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Free-living amoebae and their relationship to air quality in hospital environments: characterization of *Acanthamoeba* spp. obtained from air-conditioning systems

Débora Borgert Wopereis[1], Maria Luiza Bazzo[2], Jefferson Peres de Macedo[1], Fabiana Casara[1], Lisléia Golfeto[2], Eduardo Venancio[2], Jaquelline Germano de Oliveira[3], Marilise Brittes Rott[4] and Karin Silva Caumo[1]

[1]Universidade Federal de Santa Catarina (UFSC), Centro de Ciências da Saúde, Departamento de Análises Clínicas, Laboratório de Estudos de Protozoários Emergentes e Oportunistas, CEP: 88040-970, Florianópolis, Santa Catarina, Brazil; [2]Universidade Federal de Santa Catarina (UFSC), Centro de Ciências da Saúde, Departamento de Análises Clínicas, Laboratório de Biologia Molecular, Sorologia e Micobactérias, CEP 88040-900, Florianópolis, Santa Catarina, Brazil; [3]Fundação Oswaldo Cruz, Centro de Pesquisas René Rachou, Laboratório de Imunologia Celular e Molecular, CEP: 30.190-002, Belo Horizonte, Minas Gerais, Brazil and [4]Universidade Federal do Rio Grande do Sul (UFRGS), Instituto de Ciências Básicas da Saúde. Departamento de Microbiologia, Imunologia e Parasitologia, CEP 900035-190, Porto Alegre, Rio Grande do Sul, Brazil.

Research Article

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Author for correspondence:
Karin Silva Caumo,
E-mail: k.caumo@ufsc.br

Abstract

Free-living amoebae (FLA) are widely dispersed in the environment, can cause opportunistic and non-opportunistic infections in humans and other animals. The aim of the present study was characterize FLA obtained from air-conditioners of a public hospital in the city of Florianópolis, SC, Brazil. Fifty-four dust samples were collected of air conditioners, and were inoculated on 1.5% non-nutrient agar, overlaid with layers of *Escherichia coli*. Subsequently the isolates were axenised in PYG growth medium. The morphological and molecular characterization of the isolates was performed, as well as the tolerance (physiological) assays were used to evaluate the pathogenic potential. The results revealed the presence of FLA in 42 (77.8%) of the collected samples. Of these, 39 (92.9%) axenic isolates of FLA were obtained for morphological and genotypic studies. All the isolates characterized belong to the genus *Acanthamoeba*. Nineteen (48.7%) isolates belong to the genotype T4, 16 (41.0%) to the T5 genotype and 4 (10.3%) to genotype T11. Seven (18.0%) isolates were considered potentially pathogenic in tolerance assays. These findings require attention, considering the isolation environment and immunocompromised characteristics of many hospitalized patients.

Introduction

Air conditioning systems can harbour bacteria, fungi, viruses and protozoa, such as *Acanthamoeba* spp. (Ross *et al.*, 2004; Ooi *et al.*, 2017). These microorganisms may remain in these locations for a long time and be dispersed in the environment through air currents (Silva *et al.*, 2013). The exchange of air in indoor environments does not always occur in a satisfactory way which can favour the development of microorganisms, which can eventually affect humans causing infections (Graudenz and Dantas, 2007). Indoor air quality control plays an important role in preventing infections at these sites, particularly important in hospital settings, since immunocompromised individuals are more susceptible to infections (Alves *et al.*, 2012; Santana and Fortuna, 2012).

The critical care areas of hospitals are those that show a greater probability of transmitting hospital infection, either through invasive procedures or the presence of immunocompromised patients, such as in surgical centres (SCs), intensive care unit (ICU) haemodialysis rooms, chemotherapy, transplantation, among others. The transmission occurs through direct contact with the hospital staff, from one patient to another through fomites (objects such as gloves, tools and utensils) and the hospital ventilation system (Afonso *et al.*, 2004; Leung and Chan, 2006; Silva *et al.*, 2013).

Acanthamoeba spp. are among the most common protozoa in nature and identified as agents of granulomatous amoebic encephalitis (GAE), cutaneous lesions, pulmonary and kidney infections, primarily in immunocompromised patient and *Acanthamoeba* keratitis in immunocompetent individuals (Trabelsi *et al.*, 2012). Furthermore, *Acanthamoeba* spp. have been described as vehicles of pathogenic microorganisms including *Legionella pneumophila*, *Mycobacterium* spp. and *Pseudomonas* spp. (Marciano-Cabral *et al.*, 2010; Maschio *et al.*, 2015; Balczun and Scheid, 2017).

Species of *Acanthamoeba* have two stages: trophozoite, metabolically active form and cyst, stage of dormancy. Identification of *Acanthamoeba* at the genus level is relatively easy due to the presence of characteristics such as acanthopodia in trophozoites and double wall of cysts

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(Visvesvara, 2013). Pussard and Pons (1977) divided the genus into three groups according to the size and shape of cysts; however, this classification is uncertain because the morphology of the cysts can modify according to the culture conditions. The most accepted methodology for classifying *Acanthamoeba* spp. based on the smaller subunit sequences of the 18S rDNA gene, so that the genus can be divided into genotypes, which would correspond to species. Each genotype exhibits 5% or more of divergent sequences between different genotypes (Schroeder *et al.*, 2001; Trabelsi *et al.*, 2012). Currently, *Acanthamoeba* spp. differentiate into 21 genotypes (T1–T21) (Corsaro *et al.*, 2015; 2017). Several studies use tolerance assays to predict the pathogenic potential of *Acanthamoeba* environmental isolates (Khan *et al.*, 2002; Al-Herrawy *et al.*, 2013).

Due to the opportunistic nature of *Acanthamoeba* spp. and its possible role as a reservoir of pathogens of importance in health services infections, the monitoring of this protozoan in hospital environments becomes important and could be used as a quality biomarker in hospitals for the improvement of air quality in hospital settings, because in these places people are more debilitated and susceptible to infections and cysts of *Acanthamoeba* spp. are resistant to several disinfection systems, remaining in the environment for years, becoming a source of dissemination of pathogens (Ooi *et al.*, 2017). In this sense, the present study investigated the occurrence of FLA in air-conditioners of a public hospital in the city of Florianópolis, SC, Brazil, with a particular focus on isolation and genotyping of *Acanthamoeba* isolates.

Materials and methods

Samples

Fifty-four dust samples were collected from filters, flaps and diffuser of air conditioners of fifteen environments of a public hospital in the city of Florianópolis, SC, Brazil, between March 2014 and March 2015. The collection environments were: chemotherapy unit (CU), emergency (EM), gynecology (GN), haemodialysis unit (HU), ICU, medical clinic I (MCI), medical clinic II (MCII), obstetrical centre (OC), ophthalmology (OPT), outpatient surgical centre (OSC), paediatrics (PED), SC, surgical clinic I (SCI), surgical clinic II (SCII) and sterilization room (ST). Samples were collected using sterile swabs, which were placed in contact with 10 mL of Page saline solution (2.5 mM NaCl, 1 mM KH_2PO_4, 0.5 mM Na_2HPO_4, 40 mM $CaCl_2$ and 20 mM $MgSO_4$) for 30 min to promote the detachment of amoebic forms, when presents. After, the samples were centrifuged at 500 × *g* for 5 min, the supernatant was discarded and the pellet was resuspended in 200 μL of Page saline solution.

Isolation of free-living amoebae

The suspension obtained from each pellet was inoculated in the centre of 1.5% non-nutrient agar (NNA) plates, overlaid with layers of *Escherichia coli* (ATCC 25922) previously heat-inactivated (for 2 h at 56°C). The plates were sealed with Parafilm® and incubated at 30°C for up to 25 days. Three plates were prepared for each dust sampled. Each plate was examined daily under optical microscopy (at 100×) to verify the presence of amoebic forms. When the presence of cysts or trophozoites was observed, it was performed subculture from the transference of a small piece of agar containing the amoebic forms to a new plate in order to isolate it from other microorganisms. Subsequently the isolates were axenised in PYG growth medium [0.75% (w/v) proteose peptone, 0.75% (w/v) yeast extract and 1.5% (w/v) glucose with antibiotics] and incubated at 30°C. When necessary, PYG medium supplemented with 10% foetal bovine serum was used to promote amoebic development.

Morphological studies

The cysts and trophozoites of the FLA isolated from dust of air conditioners were morphologically characterized (Pussard and Pons, 1977; Page, 1988).

The size of the amoebic forms was estimated using calibrated ocular micrometre. For each isolate, 10 cysts and 10 trophozoites were measured. The results were expressed as mean ± S.D..

Molecular identification of isolates

Extraction of total DNA from each isolate (containing 10^6 trophozoites/mL) was performed using the QIAamp® DNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendations. The polymerase chain reaction (PCR) was performed with the genus-specific primers JDP1 and JDP2 according to Schroeder *et al.* (2001). The positive control included DNA from the strain *Acanthamoeba castellanii* Neff (ATCC 30010) and the negative control as a substitute for DNA template included DNA free water. The amplicons were separated by electrophoresis on 1.5% agarose gel, stained with 1μg/mL ethidium bromide and observed under a UV-light transilluminator. The PCR products were purified using the PureLink® PCR Purification Kit (Invitrogen, Carlsbad USA) according to the manufacturer's instructions. The purified amplicons were sequenced in both senses using the amplification primers and BigDye® sequencing kit in an ABI 3730 automated sequencer (Applied Biosystems, EUA).

To determine the genotypes, sequencing data was aligned with *Acanthamoeba* genotype sequences available in the GenBank database based on the DF3 using Basic Local Alignment Search Tool (BLAST) program of the US National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST) to search for the most similar sequences. The sequences obtained in this study were deposited in the GenBank database under accession numbers MF076628 to MF076666. Sequence alignments were performed using CLUSTAL W for pairwise alignments and phylogenetic tree was constructed with MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms (Kumar *et al.*, 2018) using the neighbour joining method, with the bootstrap based on 1000 random replicates.

Tolerance (physiological) assays

Thermotolerance and osmotolerance assays were performed as previously described (Caumo *et al.*, 2009). Briefly, for the osmotolerance assay 10^3 trophozoites were inoculated onto 1.5% NNA plates containing mannitol 1.0 M, each with overlaid with layers of heat-killed *E. coli* (ATCC 25922). NNA plates under the same conditions without the addition of mannitol were used as a control. All plates were incubated at 30°C for 10 days, after this incubation period, growth was evaluated in optical microscopy (at 100×). For this, the number of cysts or trophozoites visualized in about 20 mm from the inoculum site (previously demarcated) of the each plate, in five microscope fields were counted and classified, with counts of zero (−), 1–15 (+), 16–30 (++), >30 (+++).

For the thermotolerance assay, 10^3 trophozoites were inoculated onto 1.5% NNA plates, each with overlaid with layers of heat-killed *E. coli*. Plates were incubated at 30 and 40°C for 10 days. The plates, submitted to 30°C, were used as control in growth assessment. The growth evaluation after the incubation period was done as in the osmotolerance assay. All assays were carried out in triplicates.

The isolates were classified into three groups according to their growth in the tolerance tests. Isolates that were able to

develop in the hyperosmolar medium and the temperature of 40° C were classified as potentially pathogenic. Isolates that developed in one of the tests were classified as being of low pathogenic potential and when it was not able to develop against none of the adverse conditions were classified as probably non-pathogenic.

Results

Of the 54 dust samples obtained from air conditioners of hospital environments, 42 (77.8%) were positive for FLA. Of these three (7.2%) were not able to develop in axenic medium. Therefore, 39 (92.9%) axenic isolates of FLA were obtained for

Table 1. Morphological identification of *Acanthamoeba* isolates obtained from dust from air conditioners of a public hospital in Florianópolis

		Mean (s.d.) diameter μM		
Hospital environment	Isolated	Trophozoite	Cyst	Morphological group
CU	CU – Chemotherapy (CF)	33.8 (3.4)	13.8 (1.3)	II
	CU – Doctor's office 01 (D)	30.2 (2.2)	17.0 (1.6)	II
	CU – Doctor's office 02 (D)	32.0 (2.6)	15.5 (2,0)	II
	CU – Procedures room (D)	33.5 (1.8)	14.5 (1.6)	II
EM	EM – Left Ward (F)	35.2 (1.9)	16.8 (1.7)	II
	EM – Right Ward (F)	40.3 (2.2)	17.5 (1.6)	II
	EM – Resuscitation room (F)	32.0 (2.7)	17.5 (1.7)	II
GN	GN – Doctor's office 02 (Fp)	32.0 (1.0)	15.8 (1.6)	II
	GN – Doctor's office 02 (F)	33.6 (1.8)	17.0 (1.6)	II
	GN – Room 202 (Fp)	28.7 (1.8)	16.0 (1.3)	II
	GN – Room 202 (F)	31.8 (1.2)	16.3 (1.3)	II
HU	HU – Central procedure room 1 (D)	36.3 (1,3)	19.0 (1.3)	II
	HU – Central procedure room 2 (D)	37.0 (2.0)	19.5 (1.7)	II
	HU – Room of repose (F)	36.0 (2.1)	18.3 (1.2)	II
ICU	ICU – Intensive care unit (CF)	26.3 (2.7)	18.3 (1.2)	II
MCI	MCI – 309 (F)	28.0 (1.0)	16.5 (1.3)	II
	MCI – 310 (Fp)	27.2 (2.6)	15.5 (2.0)	III
	MCI – 310 (F)	29.6 (2.0)	18.8 (1,4)	II
	MCI – 311 (Fp)	28.0 (1.0)	15.5 (1.0)	II
	MCI – 311 (F)	30.0 (1.2)	16.8 (1.2)	II
	MCI – Medical clinic I (CF)	33.5 (1.1)	16.5 (1.3)	II
MCII	MCII – 314 (Fp)	30.3 (1.4)	17.5 (1.6)	II
	MCII – 324 (Fp)	32.6 (2.3)	19.3 (1.7)	II
OC	OC – Obstetrical centre (CF)	34.5 (1.6)	15.0 (1.7)	II
OPT	OPT – Doctor's office 02 (F)	33.3 (2.1)	16.0 (1.3)	II
	OPT – Waiting room (F)	32.0 (2.0)	17.5 (1.7)	II
OSC	OSC – Doctor's office 01 (F)	34.5 (2.6)	18.5 (2.1)	II
	OSC – Doctor's office 04 (F)	30.0 (1.6)	16.3 (1.7)	II
PED	PED – Special care room (Fp)	27.3 (1.8)	16.6 (1.6)	II
	PED – Special care room (F)	28.8 (1,3)	15.5 (1.0)	II
SC	SC – Roon 01 (D)	29.8 (1.9)	14.5 (2.0)	II
	SC – Roon 02 (D)	29.0 (1.7)	17.0 (2.0)	II
	SC – Roon 04 (D)	30.0 (1.7)	14.3 (1.2)	II
SCI	SCI – Bed 406 (D)	30.8 (2.1)	15.5 (1.6)	II
	SCI – Bed 410 (D)	29.0 (2.1)	14.6 (2.2)	II
	SCI – Procedure room (D)	29.6 (1.6)	17.3 (1.8)	II
SCII	SCII – Curative room (D)	28.8 (2.4)	15.3 (0.8)	II
ST	ST – Preparation room (F)	27.3 (1,8)	16.9 (1.2)	II
	ST – Storage room (F)	37.3 (1,8)	19.5 (2.0)	III

Central filter (CF); diffuser (D); filter (F); flaps (Fp).

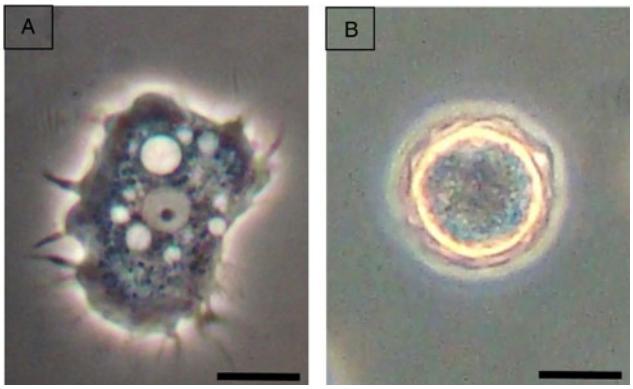

Fig. 1. Trophozoite of *Acanthamoeba* spp. presenting acanthopodia, and a nucleus with well-defined central nucleolus (a) and cyst compatible with group II (b). The bars represent 10 μM.

morphological and genotypic studies. All the amoeba isolates in this study were identified morphologically (Table 1) as belonging to the genus *Acanthamoeba*. The trophozoites presented acanthopodia, and a nucleus with well-defined central nucleolus (Fig. 1a). Thirty-seven isolates presented characteristics compatible with group II (Fig. 1b), and two isolates to morphological group III. No isolate presented group I characteristics. All the measurements of cysts and trophozoites presented size expected for genus according to Pussard and Pons (1977) and Page (1988).

The PCR using genus-specific primers (JDP1 and JDP2) confirmed that the 39 isolates from the study belonged to the genus *Acanthamoeba*. The expected amplification product (ASA.S1 18S rDNA) of ~500 bp was observed (Fig. 2). Sequencing of PCR products revealed that 19 (48.7%) isolates belonged to the genotype T4, 16 (41.0%) to the T5 genotype and 4 (10.3%) to genotype T11 (Table 2) when compared to the reference sequences deposited at GenBank. The percentage of identity between the sequences of this study and those used as reference ranged from 97 to 100%.

The sequences from *Acanthamoeba* spp. isolates were used to construct the phylogenetic tree to illustrate the relationships between the isolates obtained and reference sequences of *Acanthamoeba* genotypes T1–T20 retrieved from GenBank. The relationships among these isolates were examined by using the neighbour-joining method as showed in Fig. 2. The tree showed that 19 isolates are strictly related with *Acanthamoeba* T4 genotype chosen as references with 98% of identity, 16 isolates T5 genotype with 100% of identity with the T5 sequence references. Four of the 39 isolates analysed showed a strict correspondence with the deposited sequences for the genotype T11, with 100% of identity. The association of obtained isolates in this study with individual genotypes was supported by significant bootstrap values (Fig. 2).

Of the 39 isolates of *Acanthamoeba* submitted to the osmo and thermotolerance assays, seven (18.0%) isolates were considered potentially pathogenic, because it had concomitant growth in hyperosmolar medium and at elevated temperature of 40°C. Isolates that developed only at elevated temperature 25 (64.1%) and only in hyperosmolar 2 (5.2%) were classified as low pathogenic potential. Among the isolates, 5 (12.8%) presented no growth at 1.0 M mannitol and at 40°C and were considered probably non-pathogenic isolates (Table 3).

Discussion

Studies of FLA isolation in hospital environments are scarce, despite the importance of these microorganisms as potential causers of opportunistic infections and as vehicles and reservoirs of pathogens. Some reports of isolation of these amoebas in hospital environments have been described from water systems (Trabelsi *et al.*, 2016; Muchesa *et al.*, 2017), dust and biofilm (Silva and Rosa, 2003; Carlesso *et al.*, 2010; Costa *et al.*, 2010). Reports of FLA isolation from air conditioners have been described in some countries such as Chile (Astorga *et al.*, 2011) and Malaysia (Chan *et al.*, 2011), however, in hospital environments the presence of these amoebae in air conditioners is still poorly investigated. In the present study, a high culture rate of FLA was observed, being higher than 70%, indicating the high prevalence of these amoebae in air conditioning units in the investigated hospital.

All isolates were characterized as belonging to the genus *Acanthamoeba*. The morphological identification of the isolates of the present study showed the presence of double-walled cysts with characteristics compatible with group II and III. The morphological group II harbours *Acanthamoeba* species commonly isolated from environmental and clinical samples, described as responsible for most cases infection in humans, such as amoebic keratitis and GAE (Walochnik *et al.*, 2000).

Currently molecular methods for the detection of *Acanthamoeba* spp. are being increasingly used due to the high sensitivity and specificity of these methods (Visvesvara *et al.*, 2007). The PCR using primers that amplify a conserved region of the 18S rDNA gene is the most used, since the sequencing of the fragment obtained in the PCR allows the determination of the genotype (Fuerst *et al.*, 2015). The three genotypes of *Acanthamoeba* spp. identified in this study (T4, T5 and T11) have a wide environmental distribution, being reported the isolation of these from samples from water (Sente *et al.*, 2016), soil (Todd *et al.*, 2015) and dust (Niyyati *et al.*, 2009). The prevalence of the T4 genotype in environmental samples, reported in other studies (Geisen *et al.*, 2014). Rahdar *et al.* (2012) verified the predominance of this genotype in isolates obtained from soil and water from a province of Iran. Similarly, Geisen *et al.* (2014) reported the predominance of the T4 genotype in *Acanthamoeba* isolates from soil samples from three distinct locations, the Netherlands, Sardinia and Tibet. This is the genotype most associated with cases of keratitis and amoebic encephalitis, as well as other opportunistic infections caused by this protozoan (Siddiqui and Khan, 2012).

The T5 genotype was the second most found. In the study by Booton *et al.* (2005), which included 200 isolates of *Acanthamoeba*, this genotype was identified as the second most prevalent among environmental isolates, as well as second in the study by Ledee *et al.* (2009) that included isolates of amoebic keratitis. The T5 genotype is associated with cases of amoebic keratitis and encephalitis (Siddiqui and Khan, 2012).

Some studies relate the T11 genotype to cases of amoebic keratitis (Hajialilo *et al.*, 2016; Jercic *et al.*, 2019). This was one of

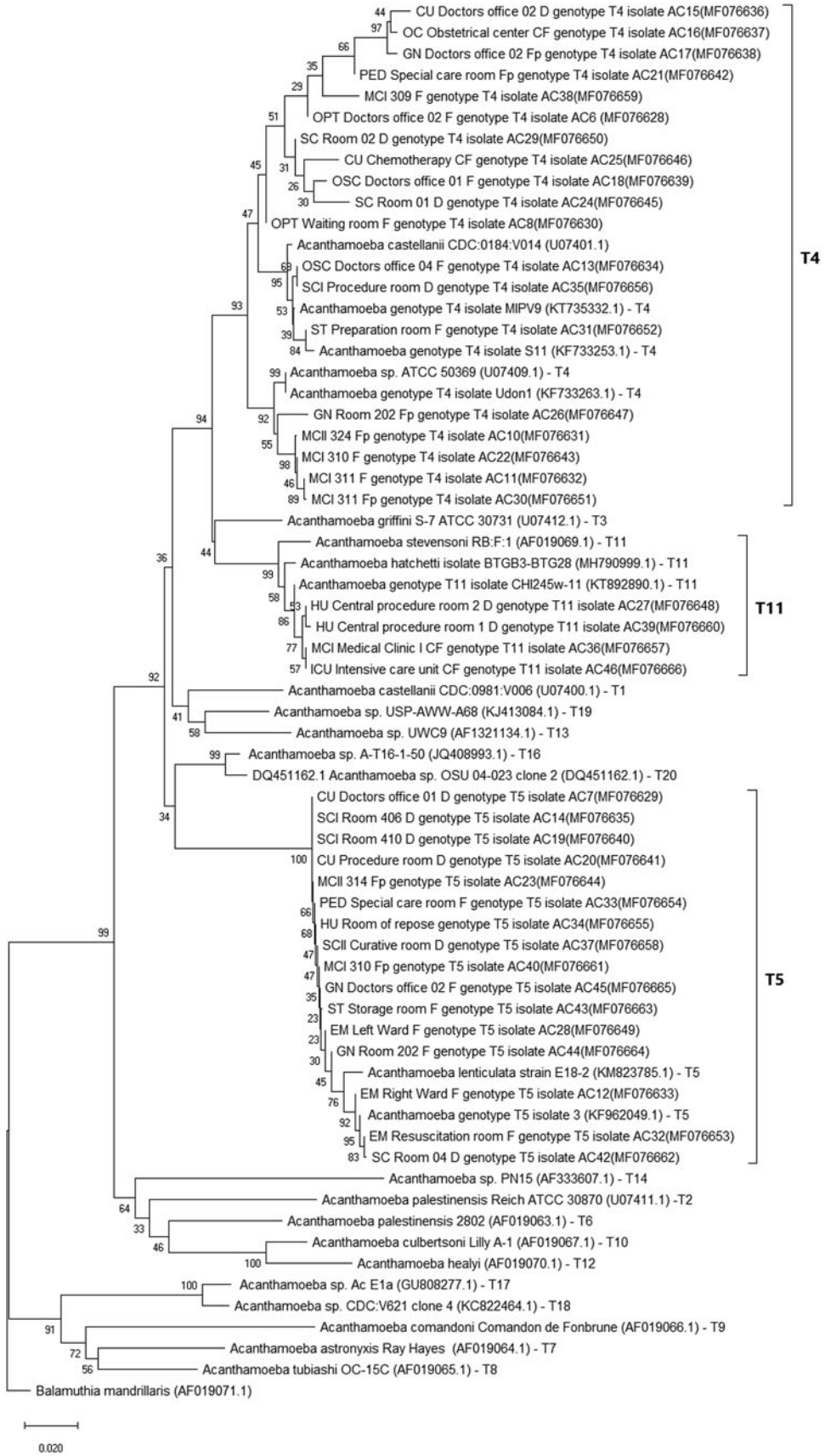

Fig. 2. Neighbour-joining 18S rDNA tree of genotype *Acanthamoeba* spp. (MEGA X program). Test isolates including reference strains representing T1–T20 genotypes. Numbers at the nodes are percentage-bootstrapping values on 1000 replicates. *Balamuthia mandrillaris* was used as the outgroup. Bar 0.02 substitutions per nucleotide position.

Table 2. Genotypic identification of *Acanthamoeba* isolates obtained from dust from air conditioners of a public hospital in Florianópolis

				Reference sequences	
Hospital environment	Site of isolation	GenBank accession no.	Genotype	GenBank accession no.	Identity (%)
CU	CU –Chemotherapy (CF)	MF076646	T4	KF733253	99
	CU – Doctor's office 01 (D)	MF076629	T5	KF962049	100
	CU – Doctor's office 02 (D)	MF076636	T4	U07409	98
	CU – Procedures room (D)	MF076641	T5	KF962049	100
EM	EM – Left Ward (F)	MF076649	T5	KF962049	100
	EM – Right Ward (F)	MF076633	T5	KF962049	100
	EM – Resuscitation room (F)	MF076653	T5	KF962049	99
GN	GN – Doctor's office 02 (Fp)	MF076638	T4	U07409	98
	GN – Doctor's office 02 (F)	MF076665	T5	KF962049	100
	GN – Room 202 (Fp)	MF076647	T4	U07409	98
	GN – Room 202 (F)	MF076664	T5	KF962049	100
HU	HU – Central procedure room 1 (D)	MF076660	T11	KT892890	99
	HU – Central procedure room 2 (D)	MF076648	T11	KT892890	99
	HU – Room for resting (F)	MF076655	T5	KF962049	100
ICU	ICU – Intensive-care unit (CF)	MF076666	T11	KT892890	99
MCI	MCI – 309 (F)	MF076659	T4	U07409	99
	MCI – 310 (Fp)	MF076661	T5	KF962049	100
	MCI – 310 (F)	MF076643	T4	KF733263	100
	MCI – 311 (Fp)	MF076651	T4	U07409	100
	MCI – 311 (F)	MF076632	T4	KF733263	100
	MCI – Medical clinic I (CF)	MF076657	T11	KT892890	99
MCII	MCII – 314 (Fp)	MF076644	T5	KF962049	100
	MCII – 324 (Fp)	MF076631	T4	KF733263	100
OC	OC – Obstetrical centre (CF)	MF076637	T4	U07409	97
OPT	OPT – Doctor's office 02 (F)	MF076628	T4	U07409	99
	OPT – Waiting room (F)	MF076630	T4	KF733253	99
OSC	OSC – Doctor's office 01 (F)	MF076639	T4	KF733253	97
	OSC – Doctor's office 04 (F)	MF076634	T4	KT735332	100
PED	PED – Special care room (Fp)	MF076642	T4	U07409	99
	PED – Special care room (F)	MF076654	T5	KF962049	100
SC	SC – Room 01 (D)	MF076645	T4	U07409	98
	SC – Room 02 (D)	MF076650	T4	KF733253	98
	SC – Room 04 (D)	MF076662	T5	KF962049	100
SCI	SCI – Bed 406 (D)	MF076635	T5	KF962049	100
	SCI – Bed 410 (D)	MF076640	T5	KF962049	100
	SCI – Procedure room (D)	MF076656	T4	KT735332	100
SCII	SCII – Curative room (D)	MF076658	T5	KF962049	100
ST	ST – Preparation room (F)	MF076652	T4	KF733253	99
	ST – Storage room (F)	MF076663	T5	KF962049	100

Central filter (CF); diffuser (D); filter (F); flaps (Fp).

the genotypes described as causing this infection in a large research carried out in Austria that included cases of *Acanthamoeba* infections in the last 20 years (Walochnik *et al.*, 2015).

Studies that evaluated the presence of *Acanthamoeba* in dust and soil samples showed similar results to the present study, reporting the presence of T4, T5 and T11 genotypes, with T4 genotype predominating (Niyyati *et al.*, 2009; Todd *et al.*, 2015). *Acanthamoeba* isolation studies from air conditioners have been performed in some countries such as Chile (Astorga *et al.*, 2011) and Malaysia (Chan *et al.*, 2011), showing the presence of T3, T4, T5 and T11.

Table 3. *In vitro* growth of the *Acanthamoeba* isolates in the osmotolerance and thermotolerance assays

		Osmotolerance	Thermotolerance
Hospital environment	Isolated	Growth* 1.0 M mannitol	Growth* 40°C
CU	CU – Chemotherapy (CF)	–	++
	CU – Doctor's office 01 (D)	–	+++
	CU – Doctor's office 02 (D)	–	+++
	CU – Procedures room (D)	–	+
EM	EM – Left Ward (F)	–	++
	EM – Right Ward (F)	++	+++
	EM – Resuscitation room (F)	–	+++
GN	GN – Doctor's office 02 (Fp)	–	+++
	GN – Doctor's office 02 (F)	–	++
	GN – Room 202 (Fp)	–	+
	GN – Room 202 (F)	–	–
HU	HU – Central procedure room 1 (D)	–	+++
	HU – Central procedure room 2 (D)	–	++
	HU – Room for resting (F)	–	++
ICU	ICU – Intensive-care unit (CF)	–	+
MCI	MCI – 309 (F)	–	++
	MCI – 310 (Fp)	–	+++
	MCI – 310 (F)	+	–
	MCI – 311 (Fp)	–	–
	MCI – 311 (F)	–	+++
	MCI – Medical clinic I (CF)	–	–
MCII	MCII – 314 (Fp)	–	++
	MCII – 324 (Fp)	+	–
OC	OC – Obstetrical centre (CF)	–	–
OPT	OPT – Doctor's office 02 (F)	+++	+
	OPT – Waiting room (F)	+++	++
OSC	OSC – Doctor's office 01 (F)	+	+++
	OSC – Doctor's office 04 (F)	–	–
PED	PED – Special care room (Fp)	–	+
	PED – Special care room (F)	–	+++
SC	SC – Room 01 (D)	–	+++
	SC – Room 02 (D)	–	++
	SC – Room 04 (D)	+++	++
SCI	SCI – Bed 406 (D)	++	+
	SCI – Bed 410 (D)	–	++
	SCI – Procedure room (D)	–	++
SCII	SCII – Curative room (D)	+++	+++
ST	ST – Preparation room (F)	–	+++
	ST – Storage room (F)	–	++

Central filter (CF); diffuser (D); filter (F); flaps (Fp).
*Scores: without growth (–); 1–15 cysts and/or trophozoites (+); 16–30 (++) cysts and/or trophozoites and >30 cysts and/or trophozoites (+++). The assays were performed in triplicate; for each replicate, cysts and/or trophozoites were counted in five microscope fields (at 100×).

There are few reports of isolation of *Acanthamoeba* in hospital settings, despite the importance of these microorganisms as causing opportunistic infections, as well as vehicles and pathogen dispersers (Kocazeybek, 2015). Carlesso *et al.* (2010) described the presence of T4 genotype *Acanthamoeba* in dust samples and T5 genotype in biofilm samples, both from a hospital environment in Porto Alegre, Rio Grande do Sul, Brazil. An investigation conducted in Austria for the presence of AVL and bacteria in

refrigeration systems following a legionellosis outbreak in and around a hospital reported the presence of nine *Acanthamoeba* isolates belonging to the T4 genotype, which had amoeba resistant bacteria in its interior, emphasizing the importance of these amoebas as bacterial vehicles (Scheikl *et al.*, 2016).

All genotypes identified in our study are associated with cases of human infections (Siddiqui and Khan, 2012; Jercic *et al.*, 2019). These results deserve special attention from the hospital community, considering the isolation environment, the characteristic of many patients in a hospital environment, as they may be immunologically susceptible to infections, as well as the opportunistic nature of *Acanthamoeba* spp. However, further sequencing is required to obtain a better understanding of the spread of amoebae throughout the studied hospital.

Several authors report that osmo and thermotolerance assays can determine the pathogenicity of an *Acanthamoeba* isolate, since isolates capable of adapting physiologically and resisting to adverse conditions, such as growth in hyperosmolar medium and at elevated temperatures, are more adapted and can cause infections in the man and animals (Khan *et al.*, 2002).

In this study, pathogenic isolates and with low pathogenic potential were obtained from hospital settings, such as EM room, OSC, SC and surgical clinic, which are environments where patients with severe health conditions and those susceptible to opportunistic infections are found. Although most of the isolates were not classified as pathogenic, they still have significant epidemiological importance, since they can serve as vehicles and reservoirs of pathogenic microorganisms in health service settings.

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