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DE GRUYTER

CASE REPORT

Virulent T4 *Acanthamoeba* causing keratitis in a patient after swimming while wearing contact lenses in Southern Brazil

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Abstract

Several strains of free-living amoebae belonging to the genus *Acanthamoeba* can cause a painful sight-threatening disease of the cornea known as *Acanthamoeba* keratitis (AK). The numbers of AK cases keep rising worldwide mainly due to an increase in contact lens wearers and lack of hygiene in the maintenance of contact lenses and their cases. We report a case of AK in a healthy young woman admitted to the Hospital de Clinicas in Porto Alegre, southern Brazil. Corneal scrapings were examined for the presence of *Acanthamoeba* strains. The initial isolate was characterized by morphological and genotypic properties. The isolate belonged to group III according to Pussard and Pons' cyst morphology. Analysis of its 18S rDNA sequence identified the isolate as genotype T4. The T4 genotype is the most commonly reported among keratitis isolates and the most common in environmental samples.

Keywords

Free-living amoebae, *Acanthamoeba*, keratitis, contact lenses

Introduction

Acanthamoeba keratitis (AK) is a severe, sight-threatening ocular ailment caused by a free-living, opportunistically pathogenic amoeba, *Acanthamoeba* spp. (Visvesvara *et al.* 2007; Gupta *et al.* 2015). This species is a unicellular protozoan that has the ability to form amoeba cysts, being present in many different environmental compartments, such as the air, soil, and fresh water, including baths, swimming pools, and stagnant water bodies. *Acanthamoeba* spp. are of major concern in public health and clinical settings, since they are resistant to many disinfecting agents as well as to changes in temperature and dry environments.

The first reported case of an *Acanthamoeba* ocular infection occurred in 1974 (Naginton *et al*. 1974; Arance-Gil *et al*. 2014). However, the incidence of ailments caused by *Acanthamoeba* spp. has risen alarmingly in the past decades, probably due to the increased use of contact lenses as well as improved diagnostic methods, which, in turn, lead to increased awareness of this issue. Over the past few decades, it has also become apparent that contact lens wearers have an increased risk of developing corneal infections, and studies have estimated that about 90% of patients with AK are contact lens wearers (Radford *et al*. 1998; Kobayashi *et al*. 2011; Siddiqui *et al*. 2014).

The clinical diagnosis of AK is challenging, since it usually presents with nonspecific signs and symptoms, including, but not limited to, severe pain, photophobia, and blindness. These conditions, however, may be present in different types of keratitis, such as those caused by viral, bacterial, and

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fungal agents. This often leads to misdiagnosis and delayed treatment, which may have a profound effect on patients, since early diagnosis and prescription of appropriate treatment are essential for a good prognosis given that AK is unresponsive to commonly prescribed antimicrobials (Gupta *et al.* 2015).

Conventionally, species comprising the genus *Acanthamoeba* have been classified according to their morphological characteristics, such as cyst size and shape, being divided into three morphological groups or species (Pussard and Pons 1977; De Jonckheere 1987; Page 1988). Although morphological characterization is still applied to isolate identification, newer molecular approaches focusing on the sequences of small-subunit nuclear 18S rRNA genes are currently the gold standard for the taxonomic characterization of *Acanthamoeba* species (Duarte *et al*. 2013). Twenty different *Acanthamoeba* genotypes have been established based on sequence variation, namely T1–T12 (Stothard *et al*. 1998), T13 (Horn *et al*. 1999), T14 (Gast 2001), T15 (Hewett *et al*. 2003), T16 (Corsaro 2010), T17 (Nuprasert *et al*. 2010), T18 (Qvarnstrom *et al*. 2013), T19 (Magnet *et al*. 2014), and T20 (Corsaro *et al*. 2015). T4 is the most abundant of these genotypes in the environment and includes many pathogenic strains, which have been associated with neurological and eye diseases (Lorenzo-Morales *et al*. 2015; Omaña-Molina *et al*. 2016).

In this context, we report a case of AK that occurred in Rio Grande do Sul, the southernmost state of Brazil, and characterize the *Acanthamoeba* isolated from the patient's cornea.

Case Report

In July 2015, a 27-year-old woman sought medical attention for severe ocular pain, blurry vision, photophobia, and a foreign body sensation in the left eye. She had been wearing disposable soft contact lenses for 8 years. The patient reported that, at first, she used the cleaning solution correctly, but then started using tap water for contact lens rinsing. She used to wear contact lenses while swimming in the pool and sea before symptom onset. She also reported wearing her contact lenses for many hours, including during sleep.

The clinical diagnosis was based on microscopic findings, including corneal edema, punctate epithelial injuries, and stromal ring infiltrate (Fig. 1). Patient history and clinical examination suggested the diagnosis of AK, which was later confirmed by laboratory tests. For 6 months, the patient was treated with available antiamoebic drugs (Brolene, biguanide, and chlorhexidine). Because of the extent of corneal involvement, the doctors decided to begin with medical treatment, followed by subsequent corneal transplantation. Her initial visual acuity was counting fingers. The patient underwent a successful corneal transplant, and her visual acuity improved to 20/100 after surgery. After transplantation, there was no recurrence of the disease and the patient continues to perform the medical follow-up.

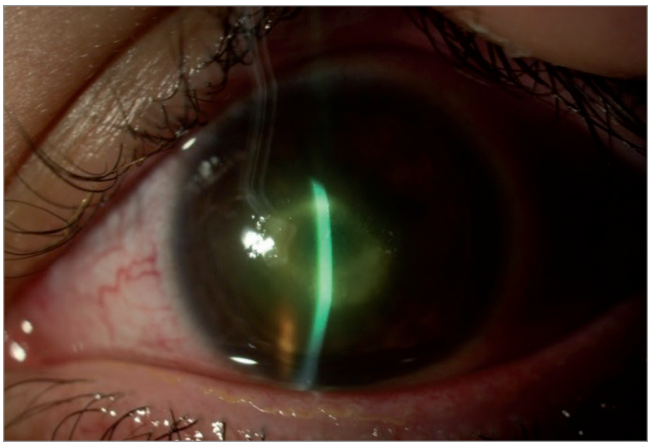

Fig. 1. Injuried eye

Materials and Methods

Acanthamoeba sample

The strain reported in this case was isolated from corneal scrapings of a patient admitted to the Hospital de Clínicas in Porto Alegre, southern Brazil, with a diagnosis of AK. Primary isolation of the *Acanthamoeba* strain was performed by transferring amoebae to 1.5% non-nutrient agar (NNA) plates seeded with a layer of heat-killed *Escherichia coli*. The clinical sample was then streaked onto the NNA plates and incubated at 30°C. Trophozoites were visualized in less than 24 hours.

Morphological identification

Cysts were harvested from culture plates by washing them with sterile saline solution (2 mL) under a laminar air-flow hood and mounted between a slide and a coverslip for examination using a light optical microscope at 1000x magnification. The morphological characteristics of trophozoites and cysts were analyzed as described by Page (1988). We measured 10 cysts of the isolate using an ocular micrometer scale under a light optical microscope. The *Acanthamoeba* isolate was classified according to the three known groups (I, II, and III) based on cyst size and shape (Pussard and Pons 1977; Khan 2006; Visvesvara *et al.* 2007).

DNA extraction, PCR and DNA sequencing

DNA was extracted and purified using a PowerSoil® DNA Isolation Kit (MO BIO) following the manufacturer's instructions. The primers JDP1 and JDP2 were used to amplify the ASA.S1 region of the gene (Rns) coding for the amoeba's nuclear small-subunit ribosomal RNA (Schroeder *et al.* 2001). The PCR products were subsequently purified and sequenced. DNA sequencing was performed on an ABI-

Table I. Morphological and genotypic characterization of *Acanthamoeba* from Porto Alegre, Rio Grande do Sul, Brazil

Isolate	Source	Cyst size (µm)[a]	Group[b]	*Rns* genotype	Similiarity %/reference[c]	
C1	Keratitis	10.78 ± 2.35	III	T4	98%	EU146071.1

[a]Average ± standard deviation.
[b]Pussard and Pon's criteria (1977).
[c]Reference strains retrieved from GenBank

Prism 3500 Genetic Analyzer sequencer (Applied Biosystems).

In order to classify the *Acanthamoeba* isolate, the 18S rDNA gene sequence was uploaded into the 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 sequence. The sequence for the new isolate described in this study is available in GenBank (accession number: KX578215).

Results

Cysts of the *Acanthamoeba* isolate ranged from 9.24 to 16.94 µm in size (Table I), (Fig 2), being regarded as typical of morphological group III according to Pussard and Pons (1977). Trophozoites showed typical contractile vacuoles and filiform pseudopodia (acanthopodia) (Fig. 3).

The 18S rDNA gene sequence analysis indicated that the *Acanthamoeba* isolate showed at least 98% identity with *Acanthamoeba* belonging to the T4 genotype, the most commonly reported among keratitis isolates (Marciano Cabral and Cabral, 2003) (Table I).

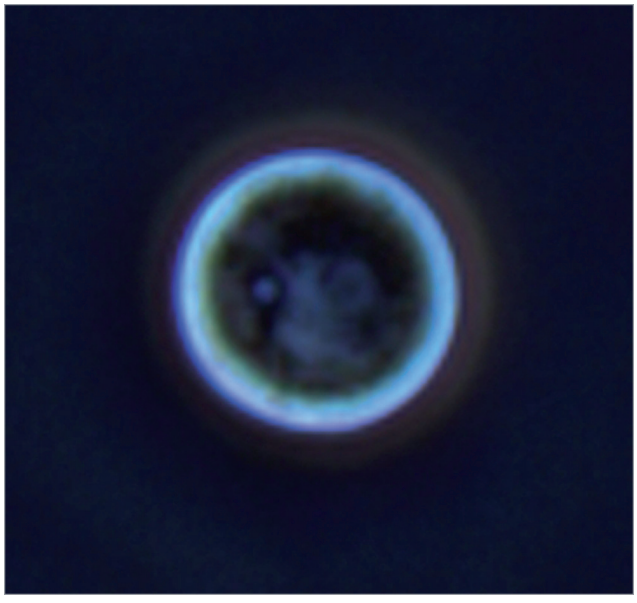

Fig. 2. Cyst of *Acanthamoeba*

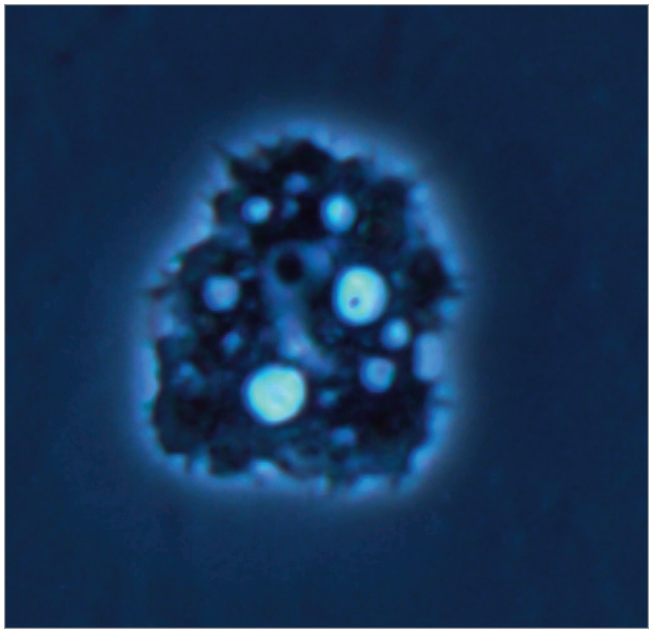

Fig. 3. Trophozoite of *Acanthamoeba*

Discussion

AK was first reported in 1974 (Naginton *et al*. 1974), being initially documented as an extremely rare form of infectious keratitis ensuing from ocular trauma. During the 1980s, a dramatic upsurge in the incidence of this condition was observed among contact lens wearers, subsequently acknowledged as being associated with the use of contact lenses (Hirst *et al*. 1984; Moore *et al*. 1985; Mutoh *et al*. 2010). Typically, only one eye is affected, although some cases of bilateral AK have been reported in the literature (Wilhelmus *et al*. 2008; Lorenzo-Morales *et al*. 2013; Omaña-Molina *et al*. 2016).

Identification of the etiological agent of AK up to the genus level is appropriate for diagnostic and therapeutic purposes. However, for epidemiological studies, it is necessary to both identify and characterize the species in order to determine whether it is more likely to be parasitic and to establish its probable niche. In the present study, the *Acanthamoeba* isolate obtained from the patient with AK was classified as belonging to group III according to Pussard and Pons' criteria (Duarte *et al*. 2013). Sequence analysis of the ASA-S1 fragment indicated that the isolate belonged to the T4 genotype, which is the most commonly reported among keratitis isolates (Marciano-

Cabral and Cabral 2003; Booton *et al*. 2005) as well as the most common in environmental samples (Booton *et al*. 2004, 2005).

Our patient with AK reported wearing soft contact lenses while swimming both in the pool and sea. Contact lens use in these environments has been acknowledged as a potential risk factor for developing ocular infection (Radford *et al*. 2002; Choo *et al*. 2005; Arance-Gil *et al*. 2014).

Early AK diagnosis is crucial to minimize irreversible damage. Accordingly, awareness should be raised when suspected herpetic keratitis does not respond to antiviral therapy (Page and Mathers 2013; Lorenzo-Morales *et al*. 2015; Omaña-Molina *et al*. 2016). Most AK cases are initially misdiagnosed as herpetic keratitis or, less frequently, as bacterial or fungal keratitis because AK signs and symptoms may be nonspecific (Hsieh and Dornic, 1989). Negative bacterial and fungal cultures that do not improve with medical treatment should be further and immediately investigated. Confocal microscopy is useful to confirm the diagnosis of AK, considering that *Acanthamoeba* cultures usually show negative results (Choo *et al*. 2005; Vaddavalli *et al*. 2011; Arance-Gil *et al*. 2014).

Conclusion

Early diagnosis is important for effective treatment of AK. Improved education of contact lens wearers is needed to prevent such infections. Surgical debridement may be a useful treatment adjunct for AK.

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