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■ Note ■

Isolation and Identification of *Acanthamoeba* in a Contact Lens Storage Case

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Acanthamoeba is a free-living amoeba that causes human infections, and recently the incidence of amoebic keratitis has increased among contact lens wearers. In order to investigate *Acanthamoeba* contamination of contact lens storage cases, a short survey was performed on 57 contact lens wearers, and *Acanthamoeba* was found in one contact lens storage case. To diagnose *Acanthamoeba*, the 18s small subunit ribosomal DNA (18s rDNA) gene was amplified by polymerase chain reaction (PCR), and subsequently, the isolate was identified as *A. lugdunensis*. This species was originally isolated from a freshwater pool in France, and was reported recently to be a cause of amoebic keratitis. This observation indicates the need for a large survey to investigate the extent of *Acanthamoeba* contamination, and suggests that contact lens wearers be aware of the importance of hygiene and of the implications of *Acanthamoeba* infection.

Key Words: *Acanthamoeba*, Contact lens storage case, 18s rDNA

The genus *Acanthamoeba* is the causative agent of granulomatous amoebic encephalitis (GAE) and amoebic keratitis (AK) (Marciano-Cabral and Cabral, 2003). Corneal infection by *Acanthamoeba* is rare but is an emerging issue principally among contact lens wearers. Researchers at the U.S. Center for Disease Control and Prevention (CDC) and others have noted sporadic outbreaks of AK among contact lens wearers. The first amoebic infection of the eye was reported in 1974 (Naginton et al., 1974), and since, a large number of AK cases (208 patients) have been reported in the United States, and furthermore, more than 85% of patients were contact lens wearers (Stehr-Green et al., 1989). The increasing incidence of AK patient among contact lens wearers has also been researched in the UK (Ficker, 1988), where an incidence of *Acanthamoeba* keratitis of one per 30,000 contact lens wearers per year was determined based on the findings of three cohort and three questionnaire based surveys (Seal, 2003). These findings suggest that contact lenses can serve as a vector for disease transmission.

This study was conducted to investigate *Acanthamoeba* contamination of contact lens storage cases among students attending the Department of Clinical Laboratory Science at Daegu Haany University. Fifty-seven students were interviewed and provided their contact lens storage cases for investigation (Table 1). Fifty-two students used soft contact lenses and 5 students used hard contact lenses. Most washed contact lenses daily or every 2~3 days using disinfecting solution, but 6 used saline, and 1 student used tap-water. As many as 53 students (93%) complained of symptoms, such as, redness, tearing, blurred vision, or a feeling that something was in their eyes.

Contact lens storage cases were wiped with a sterile cotton swab, and samples were cultured on agar plates, which had previously been spread with a lawn of heat treated (at 65℃ for 1 hr) *Escherichia coli*. Plates were then incubated in an incubator (Sanyo, San Diego, California, USA) at 25℃ for up to 7 days, and examined daily. *Acanthamoeba* was cultured from one contact lens storage case (sample No. 24). *Acanthamoeba* has two distinct stages, that is, the trophozoite and cyst stages. Cysts are formed from trophozoites under unfavorable conditions and are resistant to various biocides, pH, and high temperature

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(Coulon et al., 2010). Fig. 1 showed trophozoites and cysts of *Acanthamoeba* identified in the contact lens storage case.

Table 1. Survey of contact lens wearers

	Title	No. (%)	Total
Type of contact lens	Soft	52 (91.2)	57 (100)
	Hard	5 (8.8)	
Period of use	Less than 1 year	41 (71.9)	57 (100)
	1~3 years	14 (24.6)	
	More than 3 years	2 (3.5)	
Frequency of cleaning	Daily	37 (64.9)	57 (100)
	Once a 2~3days	12 (21.1)	
	Once a week	2 (3.5)	
	Once over a week	6 (10.5)	
Washing solution	Disinfecting solution	50 (87.7)	57 (100)
	Saline	6 (10.5)	
	Tap water	1 (1.8)	
Symptoms	Yes	53 (93.0)	57 (100)
	No	4 (7.0)	

Cultured amoebae were identified by sequence analysis of 18s small subunit ribosomal DNA (18s rDNA) (Yu et al., 2004). The amplified PCR product (2,293 bp) was cloned into the T/A cloning vector pGEM-T easy System (Promega, Madison, Wisconsin, USA). DNA sequencing was per-

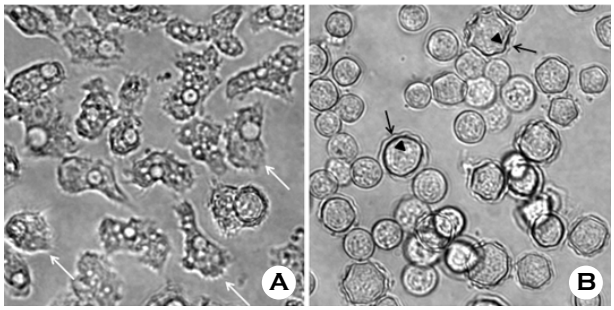

Fig. 1. *Acanthamoeba* identified from contact lens storage case sample No. 24. Showing (A) a trophozoite (an indeterminate form with acanthopodia (white arrow)). (B) a cyst (a mature cyst has complete double walls, an endocyst (black arrow head) and ectocyst (black arrow), and dense cytoplasm).

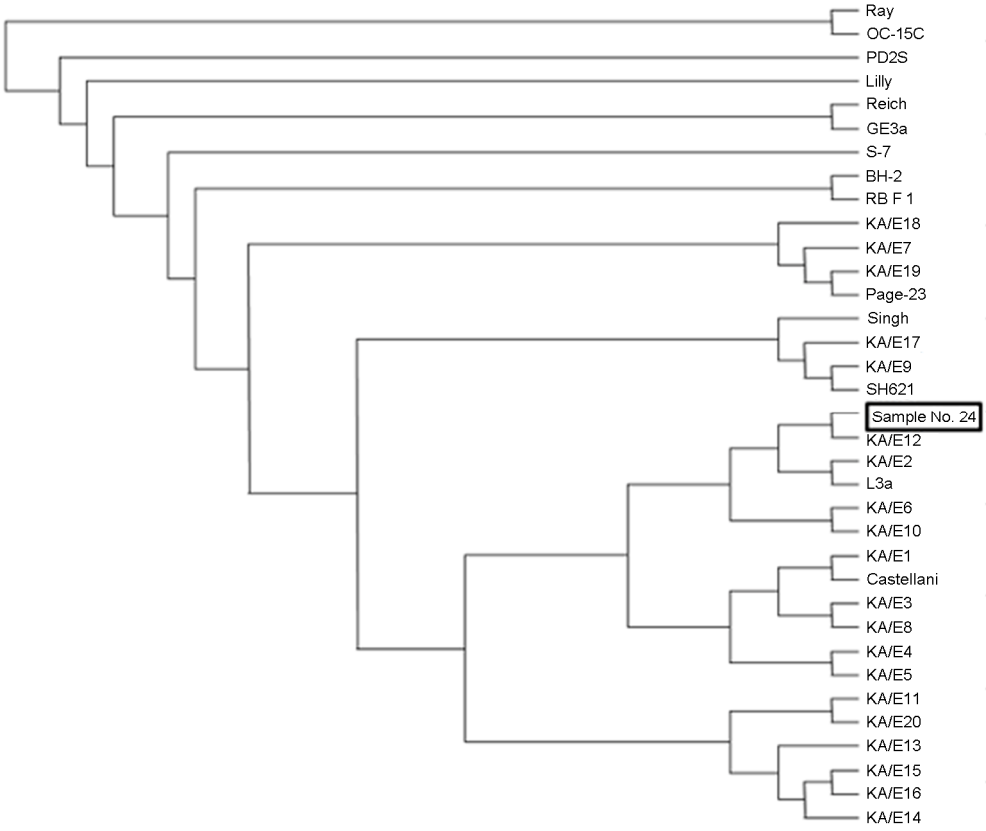

Fig. 2. Phylogenetic tree derived by aligning the 18s rDNA sequence of the isolate with other that of 14 reference strains and 20 clinical isolates. The results obtained showed that its 18s rDNA sequence shared considerable homology with *A. lugdunenesis* L3a group.

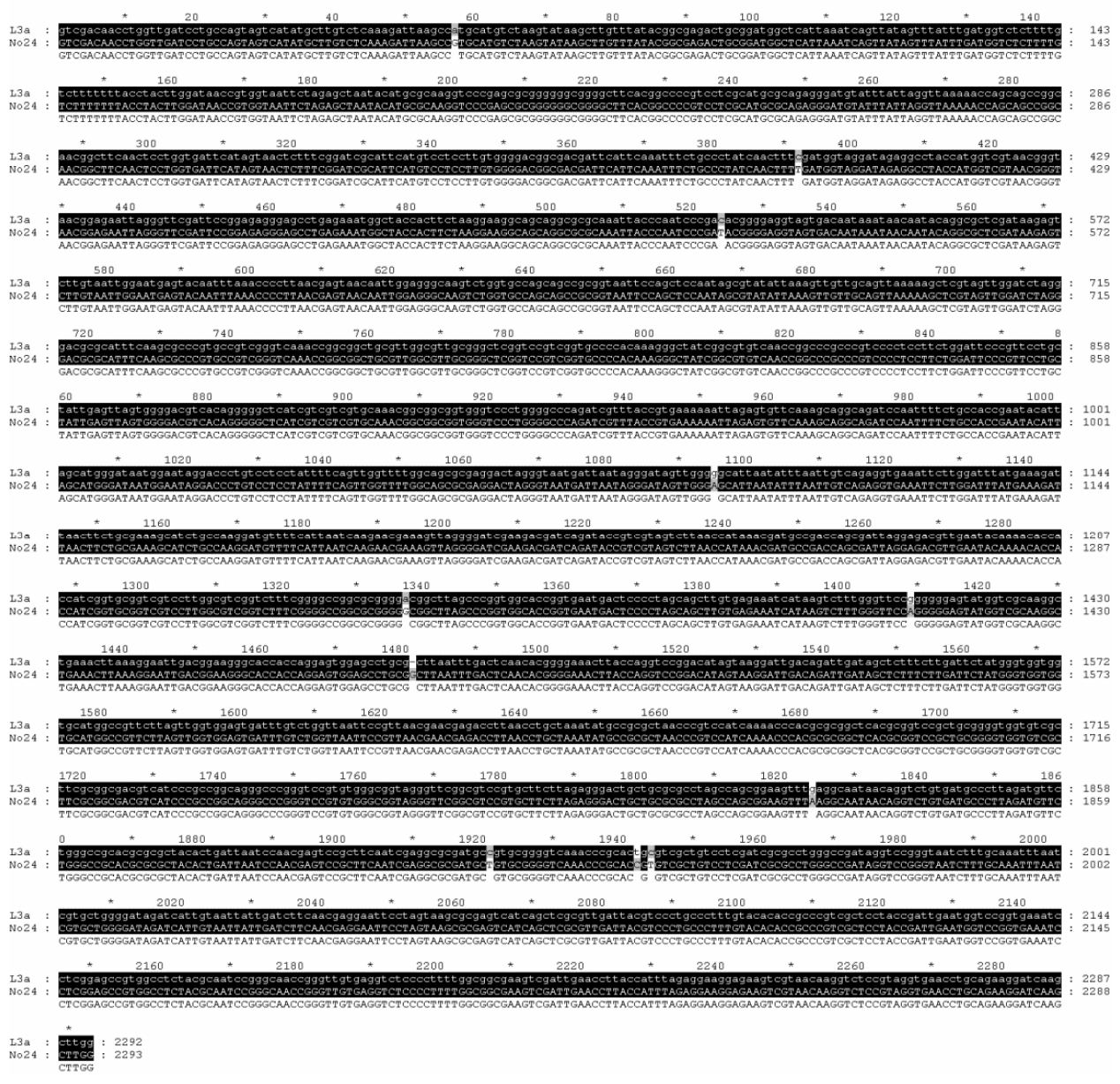

Fig. 3. Alignment of the 18s rDNA sequence of *A. lugdunensis* L3a with that of the isolate. Black backgrounds indicate the same base as the bottom sequence shown.

formed by Macrogen company (Seoul, Korea). Phylogenetic tree was derived from the alignment of 34 kinds of *Acanthamoeba* spp. using ClustalX program version 1.82 with a low gap penalty, and drawn using TreeView software. Fig. 2 shows the phylogenetic tree of the 18s rDNA produced by the *Acanthamoeba* identified in the contact lens storage case and other 18s rDNA sequences, including 14 reference strains and 20 clinical isolates. This result shows that *Acanthamoeba* identified in sample No. 24 was most closely related to *A. lugdunensis* L3a (ATCC 50240). When the 18s rDNA sequence of sample No. 24 was compared with that of *A. lugdunensis* L3a, they were found to share 99% sequence homology (2,282 bp/2,293 bp) (Fig. 3). Species of four *Acanthamoeba* previously isolated (KA/E2, KA/E12, KA/E15 and KA/E16) from the corneas of patients with keratitis were identified as *A. lugdunensis* L3a (Yu et al., 2004). These results indicate that individuals wearing contact lenses are exposed to the possibility of

Acanthamoeba spp. contamination.

The results of this study indicate that a large-scale survey is required to determine the extent *Acanthamoeba* contamination. Furthermore, this investigation provides clues regarding the pathogenesis and epidemiology of keratitis among contact lens users, and cautions contact lens wearers that proper hygiene practices are required to reduce the risks of *Acanthamoeba*, bacterial and fungal eye infections.

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