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Kertas Asli/*Original Article*

Ability of *Acanthamoeba* Cyst to Excyst at Different Temperatures

(Kebolehan Sista *Acanthamoeba* Bereksistasi pada Suhu Berbeza)

NURUL FARIZA ROSSLE, 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

ABSTRAK

Kajian ini dijalankan untuk memerhatikan kebolehan Acanthamoeba spp. untuk bertoleransi terhadap haba. Sebanyak 32 isolat Acanthamoeba yang diperolehi daripada paip air, sinki, kolam renang dan air laut digunakan. Trofozoit Acanthamoeba spp. diinokulasi ke atas piring agar tanpa nutrien (NNA) yang dilapisi dengan Escherichia coli matian haba secara aseptik dan dieram pada suhu 30°C selama 14 hari untuk mendapatkan sista. Sista matang Acanthamoeba disubkultur pada piring NNA baru yang dilapisi dengan E. coli matian haba dan dieram pada suhu 37°C dan 42°C untuk ujian toleransi terhadap haba. Pemeriksaan piring dilakukan sehingga ke 96 jam selepas pengeraman bagi eksistasi Acanthamoeba sebelum disahkan negatif. Sebanyak 81.25% sampel dapat bereksistasi pada suhu 37°C manakala 37.5% dapat bereksistasi pada suhu 42°C. Acanthamoeba yang bertoleransi terhadap haba dikaitkan dengan keupayaan kepatogenan yang tinggi.

Kata kunci: Bertoleransi haba, Sista Acanthamoeba, Eksistasi

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 1. Isolates of *Acanthamoeba* spp. capable of excystation at temperatures 30°C, 37°C and 42°C

Type of samples	Number and percentage of Acanthamoeba spp. able to excyst		
	30°C	37°C	42°C
Water tap swabs	2/2 (100)	1/2 (50)	0/2 (0)
Sink swabs	10/10 (100)	9/10 (90)	3/10 (30)
Seawater	8/8 (100)	6/8 (75)	2/8 (25)
Swimming pool swabs	12/12 (100)	10/12 (83.3)	7/12 (58.3)

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 refering 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.

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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@fskb.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