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Molecular and Morphological Identification of *Acanthamoeba* in Hospital Dust from Ahvaz, Iran

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Abstract

Background and Objective: *Acanthamoeba*, a free-living amoeba found in various environments, is an opportunistic pathogen, especially affecting immunocompromised patients with conditions like granulomatous amoebic encephalitis and *Acanthamoeba* keratitis. Despite the global significance of this pathogen, data on its prevalence in hospital dust in Iran is limited.

Methods: This study assessed *Acanthamoeba* in hospital dust from three major hospitals affiliated with Ahvaz Jundishapur University of Medical Sciences, Iran. A total of 96 dust samples were collected from various wards. Morphological analysis using non-nutrient agar culture identified cyst-like forms, followed by PCR to confirm and quantify *Acanthamoeba* presence.

Findings: Six samples showed *Acanthamoeba*-like cysts, with PCR confirming DNA in 16 samples (16.6%), indicating notable prevalence in hospital dust. Positive samples were mainly from corridors (68.75%), general care rooms (25%), and ICUs (6.25%), with no detection in surgical wards. A significant association was observed between location and *Acanthamoeba* presence ($p < 0.001$).

Conclusion: These findings highlight the importance of monitoring *Acanthamoeba* in hospitals, given its capacity to cause severe infections in vulnerable patients. Enhanced sanitation and environmental monitoring may help minimize exposure and reduce infection risk.

Keywords*:* Hospital-acquired infections, Environmental surveillance, Opportunistic pathogens, Molecular detection, *Acanthamoeba*.

Introduction

Free-living amoebae (FLA) are protozoans found in various environments, including water, soil, and air. *Acanthamoeba*, *Naegleria*, and *Balamuthia* are the most notable FLAs, as they can cause severe infections in humans, particularly impacting the central nervous system (CNS) and eyes (1). *Acanthamoeba* is of particular concern due to its ability to cause amoebic keratitis (AK), a corneal infection that is especially dangerous for contact lens users, and granulomatous amoebic encephalitis (GAE), a life-threatening condition affecting immunocompromised individuals (2).

FLAs are found globally and have adapted to survive in harsh conditions, making them resilient pathogens (3). In addition to water sources, they can inhabit hospital environments, posing risks to patients, particularly those with weakened immune systems (4). Recent studies in Iran report the presence of *Acanthamoeba* in various environmental sources, with certain genotypes, especially T4, being more pathogenic (5).

In hospital settings, dust may act as a vector for these amoebae, which could increase the risk of nosocomial infections (6). The presence of *Acanthamoeba* in hospital dust has been previously observed in Tehran and other regions (5, 7-9), but data from Ahvaz is limited. Given the potential health risks, especially in areas with immunocompromised patients, this study investigates the prevalence of *Acanthamoeba* in the dust of Ahvaz hospitals to better understand its distribution and potential impact on public health in clinical settings.

Methods

Ethical considerations

This study received ethical approval from the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Approval Code: IR.AJUMS.MEDICINE.REC.1400.004). All methods were performed in accordance with the relevant guidelines and regulations.

Sample collection

This study involved collecting dust samples from various wards in three hospitals affiliated with Ahvaz Jundishapur University of Medical Sciences in Ahvaz, Khuzestan, Iran. The sampling process was conducted under strict sterile conditions to prevent contamination. A total of 96 samples were collected from high-risk hospital areas, including corridors, general care departments, intensive care units (ICUs), and surgical wards, each receiving an equal number of 24 samples.

To ensure consistency and reliability, samples were collected using sterile swabs. Each swab was moistened with sterile distilled water and then used to wipe dust from designated surfaces, such as air vents, windowsills, and equipment surfaces in patient-care areas. The collected samples were immediately placed into sterile containers, sealed, and labeled with the location and date of collection for accurate traceability. Samples were processed within 24 hours of collection to preserve the integrity of any microorganisms present.

Non-nutrient agar culture

To culture *Acanthamoeba*, sterile swabs containing the dust samples were first immersed in distilled water for two hours to rehydrate any dormant amoeba cysts (10). Afterward, 1.5% non-nutrient agar (NNA) plates were prepared using Page's Amoeba Saline solution, which included 2.5 mM NaCl, 1 mM KH_2PO_4, 0.5 mM Na_2HPO_4, 40 µM $CaCl_2{\cdot}6H_2O$, and 20 µM $MgSO_4{\cdot}7H_2O$. The pH of this medium was adjusted to approximately 6.9 using KOH (11).

Once prepared, the NNA plates were inoculated by placing filters with the dust samples upside down over the medium near a flame to minimize contamination. The culture plates were overlaid with a heat-inactivated standard strain of *Escherichia coli*, which served as a nutrient source for *Acanthamoeba*. The plates were then sealed with parafilm to maintain humidity and incubated at 25–30°C. Cultures were monitored from the second day of incubation and examined regularly over a two-week period for the presence of *Acanthamoeba*. Positive samples were identified based on the distinctive morphological characteristics of *Acanthamoeba* cysts and trophozoites. The appearance of transparent dots on the culture medium was used as an initial indicator of *Acanthamoeba* growth, which was then confirmed through microscopic examination (12).

Cloning of the Amoeba positive plates

Acanthamoeba was identified by the presence of Acanthopodia (false feet) in its trophozoite form and a double-layered polygonal wall in its cyst form. When amoebic growth was observed, a section of the NNA plate with minimal fungal contamination was transferred to a fresh culture medium. The samples were incubated at 30°C and monitored for two months until cyst formation occurred. After cyst development, cultures were washed with a PBS buffer at pH 7.2. Both the upper and lower surfaces of the basic agar medium were gently scraped with a blade to collect *Acanthamoeba* cysts, which were then concentrated by centrifugation (11, 13).

DNA extraction and polymerase chain reaction (PCR) amplification

DNA extraction was performed using the Sinapore DNA extraction kit (Cat. No: EX6011). Specific *Acanthamoeba* primers, JDP1 (forward: 5′-GGCCCAGATCGTTTACCGTGAA-3′) and JDP2 (reverse: 5′-TCTCACAAGCTGCTAGGGAGTCA-3′), were utilized for amplification (14). The PCR reaction mixture had a total volume of 25 µL, including 12.5 µL of Ampliqon, 1 µL each of the forward and reverse primers, 3 µL of DNA template, and 8.5 µL of double-distilled water.

The PCR protocol consisted of 35 cycles with three steps: initial denaturation at 96°C for 1 minute, followed by denaturation at 94°C for 35 seconds across 35 cycles. Annealing temperatures were set at 56°C, 58°C, and 50°C for 45 seconds in successive stages. The extension step was conducted at 72°C for 1 minute (10, 14, 15).

Gel electrophoresis

PCR products were stained with ethidium bromide and loaded onto a 1.5% agarose gel. The gel was then examined under UV light to visualize the DNA bands (10, 15).

Statistical analysis

A chi-squared test was performed to determine the association between sample location and the presence of *Acanthamoeba*. Results with a p-value less than 0.001 were considered statistically significant, indicating a meaningful association between sampling locations and *Acanthamoeba* presence.

Results

A total of 96 dust samples were collected from various wards in three hospitals, including corridors, general care departments, intensive care units, and surgical wards, with 24 samples obtained from each area. *Acanthamoeba* was detected through both morphological and molecular methods. Morphological analysis using Giemsa staining identified 6 samples exhibiting cyst-like structures characteristic of *Acanthamoeba*, distinguished by their polygonal double-layered walls (Figure 1).

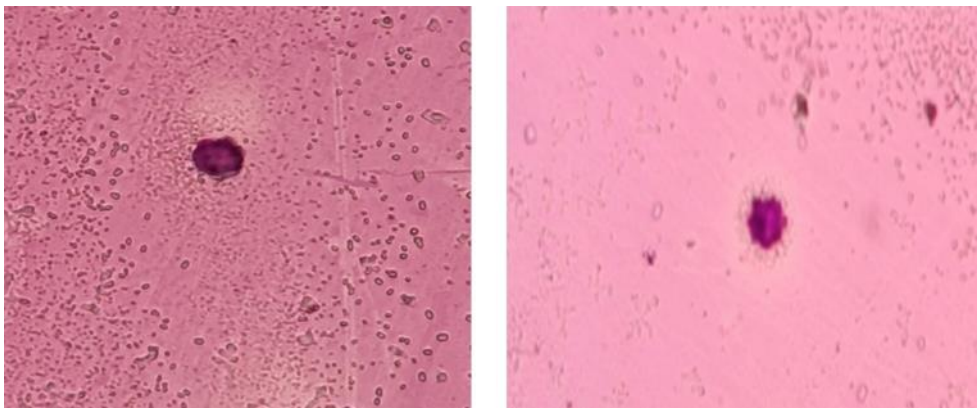

Figure 1. *Acanthamoeba* cysts observed under a light microscope. The cysts exhibit characteristic polygonal double-layered walls at a magnification of 40×.

For molecular confirmation, PCR analysis was conducted, revealing that 16 samples (16.6%) were positive for *Acanthamoeba* DNA. This was evidenced by the presence of a 480 bp amplified fragment observed on a 1.5% agarose gel under UV light (Figure 2).

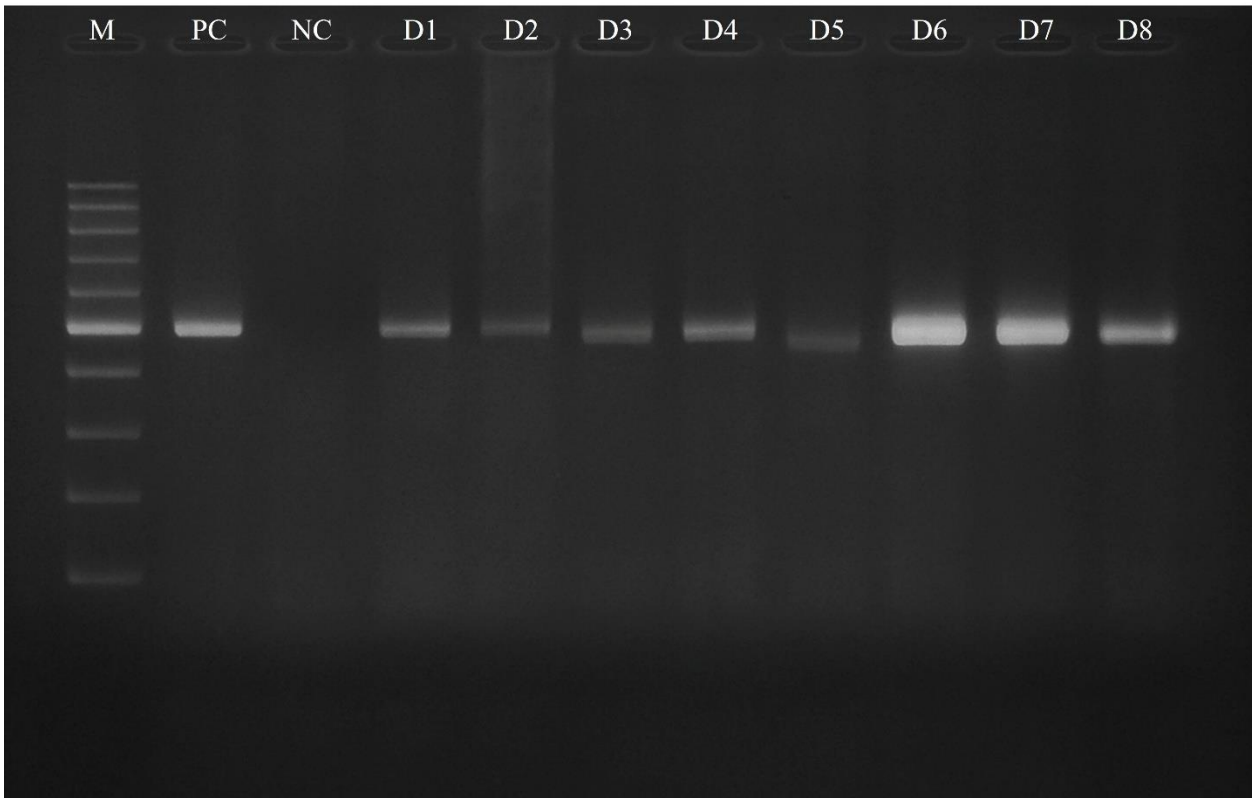

Figure 2. PCR amplification of *Acanthamoeba*-specific 480 bp fragment visualized on 1.5% agarose gel. Lane M: 100 bp DNA ladder; PC: positive control; NC: negative control; D1-D8: positive samples from dust specimens.

The distribution of *Acanthamoeba*-positive samples was as follows: 11 samples from the corridors of the general care department, 4 from general care rooms, and 1 from ICUs. Notably, no positive samples were detected in surgical wards. The detailed distribution of *Acanthamoeba*-positive samples across different hospital areas is presented in Table 1.

Table 1. Distribution of *Acanthamoeba* positive samples by location in hospital wards.

Location	Total Samples	Positive Samples	Positive Percentage
Corridors	24	11	68.75%
General Care Rooms	24	4	25.00%
Intensive Care Units	24	1	6.25%
Surgical Wards	24	0	0.00%

The chi-square test for independence revealed a statistically significant difference in *Acanthamoeba* prevalence across the hospital departments ($p < 0.001$), indicating a non-uniform distribution of *Acanthamoeba* within the tested hospital environments.

Discussion

This study presents significant findings regarding the presence and distribution of *Acanthamoeba* within hospital environments. These findings reveal important patterns that warrant careful consideration for hospital hygiene and patient safety protocols. Our investigation detected *Acanthamoeba* in 16.6% of the total samples collected, with notably variable distribution patterns across different hospital areas. Our findings can be contextualized within the broader landscape of FLA research in Iranian healthcare settings. Previous studies in various regions of Iran have reported varying prevalence rates: 20% in Khomein (16), 52.9% in Tehran's immunodeficiency wards (7), and ranging from 41.6% to 52.6% in different hospital departments in central Iran (5). Notably, a highly comparable study in Guilan province detected a 16% PCR positivity rate in hospital dust samples, which is remarkably similar to our findings of 16.6% (8). This consistent prevalence across geographically and climatically distinct regions suggests that this rate represents a stable baseline for *Acanthamoeba* contamination in well-maintained hospital environments. The notably lower prevalence rates in both our study and the one conducted in Guilan compared to other Iranian studies might be attributed to several factors, including differences in sampling methodologies, variations in detection techniques, regional climatic differences, or potentially more effective cleaning protocols in the studied hospitals.

The striking variation in *Acanthamoeba* prevalence across different hospital zones ($p < 0.001$) suggests that environmental and human factors significantly influence the organism's distribution. The highest concentration of positive samples in corridor areas (68.75%) likely reflects these spaces' role as high-traffic zones with frequent human movement and potential exposure to outside contamination. The decreasing gradient of positive samples from corridors to general care rooms (25%) and ICUs (6.25%) suggests that existing hygiene protocols may be more stringently applied in patient care areas. The complete absence of *Acanthamoeba* in surgical wards is particularly noteworthy and likely reflects the rigorous sterilization procedures and environmental controls implemented in these critical areas. These findings can be contextualized within the broader literature on hospital-based *Acanthamoeba* contamination in Iran. Niyyati et al. found a different distribution pattern in ICU and CCU environments, with high presence in CCU areas (11 positive samples), limited detection in Surgical ICU and General ICU (1 positive sample each), and none in Open Heart ICU (9). Further, Mohammady et al.'s study in Markazi Province revealed widespread contamination across different hospital zones, with 20 positive cases in general wards, 15 in specialty wards, and 5 in service departments, demonstrating a total contamination rate of 44.7% in hospital samples (5). The variation in contamination patterns across these studies suggests that local environmental factors and facility-specific practices significantly influence *Acanthamoeba* distribution.

The combined findings from these studies highlight the need for location-specific risk assessment and targeted prevention strategies. While our study demonstrates the effectiveness of stringent sterilization protocols in surgical areas, the high contamination rates in general and specialty wards reported by Mohammady et al., alongside Niyyati et al.'s findings regarding CCU contamination, highlight potential vulnerabilities in various hospital settings. This is particularly concerning for immunocompromised patients, who are especially susceptible to infection. These findings collectively suggest that while current hospital hygiene protocols may be effective in certain areas, there is a need for enhanced monitoring and control measures across different hospital zones, with particular attention to high-traffic areas and units housing vulnerable patients.

The disparity between morphological (6 positive samples) and molecular detection methods (16 positive samples) highlights the superior sensitivity of PCR-based approaches for *Acanthamoeba* detection. The molecular approach's higher sensitivity suggests that hospital environmental monitoring programs might benefit from incorporating PCR-based screening protocols to ensure more accurate detection of potential pathogens.

The detection of *Acanthamoeba* in hospital settings presents public health concerns due to its potential role as a vector for various pathogens, including resistant bacteria, fungi, and viruses (17). While its presence does not imply an immediate health threat, *Acanthamoeba* is known as a “Trojan horse” for harboring bacteria such as *Chlamydia* spp., *Klebsiella* spp., *Legionella* spp., and *Pseudomonas* spp., all of which are resistant to standard water treatments and disinfectants used in hospitals (17, 18). These bacteria can survive and even replicate within the amoeba by avoiding the typical phagocytic pathway, establishing an intracellular niche that allows their persistence and eventual release into the environment (19-21).

Legionella pneumophila and *Pseudomonas aeruginosa*, for example, have been shown to modify the amoeba's intracellular environment, preventing phagosome-lysosome fusion and thereby enhancing their survival and replication (21). This capacity for bacterial endosymbiosis allows the amoeba to serve as a reservoir for pathogens, which may then cause serious infections, such as pneumonia or even tuber-

culosis, particularly in vulnerable, hospitalized patients with indwelling invasive devices like catheters (22). Additionally, the detection of *Acanthamoeba* in hospital environments may signal conditions that favor other opportunistic pathogens, and this highlights the need for rigorous and ongoing environmental monitoring programs to mitigate potential health risks.

Our findings suggest that targeted intervention strategies in hospital settings may help mitigate potential health risks. Environmental management could be beneficial; for example, high-traffic areas, particularly corridors, might benefit from enhanced cleaning protocols. Ventilation systems and other potential points of environmental contamination could be regularly monitored, and maintaining optimal humidity levels may help reduce amoebic survival. Surveillance programs might benefit from integrating molecular detection methods into routine environmental checks, with high-risk areas undergoing regular assessment and standardized sampling protocols. Staff training is also potentially important: increasing awareness of environmental pathogens, promoting the proper application of preventive measures, and providing regular updates on cleaning and disinfection protocols may contribute to improved infection control practices.

For future research, longitudinal studies would provide a better understanding of *Acanthamoeba* distribution over time. Investigations into potential reservoirs and transmission pathways within hospitals could identify sources and improve control measures. Research on the efficacy of various cleaning and disinfection protocols, combined with molecular characterization of isolated strains, would clarify the pathogenic potential of *Acanthamoeba* and inform infection control strategies. Finally, assessing correlations between the presence of *Acanthamoeba* and other opportunistic pathogens could reveal broader implications for hospital hygiene and patient safety.

Study limitations

This study has several limitations that should be considered when interpreting the findings. As a cross-sectional investigation, it provides only a snapshot of contamination and may not reflect seasonal or temporal variations in *Acanthamoeba* distribution. Moreover, PCR detection confirms the presence of *Acanthamoeba* DNA but does not differentiate between viable and non-viable organisms.

Conclusion

This study provides compelling evidence for the non-uniform distribution of *Acanthamoeba* within hospital environments, with significant implications for infection control and prevention strategies. The marked variation in contamination levels across different hospital areas suggests that current hygiene protocols may be variably effective and could benefit from targeted enhancement in high-risk areas. These findings contribute to our understanding of environmental pathogens in healthcare settings and provide a foundation for developing more effective control strategies to protect vulnerable patient populations.

Footnotes

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Authors' Contribution: Forough Kord-zanganeh: Investigation, Data Curation.
Mahmoud Rahdar: Supervision, Methodology, Validation.
Mohammad Javad Boozhmehrani: Formal Analysis, Data Interpretation, Writing – Original Draft, Writing – Review & Editing.
Mehdi Tavalla: Conceptualization, Supervision, Project Administration, Writing – Review & Editing.

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