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PREVALENCE OF ACANTHAMOEBA IN MALAYSIAN WATER SOURCES AND ITS PUBLIC HEALTH IMPLICATIONS: A SCOPING REVIEW

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ABSTRACT

Acanthamoeba, a free-living amoeba, poses significant public health risks, causing infections like *Acanthamoeba* keratitis (AK) in contact lens users and granulomatous amoebic encephalitis (GAE) in immunocompromised individuals. This scoping review examines *Acanthamoeba* prevalence in Malaysian water sources using a PRISMA-ScR-guided methodology. Literature searches from 2010 to 2024 identified studies focused on natural and artificial water sources, employing microscopy, culture, and molecular techniques for detection and genotyping. The findings revealed high contamination rates: 54.4 % in swimming pools, 90.2 % in drinking water, 100.0 % in recreational rivers, 86.7 % in lakes, 76.0 % in hot springs, and 72.0 % in marine waters, with the pathogenic T4 genotype predominating. *Acanthamoeba*'s role as a "Trojan horse," harbouring amoeba-resistant bacteria (ARBs), amplifies public health concerns. Prevalence in Malaysia exceeds neighbouring countries, influenced by factors such as climate and water management practices. Key research gaps include the lack of longitudinal studies and insufficient exploration of environmental influences on *Acanthamoeba* virulence. The review highlights the need for improved water treatment protocols, advanced diagnostics, and targeted public health measures. Effective strategies should prioritize thorough monitoring, public education, and enhanced water safety practices to mitigate risks and protect high-risk populations.

1.0 INTRODUCTION

Acanthamoeba, first described by Castellani in 1930, is a common free-living amoeba (FLA) that poses significant health risks, particularly to contact lens users and immunocompromised individuals [1–2]. It is associated with severe infections, such as Acanthamoeba keratitis (AK), which damages the cornea, and granulomatous amoebic encephalitis (GAE), a rare but often fatal brain infection [3–4]. AK cases are well-documented in Malaysia, whereas GAE has never been reported in the country [2]. *Acanthamoeba* life cycle as in Figure 1 includes two stages: the trophozoite, responsible for feeding and reproduction, and the resilient cyst, which allows survival in extreme conditions, including chlorination and desiccation [5]. This adaptability enables *Acanthamoeba* to persist in various habitats, such as swimming pools, hot springs, drinking water systems, and recreational lakes, as well as in biofilms, soil, and dust, often linked with organic matter and microbial communities [6–10]. The trophozoite stage (25–40 µm) is active, moving and feeding using pseudopods, while cysts (13–20 µm), which form under stress, are highly resilient due to their double-layered wall [11–15]. Cysts are grouped by size and ectocyst appearance: Group I (large and smooth), Group II (smaller and wrinkled), and Group III (small and rippled) [13, 16–17]. Molecular classification, based on 18S rRNA sequences, includes 23 genotypes (T1–T23), with the T4 genotype being

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the most commonly found in environmental samples and linked to the most severe clinical cases of *Acanthamoeba* infections [18–20]. Genotypes such as T2, T4, T5, T6, T10, T11, T12, and T15 are linked to AK, while T1, T2, T4, T5, T10, T11, and T12 are associated with GAE [19–21].

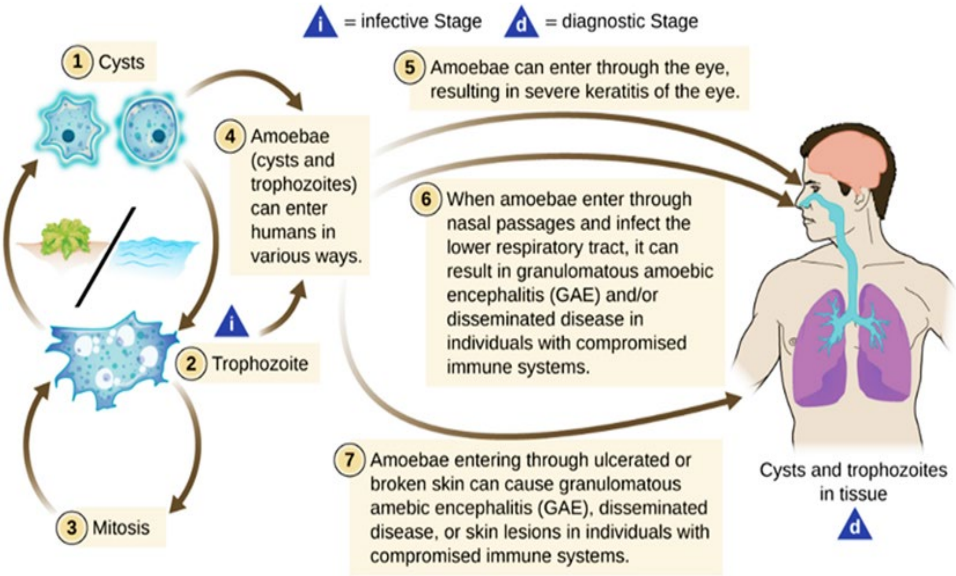

Figure 1. *Acanthamoeba* are waterborne parasites commonly found in unchlorinated aquatic environments

Acanthamoeba acts as a reservoir or "Trojan horse," harboring various pathogens such as *Esherichia coli, Salmonella spp., Shigella spp., Vibrio cholerae, Legionella pneumophila, Mycobacterium avium complex, Parachlamydia spp.,* and *Chlamydia pneumoniae* [22–25]. These bacteria, known as amoeba-resistant bacteria (ARBs), survive within *Acanthamoeba* by manipulating intracellular trafficking pathways to evade its defense mechanisms, thereby increasing their resistance and pathogenicity [22–23, 25]. As a biological reservoir, *Acanthamoeba* provides a protective environment that shields these bacteria from immune responses and antimicrobial agents, enabling their replication, persistence, and environmental transmission. The term "Trojan horse" aptly describes *Acanthamoeba*'s ability to harbor bacteria that survive within its cells for protection rather than replication, allowing them to endure harsh environmental conditions and enhancing their capacity to spread [22, 26]. Internalized bacteria, including ARBs, exploit *Acanthamoeba*'s resistance to environmental stressors such as chlorine, making them particularly challenging to eliminate from water systems [27–28]. This dual role of *Acanthamoeba* significantly amplifies the risk these pathogens pose to human health, particularly in healthcare settings [26–28]. *Acanthamoeba* is highly prevalent in Malaysia's water sources, posing significant risks to contact lens users and immunocompromised individuals. The warm climate and frequent human activity in contaminated water bodies, such as pools and lakes, facilitate its transmission [6–9, 24]. Studies reveal high contamination rates, emphasizing the need to assess pathogenic genotypes, water treatment efficacy, and strategies to mitigate exposure due to its resistance to standard disinfection [29–33].

In Malaysia, waterborne diseases such as cholera and typhoid, which may be linked to *Acanthamoeba* contamination, often go undetected due to underreporting and insufficient research efforts. This scoping review aims to survey existing research on *Acanthamoeba* in Malaysian water environments, identify research gaps, and clarify limitations in understanding its pathogenicity and adaptability. By synthesizing findings, analysing contamination patterns, and exploring factors influencing prevalence, this review seeks to inform public health policies and enhance water treatment protocols, ultimately contributing to comprehensive monitoring and mitigation strategies that address the health risks posed by *Acanthamoeba* in Malaysia.

2.0 METHODS AND MATERIAL

This study followed the framework provided by the 2018 PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews) Guideline. Key steps included formulating the research question, sourcing relevant literature, selecting suitable studies, charting the data, and finally, collating, summarizing, and reporting the findings. The literature search employed keywords

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like "*Acanthamoeba*," "Malaysia," "water contamination," and "public health risk" across databases such as PubMed, Scopus, and Google Scholar, covering the period from January 2010 to December 2024. This scoping review included studies examining the detection and prevalence of *Acanthamoeba* in both natural and man-made water sources across Malaysia. Only peer-reviewed journal articles, conference proceedings, and official reports published in English from 2010 onwards were considered. Studies were excluded if they focused on regions outside of Malaysia or lacked specification of the water sources investigated. Non-English publications and grey literature, such as conference papers and abstracts, were excluded to ensure methodological rigor and reliability, as grey literature can vary in quality and often lacks peer review. The literature search was performed across PubMed, Scopus, and Google Scholar with predefined keywords. Covidence, an online software platform, was employed to efficiently identify and remove duplicate records in a systematic manner [34]. The findings from the literature search were collaboratively reviewed by team members, and to ensure the reliability of the data, only studies employing microscopy, culture techniques, or molecular methods such as polymerase chain reactions (PCR) or DNA sequencing were considered for inclusion. Data from each study were systematically charted to capture key variables, including study characteristics, locations, prevalence of *Acanthamoeba* in water sources, laboratory detection methods, sources of contamination, and public health outcomes.

Details such as the type of study, tested locations (e.g., swimming pools, beaches), and diagnostic methods were recorded to ensure consistent data extraction and enable comparisons across studies. Charting also documented *Acanthamoeba* prevalence and genotypes, categorizing contamination levels across recreational rivers, drinking water treatment facilities, hot springs, marine waters, and swimming pools, while highlighting higher-risk genotypes. The results were organized into key themes, such as contamination sources, locations, diagnostic methods, and public health implications. Each theme provided a summary of essential data, emphasizing the prevalence of *Acanthamoeba* in recreational rivers, drinking water treatment facilities, hot springs, marine waters, and swimming pools. Patterns of contamination across these various water sources were analysed, and prevalence rates were calculated to highlight associated risks. Key findings were synthesized to illustrate trends in *Acanthamoeba* contamination, including its role as a reservoir or "Trojan horse". The results were presented in tables to identify research gaps and suggest improvements in infection control practices.

3.0 RESULTS

3.1 Characteristics of Prevalence Acanthamoeba in Water Sources

Table 1 and Table 2 provide a summary of the articles and their key findings. The search process initially identified 179 articles. After removing 16 duplicate studies, Figure 2 illustrates the PRISMA flowchart detailing the article screening and selection process. Around 153 articles were excluded during the screening phase, resulting in 10 articles proceeding to full-text screening. of these, 7 studies met the eligibility criteria and were included in the final analysis. The prevalence of *Acanthamoeba* in various water sources across Malaysia shows considerable variation depending on the water environment type and the regions studied as in Table 1. The prevalence of *Acanthamoeba* in various water sources across Malaysia shows considerable variation depending on the water environment type and the regions studied.

Table 1. The prevalence of *Acanthamoeba* in natural and man-made water source in Malaysia

Type of Study	Location	Water Sampling Location	Laboratory Investigation	Prevalence of *Acanthamoeba*	Species of *Acanthamoeba*	Ref
Cross-sectional	Petaling Jaya & Kuala Lumpur	14 public swimming pools	Culture and microscopy	54.4% (457/840)	*Acanthamoeba* spp	[6]
Cross-sectional	Selangor & Kuala Lumpur	15 recreational rivers (Sg Pangsun, Sg Congkak, Sg Lopo, Sg Kancing, Sg Sendat, Sg Rumput, Sg Tekala, Sg Serendah, Sg Kedondong, Sg Chiling, Sg Tua, Sg	Culture, microscopy, PCR and sequencing	100% (15/15)	T3, T4 (most prevalent), T5 and T15	[35]

Type of Study	Location	Water Sampling Location	Laboratory Investigation	Prevalence of *Acanthamoeba*	Species of *Acanthamoeba*	Ref
		Gabai, Sg Kemensah, Sg Templer, Sg FRIM)				
Cross-sectional	Peninsular Malaysia	11 states across Peninsular Malaysia (tap water, lakes, waterfalls, and paddy fields)	Culture, microscopy, PCR and sequencing	36.4% (91/250)	*Acanthamoeba* spp	[36]
Cross-sectional	Peninsular Malaysia	5 recreational hot springs (Sg Klah, Selayang, Hulu Tamu, Bentong, Gadek)	Culture, microscopy, PCR and sequencing	76% (38/50)	T3, T4, T5, T11, T15 and T17	[9]
Cross-sectional	Peninsular Malaysia	5 recreational beaches (Teluk Batik, Morib, Teluk Kemang, Tanjung Bidara, Teluk Cempedak)	Culture, microscopy, PCR and sequencing	72% (36/50)	T4 (most prevalent), T5, T11, T15 and T18	[7]
Cross-sectional	Sarawak	2 major drinking water treatment plants	Culture, microscopy and PCR	90.2% (55/61)	T3 and T4 (most prevalent)	[24]

The rates of *Acanthamoeba* contamination appear alarmingly high in both natural and man-made water sources, raising significant public health concerns due to the pathogenic potential of several *Acanthamoeba* genotypes. A study analysing water from swimming pools in Kuala Lumpur and Petaling Jaya documented a 54.4% overall prevalence, with platforms around the pool showing 100% positivity and water samples ranging from 19.4% to 64.7% positivity, illustrating the heterogeneity of *Acanthamoeba* distribution even within controlled environments like swimming pools [6]. Another investigation in Sarawak reported a striking 90.2% prevalence in samples from two drinking water treatment plants. All raw water samples were contaminated, although prevalence decreased in treated water and distribution systems, indicating the partial effectiveness of water treatment processes in mitigating contamination risks, though not completely eliminating them [24]. The scenario is equally concerning for natural water sources. A study in Selangor and Kuala Lumpur recorded a 100% prevalence of *Acanthamoeba* across 15 recreational rivers. Identified genotypes included T3, T4, T5, and T15, with T4 being the most dominant and often associated with higher pathogenic potential [35]. This finding aligns with another investigation that analysed a wide range of water sources, including tap water, lakes, waterfalls, and paddy fields, across Peninsular Malaysia. The overall prevalence in this case was 36.4%, with recreational places showing a higher contamination rate of 70.2% compared to 24.9% in tap water. Water dispensers and paddy fields also demonstrated high contamination rates, while filtered water systems and drains had no detectable *Acanthamoeba* [36]. These findings highlight the diverse contamination landscape, suggesting that recreational waters are more prone to contamination than municipal water systems.

Recreational hot springs also exhibited high contamination levels. An investigation across several hot springs in Peninsular Malaysia found a 76% overall prevalence, with individual springs like Hulu Tamu showing 100% contamination. The study identified prevalent genotypes such as T4 and T15, underscoring the potential health risk associated with these natural thermal waters [9]. Coastal marine waters are similarly affected. A study in various beaches across Peninsular Malaysia reported a 72% overall prevalence, with sites like Pantai Morib reaching 90% positivity. The T4 genotype was predominant, though several other genotypes, including T5, T11, and T15, were also found, showcasing the genetic diversity of *Acanthamoeba* in marine environments [7]. Lastly, research on recreational lakes in Peninsular Malaysia found an 86.7% prevalence, with Biru Lake showing 100% contamination. The genotypes identified included T4, T5, T9, and T11, with T4 being the most frequent [8]. Comparing natural and man-made water sources reveals a generally higher prevalence of *Acanthamoeba* in natural environments. Recreational rivers and lakes, often characterized by stagnant or slow-moving water, provide a conducive environment for *Acanthamoeba* growth and survival, as evidenced by consistently high contamination

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rates across studies [8, 35]. Man-made water systems, such as swimming pools and drinking water facilities, exhibited variable contamination levels. The efficacy of water treatment measures is evident from the decreased prevalence rates at different stages of water processing, as seen in Sarawak's treatment plants [24]. However, the persistence of *Acanthamoeba* even after treatment highlights the pathogen's resilience and the potential for exposure if water quality controls fail. In swimming pools, the highest contamination rates were associated with platforms and debris, suggesting that while water filtration systems may reduce pathogen load, areas around pools remain significant reservoirs for *Acanthamoeba* [6].

Regional variations within Malaysia are also noteworthy and may reflect differences in environmental conditions, water management practices, and human activity. High prevalence rates in urban areas like Kuala Lumpur and Selangor may be attributed to extensive human activity and urbanization, while the high contamination levels in places like Sarawak underscore the challenges in maintaining pathogen-free water in large-scale treatment facilities [6, 24].

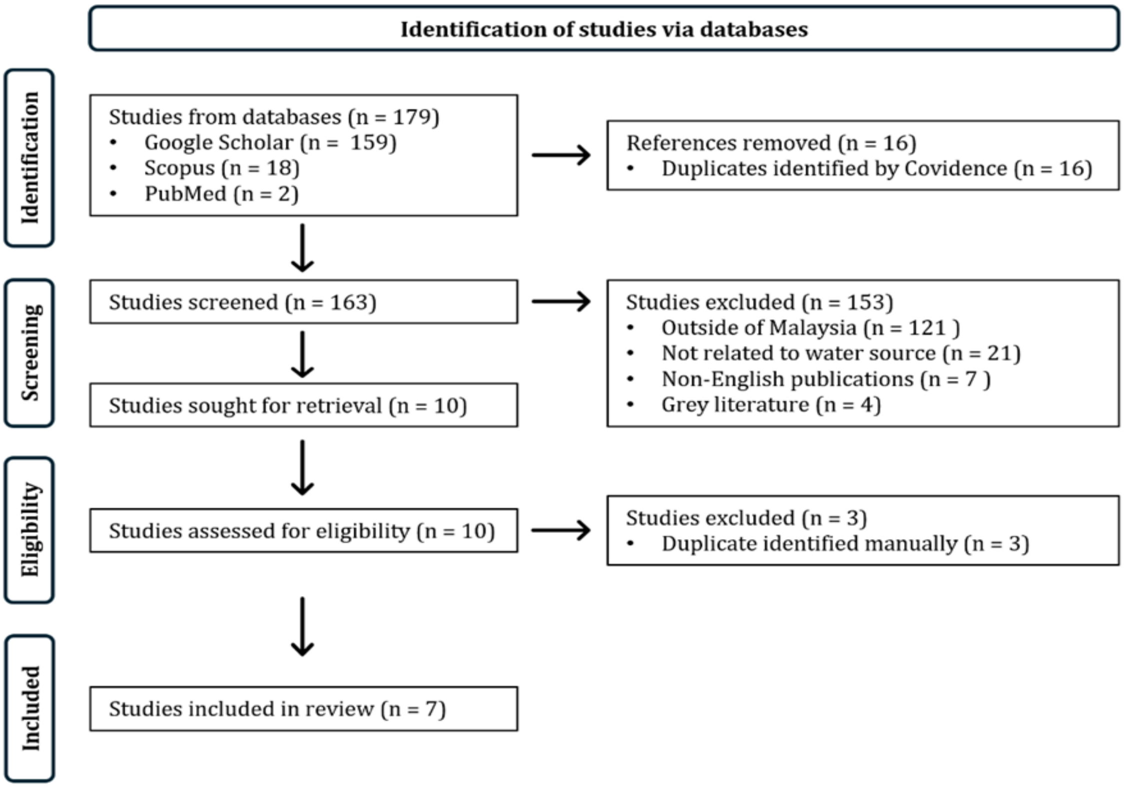

Figure 2. Flow chart illustration of steps for article selections using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) 2018 Guideline

3.2 Methodologies used in identifying *Acanthamoeba*

The detection of *Acanthamoeba* in Malaysian water sources involves several methodologies crucial for accurate identification and risk assessment (Table 1). Common techniques include culture methods, microscopic examination, and molecular approaches such as polymerase chain reaction (PCR). Culture-based methods typically involve inoculating samples onto non-nutrient agar plates seeded *with Escherichia coli* to promote the growth of *Acanthamoeba* trophozoites and cysts, which are then identified using light or inverted microscopy. In studies on swimming pools in Petaling Jaya and Kuala Lumpur, samples from platforms, walls, and water were cultured on E. coli-seeded agar and examined microscopically for cysts and trophozoites [6]. Although culture methods are simple and cost-effective, they require expertise and can be time-consuming. Molecular techniques like PCR have become vital, offering rapid and precise identification of *Acanthamoeba* species and genotypes, even in low concentrations. For example, drinking water studies in Sarawak used PCR after initial culture and microscopy to confirm genotypes, including T3 and T4 [24]. In Selangor and Kuala Lumpur's recreational rivers, PCR amplification identified prevalent genotypes, with T4 being the most frequent [35].

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PCR's high sensitivity and specificity enable differentiation between pathogenic and non-pathogenic strains, though its cost and need for specialized equipment limit accessibility in some settings. Advanced methods often combine culture and PCR for greater accuracy. Research on marine water in Peninsular Malaysia, for instance, used this dual approach, identifying diverse genotypes like T4, T5, and T11 [7]. Similar strategies are applied in hot springs and recreational lakes, where studies have highlighted the presence of pathogenic genotypes, such as T3, T4, and T11, with significant public health implications [9, 36]. Combining these methods enhances the reliability of detecting *Acanthamoeba* and underscores the need for methodological advancements to protect public health effectively.

3.3 Implications for Public Safety

Acanthamoeba-related infections pose a significant public health concern in Malaysia, particularly given the organism's ability to thrive in various natural and man-made aquatic environments. The amoeba is notorious for causing severe health conditions such as AK, which primarily affects contact lens users, and GAE, a potentially fatal condition in immunocompromised individuals [4, 37]. Studies conducted across diverse settings in Malaysia have consistently highlighted a high prevalence of *Acanthamoeba*, often associated with environmental factors that facilitate amoebic survival and pathogenicity (Table 2). For instance, a study by Init [6] detected *Acanthamoeba* in 100% of surveyed public swimming pools in Petaling Jaya and Kuala Lumpur, emphasizing the amoeba's resilience to chlorination and the heightened contamination risk posed by biofilms on pool walls and dust from platforms. These findings highlight the need for more effective pool maintenance practices to mitigate infection risks and warrant further molecular research to understand the virulence of detected strains [6]. Similarly, research on water treatment facilities in Sarawak revealed that 90.2% of raw and treated water samples tested positive for *Acanthamoeba*, with pathogenic genotypes like T4 frequently identified. This raises concerns about the efficacy of existing treatment protocols, suggesting a need for robust disinfection measures and additional studies to evaluate seasonal variations in amoebic prevalence [24]. The environmental persistence and adaptability of *Acanthamoeba* are further corroborated by findings from Basher [35], who reported an 85% detection rate in various environments, including soil, river water, and dust, with genotype T4 predominating.

The study pointed out research gaps in understanding environmental factors that influence amoebic virulence and the potential for human exposure, advocating for in-depth analyses of amoebic survival mechanisms and public awareness initiatives to reduce infection risks. Gabriel et al. [36] expanded this research by surveying untreated and inadequately treated water sources across Peninsular Malaysia, where 64% of samples harboured *Acanthamoeba*. The high prevalence in recreational waters poses a significant threat to public health, especially given the potential for waterborne disease outbreaks if preventive measures are not effectively implemented [36]. Similarly, studies on recreational hot springs highlighted a 76% prevalence of *Acanthamoeba*, with notable correlations between amoebic presence and environmental factors like sulphate levels and water temperature [9]. The identification of highly pathogenic strains accentuates the necessity for stringent monitoring and public health interventions, including posting warning signs in high-risk recreational areas [8–9]. A study on marine waters at popular recreational beaches also reported a concerning 72% prevalence of *Acanthamoeba*, with genotype T4 being the most common. The data imply significant health risks for people engaging in water-based activities, calling for consistent monitoring and more effective safety guidelines [7]. Lastly, recent research on recreational lakes in Peninsular Malaysia identified *Acanthamoeba* in 86.7% of samples, with T4, T17, and T18 genotypes showing thermo- and osmotolerance. These findings highlight critical gaps in understanding how *Acanthamoeba* adapts to environmental stressors and emphasize the urgent need for public education and preventive strategies to mitigate infection risks in high-use recreational areas [8].

3.4 Research Gaps and Limitations in Literature

A scoping review of existing literature on *Acanthamoeba* contamination across various environments, such as swimming pools, drinking water sources, hot springs, and recreational marine and lake waters, reveals significant research gaps and limitations that hinder comprehensive understanding and effective management of associated public health risks. Despite numerous studies confirming the widespread presence of *Acanthamoeba* and identifying pathogenic genotypes like T3, T4, and T11, several critical gaps remain as in Table 2. First, many studies fail to explore the long-term environmental persistence of *Acanthamoeba* or the specific mechanisms underlying its resistance to standard disinfection practices. For instance, research on swimming pools highlights the high survival rates of cysts despite chlorination, yet

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the virulence of these strains and the efficacy of various cleaning protocols remain inadequately investigated. Molecular studies are suggested to determine the pathogenic potential of detected strains and evaluate alternative water treatment methods to eliminate amoebic threats effectively [6]. Similarly, research on drinking water in Sarawak indicates high contamination levels, even post-treatment, but long-term monitoring and an in-depth assessment of seasonal fluctuations are lacking. The interplay between environmental variables and *Acanthamoeba* survival, especially the factors contributing to its resilience in treatment processes, requires further exploration [24].

Table 2. *Acanthamoeba* in the environments and man-made water sources and its implications for public health

Source of Contaminations	Public health threat or public safety implications	Key Findings	Research gaps and Limitations	Future Research	Ref
Swimming pool water and surfaces in 14 public pools in Petaling Jaya and Kuala Lumpur, Malaysia, were contaminated by biofilms, platform dust, and human activity.	*Acanthamoeba* risks include AK and GAE, especially for contact lens users and immunocompromised individuals, worsened by its cysts' resistance to chlorination.	*Acanthamoeba* was found in all 14 pools, with higher prevalence on pool walls (76.2%) and near platforms in dry, warm areas. Its cysts resisted chlorination and desiccation, thriving in favourable conditions.	The virulence of *Acanthamoeba* strains remains unconfirmed, requiring molecular studies. Improved evaluation of disinfection protocols is needed to eliminate amoebae from public pools.	Future studies should identify pathogenic *Acanthamoeba* genotypes and evaluate water treatment methods and environmental impacts on amoebic survival.	[6]
Raw river water serves as the primary source for two treatment plants (A and B) in Sarawak. Contamination stems from human and animal activities, introducing faecal and organic matter.	*Acanthamoeba* can cause severe infections like AK and GAE, especially through untreated water contact. Its presence in treated water highlights the need for better treatment processes.	90.2% of water samples contained *Acanthamoeba* (T3, T4) and Naegleria, with pathogenic T4 dominant. Contamination across treatment stages shows their resilience to standard processes.	The study lacked data on *Acanthamoeba*'s long-term persistence, seasonal prevalence, and the efficacy of current treatment protocols in complete elimination.	Future research should examine seasonal contamination patterns, test alternative disinfection methods, and study amoebic genetics to understand adaptation and pathogenicity.	[24]
Marine water from recreational beaches: Pantai Teluk Batik, Pantai Morib, Pantai Teluk Kemang, Pantai Tanjung Bidara, and Pantai Teluk Cempedak in Malaysia.	Pathogenic *Acanthamoeba* genotypes risk infections like AK and GAE, especially in immunocompromised individuals or water activity participants.	*Acanthamoeba* was found in 72% of marine samples, with T4 (75%) most common. Presence correlated with coliforms and pH, and 8.3% of isolates were	Limited knowledge exists on factors driving *Acanthamoeba* pathogenicity in marine waters and its interactions with microbial communities in disease transmission.	Future research should explore long-term monitoring, *Acanthamoeba* ecology, and genomic pathogenicity factors, while developing public health	[7]

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Source of Contaminations	Public health threat or public safety implications	Key Findings	Research gaps and Limitations	Future Research	Ref
		highly pathogenic.		guidelines for safer water recreation.	
Environmental sources included dust, soil, river water, and debris from recreational areas, playgrounds, animal shelters, and indoor/outdoor locations at the University of Malaya Medical Faculty.	*Acanthamoeba* in various environments risks severe infections like AK and GAE, particularly for immunocompromised individuals and contact lens users.	The study found 85% of environmental samples contained *Acanthamoeba*, with T4 (69.27%) most common. Genotypes T5 and T15 were also present. Corneal swabs matched environmental genotypes, linking exposure to animal infections.	The study lacked detailed analysis of human exposure to *Acanthamoeba*. More research is needed on environmental persistence, virulence factors, and real-world prevalence.	Future studies should use long-term monitoring, RT-PCR, and explore environmental factors influencing *Acanthamoeba* virulence, human infection risks, and control measures.	[35]
Contamination stemmed from untreated or poorly treated water in recreational areas, paddy fields, and tap water, linked to organic matter and biofilms.	*Acanthamoeba* poses risks of AK and GAE, particularly in immunocompromised individuals or those exposed to contaminated water, with high prevalence signalling outbreak risks without prevention.	*Acanthamoeba* was found in 64% of samples, with higher prevalence in recreational water than treated water, across 11 Malaysian states. PCR proved more sensitive and reliable than centrifugation for detecting pathogenic amoebae.	Limited data on seasonal variations, environmental impacts, human exposure risks, and genetic diversity of *Acanthamoeba* strains in water sources.	Future studies should examine seasonal dynamics, environmental factors, strain genetics, and pathogenicity, while exploring improved water treatment and safety measures to reduce infection risks.	[36]
Bacterial biofilms on vegetation, stones, and sediments promote *Acanthamoeba* growth, while human activity around lakes adds stressors and nutrients.	Pathogenic *Acanthamoeba* genotypes (T4, T9, T11) pose health risks, causing AK and AGE, especially in immunocompromised or exposed individuals.	*Acanthamoeba* was detected in 86.7% of lake samples, with T4 most prevalent. Thermo- and osmo-tolerant isolates showed high pathogenic potential, correlating with ORP, pH,	Limited knowledge exists on *Acanthamoeba*'s adaptation to lake stressors. Further research is needed on pathogenicity mechanisms and ecological interactions.	Future research should monitor *Acanthamoeba* in water bodies, study pathogenicity factors, and develop effective water safety protocols.	[8]

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Source of Contaminations	Public health threat or public safety implications	Key Findings	Research gaps and Limitations	Future Research	Ref
		and temperature.			
Hot spring biofilms promote *Acanthamoeba* growth, with conditions supporting its survival and pathogenicity.	*Acanthamoeba* poses a public health risk, causing AK and GAE, particularly in immunocompromised individuals and hot spring users.	*Acanthamoeba* was detected in 76% of hot spring samples, with pathogenic T4 most prevalent. Presence correlated with sulphate, temperature, and *E. coli*, and some isolates showed high thermo- and osmotolerance, increasing risk.	More research is needed on environmental factors influencing *Acanthamoeba* prevalence, adaptation, and virulence in hot springs.	Future research should include long-term monitoring, genetic studies on pathogenicity, environmental adaptation mechanisms, and in vivo testing for pathogenicity and interventions.	[9]

Furthermore, studies of recreational water sources, including lakes and hot springs, emphasize the need for advanced monitoring techniques and genetic characterization to ascertain the extent of public health risks. For example, findings from hot spring investigations indicate a notable presence of pathogenic *Acanthamoeba* genotypes that exhibit high thermo- and osmotolerance, yet the precise environmental stressors driving this adaptation are poorly understood. Research must focus on uncovering these adaptive mechanisms and their implications for human exposure and infection risks [9]. The literature also highlights the limitations in assessing human exposure routes and the potential for disease outbreaks, as studies often rely on in vitro findings that may not reflect real-world conditions. In addition, there is insufficient information on the genetic diversity and ecological interactions of *Acanthamoeba* with other microorganisms, which could influence disease transmission and pathogenicity [7, 36]. Efforts to monitor and mitigate *Acanthamoeba* contamination in marine and freshwater environments are further complicated by the limited understanding of how seasonal and environmental variables impact amoebic prevalence and virulence.

3.5 Future Recommendations from Literature

The reviewed literature emphasizes critical future recommendations to advance our understanding and mitigate the public health threats posed by *Acanthamoeba* contamination in various water sources and environments. One consistent theme across studies is the urgent need for comprehensive and long-term monitoring of *Acanthamoeba* prevalence in different ecological settings, including public swimming pools, recreational lakes, drinking water treatment facilities, and marine waters. Future research should employ advanced and highly sensitive diagnostic methods, such as real-time PCR, to improve the detection and characterization of *Acanthamoeba* genotypes, which are crucial for assessing infection risks and understanding amoebic distribution patterns. For instance, molecular studies are needed to differentiate between pathogenic and non-pathogenic strains, particularly in swimming pool environments, where *Acanthamoeba* cysts exhibit notable resistance to conventional disinfection methods like chlorination. Researchers recommend longitudinal studies that analyze the effectiveness of alternative water treatment strategies and explore the influence of environmental variables, such as temperature, pH, and nutrient levels, on amoebic survival and virulence [6]. In drinking water systems, there is a call for investigations into the seasonal dynamics of *Acanthamoeba* contamination and the efficacy of enhanced filtration and disinfection techniques. Studies should also consider the impact of human and animal activities on water sources, as these can introduce fecal and organic contaminants that facilitate amoebic growth. Genetic research is imperative to elucidate the mechanisms by which *Acanthamoeba* adapts to environmental stressors and to develop more robust water safety protocols [24]. The high prevalence of pathogenic *Acanthamoeba* genotypes in soil, dust, and river water samples suggests a pressing need for research into

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the environmental factors that promote amoebic virulence and the pathways through which humans may become exposed. Studies emphasize exploring the correlation between *Acanthamoeba* in natural habitats and cases of amoebic infections, using genetic matching techniques to establish causative links.

Such investigations should also address the limitations of in vitro studies, which may not accurately represent natural conditions, and instead consider in vivo research to validate pathogenicity and evaluate intervention strategies [35]. Hot springs and marine waters require specific attention due to their natural conditions that favor amoebic survival. Future work should investigate the role of environmental stressors, such as osmotic pressure and thermal fluctuations, in enhancing amoebic pathogenicity and exploring public health interventions like warning signs and safety guidelines for recreational areas. Studies should also investigate the interactions between *Acanthamoeba* and microbial biofilms, as these relationships may influence amoebic resilience and pose additional risks to public health. The literature calls for a holistic approach, integrating ecological, molecular, and public health perspectives to develop effective prevention and mitigation strategies [9, 36]. Marine water studies recommend understanding the ecological interactions between *Acanthamoeba* and other microbial communities and their role in disease transmission. This includes exploring how environmental parameters like coliform levels and water chemistry impact amoebic pathogenicity. The genetic and physiological characterization of *Acanthamoeba* isolates should also be a research priority to identify key virulence factors and guide the development of targeted control measures [7]. Furthermore, the widespread detection of *Acanthamoeba* in Malaysian lakes and its association with environmental parameters, such as oxidation-reduction potential (ORP) and temperature, underscore the necessity of developing water safety and risk assessment frameworks. Such protocols should address the environmental adaptation mechanisms of *Acanthamoeba* and incorporate public awareness campaigns to reduce exposure risks, especially among vulnerable populations like contact lens users and immunocompromised individuals. These integrated efforts are essential to mitigate the significant health risks posed by waterborne and environmental *Acanthamoeba* contamination [8].

4.0 DISCUSSION

4.1 Environmental Resilience and Adaptation Mechanisms

Acanthamoeba's resilience in harsh environmental conditions is another area of study that remains inadequately explored in Malaysia. The organism's cyst stage, which is highly resistant to standard chlorination and other disinfectants, enables it to survive in treated water systems, as observed in Spanish drinking water treatment plants [38]. This review finds that while Malaysian studies have reported high contamination rates in treated water, they lack data on how *Acanthamoeba* adapts to specific conditions in Malaysian water systems [24, 39]. Studies from Australia's coastal lagoons indicate that environmental factors such as turbidity, nutrient levels, and microbial interactions contribute significantly to *Acanthamoeba* prevalence and survival [40]. In Malaysia, biofilm formation in water treatment facilities may play a critical role in harbouring *Acanthamoeba* cysts and trophozoites, allowing them to evade disinfection protocols. A deeper investigation into the role of biofilms in *Acanthamoeba* survival and distribution across various water systems such as rivers, lakes, and marine environments would enhance understanding of how these organisms persist despite harsh environmental and chemical conditions.

4.2 Standardization and Advancements in Detection Methods

There is significant methodological variability in *Acanthamoeba* detection, with Malaysian studies relying primarily on traditional culture and PCR-based identification [7–9, 24, 35–36]. While PCR provides high specificity, it lacks sensitivity for detecting low-level contamination in large water bodies, limiting its effectiveness in comprehensive environmental surveillance. Comparative studies from Europe and Australia have incorporated advanced molecular techniques, such as metagenomics and 16S rRNA sequencing, to achieve a more complete analysis of *Acanthamoeba*'s microbial associations and overall diversity [38, 40–41]. Moreover, studies in countries like Thailand and the Philippines have highlighted the utility of combining morphological and molecular methods to improve detection rates [29, 32, 42–44]. Malaysia could benefit from the integration of these multi-faceted approaches to enable accurate assessment of *Acanthamoeba* distribution, especially in low-contamination environments like swimming pools and coastal waters. Additionally, developing standardized detection protocols would facilitate comparisons across regions, enabling meta-analyses that could inform broader public health strategies.

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4.3 *Acanthamoeba*-ARBs Interactions

One of *Acanthamoeba*'s unique roles in the ecosystem is its capacity to act as a reservoir or "Trojan horse", harbouring ARBs and other pathogenic microorganisms that can survive within the amoeba's protective cysts [21, 25]. Studies in Spain have documented interactions between *Acanthamoeba* and pathogens like *Legionella* and *Pseudomonas aeruginosa*, with these bacteria being released into the environment from *Acanthamoeba* hosts, potentially increasing public health risks [38]. In Malaysia, limited research exists on the prevalence and types of ARBs associated with *Acanthamoeba*. Understanding these interactions is crucial, as ARBs can compromise water safety and contribute to treatment-resistant infections. Research from Australia's coastal lagoons found positive correlations between *Acanthamoeba* and bacterial species like *Vibrio* and *Pseudomonas*, suggesting that bacterial presence may enhance *Acanthamoeba* survival or proliferation [40]. Similar investigations in Malaysia could reveal whether *Acanthamoeba* contributes to the persistence of ARBs and other pathogens in Malaysian water systems, thus elevating the risks associated with recreational and drinking water exposure.

4.4 Seasonal and Temporal Dynamics of *Acanthamoeba* Prevalence

Seasonal variations in *Acanthamoeba* prevalence have been observed in other tropical and subtropical countries, where warmer seasons correlate with higher contamination rates in water [34, 39-40, 45]. However, there is limited data on how Malaysia's monsoon seasons and fluctuating water temperatures influence *Acanthamoeba* prevalence and genotype diversity. Australian studies have documented increased *Acanthamoeba* presence in coastal lagoons during the summer, likely due to elevated temperatures and increased nutrient availability [39]. In contrast, studies from the Philippines indicate that seasonally dry periods may concentrate *Acanthamoeba* in smaller water bodies, increasing the likelihood of human exposure in recreational sites [32, 43–44]. Malaysian research should focus on conducting longitudinal studies to track seasonal changes in *Acanthamoeba* prevalence across different water sources, including rivers, hot springs, and marine environments. Identifying peak periods for *Acanthamoeba* contamination would provide valuable data to develop temporal intervention strategies that align with environmental conditions.

4.5 Underexplored Water Sources: Marine and Coastal Environments

While *Acanthamoeba* has been extensively studied in freshwater sources, there is limited research on its prevalence in marine and brackish waters in Malaysia. Studies from the Philippines have isolated *Acanthamoeba* from coastal areas, identifying pathogenic strains like T4 and T5, which are typically found in freshwater [32]. Similarly, research in Australia's coastal lagoons has highlighted *Acanthamoeba*'s adaptability to saline environments, particularly in urbanized coastal regions [40]. In Malaysia, the impact of coastal activities, such as tourism and fishing, on *Acanthamoeba* contamination levels remains unknown. To address this gap, future studies should assess *Acanthamoeba* prevalence in Malaysia's coastal and marine environments, evaluating the influence of salinity, pollution, and human activity on contamination rates. Understanding the organism's adaptability to these environments would inform potential risks associated with marine recreational activities.

5.0 LIMITATIONS OF THE REVIEW PAPER

This scoping review highlights several limitations that undermine the depth and reliability of its findings on *Acanthamoeba* prevalence in Malaysia's water sources. A key limitation lies in the variability of study designs, with most relying on cross-sectional approaches that lack longitudinal tracking. This methodological shortfall hinders the ability to monitor seasonal or long-term fluctuations in *Acanthamoeba* presence, limiting insights into contamination patterns and the effects of environmental changes on amoebic prevalence. Another notable issue is the geographic bias in research, which predominantly focuses on urban or frequently accessed areas, such as Kuala Lumpur and Selangor, while data from rural or remote regions remain sparse. This imbalance restricts the generalizability of findings, as rural areas with differing water management practices and environmental conditions may exhibit distinct contamination profiles. Furthermore, the review highlights a lack of studies examining *Acanthamoeba*'s interactions with other microorganisms, such as ARBs which are critical to its pathogenicity and resistance to disinfection. Additionally, the scarcity of comprehensive public health data on exposure risks for vulnerable populations, including contact lens users and immunocompromised individuals, further constrains the assessment of *Acanthamoeba*'s true community health impact.

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6.0 CONCLUSION

The findings of this scoping review reveal the widespread prevalence of *Acanthamoeba* in various water sources across Malaysia, highlighting significant public health concerns. The review highlights high contamination rates in recreational rivers, drinking water facilities, swimming pools, and natural water bodies such as hot springs and marine waters. Pathogenic genotypes like T4 dominate, posing serious risks to vulnerable populations, including contact lens users and immunocompromised individuals. The review also highlights the organism's resilience, particularly its ability to survive standard disinfection processes like chlorination, pointing to the inadequacy of existing water treatment protocols. Additionally, *Acanthamoeba*'s role as a reservoir or "Trojan horse" for ARBs further amplifies the risks of waterborne infections.

7.0 CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

8.0 AUTHORS CONTRIBUTION

Abdul Hamid, M. W. (Conceptualization; Literature review; Writing - original draft)
Abd Majid, R. ((Writing - critical revision of the article for important intellectual content; Supervision)
Knight Victor Ernest, V. F. (Resources; Supervision)
Mohamed Shakrin, N. N. S. (Resources; Supervision)
Mohamad Hamzah, F. (Resources; Supervision)
Che Roos, N. A. (Resources; Supervision)

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