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Isolation of *Acanthamoeba* sp. from conjunctival sac of healthy individuals using swab

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Abstract. Is *Acanthamoeba* sp. normally found in the eyes? A study was carried out to establish the possibility of *Acanthamoeba* sp. as a part of the normal conjunctival flora. Conjunctiva swabbing were carried out in 286 healthy Orang Asli school children using sterile cotton swab. The swab was then inoculated onto non-nutrient agar (NN-A). Heat killed *Escherichia coli* that was used as food source for the growth of the amoebae was pipetted onto and away from the smear. The plates were incubated at 30°C and examined daily using an inverted microscope for 14 days. Morphology of the trophozoites and cysts of the amoebae were used as the taxonomic criteria for identification. Positive-controls and negative-controls were done to check for the consistency of the technique used and monitoring of contamination respectively. None of the conjunctiva swab cultured was positive for *Acanthamoeba* sp. This finding may indicate that *Acanthamoeba* sp. is not part of normal conjunctival flora or conjunctiva swab is an insensitive technique to isolate the organism. However, a more extensive research is needed to investigate these possibilities.

INTRODUCTION

Acathamoeba sp. is a pathogenic free-living amoeba, which is abundant in soil, dust and water (Mergeryan, 1991). These organisms have been successfully isolated from fresh water, seawater (Davies *et al.*, 1978; Anisah *et al.*, 2004), domestic water tap (Anisah *et al.*, 2003), swimming pool (Cerva, 1971), soil, sewage and air born dust (Rivera, 1991). It can exist in two forms; in favorable environmental conditions, it exists mainly as an active trophozoite form while in adverse environmental conditions it has the ability to encyst.

Acanthamoeba sp. is important medically because they can invade human's central nervous system, skin and eye causing granulomatous amoebic encephalitis (GAE), *Acanthamoeba* cutaneous, both in immunocompromised individuals and *Acanthamoeba* keratitis respectively. However studies have shown that corneal infection is much more important and has been described as a recent epidemic (Schaumberg *et al.*, 1998). In Malaysia, Mohamed Kamel and Norazah reported the first case of *Acanthamoeba* keratitis in 1995 involving a female contact lens wearer. Recently *Acanthamoeba* Laboratory, Department of Parasitology, Faculty of Medicine, Universiti Kebangsaan Malaysia has been successful in isolating *Acanthamoeba* sp. from corneal scraping of 44 keratitis patients (Kamel *et al.*, 2003).

It has been generally agreed that patients with *Acanthamoeba* keratitis acquire the disease through exogenous sources. It also has been suggested that commensal bacteria found in eyelids, conjunctiva and tear film may have a role in the pathogenesis of *Acanthamoeba* keratitis (Larkin & Easty, 1990). Majority of *Acanthamoeba* keratitis occurs in contact

lens wearers, probably due to tap water contamination of the lens care systems. Evidence from several studies has suggested soft contact lens wear as the greatest risk factor (Buehler *et al.*, 1992). Other risk factors include corneal trauma either by vegetative matter, stone, dust or splashing unclean water into the eyes (Savitri Sharma *et al.*, 2000; Cardine *et al.*, 2002).

Acanthamoeba has been isolated from the nasal mucosa and nasopharynx of healthy individuals as well as patients with upper respiratory tract infection (Martinez, 1980; Rivera *et al.*, 1984). Is it possible that *Acanthamoeba* can exist in healthy human eye as a normal flora and invade the corneal epithelium following injury or when their colonies are substantial enough to cause pathology? The objective of this study was to establish the possibility of isolating *Acanthamoeba* sp. from conjunctival sac of healthy population who are exposed to various environmental sources of *Acanthamoeba*.

MATERIALS AND METHODS

Isolation of Acanthamoeba from conjunctiva

Sterile cotton swab was damped using Page Amoebic Saline (PAS). The lower conjunctiva of the right eye was then swab with a rolling motion from the lateral cantus toward the medial cantus.

Culturing of Acanthamoeba from conjunctiva

Cotton swab was then inoculated onto non-nutrient agar (NN-A). Heat killed *Escherichia coli* that was used as food source for the growth of the amoebae was pipetted onto and away from the smear. The plates were incubated at 30°C and examined daily using an inverted microscope for 14 days. Trophozoites and cysts were examined using a light microscope (x 100 magnification) and the morphology was used as the taxonomic criteria for identification. Positive-controls and negative-controls were done to check for the consistency of the technique used and monitoring of contamination respectively.

Subjects of study

A total of 286 subjects participated in this study. All of them were Orang Asli school children from Sekolah Kebangsaan Air Banun and Sekolah Kebangsaan Dala, Grik Perak. They were non-contact lens wearers. These children were known to have a very close contact with possible sources of *Acanthamoeba* sp. from the environment.

RESULTS AND DISCUSSION

A total of 286 subjects participated in this study. None of the specimen obtained from the subjects was positive for *Acanthamoeba* after an observation period of 14 days.

Acanthamoeba sp. is a free-living ubiquitous organism and has been successfully isolated from soil and dust, freshwater, seawater and air. All published studies agreed that patients with *Acanthamoeba* keratitis acquire the disease through exogenous sources and majority of it occurs in contact lens wearers, probably due to tap water contamination of the lens care systems. Although *Acanthamoeba* has been isolated from the nasal mucosa and nasopharynx of healthy individuals, so far there is no published report of the presence of *Acanthamoeba* on the surface of healthy human eye.

This study was carried out to establish the possibility of *Acanthamoeba* sp. being isolated from conjunctiva sac of healthy population. A study to explore this possibility was carried out before among 141 healthy medical students (Khoo, 1999). Contrary to the traditional method of using cotton swab, sampling was done by irrigating the eye with 10 ml normal saline. The researcher postulated that this technique has the advantage of thoroughly dislodging the organisms. The result was negative and several possibilities were

discussed to explain this. One of the weaknesses of the study was the choice of subjects. Medical students are usually assumed to practice good personnel hygiene and they are not often exposed to environmental sources of *Acanthamoeba*.

Based on this, Orang Asli school children were chosen because they were exposed to variety of environmental sources of *Acanthamoeba* such as soil and dust, freshwater and air. The children chosen were known to play and swim in the river located along side their village after school and in their time of leisure. A construction activity was also observed in one of the village, creating a very dusty environment. Beside that, their personnel hygienic practices were also poor. However our result was also negative and it confirmed the previous study carried out in Malaysia (Khoo, 1999). This negative result can imply several possibilities: 1) regardless whether human are exposed to the source in the environment or not, *Acanthamoeba* does not exist in healthy conjunctiva. It might be because they are not able to establish and colonized the conjunctiva of healthy individuals with healthy eyes. This is supported by the fact that *Acanthamoeba* cannot cause disease in intact corneal epithelium 2) the organisms does exist in the eye but the technique employed was not sensitive enough 3) the organisms does exist in the eye but the sample size and selection are not adequate and appropriate. A more systematic study should be carried out to explore this possibility.

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