Original Article:

First report of isolation and microscopic identification of Acanthamoeba species from Sandstorm Dust in Al Zulfi, Saudi Arabia

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Abstract

Background and aim:

Sandstorms in arid regions pose health risks owing to the presence of microbial dust particles. While microbial dust has been studied, the free-living parasite Acanthamoeba, found in air, water, and dust causing infections like Acanthamoeba keratitis and rare encephalitis, has not been investigated. This study aims to isolate and identify Acanthamoeba species from indoor and outdoor dust samples collected during sandstorm events at Majmaah University.

Methods:

In this study, a novel passive air sampling method was used with sterile aluminium foil plate containers with electrostatic dust cloth devices for outdoor samples and swab techniques for indoor samples. Samples were collected from various locations, including classrooms and laboratories. Standard microbiological techniques were applied, with samples inoculated onto non-nutrient agar with E. coli culture and incubated at 30˚C for 14 days. Acanthamoeba species were identified through microscopic observation.

Results:

Out of 108 analyzed samples, outdoor environments had a higher Acanthamoeba positivity rate (75.0%) compared to indoor samples (44.6%). The highest positivity rates in outdoor sites were found in car parking areas (81.8%), exterior college tables (75.0%), and residential area air samples (73.3%). Indoor locations showed varied results, with lab corridors (62.5%) and areas near lab windows (58.3%) having higher detection rates.

Conclusion:

This pioneering study is the first to investigate Acanthamoeba in sandstorm dust, revealing a 75.4% positivity rate in outdoor environments. The findings highlight the need for increased awareness and preventive measures, especially for vulnerable populations, and call for comprehensive research across desert regions.

Keywords:

Acanthamoeba species, Sandstorm, dust, Non nutrient agar, Trophozoites, Cysts

Introduction:

Sandstorms, also known as dust storms, or 'haboob' in Arabic, occur frequently and periodically due to strong winds that blow dust across the deserts worldwide, particularly in Middle Eastern countries. These natural events are seasonal, with notable dust hot spots in the Kingdom of Saudi Arabia and the Middle East, where most atmospheric transport of air movement occurs during the summer months from February to May. [1]

According to recent reports, sandstorms have a significant impact on transportation systems and cause numerous disruptions to normal outdoor activities. The construction of Saudi Arabia's railway network faces significant technical challenges due to sandstorms that create shifting dunes.[2] Another major concern is that dust storms degrade air quality and carry anthropogenic pollutants, leading to the deaths of more than 7 million people annually and seriously affecting children and the elderly. [3] Sandstorm dust particles carry a wide range of microbial components and allergens. This creates significant health impacts, studies have shown that dust storms increase hospital admissions for respiratory issues, including asthma and pneumonia, and deteriorate pulmonary function. Additionally, dust events are associated with cardiovascular illnesses, such as ischemic heart disease and hypertension, and can exacerbate conditions like meningitis, conjunctivitis, and skin infections. [3] Therefore, performing microbiological analysis on sandstorm dust samples is crucial for identifying the exact causative agents of diseases and allergies. Many scientists have focused on this area of research, performing microbiological analyses of sandstorm dust samples and reporting their health impacts worldwide.[4-7] Most of these studies have analyzed bacterial and fungal isolates from sandstorm dust. However, none have investigated protozoa, particularly free-living amoebae.

Acanthamoeba species are free-living, opportunistic protozoan pathogens that are ubiquitous in nature and found in soil, dust, air, and various water sources sources.[8,9] Unlike parasitic amoebae, Acanthamoeba do not rely on a host for survival. However, they can sometimes cause serious infections in humans, particularly in individuals with weakened immune systems. These amoebae can enter the body through cuts, inhalation, or contact with the eyes, potentially leading to infections of the brain (granulomatous amoebic encephalitis), skin, eyes, and sinuses. One notable infection of this parasite is Acanthamoeba keratitis, which affects the eyes and is often associated with contact lens use. [10,11]

Numerous research studies have

been conducted by various researchers, isolating Acanthamoeba from different environmental water sources, including public water supplies, ponds, seas, stagnant water, rivers, and bottled water.[8-14] Although some studies have analyzed dust samples from hospital and housing environments and reported the presence of Acanthamoeba, no attempts have been made to analyze sandstorm dust samples. [12,13] In the past two decades, Acanthamoeba species have become increasingly recognized as important microbes due to their pathogenicity. They play an important role in ecosystems and act as carriers and reservoirs for other prokaryotes. Therefore, investigating the presence of Acanthamoeba in sandstorm dust is highly significant, particularly in hotspot regions of Saudi Arabia.

Based on these facts, the aim of the present study is to investigate the isolation and identification of Acanthamoeba species from sandstorm dust collected from indoor and outdoor environments in the Central desert region of the city of Zulfi, Saudi Arabia.

Materials and methods:

Study location:

Sandstorm dust samples were collected from various locations of student classrooms and biology laboratories in the College of Science at Majmaah University, Al Zulfi, Riyadh, Saudi Arabia from February to April 2023. Al Zulfi, a region frequently affected by sandstorm dust, is in Riyadh province in the central desert region of Saudi Arabia.

Inclusion criteria for sandstorm dust samples

All sterile sampling devices and microbiological media were prepared in advance, utilizing meteorological forecasts to anticipate sandstorm events. Certain criteria were followed to select the samples; only extreme sandstorm episodes (> 20 km/ hour wind speed) were considered for sample collection. Outdoor samples were collected using passive air sampling during sandstorm episodes.[15] Indoor samples were collected via swab method after dust had settled on flat surfaces following the completion of sandstorm episodes.[16] The disinfection data for the indoor sampling area were recorded before data collection. The detailed sampling devices and procedures are provided below.

Sampling device for outdoor and indoor samples

As per our previous studies an established method of a novel passive sampling device using aluminum foil plate (AFP) container

used in sandstorm dust collection for outdoor samples.15 Briefly, a single electrostatic dust cloth (EDC) (Swiffer, 26.5 x 20.3 cm) was positioned into a circular shape aluminum container (inner diameter 15.3 cm, outer diameter 17.3). The container's lid had an environmental exposure opening of 0.023 square meters. The device was sterilized before exposure as per methodology mentioned in our previous studies.[15] For indoor samples, a sterile swab was used to collect dust from flat surfaces. [16]

Sampling locations and methods

Outdoor samples

During sandstorm episodes the AFP-EDC device was positioned horizontally with fixed by 3M adhesive tape (3M, USA), the sample was collected at a height of 1.5-meters above the ground level with a minimum exposure time of 4 hours. After exposure the sampling device was placed clean zip lock cover and closed and sent it for microbiological analysis. Outdoor sampling sites included the residential area, car parking area and College of Science campus, Majmaah University, Al Zulfi, Saudi Arabia [Table.1].

Table 1: Sampling locations of sandstorm dust samples.

Type	Sampling locations	Exposure to disinfectants
Indoor	Students class rooms – on working table	Weekly
	Students classroom – Near windows	Weekly
	Laboratory – working space	70% Alcohol, QAC based disinfectant [once in 2 days]
	Laboratory – near Windows	QAC based disinfectant [once in 2 days]
	Laboratory – Corridor's	Regular floor cleaning agent (Lizol and Dettol)
Outdoor	Waiting Tables - Outside of College	Nil
	Car Parking area	Nil
	Air sample near college entrance	Nil
	Air samples near residential area, Housing complex, Majmaah University, Al Zulfi, Saudi Arabia	Nil

Indoor samples

For indoor sampling, sterile saline-moistened swabs were used to collect dust from areas with significant dust accumulation, such as near windows, doors, and corridors of students classroom and biology laboratory, College of science, Majmaah university, Al Zulfi campus, Saudi Arabia. The swab collection area with minimum of 0.025 m^2 on flat surfaces. After sampling, the swabs were recapped and prepared for laboratory analysis.

Acanthamoeba culture

Collected dust samples were analyzed as per supportive protocol by Caumo et al., 2009.[14] In briefly, the AFP-EDC device was opened in an aseptically and transferred electrostatic cloth and cut into 6 pieces and transferred into 50 mL of sterile distilled water. Similarly, the swabs mixed thoroughly in 50 mL of sterile distilled water. The contents were centrifuged at 250X g for 15 minutes. The supernatant discorded and pellets were suspended in Page's Amoeba Saline (PAS) solution. The mixed suspension in PAS is transferred to an 90mm wide plate of 1.5% non-nutrient agar (NNA) containing an over layer of an Escherichia coli (ATCC 25922) culture. All culture plates were sealed with Parafilm and incubated at 30°C for the duration of up to 14 days. Duplicate plates were performed for each sampled pool. Each plate was observed daily under a light microscope at 100X magnification to check for the presence of trophozoites and cysts. Simultaneously, the quality control strain of A. castesllanii (ATCC 50492) was monitored microscopically for approximately 14 days for the growth comparison of trophozoites and cysts. This is considered positive media control and test methods.

Microscopic identification:

Acanthamoeba spp. was identified through careful microscopic examination on their distinctive morphological characteristics of both trophozoites and cysts. Once sufficient growth was confirmed, 1-2 ml of sterile distilled water gently poured on the surface of NNA culture plate and then scraped to collect the liquid for wet mount analysis and lacto phenol cotton blue staining as per standard procedure. [17] Microscopic observations at 40X magnification revealed characteristic features: trophozoites displayed finger-like tapering pseudopodia, while cysts appeared with a ten-sided polygonal inner wall and a wrinkled.[14,17] The culture positive samples were then systematically recorded and tabulated.

Statistical analysis:

Chi-Square test was used to determine the association between sampling locations and Acanthamoeba positive rates. $p < 0.05$

is considered as there is a statistically significant association between location and positivity rates.

Results:

A total of 108 sandstorm dust samples were collected and analyzed for the presence of Acanthamoeba using conventional microbiology NNA culture methods. After incubation period the plates were observed based on morphological characteristics of trophozoites and cysts were identified (Fig.1 and Fig.2). Among 18 samples,56 indoor samples collected by swab method and 52 outdoor samples were collected passive sampling method The overall positive rate of Acanthamoeba spp. for indoor samples was 44.6% (25 out of 56), while the positive rate for outdoor samples was significantly higher at 75.0% (39 out of 52).

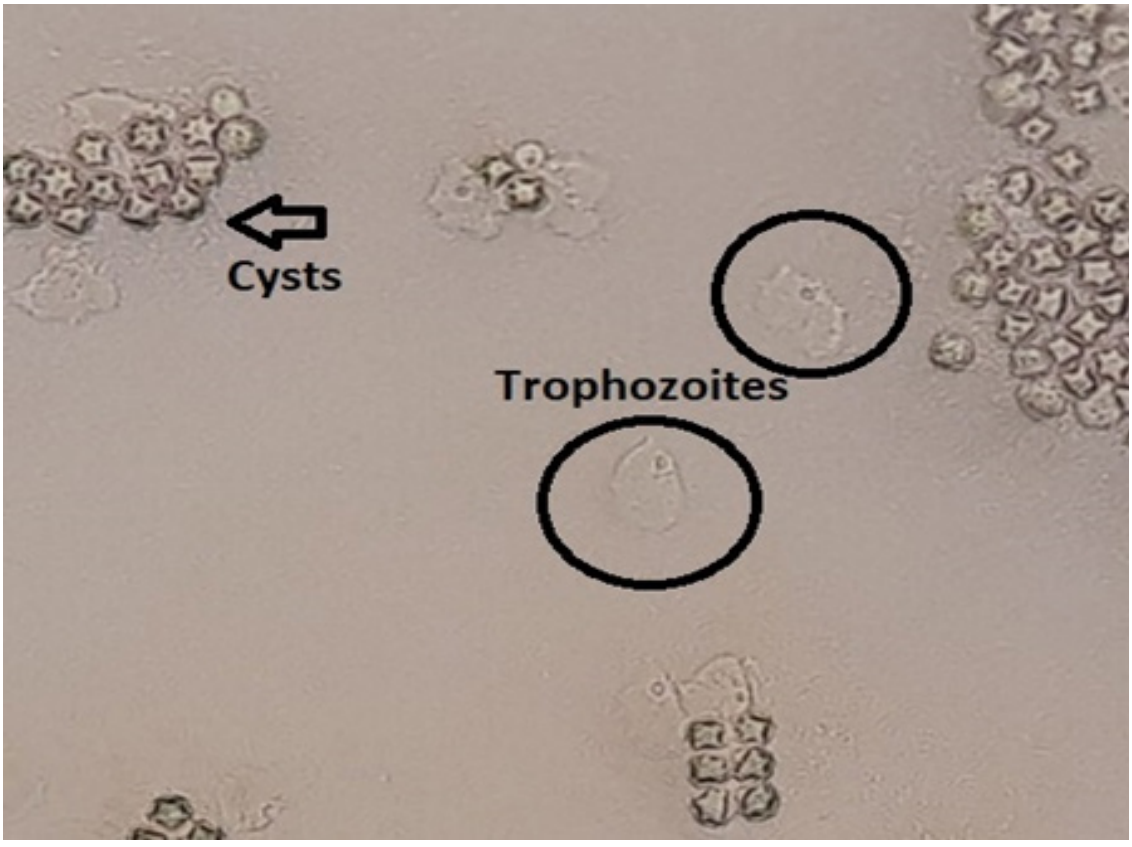


Fig.1: Direct observation of NNA culture plate under microscope to visualize trophozoites and cysts.

Specifically, the positive rates for indoor locations were as follows: students' classrooms on working tables, near windows, laboratory working tables and laboratory windows were 33.3,50,28.6 and 58.3% respectively. The highest positive rate was observed at lab corridors 62.5% (5 out of 8 samples) among indoor samples (Table.2)

Table 2: Positivity rate of Acanthamoeba spp. isolated from sandstorm dust in various locations samples.

Sampling types and locations	Total (Tested = 108)	Culture Positive	%
Indoor (56 samples)			
Students class rooms – on working table	12	4	33.3
Students classroom – Near windows	10	5	50.0
Students Lab – working space	14	4	28.6
Lab – near Windows	12	7	58.3
Lab – Corridor's	8	5	62.5
Total (Indoor samples)	**56**	**25**	**44.6**
Outdoor (52 samples)			
Tables - Outside College	12	9	75.0
Car Parking area	11	9	81.8
Air sample near college	14	10	71.4
Air samples near residential area	15	11	73.3
Total (outdoor samples)	**52**	**39**	**75.0**

In the case of outdoor locations, the positive rates of Acanthamoeba spp. from all locations were greater than 75%. Samples were collected from the car parking areas showed that highest positive rate of 81.8% (9 out of 11 samples). Other locations such as air samples near the college had a positive rate of 71.4% and residential areas had a positive rate of 73.3% (11 out of 15 samples).

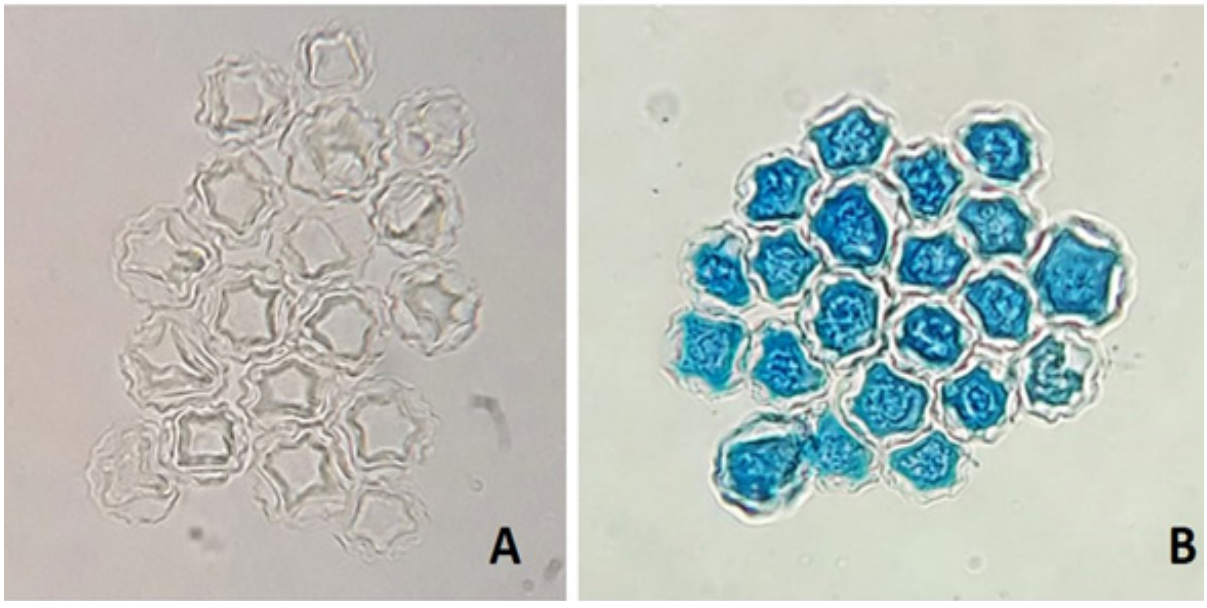


Fig. 2: Microscopic observation of Acanthamoeba cysts; Wet mount (A – no staining; B - stained with lacto phenol cotton blue) showing polygonal shapes and double cell walled

Analysing the data using a chi-square test revealed a chi-square value of 11.0461 and a p-value of 0.000892, indicating that the difference between indoor and outdoor locations is highly statistically significant ($p < 0.001$). These findings suggest that outdoor locations have a substantially higher positivity rate for Acanthamoeba compared to indoor locations.

Discussion:

The Central region of Saudi Arabia and the study location in Zulfi considered as one of major hit place of sandstorm dust due to this city surrounded by sand dunes to its north and west, which are known locally as the Al-Thoyr sands. The health impacts of sandstorm dust in Saudi Arabia and the Middle East are well-documented and significant. The microbial analysis of sandstorm dust was conducted by various researchers in Saudi Arabia and already we had reported the fungal allergens, and bacterial endotoxin loads from the sandstorm dusts.[14,18] From this series, the present study analyzed the distribution of free living Acanthamoeba in sandstorm dust samples.

Life cycle Acanthamoeba has two stages of dormant cyst stage and active trophozoite stages. It can withstand the harsh environment even it is commonly found in soil and water samples. Acanthamoeba spp. has been isolated from many environmental resources such as water, dust, soil, and hospital equipment's and environment.[14,17-20] However none of them checked in sandstorm dust, the present study is first study to explain this. To our best of knowledge, this is the first study examined sandstorm dust samples and analyzed the positivity rate of Acanthamoeba in the educational and residential environment of Saudi Arabia.

In this study 25 out of 56 samples of indoor samples were showed presence of Acanthamoeba was 44.6%. Among the indoor samples high percentage of incidence observed sample laboratory corridors (62.5%) and followed by near windows (58.3%), These locations can access dust materials from outside during sandstorm episodes easily diffused through the minute gaps of windows and doors. This is comparable with study done by Wopereis and his co-workers, they found that 77.8% of Acanthamoeba spp.,collected from the dust samples of air-conditioning systems in public hospital in Florianópolis, Brazil.[19] From the overall data, the least percentage was observed in laboratory working areas (28.6%), these areas were frequently cleaned using disinfectants and 70% alcohol this may be the reason for getting the low percentage. This evident by the recent study by Wekerle and his associates demonstrated that

Acanthamoeba trophozoites and cysts are susceptible to alcohol based and quaternary ammonium chloride disinfectants.[21] When comparing student's classroom working area this practices are more frequently done in the laboratories, this showed that least percentage was observed in the laboratory than classroom.

In the analysis of outdoor samples, the positivity rate was high and recorded as more than 70% from all the locations. There is a clear distinction between indoor and outdoor environments. Outdoor locations show approximately 30 percentage points higher positivity rates compared to indoor locations. Car parking areas in college of science Zulfi location has the highest positivity rate (81.8%), potentially due to higher concentrations of the agent being tested for (possibly pollutants, allergens, or microorganisms). Sandstorm dust samples were collected from both college and residential areas show similarly high positivity rates (71.4% and 73.3%), indicating widespread presence of the Acanthamoeba spp. in ambient air. So far there is no reports available, based on our knowledge hence comparing the presence of Acanthamoeba in sandstorm dust outdoor samples is very difficult. However, this positivity rate is comparable with other available studies who analyzed other environmental dust samples in hospitals, wards which were ranging from 40 to 80%.[13,19,22]

Apart from the dust samples, there are many reports available worldwide about the distribution of Acanthamoeba in various environmental and water sources.[10] If comparing the soil source which are close to sandstorm dust, Mahmoudi et al [23] analyzed the public park samples and reported 100% of positivity rate, when compare to our data was 75%. The presence of Acanthamoeba in dust samples across various environments highlights the potential health risks associated with this free-living amoeba. Our findings align with previous studies, such as those conducted in Tehran, Iran, and Ituiutaba, Brazil, which reported prevalence rates of 46.25% and 35.4%, respectively.[13,19] Moreover, the isolation of Acanthamoeba spp. in 20% of soil and dust samples collected from hospital environments in Khomein city, Iran, indicates that the soil and dust in this area provide suitable conditions for the growth of Acanthamoeba spp.[24]

Concerning the health impacts to local community about the presence of Acanthamoeba cysts in sandstorm dust. Currently, there is no direct evidence suggesting that inadequate indoor air quality could be a source of Acanthamoeba infections. Nevertheless, prolonged exposure of contaminated

indoor air outdoor of sandstorm dust can cause severe respiratory infections and cardiac illness.[4-7] High percentage of hospitalization, Acanthamoeba in dust exposed to human who already wearer contact lenses. [25] Acanthamoeba species can enter into minute scratches of cornea and cause keratitis. Based on ophthalmic data the most common risk factor of Acanthamoeba keratitis is corneal trauma or direct exposure to contaminated waters and dust.[10,26]

If people who contact lens wearers exposed to sandstorm dust, if it enters the eye this may get chance of Acanthamoeba keratitis. There are numerous studies available transmission of Acanthmoeba by poor handling contact lens and poor hygienic. Hence there is high chance of getting dust exposure of Acanthamoeba from the dust to enter eyes by rubbing and contact lens wearers are highly prone to get infections.[8,10]

A recent study by Akhlaghi et al. conducted in the ophthalmology wards and operating rooms in southern Iran found that 9.1% of dust samples collected from indoor environments contained Acanthamoeba.[24] Compared to our current findings, this percentage is lower, likely due to the frequent sanitization practices in hospital settings, which are more rigorous than those in educational institutions. Another study by researchers in Malaysia reported that 40.2% of dust swabs collected from supplying air diffusers and blowing fans contained Acanthamoeba,[27] which is comparable to our results because students working table (33.3%) and laboratory working spaces (28%). This low percentage of incidence due to proper disinfectant use cleaning on the working spaces of classroom table and laboratory working spaces.

The study's findings reveal a significant 75.4% prevalence of Acanthamoeba in outdoor dust samples collected during sandstorms. This high rate underscores the critical importance of raising public awareness and implementing preventive strategies, especially for vulnerable populations like contact lens wearers and individuals with compromised immune systems, who face elevated risks of serious conditions such as Acanthamoeba keratitis and granulomatous amebic encephalitis. This is the first study analyzed the 'Acanthamoeba' in sandstorm dust and more studies needed to identify the prevalence of various desert regions. These results emphasize the importance of maintaining stringent hygiene practices and regular monitoring of environmental samples of sandstorm dust to mitigate the risk of Acanthamoeba-related infections.

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Conflict of interest:

There is no conflict of interest with regard to the publication of this manuscript.

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