to the Parasitology Laboratory of İstanbul University Faculty of Medicine under appropriate conditions were filtered with a vacuum device for the culture method, and one half of the filter was inverted onto NNA-*E. coli* and the other half was placed upside down on BCYE medium. The NNA-*E. coli* was incubated at 30 °C, and BCYE was incubated at 37 °C. The NNA-*E. coli* media were incubated for up to 14 days and growth was checked at regular intervals. Since the BCYE medium provided the best growth results between 66 and 72 h and started drying after day 10, the Petri dishes were stored for 10 days. The cyst and trophozoite shapes were observed under a microscope. The examination of the culture results of 100 samples showed that *Acanthamoeba* spp. was detected in 27 water samples regardless of the medium. While 15 cysts and 7 trophozoites belonging to Acanthamoeba spp. were observed in the NNA-*E. coli* medium, 9 cysts and 10 trophozoites were detected in the BCYE medium. Cysts/trophozoites were detected in 14 samples in common in both media. Eight samples

were only detected in the NNA-*E. coli* medium, while cysts and trophozoites were observed in five samples in the BCYE medium. The BCYE medium proved to be more effective for trophozoite production.

Acanthamoeba spp. growing in NNA-*E. coli* medium and BCYE medium were statistically compared. The number of samples growing in the two media was calculated by chi-square and a significant degree of concordance was found between the NNA-*E.coli* medium and the BCYE medium (Table 1).

Acanthamoeba spp. were cultured in NNA-*E. coli* medium after identifying the presence of cysts or trophozoites in the sample. Following the passage, the cysts and trophozoites of *Acanthamoeba* were observed again. The cysts were then stained with Lactophenol cotton blue (Figure 2). As illustrated in the figure, the amoeba cyst wall was revealed and stained a darker blue than the surrounding areas.

After the examination of the NNA-*E. coli* and BCYE media, the samples with *Acanthamoeba spp.* cysts were evaluated in accordance with their morphological criteria. In the classification based on the size and shape of the cysts, the samples identified predominantly belonged to Group II and Group III.

Table 1: Comparison of the positivity of *Acanthamoeba* spp. in NNA-*E. coli* and BCYE Medium

NNA Medium/ *E.coli*	BCYE Medium				χ2	P
	No		Yes			
	n	%	n	%		
No	73	93.6	5	6.4	36.515	p<0.001
Yes	8	36.4	14	63.6		

NNA: Non-Nutrient Agar, BCYE: Buffered Charcoal Yeast Extract Agar, χ2: chi square, P: chi square p signifiance value Kappa=0.602: p<0.001, 95% CI : 0.407 and 0.797

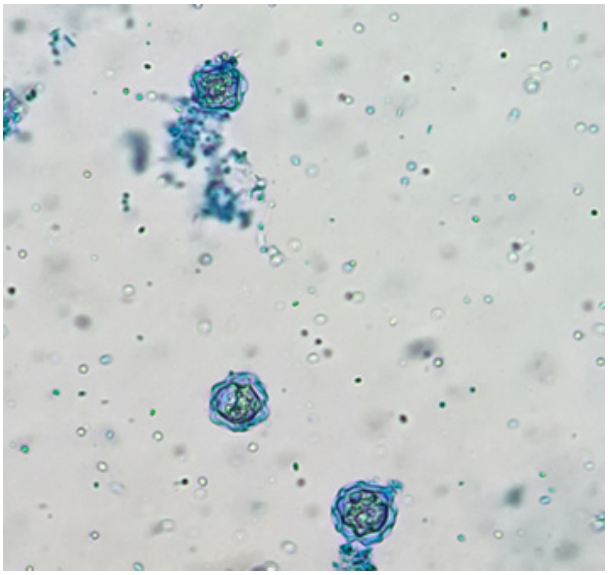

Figure 2: The *Acanthamoeba* cycsts (X40) stained with Lactophenol cotton blue in the NNA-*E. coli* medium

Table 2 shows the location-date information from which the samples were taken and the positivity used in *Acanthamoeba* spp. detection (Table 2).

RT-PCR analysis was performed after DNA isolation of the samples whose media results were observed. RT-PCR results of a total of 25 samples out of 100 were found positive.

The moderate compliance between BCYE and RT-PCR was found to be statistically significant. The compatibility of the NNA-E. coli and BCYE media with RT-PCR was evaluated by calculating the kappa coefficient. The detected coefficients showed moderate and potentially significant compliance ranging from 0.30 to 0.60 were found to be statistically significant (p<0.001) (Table 3-4).

DISCUSSION

A total of 100 water samples from various cities in Türkiye, which have intense human contact, were analysed to identify *Acanthamoeba* spp. in environmental water sources. *Acanthamoeba* spp. typically reproduced within an average of 5 days in the samples taken for NNA-*E. coli* and BCYE cultures. The presence of *Acanthamoeba* spp. was detected in 27% of the water samples using both culture methods. The NNA-*E. coli* medium (22/100) showed similar results to the BCYE medium (19/100) in the production of *Acanthamoeba*. Penland and Wilhelmus reproduced the *Pseudomonas aeruginosa* ATCC 27853, *E. aerogenes* ATCC 13048, *Stenotrophomonas maltophilia* ATCC 13637, *E. coli* ATCC 25922, *Staphyloccucus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 13883 and *Serratia marcescens* B1523 bacteria as alive and dead in non-nutrient medium, and in BCYE and TSA(rabbit blood, sheep blood, horse blood, human blood) in patient samples who were found positive for *Acanthamoeba spp.*, and found that the BCYE medium provided more successful results in the production of trophozoites as similarly with our study (7). In our study, we observed 10 trophozoites and 9 cysts of *Acanthamoeba* spp. in the BCYE medium, whereas 15 cysts and 7 trophozoites were found in the heat-killed NNA-*E. coli* medium. There are few studies comparing the growth of *Acanthamoeba* in different culture media. This suggests that new and more accessible culture media could be discovered for cultivating this amoeba.

Tawfeek et al. collected 75 environmental samples and cultured NNA-*E. coli* medium in their study aiming at the genotypic, physiological and biochemical characterisa-

Table 2: The location and date of collection of samples and the comparison of positivity of the methods used in the detection of *Acantahamoeba* spp.

Sample no	Source of the water	Type of water	Sampling date	Sampling season	NNA medium/ *E. coli*	BCYE medium	RT-PCR (C_t)
1	Mardin	Brackish	27.08.2021	Summer	-	-	-
2	Mardin	Natural spring	27.08.2021	Summer	+	-	+ 23.45
3	Mardin	Tap	27.08.2021	Summer	+	+	+ 21.34
4	Şanlıurfa	Lake	28.08.2021	Summer	+	+	+ 21.12
5	Şanlıurfa	Tap	28.08.2021	Summer	-	-	-
6	Şanlıurfa	Brackish	28.08.2021	Summer	+	+	+ 26.99
7	Şanlıurfa	Dam	28.08.2021	Summer	+	+	+ 29.32
8	Diyarbakır	Thermal	29.08.2021	Summer	+	-	+ 32.21
9	Diyarbakır	Thermal	29.08.2021	Summer	+	+	-
10	Diyarbakır	Natural waterfall	29.08.2021	Summer	+	+	-
11	Diyarbakır	Tap	29.08.2021	Summer	-	-	-
12	Diyarbakır	Stream	29.08.2021	Summer	+	+	-
13	Diyarbakır	River	29.08.2021	Summer	-	-	-
14	Diyarbakır	River	30.08.2021	Summer	-	-	-
15	Diyarbakır	River	30.08.2021	Summer	+	+	-
16	Diyarbakır	Natural spring	30.08.2021	Summer	-	-	-
17	Diyarbakır	Dam	30.08.2021	Summer	-	-	-
18	Diyarbakır	Tap	31.08.2021	Summer	-	-	-
19	Diyarbakır	Tap	31.08.2021	Summer	-	-	-
20	Diyarbakır	Tap	31.08.2021	Summer	-	-	-
21	Diyarbakır	Swimming pool	31.08.2021	Summer	-	-	-
22	Diyarbakır	Tap	31.08.2021	Summer	-	-	-
23	Eskişehir	Tap	25.09.2021	Autumn	-	-	-
24	Eskişehir	Tap	25.09.2021	Autumn	-	-	-
25	Eskişehir	Stream	25.09.2021	Autumn	-	-	-
26	Eskişehir	Bath	25.09.2021	Autumn	-	+	-
27	Antalya	Sea	27.06.2022	Summer	+	+	-
28	Tokat	Tap	22.08.2022	Summer	-	-	-
29	Tokat	Tap	22.08.2022	Summer	-	-	-
30	Tokat	Natural spring	22.08.2022	Summer	-	-	-
31	Afyonkarahisar	Thermal	02.04.2022	Spring	-	-	-
32	Manisa	Tap	03.04.22	Spring	-	-	-
33	Zonguldak	Natural spring	26.08.2022	Summer	-	-	-
34	Zonguldak	Earthy	26.08.2022	Summer	-	-	-
35	Diyarbakır	Earthy	30.08.2021	Summer	-	+	-
36	Bolu	Tap	08.09.2022	Autumn	-	-	-
37	Bolu	Tap	08.09.2022	Autumn	-	-	-
38	Bursa	Tap	18.09.2021	Autumn	-	-	-

Table 2: Continue

Sample no	Source of the sample	Type of water	Sampling date	Sampling season	NNA medium/*E. coli*	BCYE medium	RT-PCR (C_t)
39	İstanbul	Hospital tap	22.09.2022	Autumn	-	-	-
40	İstanbul	Tap	07.10.2022	Autumn	-	-	-
41	İstanbul	Hospital tap	10.10.2022	Autumn	-	-	-
42	İstanbul	Hospital tank	10.10.2022	Autumn	+	-	-
43	İstanbul	Hospital tank	10.10.2022	Autumn	-	-	-
44	İstanbul	Drain	10.10.2022	Autumn	-	-	-
45	İstanbul	Potted	13.10.2022	Autumn	+	+	+ 32.39
46	İstanbul	Potted	13.10.2022	Autumn	+	-	+ 20.86
47	İstanbul	Bath	25.10.2022	Autumn	+	+	-
48	İstanbul	Bath	25.10.2022	Autumn	+	-	+ 38.45
49	İstanbul	Tap	25.10.2022	Autumn	-	-	-
50	Van	Lake	10.08.2022	Summer	-	-	-
51	Bitlis	Natural spring	14.08.2022	Summer	-	-	-
52	Siirt	Tap	14.08.2022	Summer	-	-	-
53	Diyarbakır	Tap	02.09.2022	Autumn	-	-	-
54	Diyarbakır	Natural spring	02.09.2022	Autumn	-	-	-
55	Elazığ	Natural spring	06.11.2022	Autumn	-	-	-
56	Elazığ	Lake	06.11.2022	Autumn	-	-	-
57	Malatya	Tap	06.11.2022	Autumn	-	-	-
58	Malatya	Tap	06.11.2022	Autumn	-	-	-
59	İstanbul	Artificial waterfall	26.10.2022	Autumn	-	-	+ 27.65
60	İstanbul	Artificial waterfall	19.11.2022	Autumn	+	+	+ 17.10
61	İstanbul	Potted	20.12.2022	Winter	+	+	+ 30.79
62	İstanbul	Fountain	20.12.2022	Winter	+	-	+ 29.22
63	İstanbul	Fountain	20.12.2022	Winter	-	-	+ 33.77
64	Denizli	Tap	20.12.2022	Winter	-	-	+ 29,67
65	İstanbul	Artificial	04.01.2023	Winter	-	-	+ 30.82
66	İstanbul	Artificial stream	04.01.2023	Winter	-	-	+ 31.73
67	İstanbul	Swimming poll	04.01.2023	Winter	-	-	+ 30.36
68	İstanbul	Brackish	09.01.2023	Winter	-	-	-
69	İstanbul	Brackish	09.01.2023	Winter	-	-	-
70	İstanbul	Tank	10.01.2023	Winter	-	-	-
71	İstanbul	Potted	23.01.2023	Winter	-	-	-
72	İstanbul	Bath	23.01.2023	Winter	-	-	-
73	İstanbul	Bath	23.01.2023	Winter	-	-	-
74	İstanbul	Sea	24.01.2023	Winter	-	-	+ 29.76
75	Ankara	Thermal	28.01.2023	Winter	-	-	-
76	Ankara	Thermal	28.01.2023	Winter	-	-	-
77	Ankara	Thermal	28.01.2023	Winter	-	-	-

Table 2: Continue

Sample no	Source of the sample	Type of water	Sampling date	Sampling season	NNA medium/*E. coli*	BCYE medium	RT-PCR (C_t)
78	Ankara	Tap	28.01.2023	Winter	-	-	-
79	İstanbul	Sea	26.02.2023	Winter	-	-	-
80	İstanbul	Sea	26.02.2023	Winter	-	-	-
81	Bolu	Tap	29.01.2023	Winter	-	-	-
82	İstanbul	Lake	26.02.2023	Winter	-	-	-
83	İstanbul	Lake	26.02.2023	Winter	-	-	-
84	İstanbul	Sea	26.02.2023	Winter	-	+	-
85	İstanbul	Sea	26.02.2023	Winter	-	+	+ 38.81
86	İstanbul	Potted	01.03.2023	Spring	-	+	+ 34.82
87	İstanbul	Potted	01.03.2023	Spring	-	-	+ 31.48
88	İstanbul	Potted	01.03.2023	Spring	+	-	+ 29.89
89	İstanbul	Stream	01.03.2023	Spring	+	-	-
90	İstanbul	Ornamental pool	01.03.2023	Spring	-	-	-
91	İstanbul	Swimming pool	14.03.2023	Spring	-	-	-
92	İstanbul	Swimming pool	14.03.2023	Spring	+	+	+ 20.91
93	İstanbul	Ornamental pool	15.03.2023	Spring	-	-	+ 36.01
94	İstanbul	Tap	15.03.2023	Spring	-	-	-
95	İstanbul	Tap	15.03.2023	Spring	-	-	-
96	İstanbul	Bath	15.03.2023	Spring	-	-	-
97	İstanbul	Bath	15.03.2023	Spring	-	-	-
98	İstanbul	Bath	15.03.2023	Spring	-	-	-
99	İstanbul	Ornamental pool	15.03.2023	Spring	-	-	-
100	İstanbul	Tap	15.03.2023	Spring	-	-	-

NNA: Non-Nutrient Agar, BCYE: Buffered Charcoal – Yeast Extract Agar, RT-PCR, (C_t): Real-time polymerase chain reaction (cycle threshold)

Table 3: Comparison of the NNA-*E. coli* and RT-PCR positivity in the detection of *Acanthamoeba* spp.

	NNA-*E.coli*		χ^2	p
RT-PCR	**No**	**Yes**		
No	67	11	22.4	p<0.001
Yes	8	14		
Total	75	25		

NNA: Non-Nutrient Agar, RT-PCR: The real-time polymerase chain reaction, $\chi 2$: chi square, P: chi square p signifiance value Kappa=0.472, p<0.001; 95 % CI 0.270 and 0.675. The compliance between NNA-*E. coli* and RT-PCR was mostly found to be significant

Table 4: Comparison of the BCYE and RT-PCR positivity in the detection of *Acanthamoeba* spp.

	BCYE		χ^2	p
RT-PCR	**No**	**Yes**		
No	66	9	9.5	p<0.002
Yes	15	10		
Total	81	19		

BCYE: Buffered Charcoal – Yeast Extract Agar, RT-PCR: The real-time polymerase chain reaction, $\chi 2$: chi square, P: Chi square, p signifiance value Kappa=0.304, p<0.001, 95% CI 0.089 and 0.519

tion of potentially pathogenic *Acanthamoeba* isolated from the environment in Cairo, Egypt. *Acanthamoeba* spp. was found in 11 (31.4%) of the 35 water samples collected in this study (8). Similarly, in our study, a total of 27% culture-positive results were obtained. In Iran, Mahmudi and his colleagues randomly collected 80 water samples and 20 soil samples from public parks in the districts of Guilan province between May and June 2019, and detected *Acanthamoeba* in 40 (50%) of the 80 water samples (9). Since environmental sources are an important potential risk factor for human infection, examining the prevalence of *Acanthamoeba* in different environments

can be helpful for the control and prevention of the disease in humans. There are differences in the prevalence of *Acanthamoeba* spp., found in many places in the environment. The reason is considered the conditions such as water temperature and climatic conditions in countries and regions with different geographical locations.

In their study, Hajialilo et al. examined 138 corneal scrapings, contact lens samples, and equipment to isolate and genotype *Acanthamoeba* strains from patients with amoebic keratitis in Iran in 2016. They investigated all clinical samples using both direct microscopy and culturing methods. Among the samples, they classified them based on morphology and predominantly detected cysts belonging to Group II. However, in one isolate, they found a cyst of *Acanthamoeba* spp. belonging to Group I morphology (10).

In another study by Al-Herrawy et al., the presence of *Acanthamoeba* species was investigated in the Damanhour drinking water treatment plant in Egypt. Of the 48 water samples analysed, 12 (25%) were positive for *Acanthamoeba*. The results revealed *Acanthamoeba* spp. from Group I, Group II, and Group III in the morphologically classified samples. In our study, which examined 100 different environmental water samples, the morphological classification of 27 *Acanthamoeba* spp. indicated that they primarily belonged to Group II and Group III (11).

In their study, Milanez et al. examined *Acanthamoeba* spp. isolated from 63 drinking water systems in the Philippines from both epidemiological and molecular perspectives. They found a positivity rate of 14.28% (9 out of 63) in the NNA-*E. coli* medium, and the cultures were positive using *Acanthamoeba*-specific primers. Additionally, all samples tested (9 out of 9) were confirmed positive through molecular methods (12).

Meanwhile, Karimi and colleagues investigated the identification and genotyping of *Acanthamoeba* spp. in water resources in Western Iran. They collected and cultured 72 water samples, subsequently performing PCR. They discovered that all 72 samples (100%) were positive for free-living amoebae (FLA) through culture methods. However, only 17 samples (23.6%) tested positive according to the PCR results used to diagnose and identify *Acanthamoeba* from the culture samples (13). In our study, we detected culture positivity in 27 out of 100 samples, while RT-PCR results confirmed that 25 samples were positive for *Acanthamoeba* spp. Additionally, 9 out of 73 samples that did not yield positive results in culture were found to be positive through RT-PCR. Based on our findings, although the culture method is time-consuming, it remains the gold standard for detecting *Acanthamoeba* spp. The discrepancy between the culture and RT-PCR results may be because the cultured samples examined under the microscope could be Vermamoeba spp. (formerly known as Hartmannella), which have morphological similarities to *Acanthamoeba* spp. It is also worth noting that *Acanthamoeba* species exhibit numerous genotypes, with new variants continuously being discovered.

Acanthamoeba spp. are found in regions with various climatic characteristics and water resources, and their presence can be influenced by seasonal changes. In a study by Kao et al., the researchers evaluated the presence of *Acanthamoeba* spp. in the Puzih River basin in Taiwan. They collected 136 water samples between July 2009 and March 2010, spanning all four seasons. *Acanthamoeba* spp. was detected in 16 of 136 samples, representing a prevalence of 11.7%.

Seasonally, the findings showed *that Acanthamoeba* spp. was identified in the following proportions: spring (2.9%), summer (32.4%), autumn (2.9%), and winter (8.8%). The organism was most frequently detected during the summer months. In our study, which involved culturing samples collected across the four seasons, *Acanthamoeba* spp. was primarily detected in the summer, yielding 12 positive culture results.

CONCLUSION

In conclusion, 100 different water samples were cultured using NNA-*E. coli* and BCYE media. *Acanthamoeba* spp. were detected in 22 samples (22%) from the NNA-*E. coli* medium and in 22 samples (19%) from the BCYE medium. No significant difference was found between the two types of media. A total of 25 *Acanthamoeba* spp. were identified using the RT-PCR method, representing 25% of the samples. The compatibility of the media with RT-PCR showed a statistically significant result ($p<0.005$) and was considered bidirectionally acceptable when the kappa coefficient was calculated. There are few studies comparing the growth of *Acanthamoeba* in different culture media, highlighting the need to discover novel and more accessible culture options for this amoeba.

Ethics Committee Approval: Since this study was conducted on environmental water samples, ethics committee approval was not obtained.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Ö.B., Ş.E.; Data Acquisition- Ş.E., Ö.B.; Data Analysis/Interpretation – H.İ., Ö.B., Ş.E.; Drafting Manuscript- Ş.E., Ö.B.; Critical Revision of Manuscript- Ö.B., H.İ., Ş.E.; Final Approval and Accountability- Ş.E., Ö.B., H.İ.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study received no financial support.

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