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Genotyping of *Acanthamoeba* Isolates from Corneal Scrapings and Contact Lens Cases of *Acanthamoeba* Keratitis Patients in Osaka, Japan

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Free-living amoebae of the genus *Acanthamoeba* are ubiquitous in the environment and can be found in soil, dust, domestic tap water, and air-conditioning units (1,2). In humans, they are responsible for causing *Acanthamoeba* keratitis (AK), which is a very painful and sight-threatening infection (1,2). The genus *Acanthamoeba* is comprised of multiple species, but their discrimination by light microscopy is difficult because the morphology of their cysts is similar among species (1,2). Recent molecular studies indicate *Acanthamoeba* is comprised of at least 16 genotypes (T1–T16) based on sequence analysis of the 18S ribosomal RNA gene (*Rns*) (3–7), and some of these genotypes have been linked to *Acanthamoeba* spp., such as *A. lenticulata* (T5), *A. griffini* (T3), *A. castellanii*, *A. polyphaga*, *A. culbertsoni* (T4), and *A. jacobsi* (T15) (3,6). Therefore, genotype analysis of the isolates is helpful for rapid diagnosis of AK infection, accurate identification of *Acanthamoeba* isolates and for understanding the molecular epidemiology of *Acanthamoeba* infection. In this study, we showed the usefulness of genotype analysis of *Acanthamoeba* isolates using clinical isolates.

The five isolates, AC6, AC8, AC15, AC13, and AC14 from patients A and B, originated from corneal scrapings and swabs of contact lens cases of both patients (Table 1). These were isolated by the plate culture method reported previously (2) and subcultured by cutting a small piece of agar with sterile cover glasses (9 × 9 mm) and applying it centrally onto a fresh plate. For molecular analysis, the cysts of these isolates were collected using sterile scrapers and suspended into phosphate buffered saline (PBS). The other two isolates AC29 and AC28 originated from the corneal scraping and swab of the contact lens case of patient C (Table 1), and both samples were provided for molecular analysis directly without plate culture because of a request for rapid diagnosis. The DNA from five isolates and two samples mentioned above were extracted and purified using a DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions. The *Acanthamoeba*-specific *Rns* ASA.S1 region that includes the highly variable region designated diagnostic fragment 3 (DF3) was amplified using the *Acanthamoeba*-specific primers JDP1 and JDP2 reported previously (8). The PCR products were sequenced in both directions on an ABI 3130 automated sequencer (Applied Biosystems, Foster City, Calif., USA). The *Rns* genotypes of the isolates examined in this study were determined by phylogenetic analysis of the ASA.S1 sequences (460 bp) and the DF3 sequence types were compared to those of the isolates reported previously (9,10). The new numbers of DF3 sequence type identified in this study are a continuation of those of the previous reports (9,10) (Table 1, see Fig. 2). The *Rns* ASA.S1 sequences obtained in this study are available in the International Nucleotide Sequence Database (INSD, GenBank/DDBJ/EMBL) under accession nos. AB554223 to AB554229.

The diagnostic fragment was successfully amplified in all isolates. Phylogenetically, the *Rns* genotypes of all isolates were identified T4 that have been isolated most frequently in human infection (1,2) (Fig. 1). The DF3 sequences (69 bp) of all isolates from the three patients were varied (Fig. 2). Namely, the sequences of two isolates from patient A were identical to each other but different from the other DF3 sequences reported previously (Table 1, Fig. 2) (9,10). Similarly, the DF3 sequences of the other five isolates from patients B and C were identical to each other but also different from that of the isolates from patient A (Table 1, Fig. 2).

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Isolate source</th>
<th>Collection date</th>
<th><em>Rns</em> genotype/DF3 sequence type</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC6</td>
<td>Corneal scraping from patient A</td>
<td>Nov. 2006</td>
<td>T4/22</td>
</tr>
<tr>
<td>AC8</td>
<td>Right lens case used by patient A</td>
<td>Nov. 2006</td>
<td>T4/22</td>
</tr>
<tr>
<td>AC15</td>
<td>Corneal scraping from patient B</td>
<td>Dec. 2006</td>
<td>T4/23</td>
</tr>
<tr>
<td>AC13</td>
<td>Left lens case used by patient B</td>
<td>Dec. 2006</td>
<td>T4/23</td>
</tr>
<tr>
<td>AC14</td>
<td>Right lens case used by patient B</td>
<td>Dec. 2006</td>
<td>T4/23</td>
</tr>
<tr>
<td>AC29</td>
<td>Corneal scraping from patient C</td>
<td>May 2009</td>
<td>T4/23</td>
</tr>
<tr>
<td>AC28</td>
<td>Right lens case used by patient C</td>
<td>May 2009</td>
<td>T4/23</td>
</tr>
</tbody>
</table>

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The use of contact lens is one of the probable risk factors for AK infection due to poor lens hygiene and insufficient acanthamoebacidal activities of multipurpose contact lens solutions (11–13). In this study, the sequences of *Rns* ASA.S1 including the hyper variable DF3 region of the isolates from the corneal scraping and contact lens case of each patient were identical for each patient, indicating a link between contact lens contamination and AK infection. In addition, the DF3 sequence type of the five isolates from patients B and C were
identical to each other (T4/23), suggesting both patients might have been infected by the same strain. Although the early diagnosis of AK infection is essential for a successful prognosis (1,2), it is difficult to diagnose AK infection by clinical symptoms alone because the clinical symptoms of this infection resemble those of a viral infection such as herpes simplex virus, or a bacterial or fungal infection. Therefore, isolation of Acanthamoeba from clinical samples by culture is helpful for accurate diagnosis. However, culture plates inoculated with clinical samples have to be observed for several days, sometimes for 10 days or more depending on the number of amoeba present in the samples, for confirmation of the presence of Acanthamoeba trophozoites or cyst (1,2). In this study, we trialed PCR using JDP1 and JDP2 primers on clinical samples from patient C for rapid diagnosis of AK infection, and we were able to obtain the successful result mentioned above. It only took approximately 6 h from the extraction of the sample DNA till confirmation of the result by PCR. Therefore, we believe that the PCR method used in this study is a helpful tool for the rapid and accurate diagnosis of AK infection.

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Conflict of interest None to declare.

REFERENCES