Experimental Parasitology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Genotypic heterogeneity based on 18S-rRNA gene sequences among *Acanthamoeba* isolates from clinical samples in Italy

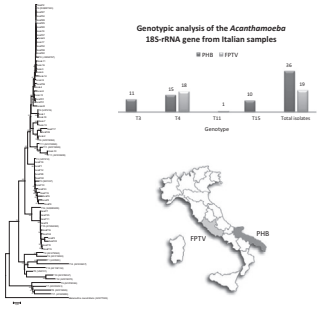
David Di Cave [a,b], Rossella D' Alfonso [c], Kodjo A. Dussey Comlavi [b], Carlo D' Orazi [b], Rosa Monno [d], Federica Berrilli [a,*]

[a] Department of Experimental Medicine and Surgery, University of Rome "Tor Vergata", Via Montpellier 1, 00133 Rome, Italy
[b] Laboratory of Parasitology, Foundation Polyclinic Tor Vergata, Viale Oxford 81, 00133 Rome, Italy
[c] Department of Systems Medicine, University of Rome "Tor Vergata", Via Montpellier 1, 00133 Rome, Italy
[d] Department of Basic Medical Science, Neuroscience and Sense Organ, University of Bari, P. G. Cesare 11, Bari, Italy

HIGHLIGHTS

- The genotype heterogeneity of *Acanthamoeba* from patients with AK was analyzed.
- Different *Acanthamoeba* genotypes were detected.
- The majority were T4 and T15; T3 and T11 were identified for the first time in Italy.
- Genotyping allows understanding the environmental circulation and disease association.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 January 2014
Received in revised form 11 April 2014
Accepted 7 May 2014
Available online xxxx

Keywords:
Acanthamoeba
Keratitis
18S rDNA
Genotype variability
Italy

ABSTRACT

Acanthamoeba keratitis (AK) is an ocular disease caused by members of a genus of free-living amoebae and it is associated predominantly with contact lens (CL) use. This study reports 55 cases of AK diagnosed in Italy. Genotype identification was carried out by PCR assay followed by sequence analysis of the 18S rRNA gene using the genus specific primers JDP1 and JDP2. Genotype assignment was based on phenetic analysis of the ASA.S1 subset of the small-subunit rRNA gene sequences. The material has been collected at the Polyclinic Tor Vergata of Rome for a total of 19 isolates and at the Polyclinic Hospital of Bari (36 isolates). Thirty-three out of the 55 genetically characterized isolates were assigned to the genotype T4. Ten isolates were identified as belonging to the genotype T15 thus confirming the first association between the genotype T15 and human amoebic keratitis previously described from the same area. We underline the occurrence of the genotype T3 and T11 identified for the first time in the country.

1. Introduction

Acanthamoeba keratitis (AK) is a potentially blinding disease, occurring mainly among users of soft contact lenses (Panjwani, 2010). The causative agents are species of the genus *Acanthamoeba*, opportunistic free-living protozoans found in the environment worldwide. *Acanthamoeba* species have been traditionally identified on the basis of their morphology (Pussard and Pons, 1977). However, since it is not always possible to unequivocally establish differences by microscopy, molecular genotyping mainly based on the analysis of 18S rRNA, allowed to establish 17 different genotypes (T1-T17) among the genus *Acanthamoeba* (Nuprasert et al., 2010). It is widely accepted that the current cut-off limit of

* Corresponding author. Fax: +39 06 72506040.
E-mail address: berrilli@uniroma2.it (F. Berrilli).

http://dx.doi.org/10.1016/j.exppara.2014.05.009
0014-4894/

similarities between all sequences of each distinct genotype from all other 18S rRNA gene sequences should be at least 5% (Stothard et al., 1998). To date genetic studies have shown that the genotype T4 represent the most commonly type found in human AK as well as in environmental isolates (Khan, 2006; Ledee et al., 2009; Magnet et al., 2012). The preponderance of genotype T4 in human AK infections could be most likely due to their potential virulence as well as to its relative presence in the environment (Khan, 2006). Recently, a high genetic diversity has been observed amongst T4 isolates and a classification of the T4 cluster in sub-genotypes is currently applied on the basis of differences in the sequence (Booton et al., 2002; Maciver et al., 2013).

Although epidemiological and molecular studies from patients with amoebic keratitis have frequently been reported in several countries worldwide including Europe (Magnet et al., 2012; Nagyová et al., 2010; Yera et al., 2008), data about the epidemiology of *Acanthamoeba* in Italy are still scarce and the pattern of genetic variation in *Acanthamoeba* isolated from the environment and from patients infected by this pathogen is poorly known (Corsaro and Venditti, 2010; Di Cave et al., 2009; Gatti et al., 2010). In the present study, the genotypes among clinical *Acanthamoeba* were determined.

2. Materials and methods

The study involved 55 *Acanthamoeba* positive specimens from symptomatic patients with suspected AK. Thirty-six samples were collected at the Ophthalmology Clinic of the Polyclinic Hospital of Bari (PHB) in Southern Italy from September 2008 to February 2012 while 19 samples were collected at the Laboratory of Parasitology of the Polyclinic Tor Vergata of Rome (FPTV) in Central Italy, between December 2009 and May 2013. Overall, 25 subjects were males and 30 females; 24 conjunctival swabs, 12 corneal scrapings, and 19 lens case solutions were analyzed. *Acanthamoeba* isolates from PHB were cultured on agar plates in according to Di Cave et al. (2009) and rinsed in phosphate-buffered saline (pH 7.4) before molecular procedure; for FPTV samples DNA was extracted from biological samples directly without plate culture. In Table 1, the samples cultured or just detected by PCR were reported in detail. The extraction procedure was performed by using the QIAamp DNA Micro Kit (Qiagen, Italy). PCR was carried out to amplify a region from the 18S-rRNA gene defined as ASA.S1 that includes Diagnostic Fragment 3 (DF3), using the genus-specific primers JDP1 and JDP2 (Schroeder et al., 2001) as described previously (Di Cave et al., 2009). The expected PCR amplicons were purified

Table 1
Acanthamoeba positive isolates tested in the present study and genotypes.

	FPTV	PHB	Total
Patients	19	36	55
Male	9	16	25
Female	10	20	30
Samples			
Conjunctival swabs	2	22*	24
Corneal scrapings	12	–	12
Lens case solutions	5	14*	19
Genotypes			
T3	–	11	11
T4	18	15	33
T11	1	–	1
T15	–	10	10

FPTV: Foundation Polyclinic Tor Vergata of Rome (Central Italy).
PHB: Polyclinic Hospital of Bari (Southern Italy).
* Cultured samples.

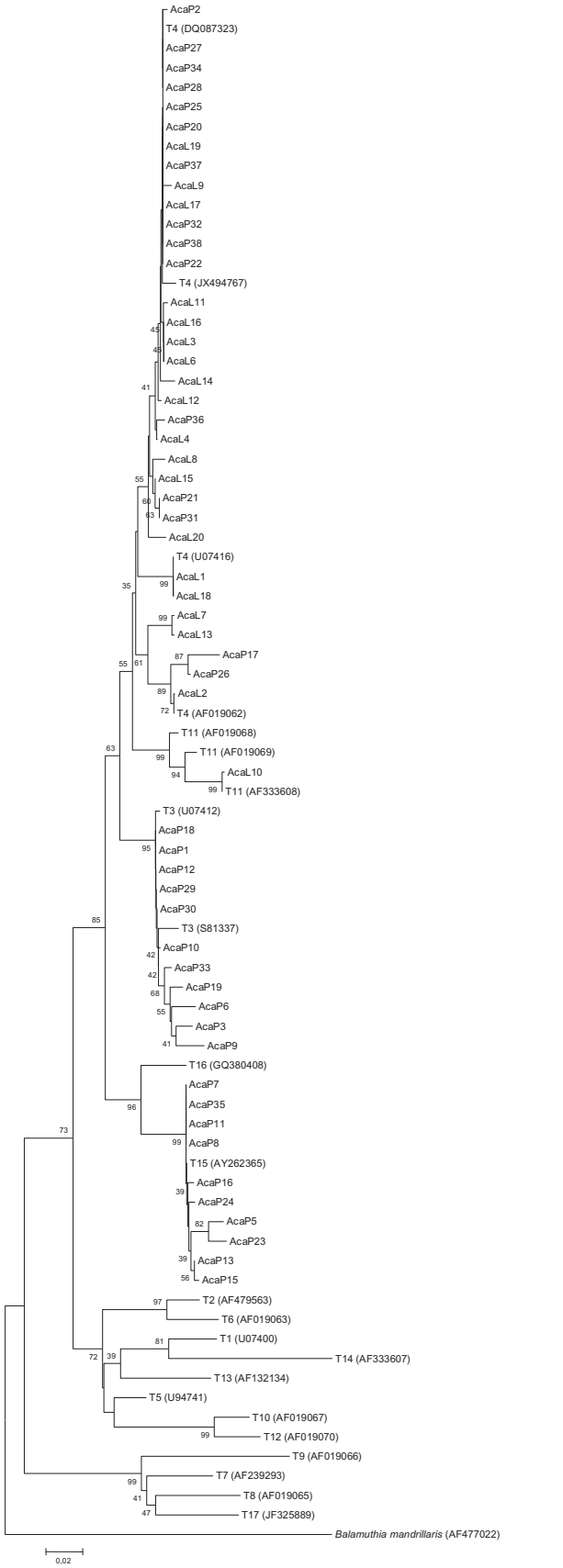


Fig. 1. Neighbor-joining tree showing the genetic relationships among 55 *Acanthamoeba* isolates examined in this study, based on ASA.S1 DNA sequences. GenBank accession numbers of reference strains are in *parentheses*.

Please cite this article in press as: Di Cave, D., et al. Genotypic heterogeneity based on 18S-rRNA gene sequences among *Acanthamoeba* isolates from clinical samples in Italy. Exp. Parasitol. (2014), http://dx.doi.org/10.1016/j.exppara.2014.05.009