The Determination of Acanthamoeba spp. from Air Ventilation System Samples from Selected Buildings in Kuantan

Muhammad Lokman Md Isa[1,2] ,Anis Amira Aznan[1], Nakisah Mat Amin[3], and Afzan Mat Yusof[1,2*]

[1]*Department of Basic Medical Sciences, Kulliyyah of Nursing, International Islamic University Malaysia, Kuantan Campus, Pahang, Malaysia*
[2]*Integrated Cellular and Molecular Biology Cluster (iMolec), International Islamic University Malaysia, Kuantan Campus, Pahang, Malaysia*
[3]*Department of Biological Sciences, Faculty of Science and Technology, University Malaysia Terengganu, Terengganu, Malaysia.*

ABSTRACT

Objective: The present study is designed to investigate the occurrence of Acanthamoeba in air ventilation and air-conditioning systems in selected buildings in Kuantan. **Methods:** Acanthamoeba was isolated from dust samples taken from filters of air ventilation systems in selected buildings in Kuantan. The dusts were collected by using sterile cotton swabs, cultured in a xenic culture medium on non-nutrient agar (NNA) plates and incubated at 37° C. The plates were examined daily for any presence of Acanthamoeba cysts up to 7 days of incubation. **Results:** Based on the results obtained, there were no cysts or trophozoites of Acanthamoeba successfully isolated from all 75 dust samples. After three days of incubation, all culture plates examined show negative findings. **Conclusions:** The negative findings of this study were probably due to the limitations of the sampling method. It is recommended that future studies use the method proposed by National Institute of Occupational Safety and Health (NIOSH) for indoor air quality monitoring.

KEYWORDS: Acanthamoeba, air, ventilation systems, dust samples, immunosuppressed.

INTRODUCTION

Acanthamoeba species or Acanthamoeba is an opportunistic protist that is ubiquitously distributed in water, soil and air environments (1). Acanthamoeba has been isolated from a number of ecological sources such as coastal water (2) river water (3), salt-water lakes (4) and freshwater ponds (5). Studies conducted throughout the years have shown that isolation of Acanthamoeba from man-made environments including tap water (6), swimming pools (7) and air-conditioning units (8) to be significant. Besides that, a study conducted in Peru proved Acanthamoeba occurs in the human body (9). Thus, it is generally accepted that Acanthamoeba is commonly encountered in the natural environment and it is suggested that we are exposed to Acanthamoeba spp. in our routine lives.

Acanthamoeba was described as being pathogenic to immunocompromised individuals in late 1960 by Douglas and Culbertsoni (2). Acanthamoeba keratitis (AK) was found in 1973 in the United States, where it was associated with A. polyphaga infection. It is a rare, parasitic, vision-threatening disease, which is most likely to affect contact lens wearers. The first case of granulomatous amoebic encephalitis (GAE) caused by Acanthamoeba was reported in a specimen of cerebrospinal fluid from a farmer diagnosed in Taiwan (10). Acanthamoeba's point of entry into the human body is known to be through the nostrils (11).

***Corresponding author:**

Email: afzan@iium.edu.my
Tel: +60129656253
Department of Basic Medical Sciences, Kulliyyah of Nursing, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia.

The presence of Acanthamoeba in air may affect the indoor air quality (12) by contaminating air ventilation systems, which distribute respiratory-related illnesses throughout the building (13). Acanthamoeba is widespread in the environment and many contact lens wearers remind us of the need for increasing the public's awareness and knowledge and to educate them about Acanthamoeba's risk to human health (3). Due to its diverse roles in ecosystems and in causing serious human infections, over the past several decades, Acanthamoeba has gained increasing attention not only from the scientific community but also from the medical community.

The present study was designed to investigate the occurrence of Acanthamoeba in air ventilation and air-conditioning systems in selected buildings in Kuantan; it aimed to determine the possible factors related to the presence of Acanthamoeba in indoor air of selected buildings. The presence of Acanthamoeba in air-conditioners and air ventilation systems was determined by using morphological techniques involving amoebic culture and microscopic examinations. Possible related factors are air ventilation systems maintenance and building occupancy, which were be determined by purposely selecting public premises as sampling sites for air ventilation dust collection and interviewing the staff in-charge of ventilation cleaning.

METHODS

Approval

This study was granted approval to collect dust samples from air ventilation systems in the selected main buildings by the authorities from all six premises involved. This study was conducted from November 2016 till July 2017.

Sample collection

A total of 75 dust samples were collected from air handling units (AHU), vents at ceilings and filters from installed air-conditioners in the rooms of selected sampling sites by using sterile cotton

swabs. The dusts collected were immediately inserted into sterile 15mL falcon tubes.
Xenic culture

The samples taken were cultured and incubated at 37° C. The culture method used in this study is xenic culture, where active Escherichia coli (E. coli) were added to the non-nutrient agar plates along with the dust samples.

Microscopic examination

The plates were directly observed under the inverted microscope at a 400X magnification and examined daily for any presence of Acanthamoeba cysts up to 7 days of incubation.

RESULTS

Microscopic examination

Based on the results obtained, no cysts or trophozoites of Acanthamoeba were isolated successfully from any of the 75 dust samples. After three days of incubation, all culture plates examined show negative findings. However, observations revealed that, despite the mentioned factors, both cyst and trophozoite forms of Acanthamoeba were absent in any of the culture plates. There was also fungal growth observed on the plates (Figure 1&2).

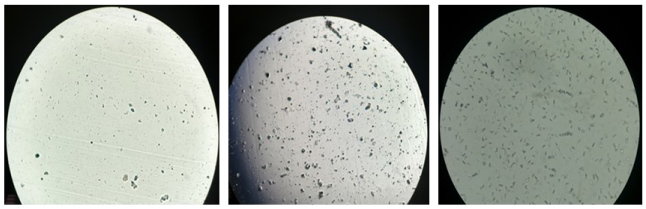

Figure 1: The absence of Acanthamoeba cysts or trophozoites in culture plates. Only debris and dusts were observed.

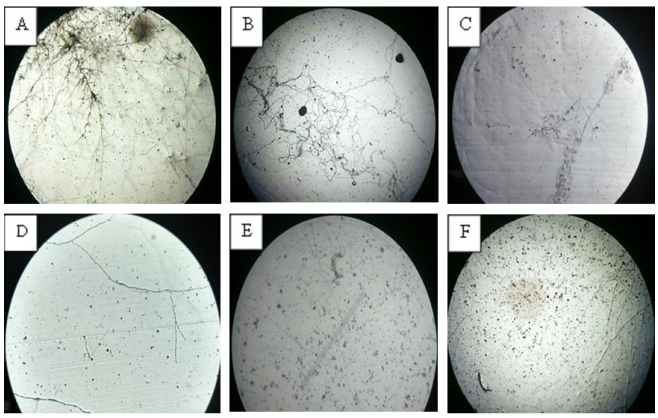

Figure 2: Significant fungal growths with debris and dusts were present in samples isolated from all six premises (Premise A – F).

DISCUSSION

Acanthamoeba reached its maximum population density in xenic medium after 72 hours of incubation (14; 15). Incubation at 37° C with sufficient growth medium may induce Acanthamoeba to change form, from cyst to trophozoite (4; 16). Fungal presence is expected since air ventilation systems have high moisture contents, which are favourable for their growth (17; 18). Although xenic (bacterised) culture with inactive E. coli is the commonly used method of cultivating Acanthamoeba in studies (19; 17), Acanthamoeba can also be cultivated readily in the presence of living bacteria on agar media containing low concentrations of nutrients (11). Thus, a re-evaluation of method was done to use active E. coli instead of heat-killed (inactive) E. coli as the bacterial lawns on NNA plates. Nevertheless, microscopic examination for the modified method also showed negative results, similar to the results from samples that had been processed using the unmodified method.

The related factors, which are the number of building occupants and air ventilation systems maintenance, were suggested to have no significant association with the presence of Acanthamoeba. The presence of Acanthamoeba could be positively correlated with fungi counts and the cleanliness of the air ventilation systems as suggested in a study, though the statistical analyses shown was only a weak correlation (12). This shows that poor maintenance of air ventilation systems may decrease indoor air quality (20) but not necessarily indicate Acanthamoeba presence. As suggested by another study, the activity of large numbers of building occupants may increase the exposure of indoor air to outdoor pollutants, which includes Acanthamoeba spp. (3). However, based on the results of this study, it is not considered significant to determine the presence of Acanthamoeba only by assessing the number of individuals entering in and out of the building. Thus, it is suggested that there is no significance difference between the number of building occupants and air ventilation systems maintenance with Acanthamoeba presence since negative findings were obtained in this study.

CONCLUSIONS

The study concluded that Acanthamoeba was absent in all 75 dust samples collected from air ventilation system samples in selected buildings in Kuantan. The two factors studied that may have accounted for Acanthamoeba had it been detected were air ventilation system maintenance and building occupancy; yet these not were found to be significantly associated to Acanthamoeba presence in air ventilation systems. The negative findings from this study suggest that the indoor air of the premises examined were not contaminated with Acanthamoeba.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare with regard to this work.

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