Kertas Asli/Original Article

Ability of Acanthamoeba Cyst to Excyst at Different Temperatures
(Kebolehan Sista Acanthamoeba Bereksistasi pada Suhu Berbeza)

NURUL FARIZA ROSSL, MOHAMED KAMEL ABD GHANI, ANISAH NORDIN, YUSOF SUBOH & NORAINA AB RAHIM

ABSTRACT
This study was carried out to observe thermotolerance ability of Acanthamoeba spp. A total of 32 Acanthamoeba spp. isolates obtained from water taps, sinks, swimming pools and sea water were used. Trophozoites of Acanthamoeba spp. were inoculated onto non-nutrient agar (NNA) seeded with heat-killed Escherichia coli using aseptic technique and incubated for 14 days at 30°C to obtain the cyst. The cysts were subcultured onto new agar plates for thermotolerance test at 37°C and 42°C. The plates were observed until 96 hours after incubation for excystation of Acanthamoeba before being declared negative. Overall, 81.25% of samples were able to excyst at 37°C while 37.5% were able to excyst at 42°C. Thermotolerant Acanthamoeba is associated with high pathogenicity potential.

Keywords: Thermotolerance, Acanthamoeba cyst, Excystation

INTRODUCTION
Since the year 1970, the species Acanthamoeba has been recognised as being medically important. This protozoon is the causal agent of a few diseases with high mortality or morbidity risk such as granulomatous amoebic encephalitis, keratitis, and cutaneous lesion (Martinez 1985).

Although the genus Acanthamoeba was first found in 1930 as a contaminant of eukaryotic cell culture (Castellani 1930), infection in human was not reported until early 1970 based on a number of deaths caused by cerebral granulomatous disease (Warhurst 1985). Ocular amoebic infection by Acanthamoeba was detected initially in 1974 in United Kingdom (Nagington et al. 1974). Later on, more than 60 cases have been detected worldwide, excluding cases that may not be reported (Epstein 1986; Wilhelmus et al. 1986).

The first Acanthamoeba keratitis case in Malaysia was reported in 1995 involving individual with a history of 15 years of contact lens usage (Mohamad Kamel & Norazah 1995). Contact lens wearer has higher risk of getting Acanthamoeba keratitis compared to non-wearer, covering 90% of reported cases (Radford et al. 1995; Radford et al. 2002).

Thermotolerance ability of trophozoite is said to influence the difference between virulent and non-virulent Acanthamoeba strains (Griffin 1972), but so far there is no study that confirmed the statement. Ability to withstand high temperature is accepted by many parties as a requirement for pathogenicity in granulomatous amoebic encephalitis because human body temperature is 37°C, but not for keratitis infection for the reason that the eye has an average temperature of only 34°C. Nevertheless, it could not be denied that clinically important Acanthamoeba strains displayed thermotolerance and accelerated growth rate compared to non-pathogenic free-living strains (Badenoch et al. 1995; Dini et al. 2000).

Hence, this study is carried out to examine the potential pathogenicity risk of Acanthamoeba sp. using thermotolerance test from previously selected environmental samples.
MATERIALS AND METHODS

SOURCE OF ACANTHAMOEBA

Acanthamoeba spp. were obtained through isolation from the environment. A total of 32 isolates were used with 2 from water taps, 10 from sinks, 8 from sea water, and 12 from swimming pools. Acanthamoeba subcultured onto non-nutrient agar seeded with heat-killed E. coli was incubated at 30°C for 14 days to attain mature cysts.

THERMOTOLERANCE TEST

Thermotolerance test assay was modified from the method used by Khan & Paget 2002 and Walochnik et al. 2000. Acanthamoeba cysts were inoculated onto non-nutrient agar seeded with heat-killed E. coli. The plate was then incubated at 37°C and 42°C and observed under inverted microscope up to 96 hours after incubation for excystation of cysts before being declared negative. Incubation at 30°C was used as positive control.

RESULTS

There were 26 Acanthamoeba spp. isolates that could undergo excystation at 37°C but only 12 isolates could excyst at 42°C. The number and percentage of samples able to excyst at 37°C and 42°C are shown in Table 1. The temperature 30°C is the average optimum temperature for the growth of Acanthamoeba spp. and is therefore used as positive control.

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Number and percentage of Acanthamoeba spp. able to excyst</th>
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<tbody>
<tr>
<td></td>
<td>30°C</td>
</tr>
<tr>
<td>Water tap swabs</td>
<td>2/2 (100)</td>
</tr>
<tr>
<td>Sink swabs</td>
<td>10/10 (100)</td>
</tr>
<tr>
<td>Seawater</td>
<td>8/8 (100)</td>
</tr>
<tr>
<td>Swimming pool swabs</td>
<td>12/12 (100)</td>
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</tbody>
</table>

DISCUSSION

In this study, thermotolerance of Acanthamoeba is defined as the ability of Acanthamoeba spp. to undergo excystation under the desired temperature (37°C or 42°C) after mature cysts were subcultured onto new NNA plates. Trophozoites possessing the ability to exist at 37°C or higher have more potential to cause infection in human (Griffin 1972). Meanwhile, trophozoites with capability to excyst at 42°C or higher are labelled as thermotolerant species.

Acanthamoeba spp. that could tolerate high temperature has the potential to become more virulent than strains that could only excyst at 30°C (De Jonckheere 1983; Griffin 1972; Khan et al. 2001; Walochnik et al. 2000).

From the results, it is evident that Acanthamoeba with the ability to grow at 37°C could be isolated from all four sampling sites, eventhough not at 100% of samples. Samples from sink have the highest positive percentage (90%) followed by swimming pool (83.3%), sea (75%), and least from water tap (50%). This suggests that there is a risk of getting infection from Acanthamoeba spp. at each sampling location especially for contact lens wearer who placed their contact lens cases over sink surface or washed and stored contact lenses using tap water. Swimming with contact lenses is not advisable due to the high prevalence of Acanthamoeba sp. in recreational water bodies such as seas and swimming pools.

The percentage of Acanthamoeba isolates that could tolerate 42°C is less than those that could tolerate 37°C, though the value could still be considered high. Samples from swimming pool have the highest positive percentage at 58.3% followed by sink (30%) and lastly sea (25%). There is no Acanthamoeba isolate capable of excystation at 42°C found from water tap samples.

The propensity of Acanthamoeba spp. to cause infection in human is dependent on numerous factors which are interconnected to each other. Corneal epithelium cells exist in conditions of high osmolarity due to the salinity of tears and temperatures exceeding those optimal to most Acanthamoeba species (Khan 2006). Meanwhile, to cause systemic infection, the infective stage of Acanthamoeba spp. which is the trophozoite must be able to proliferate at 37°C, the average human body temperature.

Nonetheless, just referring to thermotolerance ability is not enough to conclude whether a certain Acanthamoeba strain is virulent or not (Megeryan 1991). Additionally, there are a few thermotolerance strains which are not pathogenic when tested using animal model (John & Howard 1996). This study is only a preliminary observation used to predict the pathogenicity potential of isolated Acanthamoeba species before further research through in vitro method using tissue culture or in vivo method using animal model is carried out.

CONCLUSION

From the 32 Acanthamoeba isolates used, 26 of them were able to excyst at 37°C while 12 of them were able to excyst at 42°C. Acanthamoeba sp. with the ability to tolerate 37°C temperature could be garnered from all four sampling sites, though not at 100% of samples. Samples from sink have the highest positive percentage (90%) followed by swimming pool (83.3%), seawater (75%), and least from water tap (50%). More isolates from swimming pool could excyst at 42°C (58.3%) compared to other places. No thermotolerance Acanthamoeba could be found in samples.
from water tap. A precautionary guideline should be created for places with high risk for *Acanthamoeba* infection to avert the probability of its manifestation.

REFERENCES


Nurul Fariza Rossle
Mohamed Kamel Abd Ghani
Department of Biomedical Science
Faculty of Allied Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur, Malaysia

Corresponding author: Mohamed Kamel Abd Ghani
Email address: mkamal@skkb.ukm.my
Tel: 603 92897634 Fax: 603 26929032

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Anisah Nordin
Yusof Suboh
Noraina Ab Rahim
Department of Parasitology & Entomology
Faculty of Medicine
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur, Malaysia