Isolation and molecular characterization of *Acanthamoeba* genotypes in recreational and domestic water sources from Jamaica, West Indies

Cheridah D. Todd, María Reyes-Batlle, José E. Piñero, Enrique Martínez-Carretero, Basilio Valladares, Don Streete, Jacob Lorenzo-Morales and John F. Lindo

ABSTRACT

Free living amoebae (FLA) are amphizoic protozoa that are ubiquitous in nature. Infection with FLA may result in neurological, ocular and skin infections. Exposure to *Acanthamoeba* occurs frequently through water contact and knowledge of the presence of the organisms in water sources is important in understanding transmission dynamics. The distribution of *Acanthamoeba* was studied in recreational and domestic water samples collected from across Jamaica. Morphological assessment and polymerase chain reaction revealed *Acanthamoeba* spp. isolates in 50.6% (42/83) and 17.3% (14/81) of recreational and domestic water, respectively. Sequencing of the DF3 region of the 18S rDNA resulted in the identification of genotypes T3, T4, T5, T10 and T11 corresponding to *Acanthamoeba* spp: *A. griffini*, *A. triangularis*, *A. lenticulata*, *A. culbertsoni* and *A. hatchetti*. Moreover, T4 was the most frequently isolated genotype in both recreational and domestic water. Thermotolerance and osmotolerance assays indicated that most isolates were potentially pathogenic. This is the first report of T3 and T10 genotypes in the Caribbean and the first report of these *Acanthamoeba* spp. in Jamaican waters. The study shows that there is potential risk of infection to contact wearers who practise poor lens care. Further, *Acanthamoeba* should be considered as a cause of neurological infections in Jamaica.

Key words | *Acanthamoeba* spp., domestic water, genotypes, mineral springs, recreational water

Cheridah D. Todd
María Reyes-Batlle
José E. Piñero
Enrique Martínez-Carretero
Basilio Valladares
Jacob Lorenzo-Morales
University Institute of Tropical Diseases and Public Health of the Canary Islands,
University of La Laguna,
Avda Astrofísico Fco. Sánchez, La Laguna,
Tenerife,
Canary Islands,
Spain

Cheridah D. Todd
John F. Lindo (corresponding author)
Department of Microbiology,
University of the West Indies,
Kingston,
Jamaica
E-mail: *john.lindo@uwimona.edu.jm*

Don Streete
National Water Commission,
Marescaux Road,
Kingston 5,
Jamaica

INTRODUCTION

Free living amoebae (FLA) are protists that occupy a wide variety of habitats including freshwater and saltwater bodies, air and various soil types (Rivera *et al.* 1981; Brown *et al.* 1982; Lorenzo-Morales *et al.* 2005a, b, c, 2006; Khan 2009). They have also been isolated from air conditioning and ventilation systems, dialysis units, eye wash stations and the upper respiratory tract of humans (Marciano-Cabral & Cabral 2003; Astorga *et al.* 2011). The organisms are emerging as causes of chronic and debilitating disease in humans, and infections are strongly correlated with increased cases of human immunodeficiency virus (HIV), cancer, diabetes and contact lens usage (Marciano-Cabral & Cabral 2003; Khan 2006). Of interest are those of the genus *Acanthamoeba*, *Naegleria fowleri* and *Balamuthia mandrillaris*, which have been associated with various neurological, ocular and skin diseases in humans and animals (De Jonckheere 2002; Visvesvara *et al.* 2007; da Rocha-Azevedo *et al.* 2009; Sarica *et al.* 2009; Shakoor *et al.* 2011). *Acanthamoeba* is the causative agent of granulomatous amoebic encephalitis (GAE) or *Acanthamoeba* granulomatous encephalitis and amoebic keratitis (AK) (Marciano-Cabral & Cabral 2003; Khan 2006). AK is the more common of the two diseases and is mostly reported in persons after swimming while wearing contact lenses or

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inadequate contact lens care (Parija *et al.* 2001; Marciano-Cabral & Cabral 2003; Schuster & Visvesvara 2004).

Acanthamoeba can act as reservoir and Trojan horse for various bacteria, some of which may cause disease in humans. These include: *Legionella pneumophila*, and *Mycobacterium avium*, causative agents of respiratory disease (Siddiqui & Khan 2012). The amoebae provide a protective niche for the bacteria in which they reproduce, evade host defences and or chemotherapeutic drugs, and transmit throughout the environment (Khan 2006; Siddiqui & Khan 2012). Amoebae are also useful bacterial vectors because of their potential to survive harsh conditions such as extremes of temperature, pH, osmolarity and various disinfectants and chemicals including chlorine (Khan 2006; Scheid *et al.* 2008; Siddiqui & Khan 2012). This has implications for the eradication of bacterial pathogens from water supplies (Khan 2006).

Lorenzo-Morales *et al.* (2005a) reported *Acanthamoeba* from 36.1%, 26.4% and 49.6% of the tap-, river- and seawater samples, respectively, from Jamaica. Further, a single case of AK was reported from the island (Wynter-Allison *et al.* 2005). The aim of this study was to expand on previous work by including more recreational sites such as mineral springs and baths. Jamaica has many different aquatic habitats where amoebae can thrive and pose a health risk to residents and visitors. Therefore, elucidation of the distribution and pathogenic potential of these organisms will offer insight into potential risks for infection.

MATERIALS AND METHODS

Amoebae isolation

Eighty-three recreational water samples were collected from popular sites used for swimming, bathing, boating or garment laundering from the 14 parishes of Jamaica. These sites included beaches, the banks of lagoons, rivers, ponds, mineral springs and streams. Eighty-one domestic water samples were collected from the bathroom showers of randomly selected homes from across the country. Where there was no municipal running supply to the homes, water was collected from drums, storage tanks or outside taps used for bathing.

Approximately one litre of each water sample was filtered through a cellulose nitrate filter, 0.45 μm diameter (Millipore Corporation, Bedford, Madison, Wisconsin) with a weak manifold vacuum system (flow rate, 1.3 ml/min). The filters were inverted on 2% non-nutrient agar plates seeded with heat-killed *Escherichia coli* and incubated at room temperature (approximately 30 °C) for 2 weeks. All plates were then monitored microscopically for growth of amoebae. Blocks containing amoebae were removed from the plates and cultures were started by dilution. Isolated amoebae were then transferred to separate axenic cultures by placing each type of amoeba into peptone yeast glucose (PYG) 712 liquid medium (American Type Culture Collection (ATCC)), with 10 μg/ml gentamicin (Sigma, St. Louis, MO, USA). Amoebae controls from ATCC were grown without shaking in PYG (0.75% (w/v) proteose peptone, 0.75% (w/v) yeast extract and 1.5% (w/v) glucose) medium at room temperature (Lorenzo-Morales *et al.* 2006).

DNA extraction and polymerase chain reaction amplification assay

DNA was extracted by placing 1–2 ml of amoebic cultures directly into the Maxwell® 16 Tissue DNA Purification Kit sample cartridge (Promega, Madrid, Spain). Amoebic genomic DNA was purified using the Maxwell® 16 Instrument following the instructions of the kit manufacturer (Promega, Madrid, Spain). DNA yield and purity were determined using the NanoDrop® 1000 spectrophotometer (Fisher Scientific, Madrid, Spain) as previously described (Cabello-Vílchez *et al.* 2014). DNA amplification reactions were performed using genus-specific markers for *Acanthamoeba*. A volume of 30 μl containing approximately 40 ng template DNA, Buffer (1X) without $MgCl_2$, 2.5 mM $MgCl_2$, 200 μM dNTP, 2.5 pmol of each primer pair and 1.25 units of Taq DNA polymerase (Applied Biosystems, Hammonton, NY, USA), pH 8.3 was used for amplification in a Perkin-Elmer 9,600 thermocycler. The cycling conditions were: initial denaturation of 95 °C for 5 minutes; 40 repetitions of denaturation at 95 °C for 45 seconds, annealing phase at 50 °C for 45 seconds and elongation at 72 °C for 45 seconds; and final elongation at 72 °C for 7 minutes. Amplification products were fractionated using 2% agarose electrophoresis gel stained with a solution of 20.000X of REALSAFE Nucleic

Acid Staining Solution (Durviz, Madrid, Spain) and visualized under ultraviolet light. *Acanthamoeba castellanii* Neff ATCC 30010 strain was used as a positive control and distilled water was added to the reaction mixture (instead of DNA) as the negative control.

Sequencing and genotyping of strains

Polymerase chain reaction (PCR) products were purified using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions, and sequenced in both directions. The sequencing was done in a MegaBACE 1000 automatic sequencer (Healthcare Biosciences, Barcelona, Spain) using the University of La Laguna sequencing services (Servicio de Secuenciación SEGAI, University of La Laguna). Sequences were edited and aligned using the Mega 5.0 software program (Tamura *et al.* 2011). Phylogenetic analyses were carried out using the method described by Reyes-Batlle *et al.* (2014), with the Mega 5.0 software program.

The sequences for the new isolates are deposited in the Genbank database under the accession numbers shown in Figure 1.

Tolerance assays

Osmotolerance is the ability of amoebae to grow at extremities of salinity. Two percent non-nutrient agar plates containing mannitol 0.5 M and 1.0 M, and seeded with heat-killed *E. coli* were used to investigate osmotolerance. Approximately 10^3 trophozoites were inoculated onto the centre of the non-nutrient agar plates. Plates were observed for amoebae growth using an inverted microscope after 24, 48 and 72 hours. To investigate thermotolerance, the ability of amoebae to grow at temperature extremities, approximately 10^3 trophozoites were inoculated in the centre of different non-nutrient agar plates seeded with heat-killed *E. coli*. The initial isolation was done at room temperature in order to isolate all species and genotypes of *Acanthamoeba* present in the sample and not only thermotolerant *Acanthamoeba*. Amoebae were then grown at different temperatures to investigate their potential to survive under temperature extremities without forming cysts. Temperature tolerance was used as a proxy for pathogenicity, although some pathogenic amoebae may grow at low temperatures (De Jonckheere 2002; Pumidonming *et al.* 2010).

The plates were incubated at 29, 37, and 41 °C, and monitored using an inverted microscope for amoebae growth after 24, 48 and 72 hours. Approximately 10^3 trophozoites were inoculated in the centre of new non-nutrient agar plates seeded with heat-killed *E. coli* incubated at room temperature, and monitored for growth after 24, 48 and 72 hours. This plate was used as a control and the procedure was repeated using positive controls (Chan *et al.* 2011). Plates that had amoebae growth of <50 (+), 50–100 (++) and >100 (+++) outside of the point of inoculation were categorized as displaying 'low pathogenic potential', 'moderate pathogenic potential', or 'high pathogenic potential', respectively.

RESULTS AND DISCUSSION

Acanthamoeba spp. were found in 50.6% (42/83) and 17.3% (14/81) of recreational and domestic water, respectively. The sample sites were located close to the coastline of Jamaica because this was the location of most of the popular points used for recreational activity (Figure 2). *Acanthamoeba* were identified by observing the morphology of trophozoites and cysts using an inverted microscope. *Acanthamoeba* spp. with endocysts possessing three to seven arms were isolated from the water samples (Figure 3). Based on the characteristic of the endocyst, some samples contained more than one amoeba isolate.

Twenty of 42 (47.62%) of the *Acanthamoeba* isolates obtained from recreational water belonged to the T4 genotype. The remaining samples contained isolates belonging to the T5 (9.52%), T10 (2.38%) and T11 (4.76%) genotypes. Most T4 isolates displayed high pathogenicity (35%) or moderate pathogenicity (40%) at 37 °C, while most showed low pathogenicity (85%) in 1 M mannitol. Two of the four T5, the T10, and one of the two T11 isolates displayed high pathogenicity at 37 °C, while all three genotypes displayed low pathogenicity in 1 M mannitol. A total of 40.74%, 37.04% and 22.22% of the isolates displayed high, moderate and low pathogenic potential at 37 °C, respectively. While 3.71%, 7.41% and 88.89% displayed high, moderate and low pathogenicity, respectively, based on growth in 1 M

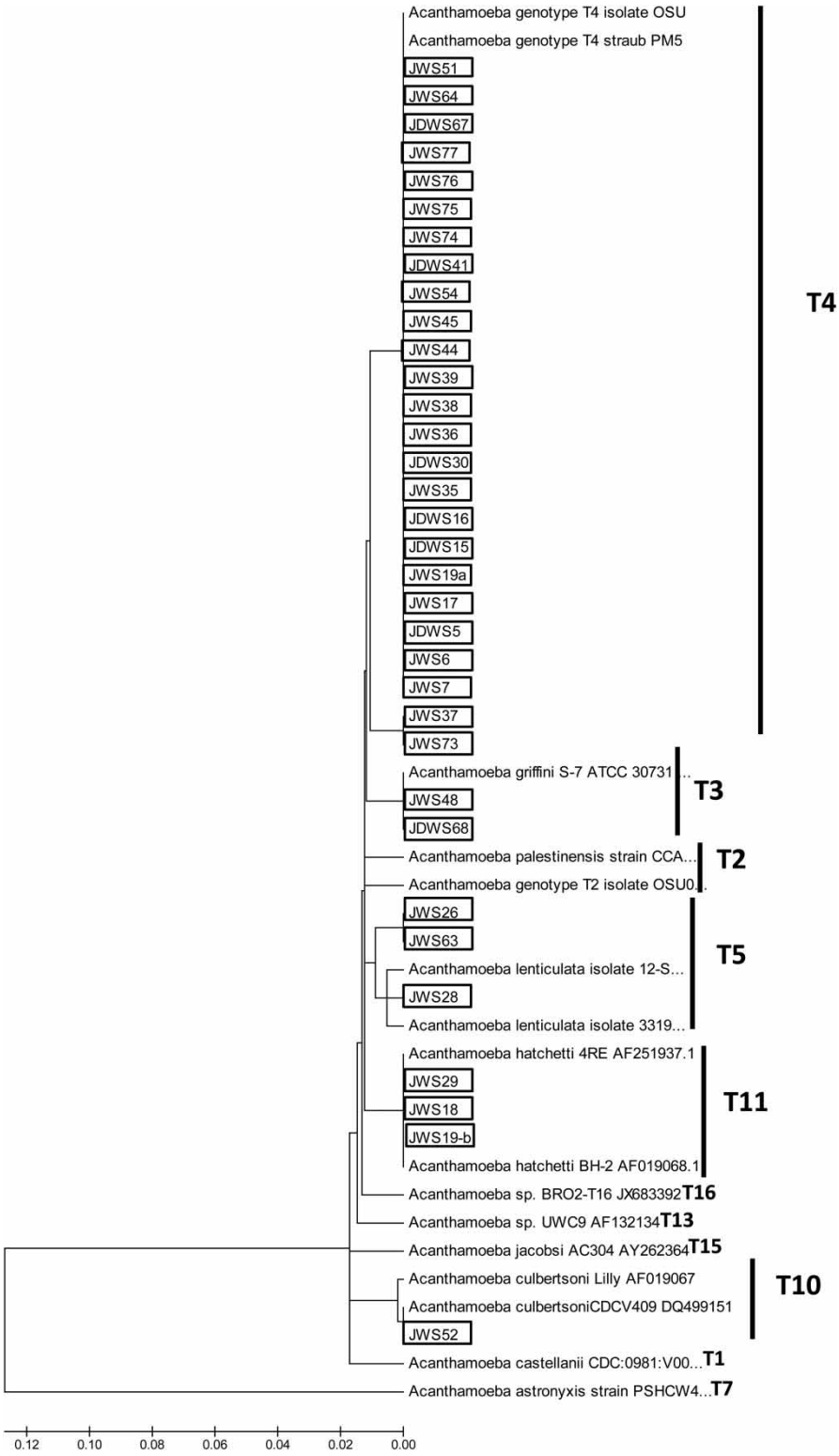

Figure 1 | 18S rDNA DF3 linearized neighbour-joining tree obtained using the Kimura two-parameter distance algorithm, produced in MEGA 5.0. The isolates obtained in the study are identified in the tree (boxes). The type sequences were taken from GenBank and accession numbers are included in the tree.

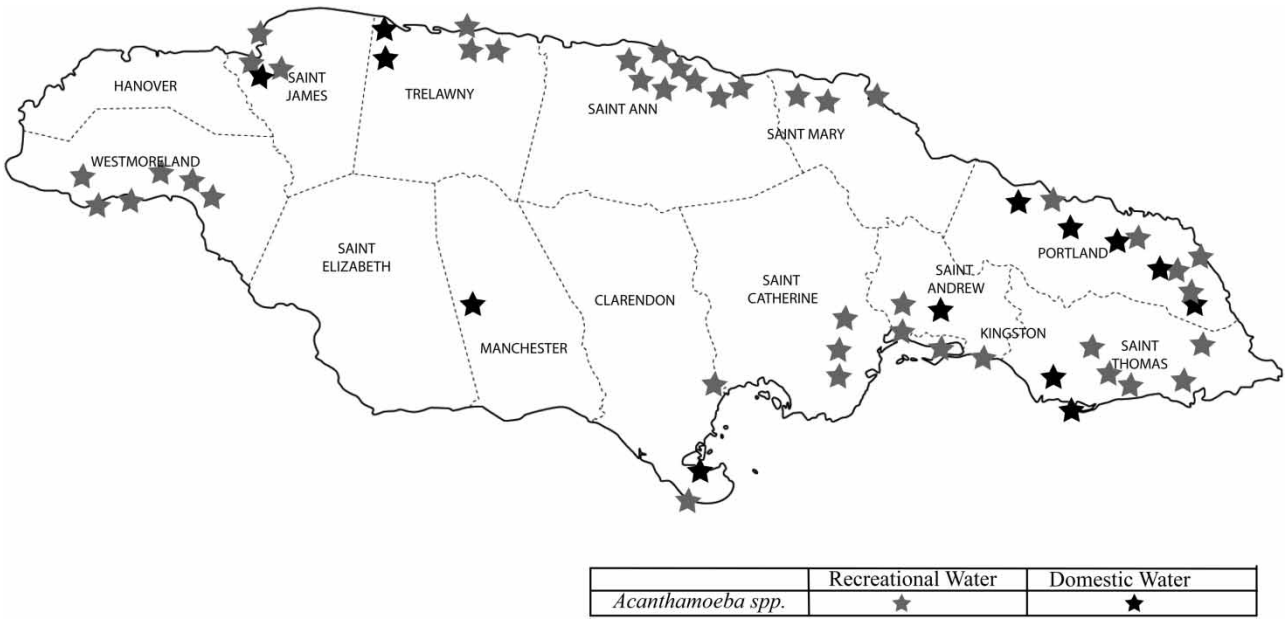

Figure 2 | Map showing the distribution of *Acanthamoeba* spp. found in recreational and domestic water samples collected from the 14 parishes of Jamaica.

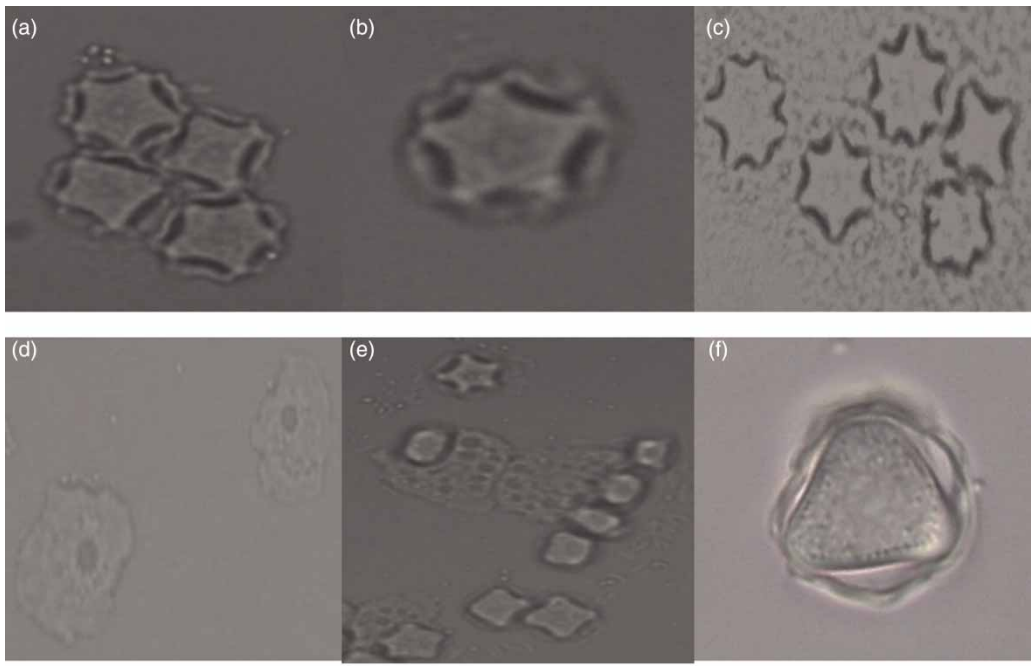

Figure 3 | *Acanthamoeba* trophozoites (d) and (e) and cysts (a)–(f) found in recreational and domestic water collected from Jamaica. Magnification of 40× (a), (c), (d) and (e) and 100× (b) and (f).

mannitol. Therefore, 77.78%, 11.11% and 7.41% were either thermotolerant, osmotolerant or both, respectively, and were considered to be potentially pathogenic in humans. Three of the T5 isolates were identified as *A. lenticulata*, and the T11 isolate was identified as *A. hatchetti*. A T4 isolate *A. triangularis* and a T10 isolate *A. culbertsoni* were also identified (Table 1). The majority of *Acanthamoeba* isolates (42.86%) obtained from domestic water also belonged

Table 1 | Temperature tolerance, osmotolerance, genotyping and speciation of *Acanthamoeba* isolates collected from soil from Jamaica

			Pathogenic potential			
			Temperature tolerance	Osmotolerance		
Parish	Source	Code	37 °C	1 M Mannitol	Genotype	Species
Kingston and St. Andrew	Port Royal Beach	JWS84	++	+	ND	*Acanthamoeba* sp.
	Gunboat Beach	JWS85	+++	+	ND	*Acanthamoeba* sp.
	Kingston Harbour	JWS7	+++	+	T4	*Acanthamoeba* sp.
	Greenwich Fishing Village	JWS6	+++	+	T4	*Acanthamoeba* spp.
	Student Residence Mona Campus, Kgn 7	JDW5	++	+	T4	*Acanthamoeba* sp.
St. Thomas	Bath Fountain Mineral Spring	JWS86	+++	+	ND	*Acanthamoeba* sp.
	Rocky Point Beach	JWS17	+++	+	T4	*Acanthamoeba* sp.
	UWI/Lyssons Beach	JWS18	+++	+	T11	*A. hatchetti*
	Morant Bay Beach	JWS19	++	+	T4, T11	*Acanthamoeba* spp.
	St. Thomas Pond	JWS87	+	+	ND	*Acanthamoeba* sp.
	Lysson's Road	JDW15	++	+	T4	*Acanthamoeba* sp.
	Rocky Point	JDW16	+++	+	T4	*Acanthamoeba* sp.
Portland	Reach Falls	JWS88	+++	+	ND	*Acanthamoeba* sp.
	Winnefred Beach	JWS89	+	+	ND	*Acanthamoeba* sp.
	San San Beach	JWS90	+	+	ND	*Acanthamoeba* sp.
	Frenchman's Cove	JWS26	+++	+	T5	*A. lenticulata*
	Sommerset Falls	JWS28	++	+	T5	*A. lenticulata*
	Wharf Lane Orange Bay	JDW100	+	+	ND	*Acanthamoeba sp.*
	Fairy Hill	JDW101	+++	+	ND	*Acanthamoeba* sp.
	St. Margaret's Bay	JDW29	+++	+	T11	*Acanthamoeba* sp.
	Hope Bay	JDW30	+++	+	T4	*Acanthamoeba* sp.
	Priestman's River	JDW102	+	+	ND	*Acanthamoeba* sp.
St. Ann	Roxborough Beach	JWS35	++	+	T4	*Acanthamoeba* sp.
	Mahogany Beach	JWS36	++	+	T4	*Acanthamoeba* sp.
	Dunn's River Falls	JWS91	+	+	ND	*Acanthamoeba* sp.
	Dunn's River Falls Beach	JWS92	+++	+	ND	*Acanthamoeba* sp.
	Puerto Seco Beach	JWS37	++	+	T4	*Acanthamoeba* sp.
	Priory Beach	JWS93	+++	+	ND	*Acanthamoeba* sp.
	Ocho Rios Bay Beach	JWS38	+++	+	T4	*Acanthamoeba* sp.
	Fire Pond	JWS39	++	+	T4	*A. triangularis*
Clarendon	Rocky Point Beach	JWS44	++	++	T4	*Acanthamoeba* sp.
	Salt River Beach	JWS45	+	+	T4	*Acanthamoeba* sp.
	Lionel Town	JDW103	+	+	ND	*Acanthamoeba* sp.
Manchester	Kingsland Meadows	JDW41	++	+	T4	*Acanthamoeba* sp.
St. Catherine	Waves Beach	JWS94	+	+	ND	*Acanthamoeba* sp.
	Helshire Beach	JWS54	+	+	T4	*Acanthamoeba* sp.

(*continued*)

Table 1 | continued

			Pathogenic potential			
			Temperature tolerance	Osmotolerance		
Parish	**Source**	**Code**	**37 °C**	**1 M Mannitol**	**Genotype**	**Species**
	Rio Cobre	JWS55	+	+	T4	*Acanthamoeba* sp.
Westmoreland	Blue Hole Mineral Spring	JWS95	+	+	ND	*Acanthamoeba* sp.
	Negril Marine Park and Beach	JWS73	+++	++	T4	*Acanthamoeba* sp.
	Bluefields Beach	JWS74	+	+++	T4	*Acanthamoeba* sp.
	Longbay Beach	JWS75	++	+	T4	*Acanthamoeba* sp.
	Bluefields River	JWS76	+	+	T4	*Acanthamoeba* sp.
	Waterway Cane River	JWS77	+++	+	T4	*A. triangularis*
St. James	Dump Up Beach	JWS96	+	+	ND	*Acanthamoeba* sp.
	Walter Fletcher Beach/Aquasol Theme Park	JWS97	+	+	ND	*Acanthamoeba* sp.
	Doctor's Cave Beach	JWS98	+	+	ND	*Acanthamoeba* sp.
	Catherine Hall	JDW67	+++	+	T4	*Acanthamoeba* spp.
	Westgreen	JDW68	++	+	T3	*A. griffini*
Trelawny	Burwood Beach	JWS63	+	+	T5	*A. lenticulata*
	Silversands Beach	JWS64	++	+	T4	*Acanthamoeba* sp.
	Bengal Beach	JWS99	+	+	ND	*Acanthamoeba* sp.
	Duke Street, Falmouth	JDW104	+	+	ND	*Acanthamoeba* sp.
	Queen's Street Falmouth	JDW105	+	+	ND	*Acanthamoeba sp.*
St. Mary	James Bond Beach	JWS51	+++	+	T4	*Acanthamoeba* sp.
	Dry River Beach	JWS52	+++	+	T10	*A. culbertsoni*
	White River Beach	JWS48	+++	+	T5	*A. lenticulata*

Key: number of trophozoites <50 (+), 50–100 (++), >1,000 (++).
ND: indicates not determined as the strains were not axenified.

to the T4 genotype. The remaining samples contained isolates belonging to the T3 (7.14%) and T11 (7.14%) genotypes. The T4 isolates either displayed high pathogenicity (3/6) or moderate pathogenicity (3/6) at 37 °C, while all showed low pathogenicity in 1 M mannitol. The T3 and T11 isolates displayed moderate and high pathogenicity at 37 °C, respectively; however, they displayed low pathogenicity in 1 M mannitol. Half of the isolates displayed high pathogenicity while the remaining half displayed moderate pathogenicity at 37 °C. Therefore, all the isolates were thermotolerant and were considered to be potentially pathogenic to humans and other animals. The T3 isolate was identified as *A. griffini* (Table 1). None of the isolates was osmotolerant, as all displayed low pathogenicity in 1 M mannitol.

Water samples were collected from beaches, rivers, lagoons, ponds, streams and mineral springs that were popularly used for recreational activities. The high percentage of *Acanthamoeba* in the recreational water, especially in seawater, indicates that these isolates might be pathogenic because they displayed osmotolerance. Seawater obtained from the Kingston Harbour contained different isolates of *Acanthamoeba* (based on the morphology of the cysts and sequencing results). The harbour is heavily polluted by poorly treated or untreated sewage, industrial effluent and impacts from shipping (Webber & Kelly 2003). The World Health Organization (WHO 2006) identified the presence of *Acanthamoeba* in seawater to be associated with sewage and waste effluent outlets. Sawyer *et al.* (1977) reported the isolation of *A. culbertsoni* from sewage-spoil dump and *A.*

hatchetti from the Baltimore Harbour in Maryland. In 2005, Lorenzo-Morales and others reported the finding of *Acanthamoeba* in 49.6% and 26.4% of sea and river water, respectively in Jamaica. In this study, *Acanthamoeba* was isolated from 64.0% and 28.6% of sea and river water, respectively.

Most of the beaches in Jamaica are used for swimming, water sport activities and fishing all year round because of the tropical climate of the country. This may result in heavy human use compared to the beaches of countries with a temperate climate. The high prevalence of *Acanthamoeba* (50.6%) in recreational water including beaches indicates that persons are exposed to them quite frequently during swimming or water sports. GAE is a rare disease and there are no reported cases from Jamaica; however, one case of AK has been reported (Wynter-Allison *et al.* 2005). There are no studies on the prevalence of contact lens wear in Jamaica or on the level of education regarding contact lens use, which may also contribute to the low incidence of AK in Jamaica. The absence of reported cases may also be due to misdiagnosis of cases due to lack of awareness, high immunity or low infectivity rates despite high osmotolerance. There are no molecular diagnostic facilities established for FLA in Jamaica. A serological survey of Jamaicans for antibodies to *Acanthamoeba* may be helpful in understanding exposure patterns associated with recreational water contact. Normally, the distribution of FLA, especially *Acanthamoeba*, is high in environmental sources with a low incidence of amoebic infections (Schuster *et al.* 2006).

Four treated and one untreated mineral baths, and three mineral springs were included in the study. Of the eight mineral water sources, three harboured *Acanthamoeba*. These three included the untreated mineral bath and two mineral springs. In Iran only one out of 28 samples from hot springs contained *Acanthamoeba* (Badirzadeh *et al.* 2011). Mineral springs and baths are frequently visited in many countries for health and wellness purposes (Badirzadeh *et al.* 2011). The Blue Hole mineral spring in Westmoreland was the only spring sampled with a limestone soil type. Mineral springs contain limescale which may provide a suitable environment for *Acanthamoeba* proliferation (Seal *et al.* 1992; Radford *et al.* 2002). Other reports on the isolation of *Acanthamoeba* from hot springs have come from Nicaragua, Taiwan, Switzerland and Iran (Leiva *et al.* 2008; Hsu *et al.* 2009; Gianinazzi *et al.* 2010; Solgi *et al.* 2012). The presence of *Acanthamoeba* in these sources indicates that these sites are risk factors for AK especially among contact lens wearers. Contact lens wearers especially should be mindful when visiting mineral springs and baths and should always remove contact lenses before swimming or washing the face to reduce the risk of infection.

Acanthamoeba was isolated from 17.3% of domestic water samples. FLA are found in treated drinking water worldwide and have been reported in 45% of treated drinking water collected from 18 different countries (Thomas & Ashbolt 2011). The percentage of domestic water samples harbouring *Acanthamoeba* was low (17.3%). In a previous study in Jamaica, Lorenzo-Morales *et al.* (2005a) reported a finding of 36.1%. Shoff *et al.* (2008) reported that the high incidence of amoebae in tap water in the UK is due to the storage of water in tanks. Delafont *et al.* (2013) reported that 14.4% of samples from municipal sources in France contained *Acanthamoeba*, which was slightly lower than the findings of this study.

The most frequently isolated genotype from both recreational and domestic water samples was the T4 genotype. These results are further supported by other findings that show that T4 genotypes are the most frequently isolated genotype from the environment (Badirzadeh *et al.* 2011; Rahdar *et al.* 2012; Solgi *et al.* 2012; Qvarnstrom *et al.* 2013; Reyes-Batlle *et al.* 2014). The isolation of genotypes T3 and T4 from domestic water is not rare as they were also reported as the most frequently isolated genotypes in tap water from Hong Kong, although they were not linked to any of the AK cases investigated (Booton *et al.* 2002). However, some T3 strains reported are pathogenic including a strain of *A. griffini*, which was the cause of AK in a patient in Scotland (Ledee *et al.* 1996). T3 and T4 cysts are highly resistant to varying concentrations of chlorine, while T11 and T5 are much more susceptible (Shoff *et al.* 2008). The recovery of the T11 genotype from the domestic water sample might be used as indication of very low chlorination at the time of sample collection. However, the chlorine level in the water was not measured. T10 and T12 genotypes are rarely isolated from the environment and are usually associated with GAE, while T11 is associated with AK and T5 is more frequently isolated from the environment (Booton *et al.* 2005). This is the first report of genotypes T3 and T10 from Jamaica and the Caribbean.

Only 14.29% and 7.14% of *Acanthamoeba* isolated from recreational and domestic water, respectively, were thermotolerants, while 33.33% of the mineral water isolates were thermotolerants. This confirms that users of mineral springs and mineral baths may be at higher risk of AK than individuals using other sources of domestic and recreational water. Similarly thermotolerant *Acanthamoeba* were found in 20% of mineral water sources in Iran, a few of which were able to grow at temperatures above 40 °C (Solgi *et al.* 2012). This supports findings by Rohr & others (1998) that *Acanthamoeba* isolates are sensitive to temperatures above 40 °C and those that can survive such extremities are rare.

All five species, *A. hatchetti*, *A. culbertsoni*, *A. lenticulata*, *A. triangularis* and *A. griffini*, were categorized as thermotolerants and considered to be potential human pathogens. *A. hatchetti*, *A. culbertsoni* and *A. griffini* are causative agents of AK in humans while *A. culbertsoni* is associated with amoebic encephalitis (Ledee *et al.* 1996; Visvesvara *et al.* 2007). Thermotolerance and osmotolerance are objective assessments of pathogenic potential and indicate the behaviour of amoebae under stressful conditions. Further assessment of the pathogenic potential of amoebae needs to be done by conducting PCR with primers that are specific for serine proteases as an indicator of pathogenicity.

CONCLUSIONS

The study showed that *Acanthamoeba* with varying levels of pathogenicity were recovered from Jamaican waters that hosted human activity for recreation and domestic use. There is potential risk of infection for contact wearers who practise poor lens care from waterborne exposure to the organisms. Further, *Acanthamoeba* should be considered as a cause of neurological infections in Jamaica. This is the first report of *A. griffini*, *A. triangularis*, *A. lenticulata*, *A. culbertsoni* and *A. hatchetti* in water sources in Jamaica and also of the T3 and T10 genotypes.

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