

Distribution of *Acanthamoeba* genotypes isolated from recreational water resources: a review

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ABSTRACT

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Acanthamoeba spp. are ubiquitously living in the environment of both natural and man-made. They are pathogenically known to cause blinding keratitis and fatal encephalitis in humans. The severity of *Acanthamoeba* infection can be life-threatening although it is rarely reported. *Acanthamoeba* spp. have been widely isolated from various environmental sources, including recreational waters, such as hot springs, rivers, swimming pools, and seawater. The presence of pathogenic *Acanthamoeba* spp. in these resources is significant as many people visit these places for recreational activities. They can be possibly exposed to the infection. Genotype T4 is known as the most pathogenic and prevalent as it has been identified from a variety of sources, including environmental and clinical samples. It also has the highest association with human infections, followed by T5. *Acanthamoeba* spp. can be identified by using culture and molecular techniques, which are polymerase chain reaction and gene sequencing. This review provided an overview of the distribution of *Acanthamoeba* genotypes from recreational waters across the world from available studies, which was aimed to describe the prevalence of genotypes isolated. Further, this review also described the genotypes associated with *Acanthamoeba* spp. infections, which are keratitis and encephalitis.

Keywords: *Acanthamoeba* spp.; genotypes; environment; recreational water; gene sequencing

1. INTRODUCTION

Acanthamoeba spp. are free-living amoebae that are known to cause life-threatening diseases to humans, which are *Acanthamoeba* keratitis (AK) and fatal granulomatous amoebic encephalitis (GAE) (Khan, 2006). They are omnipresent in the environment and have been isolated from various sources such as soils, lakes, swimming pools, sewage, seawater, domestic water supply, air-conditioners, and contact lens equipment (Chan et al., 2011; Siddiqui and Khan, 2012; Ghani et al., 2013; Sente et al., 2016; Gabriel et al., 2019; Hussain et al., 2019). This amoeba has

two stages, which are trophozoite and cyst. According to Pussard and Pons (1977), *Acanthamoeba* spp. are divided into three groups (I, II, and III), based on the morphological shape and size of the cyst. Its morphological identification can be made by culturing the amoeba on non-nutritive agar (NNA) enriched with *Escherichia coli*. Direct microscopy with several stains is used to enhance and identify the amoeba. However, morphological identification for species identification is not accurate. Therefore, a molecular technique to identify the genotypes and species of *Acanthamoeba* has been widely used, which is the gene sequencing of 18S ribosomal RNA (rRNA).

Identification of *Acanthamoeba* species and genotypes using the full length of 18S rRNA consists of long base pairs (bp), approximately ~2000 bp, which are subsequently made to a small region of the gene (Corsaro et al., 2017). This rRNA subunit (*Rns*) consists of the *Acanthamoeba*-specific amplimer (ASA.S1) region with 423-551 bp fragments, which contain a high variable sequence called diagnostic fragment 3 (DF3) (Schroeder et al., 2001; Booton et al., 2002). The molecular technique is commonly used as a diagnostic method for the genotyping of *Acanthamoeba* by using the ASA.S1 region. As of now, researchers had identified and classified 21 genotypes (T1-T21) of *Acanthamoeba* spp. (Fuerst, 2014; Corsaro et al., 2017).

T4 was reported to be the most prevalent genotype isolated from human-infected clinical samples and environmental samples (Booton et al., 2005; Hussain et al., 2019; Prithiviraj et al., 2020). Other genotypes such as T2, T3, T5, T11, T12, and T13 have also been associated with AK (Risler et al., 2013; Grün et al., 2014; Prithiviraj et al., 2020), whereas several genotypes such as T1, T2, T5, T10, T12, and T18 have been associated with GAE (Khan, 2006; Walochnik et al., 2008; Lackner et al., 2010; Qvarnstrom et al., 2013; Matsui et al., 2018). Such infection-associated genotypes have also been identified to be isolated from the environment.

Nowadays, *Acanthamoeba* spp. infection has risen across the world but rarely reported. A majority of the public frequently spend their leisure time at recreational water resources for recreational activities. Thus, they are extremely likely to get infected by the contaminated water consisting of *Acanthamoeba* spp. Corneal trauma due to injury or skin lesions may provide an entrance for the amoeba. The public awareness about the *Acanthamoeba* spp. infection is still lacking and needs improvement. The source of infection needs to be identified to prevent the infection. This is necessary as it can help the doctors to diagnose the infection and treat it properly. In addition, the authorities should be alerted on the prevalence of *Acanthamoeba* spp. from recreational water resources to maintain a safe environment for the public.

Hence, this review aims to describe and provide the prevalence of *Acanthamoeba* genotypes as well as their distribution worldwide, isolated from recreational water resources. This review also discussed the association of *Acanthamoeba* genotypes with AK and GAE infections.

2. ACANTHAMOEBA SPP.

Acanthamoeba spp. are pathogenic free-living amoebae that are commonly distributed in the environment. They are known as amphizoic amoebae as they can live inside a host or in the environment as 'free-living' (Abdul Majid et al., 2017). They have two stages in their life cycle which are vegetative trophozoite and dormant cyst. The trophozoite has a diameter of around 25 - 40 μm , with the inclusion of acanthapodia, a spine-like structure. The acanthapodia structure enables the amoeba to feed on other microorganisms and act as locomotion (Marciano-cabral and Cabral, 2003). Meanwhile, the size of the cyst is approximately 13 - 20 μm . The cyst possesses a double wall that helps it to withstand harsh conditions such as temperature and pH changes, food scarcity, and osmolarity imbalance (Khan, 2006). The species of *Acanthamoeba* cyst can be divided into three, I, II, and III, based on the morphological size and

shape of the cyst (Pussard and Pons, 1977). The cyst size for Group I exceeds 18 μm , which is greater than Group II and Group III. Group I has astral endocyst with smooth or wrinkled ectocyst, while the morphological shape of Group II can be polyhedral or globular endocyst with wrinkled ectocyst. Group III depicts round or ovoid endocyst with thin, smooth, or wavy ectocyst which can be used to distinguish the species from Group II.

The *Acanthamoeba* spp. can serve as hosts that allow other microorganisms to grow and live within them, including *Legionella* spp., *Chlamydialis*, *Rickettsialis*, *Mycobacterium avium*, *Mycobacterium bovis*, *Cryptococcus neoformans*, and adenoviruses (Steenbergen et al., 2001; Ben Salah and Drancourt, 2010; Scheid and Schwarzenberger, 2012). Furthermore, they can be a host for pathogenic bacteria as they are highly resistant and viable.

3. ACANTHAMOEBA GENOTYPES

Castellani (1930) had first identified the presence of *Acanthamoeba* spp. where he found the amoeba in a culture of *Cryptococcus pararoseus* fungus. They were classified into *Hartmannella* genus before Volkonsky (1931) put them into *Acanthamoeba* genus. According to Visvesvara et al. (2007), over 24 species of *Acanthamoeba* had been grouped based on the morphological criteria of cyst defined by Pussard and Pons (1977). Nevertheless, it can be inaccurate to classify the species of *Acanthamoeba* by morphological criteria only, as the cyst varies depending on the conditions of culture (Marciano-cabral and Cabral, 2003).

Today, molecular methods have been frequently used in identifying the genotypes and species of *Acanthamoeba*, as they are more precise and reliable. The identification of *Acanthamoeba* species is done by using the sequencing of 18S rRNA genes. Currently, the genus had been categorized into 21 distinct genotypes (T1-T21) (Fuerst, 2014; Corsaro et al., 2017). For each genotype, it has 5% or more divergent sequence between the diverse genotypes (Siddiqui and Khan, 2012). Furthermore, the identification of the amoeba can be further analyzed by molecular phylogeny analysis, which allows the existing database to cluster the *Acanthamoeba* species and genotypes. Several studies reported that most of the clinical cases involving AK or GAE are primarily associated with genotype T4, followed by other genotypes such as T2, T3, T6, T11, T12, T13, and T15 (Risler et al., 2013; Omaña-molina et al., 2016; Prithiviraj et al., 2020). T4 is also the most common genotype found in the environment. Other rare genotypes such as T8, T9, and T14 are usually not considered pathogenic and can be found in the environment. The *Acanthamoeba* species can be monophyletic or polyphyletic, which means that certain species can be identified in several different genotypes (Corsaro et al., 2017). This genotype system helps in recording the occurrence of the strains in the environment or clinical samples, as well as for an epidemiological purpose (Maciver et al., 2013).

4. EPIDEMIOLOGY OF ACANTHAMOEBA SPP. FROM RECREATIONAL WATER RESOURCES

Acanthamoeba spp. are known to be ubiquitously distributed worldwide and cause infection to humans such as AK and

GAE. They are found to be isolated from diverse sources, including recreational water resources. These recreational waters include both treated and untreated water. As shown in Table 1, several researchers had isolated *Acanthamoeba* spp. from recreational water resources and identified their genotypes. Most of the *Acanthamoeba* spp. from these studies were isolated from hot springs, swimming pools, and recreational rivers, which are frequently used by the public for recreational activities such as swimming, bathing, fishing, and kayaking. Such locations were selected as they have the opportunity to expose the public to potentially pathogenic *Acanthamoeba* spp. Due to wind dispersal, the amoeba can be dispersed into the environment in its resistant form, which is a cyst (Bunsuwansakul et al., 2019). In other words, people that do not have direct contact may even get infected with *Acanthamoeba* spp. caused by an accidental splash of the contaminated water containing pathogenic *Acanthamoeba* spp. (Abdul Majid et al., 2017).

The researchers from these studies (Table 1) used several methods to identify the *Acanthamoeba* genotypes and species, which include culture, PCR, rRNA gene sequencing, and molecular phylogenetic analysis. The data summarized from these studies gave an overview of the genotypes found in the recreational waters that are frequently used by the public from various countries. From these studies, most of them reported the presence of genotype T4 (27.42%), where the species from this genotype are known to be pathogenic. Several significant T4 species were identified in these recreational waters, which include *Acanthamoeba castellanii*, *A. polyphaga*, *A. triangularis*, and *A. royrebea* (Evyapan et al., 2014; Dendana et al., 2018; Hussain et al., 2019; Koyun et al., 2020). These pathogenic strains may pose such a threat to people visiting the places and having direct water contact. In addition to genotype T4, the other genotypes such as T2, T3, T5, T7 T9, T10, T11, T14, T15, T16, and T17 were also isolated from these recreational waters. Also, few studies were unable to identify the *Acanthamoeba* genotypes from the database (Değerli et al., 2020).

In Malaysia, genotypes T5 (100%) (Basher et al., 2018) and T4 (63.1%) (Hussain et al., 2019) are the most predominant genotypes isolated from recreational rivers and hot springs, respectively. This could pose a risk to the population in Malaysia as the genotype T4 identified is similar to *A. castellanii*, a significant genotype associated with AK (Hussain et al., 2019). As in Taiwan, hot springs are popularly used by the public for therapeutic reasons and bathing. Hot springs in Taiwan were found to have predominantly isolated T15 (44.4%) (Kao et al., 2012) and T4 (27.6%) (Ji et al., 2014). Tunisia recorded a high prevalence of genotype T4 (51%) from oasis water resources, especially in large waterfalls, followed by T14 (18%) and T5 (11%) (Dendana et al., 2018). These places are commonly visited by tourists and most of them swim in these recreational waters, hence increasing the risk of getting *Acanthamoeba* spp. infection. T14 is the second most predominant genotype in Tunisia and rarely has a high prevalence in most of the other countries. There has not been any established data yet on T14 of its pathogenicity, but it can act as a reservoir for the bacteria (Todd et al., 2015).

As expected, T4 is also the most prevalent isolated genotype from both Egypt and Jamaica, with a prevalence of 18.5% and 47.6%, respectively. In Egypt, 48.2% (27/56) of *Acanthamoeba* spp. were isolated, but only nine were

able to confirm by using genus-specific primers (Al-Herrawy et al., 2017). Meanwhile, Turkey also has a high prevalence of genotype T4 in all studies from various recreational water resources (Evyapan et al., 2014; Değerli et al., 2020; Koyun et al., 2020). Four *Acanthamoeba* spp. were not able to be identified in terms of their genotypes as they did not match any known genotype references in the GenBank database (Değerli et al., 2020).

Table 1 shows that several data were obtained from Iran in various recreational water resources. In Iran, genotype T4 was the most isolated from diverse recreational waters, except in a recent study by Paknejad et al. (2020), where T3 (36.3%) was the most prevalent genotype isolated. In other studies from Iran (data not included), genotypes T4 and T5 were reported to be the most prevalent and isolated from recreational water resources (Armand et al., 2016; Aghajani et al., 2016). Studies from swimming pool resources in Brazil (Caumo and Rott, 2011; Alves et al., 2012) identified T5 as the most isolated genotype, while from thermal swimming pools and hot tubs, Fabres et al. (2016) identified T15 as the most isolated genotype. In Brazil, the most prevalent genotypes present are T5 and T15, followed by T4 as the second most prevalent.

The data in Table 1 shows that diverse genotypes of *Acanthamoeba* spp. were isolated and identified from different countries worldwide, where these countries are located from various regions. From these data, almost all the studies from Malaysia, Turkey, and Iran have the highest prevalence of T4, which exceeded 60% from the identified *Acanthamoeba* spp., compared to other countries, which have a T4 prevalence of less than 50%. Compared to the other countries where T4 is the most isolated genotype, T5 and T15 are the most predominant genotypes isolated in Brazil (Caumo and Rott, 2011; Alves et al., 2012; Fabres et al., 2016). This shows that the *Acanthamoeba* spp. preference in an aquatic environment for every country can be different. Hence, this leads to the diversity of genotypes present from each country and recreational water resource.

Table 2 shows the prevalence of each *Acanthamoeba* genotype present in each study, where T4 has the highest prevalence (27.42%), followed by T15 (14.51%) and T5 (14.51%). Previously mentioned, many researchers have reportedly isolated T4 from various clinical and environmental samples. T4 has been mostly associated with human infections including AK and non-keratitis infections such as GAE. A case was reported where T4 was isolated from a woman having keratitis after swimming while wearing contact lenses (Fabres et al., 2018). The high prevalence of T4 isolation in human infections is probably due to its significance in virulence and the properties that improve its transmissibility (Siddiqui and Khan, 2012).

Meanwhile, T5 and T15 are the second most prevalent genotypes that had been isolated with a prevalence of 14.51%. Genotype T5 is commonly isolated from environmental sources. From these studies, the species identified for T5 and T15 were *A. lenticulata* and *A. jacobsii*, respectively (Hussain et al., 2019). Both species are known to be pathogenic and can cause AK in humans. Table 2 shows that T7, T9, T10, T14, T16, and T17 are the least genotypes isolated. Compared to T4, T5, and T15, which are the most prevalent genotypes, the other least isolated genotypes such as T7, T9, and T14 are rarely reported to be associated with infections.

Table 1. Distribution of *Acanthamoeba* genotypes isolated from various recreational water resources worldwide

Country	Type of samples	No. of sample	Positive culture/PCR		<i>Acanthamoeba</i> spp. genotypes	References
			FLA	<i>Acanthamoeba</i> spp.		
Malaysia	Recreational rivers	15	-	15/15 (100.0%)	T5 (15, 100.0%)	Basher et al., 2018
	Hot springs	50	-	38/50 (76.0%)	T3 (2, 5.3%) T4 (24, 63.1%) T5 (1, 2.6%) T11 (1, 2.6%) T15 (9, 23.7%) T17 (1, 2.6%)	Hussain et al., 2019
Taiwan	Thermal spring waters (Southern)	60	-	9/60 (15.0%)	T2 (1, 11.1%) T4 (3, 33.3%) T15 (4, 44.4%) <i>Acanthamoeba</i> spp (1, 11.1%)	Kao et al., 2012
	Hot springs	61	57/61 (93.4%)	29/57 (50.9%)	T4 (8, 27.6%) T15 (2, 6.9%)	Ji et al., 2014
Tunisia	Oasis waters (recreational waters, waterfalls)	211	-	173/211 (82.0%)	T3 (6, 4.0%) T4 (71, 51.0%) T5 (16, 11.0%) T7 (1, 1.0%) T9 (2, 1.0%) T10 (4, 4.0%) T11 (4, 3.0%) T14 (24, 18.0%) T15 (5, 4.0%) T16 (6, 4.0%)	Dendana et al., 2018
Egypt	Swimming pools	120	56/120 (46.7%)	27/56 (48.2%)	T3 (2, 7.4%) T4 (5, 18.5%) T5 (1, 3.7%) T15 (1, 3.7%)	Al-Herrawy et al., 2017
Jamaica, West Indies	Recreational waters (beaches, banks of lagoons, rivers, ponds, mineral springs, streams)	83	-	42/83 (50.6%)	T4 (20, 47.6%) T5 (4, 9.5%) T10 (1, 2.4%) T11 (2, 4.7%)	Todd et al., 2015
Turkey	Rivers	192	-	98/192 (51.0%)	T4 (95, 96.9%) T5 (3, 3.1%)	Koyun et al., 2020
	Hot springs & swimming pools	50	-	21/50 (42.0%)	T3 (1, 4.8%) T4 (19, 90.5%) T15 (1, 4.8%)	Evyapan et al., 2014
	Swimming pools	434	148/434 (34.1%)	26/102 (25.5%)	T2 (1, 3.8%) T3 (1, 3.8%) T4 (8, 30.7%) T11 (3, 11.5%) T15 (7, 26.9%) T17 (2, 7.7%) <i>Acanthamoeba</i> spp. (4, 15.4%)	Değerli et al., 2020
Iran	Rivers	55	15/55 (27.3%)	13/15 (87.0%)	T4 (12, 92.3%) T15 (1, 7.7%)	Niyyati et al., 2012
	Recreational waters:		-			Niyyati et al., 2015
	Seawater	10		4/10 (40.0%)	T4 (4, 100.0%)	
	Pools	10		2/10 (20.0%)	T4 (1, 50.0%)	
	Ponds	10		5/10 (50.0%)	T4 (5, 100.0%)	
	Waterfalls	10		1/10 (10.0%)	T4 (1, 100.0%)	
	Streams	10		3/10 (30.0%)	T4 (2, 66.6%)	
	Hot springs	24	-	11/24 (47.8%)	T4 (11, 100.0%)	Dodangeh et al., 2018
	Swimming pools & Jacuzzi tubs	166	31/166 (18.6%)	14/22 (63.6%)	T3 (8, 36.3%) T4 (4, 18.1%) T11 (1, 4.5%) <i>Acanthamoeba</i> spp. (1, 4.5%)	Paknejad et al., 2020
	Geothermal waters:	40	-	20/40 (50.0%)		Niyyati et al., 2016
	Hot springs			6/20 (30.0%)	T4 (6, 37.5%)	
	Rivers			11/20 (55.0%)	T4 (7, 43.7%)	
	Swimming pools			3/20 (15.0%)	T4 (2, 12.5%)	
					T2 (1, 6.2%)	

Table 1. (continued)

Country	Type of samples	No. of sample	Positive culture/PCR		Acanthamoeba spp. genotypes	References
			FLA	Acanthamoeba spp.		
Brazil	Swimming pools	7	-	7/7 (100.0%)	T4 (3, 42.8%) T5 (4, 51.1%)	Alves et al., 2012
	Swimming pools	65	13/65 (20%)	13/13 (100.0%)	T3 (1, 7.7%) T4 (3, 23.1%) T5 (9, 69.2%)	Caumo and Rott, 2011
	Thermal swimming pools & hot tubs	72	20/77 (27.7%)	8/20 (40.0%)	T3 (1, 12.5%) T4 (2, 25.0%) T5 (1, 12.5%) T15 (4, 50.0%)	Fabres et al., 2016

Table 2. Frequency and prevalence of *Acanthamoeba* genotypes distributed worldwide (from Table 1)

Genotypes	Frequency (n)*	Prevalence (%)
T2	3	4.84
T3	8	12.90
T4	17	27.42
T5	9	14.51
T7	1	1.61
T9	1	1.61
T10	2	3.22
T11	5	8.06
T14	1	1.61
T15	9	14.51
T16	1	1.61
T17	2	3.22
<i>Acanthamoeba</i> spp.	3	4.84

Note: *the presence of each genotype for every study in each country

5. CLINICAL SIGNIFICANCE OF ACANTHAMOEBA GENOTYPES ASSOCIATED WITH AK AND GAE

In recent years, there has been an increasing interest in *Acanthamoeba* spp. infections as they can cause major health problems to humans which are blinding AK and fatal GAE. The potential pathogenicity of *Acanthamoeba* spp. has also been discovered in several studies where the amoeba acted as a host to the pathogenic microorganisms (Ben Salah and Drancourt, 2010; Scheid and Schwarzenberger, 2012). The AK and GAE infections are mostly associated with immunocompromised patients, as well as contact lens wearers for keratitis infection (Lackner et al., 2010; Ghani et al., 2013).

5.1 *Acanthamoeba* keratitis (AK)

Acanthamoeba keratitis (AK) is a painful eye infection caused by pathogenic *Acanthamoeba* spp. and affects the vision of an individual. It can cause the individual to experience ulceration of the cornea, loss of visual acuity, and eventually blindness if it is not treated promptly (Siddiqui and Khan, 2012). The infection is commonly associated with contact lens wearers, particularly immunocompromised individuals. According to the Centers for Disease Control and Prevention (2012), the incidence of AK infection in developed countries was about 1 to 33 cases per million contact lens wearers. The usage of contact lenses has become such a risk factor of AK as there is a previous study that found *Acanthamoeba* spp. in lens cases and saline

cleaning solution (Ghani et al., 2013). AK can also infect non-contact lens wearers through corneal injury. The symptoms of AK can cause sight-threatening conditions that are similar to Herpes simplex, bacterial, or fungal keratitis, leading to misdiagnosing the infection as AK (Lorenzo-Morales et al., 2015).

The diagnosis of AK can be done by a corneal scraping or biopsy for microscopy with several stains applied. The molecular technique is performed to identify the genotype and species of *Acanthamoeba* spp. that cause the infection. As AK can be misdiagnosed, the infection can be mistreated and complicate the diagnosis. A good prognosis and treatment should be given as the amoeba cyst can be resistant to most of the drugs (Lorenzo-Morales et al., 2015). Many researchers have identified T4 as the most clinically isolated genotype associated with keratitis (Visvesvara et al., 2007; Omaña-molina et al., 2016; Prithiviraj et al., 2020). Several studies have identified and documented the other genotypes also associated with AK which are T2, T3, T5, T6, T7, T10, T11, T13, and T15 (Visvesvara et al., 2007; Risler et al., 2013; Grün et al., 2014; Hajialilo et al., 2016; Tawfeek et al., 2016). T13 was identified by Grün et al. (2014) and determined as an etiological agent of keratitis. A recent study done by Prithiviraj et al. (2020) found another genotype aside from T4 and T11, isolated from keratitis patients associated with AK, which is T12. They also stated that T12 was the first report to be associated with AK, thus it becomes another possible genotype that can cause keratitis, along with other common genotypes mentioned.

5.2 Granulomatous amoebic encephalitis (GAE)

GAE is a rare *Acanthamoeba* spp. infection that affects the central nervous system. This infection can be fatal to humans, especially to immunocompromised individuals (Sütçü et al., 2018). The infection can be transmitted through the lower respiratory route, traumatized skin, or wounds which later can lead to hematogenous spread (Siddiqui and Khan, 2012). Any complications that occur can cause brain dysfunction. Commonly, GAE patients will exhibit neurological symptoms such as headache, confusion, stiff neck, focal neurologic deficits, and signs of increased intracranial pressure (Siddiqui and Khan, 2012). The diagnosis of GAE infection is done by microscopy and culture from cerebrospinal fluid, which is the gold standard method (Bunsuwanakul et al., 2019). Other methods such as immunohistochemistry and indirect immunofluorescence are also used to diagnose GAE in patients after going through magnetic resonance imaging of the brain (Kalra et al., 2020).

In GAE infection, genotype T4 has been identified as the most prevalent clinical isolates (Booton et al., 2005; Maciver et al., 2013). Other genotypes aside from T4 were recently reported to be associated with GAE, which include T1, T2, T5, T10, T12, and T18 (Khan, 2006; Walochnik et al., 2008; Lackner et al., 2010; Qvarnstrom et al., 2013; Matsui et al., 2018). This infection is rarely reported as sometimes it is misdiagnosed or late-diagnosed. Hence, proper treatment could not be given to the patients and only a few patients were reported to be cured (Zamora et al., 2014). A review described by Bunsuwasakul et al. (2019) listed the clinical cases of *Acanthamoeba* spp. in Southeast Asian countries where GAE infection was less reported as listed in the review. A rapid and accurate prognosis for GAE infection is needed to treat the infection.

6. CONCLUSION

This review highlighted the distribution of *Acanthamoeba* genotypes that had been isolated from recreational water resources worldwide. Various genotypes were found to be isolated from recreational waters, which include T2, T3, T4, T5, T7, T9, T10, T11, T14, T15, T16, and T17. From this review, genotype T4 was predominantly found isolated from recreational water resources worldwide, followed by T5 and T15. Most of these prevalent genotypes are associated with human infections, which are AK and GAE. Hence, the responsible authorities should monitor and enforce the quality standards of recreational water resources to curb the potential infection of *Acanthamoeba* spp. to the public. Furthermore, medical officers should be aware of the possible sources of infection and improve the prognosis for a better diagnosis.

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