

ORIGINAL ARTICLE

Isolation, identification of pathogenic *Acanthamoeba* from drinking and recreational water sources in Saudi Arabia

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ABSTRACT

Objective: The present study was conducted to isolate and identify the *Acanthamoeba* species from various water sources such as drinking water, tap water, swimming pool, and other recreational water.

Materials and methods: During the study period, 57 water samples were collected from various sources such as tap water, drinking water, swimming pool, and recreational water. All samples were processed and cultured on non-nutrient agar medium (NNA) with *Escherichia coli* overlay for the isolation of *Acanthamoeba* species. Organism identified based on the microscopic morphology of cyst and trophozoites forms. The pathogenicity of *Acanthamoeba* was analyzed by thermotolerance and osmotolerance assays.

Results: *Acanthamoeba* were detected in 10 out of 57 (17.5%) examined water samples. The high percentage of positivity was observed in bore well water stored in tanks (37.5%) and in recreational water samples (26.7%). All processed drinking water samples were free from *Acanthamoeba*. Based on pathogenicity test assays, four (40%) were pathogenic and three (30%) were non-pathogenic. The observed frequency of *Acanthamoeba* spp. was compared with available literature worldwide.

Conclusion: This study is the first report showing the distribution of *Acanthamoeba* in various water sources in the central region of Saudi Arabia and confirms that the high percentage presence of pathogenic strains in recreational water could threat contact lens wearers. Further research works are required to identify the prevalence of pathogenic *Acanthamoeba* from various water sources in Saudi Arabia.

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Introduction

Acanthamoeba, an opportunistic protozoan pathogen, is omnipresent in nature. They can be present in air, soil, dust, drinking water, sea water, and recreational water (home aquaria, swimming pools) sources [1–3]. Scientists have been analyzed the presence of *Acanthamoeba* in many aquatic environmental samples which are frequently exposed to human [4]. *Acanthamoeba* has adapted to resist harsh environmental conditions by changing their phenotype (infective trophozoites form and resistant cyst form) [5]. *Acanthamoeba* species can cause nasopharyngeal and skin infections and some pathogenic strains cause serious human diseases such as corneal ulcers and granulomatous

amebic encephalitis [6,7]. The presence of *Acanthamoeba* in water samples was considered as a double danger since some of these are pathogenic and could harbor pathogenic strains of *Helicobacter*, *Pseudomonas*, and *Legionella* [8].

Saudi Arabia is the largest country in the Arabian Peninsula and is one of the driest regions in the world with no perennial rivers. However, the government provides water to industrial, municipal water consumption via desalination plants and groundwater sources [9]. Saudis frequently visit swimming pools and access recreational water parks during the summers. Even though increasing health-related problems over this parasite, there is still a lack of knowledge about scientific studies regarding

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prevalence and pathogenicity of *Acanthamoeba* in various recreational water systems in Saudi Arabia. Thus, the aim of this project is to examine, isolate, and identify the pathogenicity of *Acanthamoeba* from various water sources, especially drinking water, swimming pool water in various cities of the central region of Saudi Arabia.

Materials and Methods

Samples and sampling sites

Water samples were collected from different sources such as tap water, drinking water from houses, public swimming pools, and recreational water (artificial fountains and bore well water stored in a tank from dates farms) in Al Zulfi, Saudi Arabia, from April 2017 to March 2018. Each sample consists of two 500 ml of samples which were collected in sterile tubes and labeled with sampling details of date, time, and place of collection. All these collected samples were examined in the microbiology laboratory unit at College of Science Zulfi, Saudi Arabia.

Isolation and identification of *Acanthamoeba*

Each water sample was filtered using multiple folded sterile gauze to remove dirt and mud. Each filtrate was centrifuged at $250 \times g$ for 20 min. The supernatant discarded and sediments were dissolved in Page's Amoeba saline solution [10]. The mixed suspension was inoculated into Petridish containing 1.5% non-nutrient agar (NNA) overlaid with *Escherichia coli* (ATCC 25922) culture. All the plates were sealed tightly and incubated at 30°C for the duration of up to 2 weeks. Duplicate tests were done for each sample. All the processed culture plates were observed under the microscope on daily basis to check for the growth of trophozoites and cysts. Simultaneously, quality control strain of *A. castellanii* (ATCC 50492, keratitis, India) was examined microscopically to compare the growth of trophozoites and cysts. This is considered as a positive control of media and test methods. *Acanthamoeba* spp. was identified based on the size and morphological characteristics of both trophozoites and cysts. The presence of finger-like tapering pseudopodia was observed in trophozoites, cysts would appear as an inner wall of 10 polygonal and wrinkled outer wall [10]. The culture-positive samples were recorded and tabulated.

Pathogenicity assays

All culture-positive *Acanthamoeba* strains were selected for osmotolerance and thermotolerance assays as described by Caumo et al. (10). The positive growth media was washed with sterile phosphate buffered saline (pH 7.2) by gently scraping the surface of the agar and the content was

centrifuged for the collection of the *Acanthamoeba* trophozoites or cysts.

Osmotolerance assays

Trophozoites of *Acanthamoeba* (10^3 /plate) were transferred to the center of freshly prepared 1.5% NNA containing 0.5M, 1M concentrations of mannitol, and no mannitol as considered a control. *E.coli* suspension overlaid to each plate and incubated at 30°C for the maximum of 2 weeks. At day 7–9, the plates were observed under a microscope ($100\times$ magnification) and number of trophozoites or cysts were counted in the middle region of the cultured plate. Results of pathogenicity were scored based on the number of counts, zero count (–, non-pathogenic), 1–15 (+), 16–30 (++) , and >30 (+++).

Thermotolerance assays

For thermotolerance assay, all samples were processed as per the same protocol followed osmotolerance assay and incubated at various temperature 30°C (control), 37°C , and 42°C for 14 days. The results were recorded based on the growth at the end of the incubation period. Growth of trophozoites or cysts was scored as “positive,” and no growth was recorded as “negative.” Pathogenicity characterized by based on the assays scores, pathogenic considered as positive results obtained in both thermo and osmotolerance assays. Positive result in either one assay was considered as low pathogenic and negative results in both assays were considered as non-pathogenic.

Results

The study included 57 samples that were collected from different localities of Al Zulfi, Saudi Arabia. Ten out of the 57 samples were observed positive growth for *Acanthamoeba* spp. based on microscopic morphology characterization (Fig. 1). *Acanthamoeba* spp. were found in the high frequency of 37.5% in bore well water stored in tanks and 26.7% in recreational water samples. All processed drinking water samples were free from *Acanthamoeba*. The frequency of *Acanthamoeba* spp. in other samples was listed in Table 1. Based on pathogenicity test assays among 10 positive cultures, four (40%) were pathogenic and three (30%) were non-pathogenic. The results and scores of thermotolerance and osmotolerance assays were summarized in Table 2. Overall, the prevalence of the present study results was compared with available reports in worldwide listed in Table 3.

Discussion

Acanthamoeba research has been published by many authors in various countries and analyzed with various

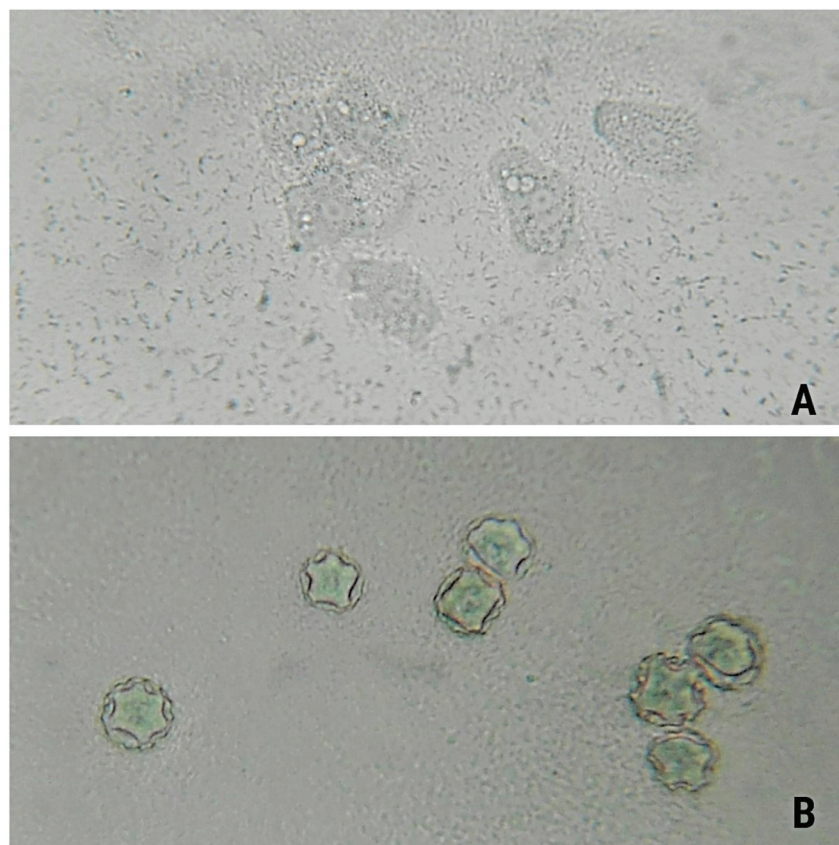


Figure 1. Non-nutrient agar culture positive for *Acanthamoeba* trophozoites (A) and cysts (B) samples from swimming pool.

Table 1. Frequency of *Acanthamoeba* spp. in various water sources.

Sample type	Number of samples processed	Number of <i>Acanthamoeba</i> positive samples in NNA culture	%
Tap water	10	1	10
Drinking water	12	0	0
Swimming pool	12	2	16.7
Recreational water (samples from water fountain, artificial waterfalls at parks)	15	4	26.7
Borewell water (Stored tanks at date farms)	8	3	37.5
Total	57	10	17.5

types of water samples. But, in the largest gulf country of Saudi Arabia, it has been not well studied even though they have many water-based recreational places such as parks, theme parks, and roadside artificial falls. Considering the ubiquitous nature of this organism, this study analyzed various water samples using conventional culture methods to isolate *Acanthamoeba* spp. In this present study, *Acanthamoeba* was found in 10% of tap water and none in drinking water. This is very similar to the report by Winck et al. [14] and they found that 9.5% from the tap

water sources in Brazil. Indeed, some studies reported that higher occurrences of 26.9% in the UK [20], 29.9% in Egypt [12], 92% in drinking water samples from various sources collected from the Karachi city, Pakistan [3]. This showed that Saudi Arabia is providing good water facility throughout the Al Zulfi city. All drinking water systems of study location are properly filtered and purified. The majority of the drinking water system is maintained by the refrigerated system and maintains 2°C to 8°C. This high quality of raw water may be influencing a very low

Table 2. Thermo and osmotolerance assays of isolated *Acanthamoeba* spp. and their pathogenicity.

Isolate identification number	Growth scores				Pathogenicity
	At 37°C	At 42°C	0.5 M Mannitol	1.0 M Mannitol	
RW1	+++	+++	++	-	Low pathogenic
RW2	+++	+++	+++	++	Pathogenic
RW3	+++	-	+	-	Non pathogenic
RW4	+++	++	++	-	Low pathogenic
TW 1	+++	++	+	-	Non pathogenic
SW1	+++	-	+	-	Low pathogenic
SW2	+++	+++	+++	++	Pathogenic
BW1	+++	-	++	-	Non pathogenic
BW2	+++	++	++	+	Pathogenic
BW3	+++	+++	+++	+	Pathogenic
QC	+++	-	++	-	Non pathogenic

Table 3. Prevalence of *Acanthamoeba* in different types of water analyzed worldwide.

Country	Type of water source	Prevalence of <i>Acanthamoeba</i>	Reference
Egypt	Swimming pools	60%	[11]
	Tap water	29.9%	[12]
Poland	Swimming pools	37.2%	[1]
USA, Oklahoma	Pond, running water	63%	[13]
USA	James river water	7%	[4]
Brazil, Porto alegre	Swimming pools	20%	[10]
Brazil, Rio Grande do sul	Tap water	9.5%	[14]
Jamaica	Tap water	59.5%	[15]
	Sea water	40%	
Korea	Domestic tap water	46.9%	[16]
Thailand	River water	35%	[17]
Saudi Arabia	River water	36.7%	[18]
Turkey	Tap water	22%	[19]
Pakistan	Drinking water	92%	[3]
Saudi Arabia	Various water samples	17.5%	Present study

percentage of occurrence of *Acanthamoeba* comparing with other reports.

In this study, we found that 16.7% of the sample showed a positive growth in swimming pool samples. This is comparable with another study from Saudi Arabia [21] and Brazil and they reported 20% [10]. However, reports from Egypt and Poland showed a high prevalence in swimming pool water samples [1,11]. The major variation observed in different studies appears due to *Acanthamoeba* contamination introduced into water in soil by humans and improper maintenance and disinfection protocol in

swimming pools. *Acanthamoeba* resistance to chlorination may also be considered as another reason for variation of prevalence.

Concerning isolation of *Acanthamoeba* spp. from the recreational water sources, in the present study, samples were collected from the water fountains located at family parks and artificial waterfalls at roadsides. Interestingly, we found that 26.7% of samples were positive for *Acanthamoeba* spp. Nevertheless, *Acanthamoeba* has been isolated from different sources such as sewage, air conditioning unit, borings, well, pond, canals, and

rivers [3,13,17,19]; so far none of the reports analyzed water from the artificial fountains and falls. Hence, comparing the present study findings with other reports is difficult; however, this will give a piece of informations in the presence of *Acanthamoeba* in different geographical locations. Finally, the bore well water stored in tanks at dates farms have a high percentage of *Acanthamoeba* spp. This can explain that tanks were kept open for long days and muddy soil can easily contaminate; hence, this sample showed a higher percentage of prevalence 37.5%.

Concerning pathogenicity of *Acanthamoeba* spp., a report from Jamaica showed that pathogenic amoebae were found in the range between 26% and 49% in various water sources, including river, sea, and tap water [15]. Based on exhibiting growth at 40°C and 1 M mannitol, in this study, it showed 40% of culture positives were highly pathogenic and 30% were non-pathogenic. Majority of the isolated *Acanthamoeba* strains (7 out of 10) were thermotolerant and grown at 42°C. Since study center is a place of the central region of Saudi Arabia of extreme hot temperature during summer and cold temperature in winter season throughout the year, thus cysts of *Acanthamoeba* spp. are tolerant to ecological conditions. Furthermore, the minimal cysticidal temperature for most *Acanthamoeba* spp. is greater than 65°C and it can tolerate dehydration condition for 20 years and above [22]. As per literature, the presence of *Acanthamoeba* in the water samples does not mean a risk factor for illness to human and animals, even though some of the strains are potentially pathogenic such as *A.castellanii*, *A. polyphaga*, and *A. culbertsoni* [6]. Conversely, the presence of pathogenic *Acanthamoeba* in water with high percentage could cause a sanitary risk for public health, particularly immunocompromised people and contact lens wearers. Because amoebal cysts are found in recreational water, it can easily spread to environment and transport through the air in aerosol as well as in dry forms. There is a high chance of transmitting cysts during strong winds and dust storm events, which is a frequent occurrence in Saudi Arabia. Thus, *Acanthamoeba* can enter into the eye via contact lenses or through a corneal cut and cause Acanthamoebic keratitis (AK) after exposure to contaminated water and aerosols. Considering these facts, this study also found that pathogenic, low pathogenic strains of *Acanthamoeba* were distributed in all types of samples (except drinking water) processed. Hence, the present study recommends that contact lens wearers can have a significant increase in the risk of infection from *Acanthamoeba* while taking bath in swimming pools and showering in recreational water of artificial falls. Since many studies suggested that *Acanthamoeba* in tap water sources would increase the incidence of AK in contact lens users and contact lens

cleaning solution contamination [16,20]. However, there are no AK reports available due to the exposure of recreational water and very low incidence of AK reported in Saudi Arabia compared to Western countries [23]. Despite this, swimming pool authorities should alarm public to give awareness by showing signboards and posters about *Acanthamoeba* exposure to control AK infection by a proactive approach.

Lastly, by reviewing the frequency of *Acanthamoeba* reports worldwide, the present study showed a very low percentage of positivity comparing USA, Egypt, Jamaica, and Pakistan. However, the major variations of the frequency of *Acanthamoeba* obtained in different studies appear to be due to several ecological conditions and methodology used for isolation and identification. In this present study, culture-based method is used for organism identification; however, many studies used molecular-based methods [3,6,19]. To our knowledge, this is the first report examining recreational water in this country and need more studies to find out the prevalence and distribution of pathogenic *Acanthamoeba* in various sources.

Conclusion

In conclusion, a high frequency of *Acanthamoeba* spp. isolated in recreational water of artificial fountains, falls and bore well water in the farms representing a sanitary risk in aquatic sources. The present study is the very first report showing the distribution of *Acanthamoeba* spp. in various water sources from the Al-Zulfi region, the central region of Saudi Arabia. This study recommends that awareness of the prevalence of *Acanthamoeba* in various water sources which public used to access in their routine lifestyle. Finally, further research works are required to know about the microbial ecology, biogeography, and spread of pathogenic *Acanthamoeba* in water sources.

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Conflict of Interest

There is no conflict of interest.

Author Contributions

Rajendran Vijayakumar is the corresponding author. He designed the research plan, collected samples, performed laboratory analysis, and wrote the manuscript.

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