

## Short communication

# A survey of Free-Living Amoebae (FLAs) in Nasal Mucosa of Healthy Individuals using Culture-based Method

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## Abstract

Free-living amoebas (FLAs) are facultative parasites, and some of them can be highly pathogenic for animals and humans. These protozoan are found in abundance in environmental resources such as freshwater and soil, and thus the possible colonization of such FLA is probable. Our previous studies showed that *Acanthamoeba* spp. can colonize nasal mucosa of immunosuppressed patients. However, there was no report of colonization of free-living amoebae in healthy individuals in Iran. The present study was conducted to investigate the possibility that FLAs are the normal flora of the nasal mucosa. Overall, 46 nasal swab samples were collected from healthy individuals and cultured on a 1.5% non-nutrient agar (NNA) plate enriched with a lawn of heat-killed *Escherichia coli*. The plates were incubated at room temperature. After one week, the plates were microscopically examined, and this was continued for one month. None of the samples were found positive for FLAs. Our findings may indicate that FLAs are not the normal flora of the nasal mucosa, or their number is so limited in the nasal mucosa that they cannot be isolated by the method used. However, supplementary and comprehensive studies are recommended.

**Keywords:** Free-living amoeba, Healthy individual, Immunocompetent, *Acanthamoeba* spp

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## Introduction

Free-living amoebas (FLAs), including *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Acanthamoeba* spp. are opportunistic protozoan parasites with wide environmental distribution around the world. FLAs have been isolated from various resources, including air, water, soil, dust, contact lenses, hospital equipment, and clinical samples<sup>1-2</sup>. The amphizoic lifestyle of these protozoans allows them to survive

in different environmental conditions and cause human infections<sup>3</sup>. Many human FLA-causing infections are correlated with *Naegleria fowleri*, *Acanthamoeba* spp. and *Balamuthia mandrillaris*<sup>4</sup>. Evidence indicate that FLAs have more tropism to the eyes, nervous system, and skin. In the central nervous system, primary amoebic meningoencephalitis (PAM) is related to *Naegleria fowleri*, while *Acanthamoeba* spp. and *Balamuthia mandrillaris* are considered as etiological agents of

granulomatous amoebic encephalitis (GAE). Also, *Acanthamoeba* spp. may cause amoebic keratitis (AK), and lung and skin complications<sup>5</sup>. Previous studies have proved that some pathogenic strains of *Acanthamoeba* are thermotolerance and osmotolerance<sup>6</sup>.

There are four main routes of entry for FLAs, including inhalation, using contact lenses (exceptionally soft lenses), damaged skin, and nasal mucosa/olfactory (for *Naegleria*)<sup>2,4,7</sup>.

Regarding the high occurrence of FLAs, especially *Acanthamoeba*, in the environmental resources of Tehran city<sup>8,9</sup>, and lack of information about the prevalence of these pathogenic agents in healthy people, the present study aimed to investigate FLAs in samples of healthy individuals.

## Material and method

In the present study, 46 nasal swab samples were collected from healthy individuals (13 to 85 years old). All of the specimens were collected using sterile swabs. Immediately, culturing was carried out on a 1.5% non-nutrient agar (NNA) plate enriched with a lawn of heat-killed *Escherichia coli*, and parafilm was used to seal the plates. Also, the demographic characteristics of the participants were recorded (table 1). All of the plates were delivered to the protozoology laboratory of the Dept. of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical sciences. The plates were incubated at room temperature, and microscopic investigation (using 100X magnification) of the plates was started one week after cultivation. The investigation lasted one month.

## Results

In the present study, a total of 46 subjects participated. After four weeks of observation, none of the samples were found positive, while in previous studies, 0.86 to 28.4% of the samples were reported positive for FLAs<sup>7,11-13</sup>.

A total of 17 (36.96%) participants were male, and 29 (63.04%) were female; the mean age of participants was 44.10 years. All participants were immunocompetent, and they did not have any underline diseases. Educational information and

**Table 1:** Sociodemographic characteristics of the participants.

Characteristic	Total No. (%)
Sex	
Male	17 (36.96)
Female	29 (63.04)
Age group	
<30	5 (10.87)
30-60	34 (73.91)
60<	7 (15.22)
Occupation	
Student	16 (34.78)
Housewife	6 (13.05)
Employee	9 (19.56)
Nurse	5 (10.87)
Other	10 (21.74)
Education	
Illiterate	1
High school degree	2
Diploma	15
Bachelor degree	7
Master degree	13
PhD	8

sociodemographic characteristics of the participants are shown in table 1.

## Discussion

FLAs are ubiquitous organisms reported from various sources, including seawater, freshwater, dust, soil, and air<sup>13</sup>. All previous studies agree that the source of infection in all diseases caused by FLAs such as AK, PAM, and GAE is exogenous.

The present study was conducted to isolate FLAs from the nasal mucosa of the healthy population. *Acanthamoeba*, as the most prevalent amoeba, has already been isolated from healthy people<sup>7</sup>. However, the result of our study was negative. Using nasal swabs to detect FLAs is common, and this method has already been used by the authors of the present study and other authors<sup>7,14</sup>. Therefore, there was no problem with the method. One of the reasons for the present

study's negative result could be sample selection. Almost all participants themselves or their family members have some connection with the School of Medicine and the Department of Parasitology, Shahid Beheshti University of medical sciences, so they take good care of personal. Also, due to their job and urban life, chosen participants are not regularly exposed to the environmental resources of FLAs, such as freshwater, soil, and dust. However, the result of the present study is consistent with the result of a study conducted in Malaysia<sup>15</sup>. The difference between the mentioned study and our study is using different samples, as they tried to isolate the FLAs from the conjunctival sac of healthy individuals.

According to the results of the present study, the probabilities are the following:

- 1) Practicing personal hygiene and lack of constant contact with environmental sources of FLAs can greatly reduce the possibility of their colonization in the nasal mucosa of healthy people.
- 2) The organism is present in limited numbers in the nasal mucosa of healthy people, but the employed method was not sensitive enough to detect.
- 3) FLAs exist in the nasal mucosa of healthy people, but the sample size was not adequate. Finally, we recommend conducting more studies with larger sample sizes from healthy individuals but with different lifestyles.

## Conclusion

Our findings may indicate that FLAs are not the normal flora of the nasal mucosa, or their number is so limited in the nasal mucosa that they cannot be isolated by the method used. However, supplementary and comprehensive studies are recommended.

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