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Co-infection of *Pseudomonas aeruginosa* and *Acanthamoeba* spp. Isolated from Dust in Eastern Thailand: Public Health Implications

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Abstract: This study investigates the prevalence of *Pseudomonas aeruginosa* and *Acanthamoeba* spp. co-infection in dust from public parks in Eastern Thailand, a region with substantial air pollution from industrial activities. 336 dust samples collected from seven provinces were analyzed using microscopy and PCR to detect *Acanthamoeba* spp., with an overall prevalence of 22.32%. Co-infection with *P. aeruginosa* was confirmed in 43 samples (57.33%). Prachinburi province had the highest co-infection rate (75%), suggesting a potential link between environmental factors and pathogen prevalence. Analysis revealed a significant association between temperature and co-infection rates ($p = 0.02$), supporting previous findings on the impact of warmer climates on pathogen survival. Humidity, $PM_{2.5}$, and PM_{10} levels did not significantly correlate with co-infection rates. These findings underscore the public health implications of dust as a reservoir for pathogenic microorganisms, particularly in areas with high pollution.

Keywords: *Pseudomonas aeruginosa*; *Acanthamoeba* spp.; Co-infection; Dust; Eastern Thailand

1. Introduction

Co-infection between bacteria and parasites is a significant public health concern, as it increases the severity of diseases and complicates treatment efforts. One medically significant bacterium is *Pseudomonas aeruginosa*, which can cause infections across multiple human systems, particularly in immunocompromised patients with cancer, diabetes, or those undergoing chemotherapy [1]. *P. aeruginosa* is a Gram-negative bacterium commonly found in the environment [2]. It is known for its high resistance to multiple antibiotics, which makes infection management more challenging [3]. This bacterium can lead to many respiratory, skin, and bloodstream infections [4]. Additionally, *P. aeruginosa* can form biofilms that enable it to more easily establish infections in various body areas, such as the respiratory tract, urinary tract, and chronic wounds [5]. Its capacity for multidrug resistance further complicates treatment, making infections more difficult to manage and posing higher risks to affected individuals [6].

On the other hand, *Acanthamoeba* spp. are free-living amoebae found in various environments, including soil, freshwater, seawater, and dust [7]. These amoebae can cause severe diseases, such as *Acanthamoeba* keratitis, an eye infection that can lead to vision loss if not promptly treated [8]. Additionally, they can cause Granulomatous Amoebic Encephalitis (GAE), a severe central nervous system infection with a high mortality rate [9]. Although infections caused by *Acanthamoeba* spp. are relatively rare, they are challenging to treat due to the amoebae's ability to thrive in various environmental conditions, making them pathogens of particular concern in medical and public health research [10]. Given these factors, *Acanthamoeba* spp. should receive considerable attention in medical and public health research due to its potential to cause significant infections.

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Airborne particulate matter, particularly $PM_{2.5}$, has become a significant issue of widespread concern in Thailand. These particles can easily penetrate the human respiratory system, leading to respiratory diseases, chronic lung conditions, and cardiovascular diseases [11]. Moreover, studies have shown that particulate matter can act as a reservoir for pathogenic microorganisms, making microbial contamination in dust a considerable public health concern, especially in industrial zones and areas with heavy traffic. The eastern region of Thailand, characterized by rapid industrial development, has seen an increase in dust contamination with pathogens that can quickly spread to the general population, particularly vulnerable groups such as children, the elderly, and individuals with chronic diseases. Dust particles can carry pathogens like *P. aeruginosa* and *Acanthamoeba* spp., leading to respiratory infections and other health complications. This presents a substantial public health risk that warrants urgent attention.

The co-infection of *P. aeruginosa* and *Acanthamoeba* spp. in areas with high levels of delicate particulate matter ($PM_{2.5}$), such as in Eastern Thailand, highlights an essential epidemiological link between air pollution and the transmission of microorganisms capable of causing severe diseases. Delicate particulate matter can act as a carrier for microbial pathogens, particularly in regions experiencing rapid industrialization, transportation, construction, and economic activities that contribute to the continuous release of dust and air pollutants. These factors create an environment conducive to the accumulation and spread of pathogens in surrounding communities. The role of dust particles as carriers of *P. aeruginosa* and *Acanthamoeba* spp., both commonly found in dust and environmental reservoirs, underscores significant respiratory infection risks and other health concerns, especially for immunocompromised populations, such as those with chronic illnesses, children, and the elderly.

This study is crucial for raising awareness about the infection risks associated with environmental dust, particularly in Eastern Thailand, where pollution levels are high. The co-infection of *P. aeruginosa* and *Acanthamoeba* spp. from dust particles found in public parks may have significant health implications at both local and national levels. The findings of this study will provide critical information to public health officials for planning disease prevention and control strategies and developing hygiene measures to mitigate health risks in areas affected by dust pollution.

2. Materials and Methods

2.1. Study Design and Environmental Monitoring for Dust Sampling

This cross-sectional study was conducted between March - May 2023 in seven provinces located in eastern Thailand: Chonburi, Rayong, Chanthaburi, Trat, Chachoengsao, Prachinburi, and Sa Kaeo. The study sites comprised public parks within each province, selected for their accessibility and potential as sources of dust-borne pathogens. Environmental data recorded during the study period included an average temperature of 34°C, relative humidity of 63.57%, wind speed of 13.23 km/h, and average particulate matter concentrations of 35.09 $\mu g/m^3$ for $PM_{2.5}$and 59.28 $\mu g/m^3$ for PM_{10}. All environmental data were collected using standardized meteorological equipment during sampling to ensure consistency. The selection of these parameters was based on their relevance to dust-borne microbial survival and dispersal, which may affect pathogen detection [12, 13].

A total of 336 dust samples were collected across three public parks in each province, with 48 samples taken from each park. Dust samples were collected from various surfaces (e.g., benches, pathways, playground equipment) to ensure a diverse and representative sample from each location [14]. Samples were collected using sterile brushes and immediately transferred into clean plastic bags to prevent contamination [15]. Each sample weighed approximately 0.1 g of dust, carefully collected with sterile tools, and stored in sterile containers to avoid contamination from external sources. The sampling sites were selected to avoid direct sunlight or excessive moisture, which could interfere with the viability of microorganisms in the dust. Once collected, each dust sample was carefully sealed, labeled, and stored under controlled conditions to prevent degradation or contamination. The samples were transported under controlled conditions to the Faculty of Physical Therapy, Srinakharinwirot University, where they were processed for microbial analysis.

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2.2. Cultivation of Acanthamoeba spp.

The Dust samples were prepared and inoculated onto 1.5% non-nutrient agar (NNA) plates pre-covered with heat-inactivated *Escherichia coli* (ATCC 25922) [7]. The *E. coli* cultures were heat-inactivated at 60 °C for 1 hour before being applied to the agar. A 0.1 mL aliquot of the *E. coli* suspension was inoculated onto each NNA plate. The plates were then incubated at 25 °C to grow Acanthamoeba trophozoites [16]. Observations for the presence of trophozoites were carried out every 48 hours for up to one week using an inverted microscope (Olympus IX71, Tokyo, Japan) [8]. Once trophozoites were identified, the samples were considered positive for Acanthamoeba spp. Positive samples were subcultured by transferring a portion of the original sample onto fresh NNA plates, following the same inoculation and incubation procedures [9]. The amoeba genera were identified based on the morphological characteristics of cysts and trophozoites, noting key factors such as the type of movement and specific morphological features [17]. Each isolate was classified into its corresponding morphological group according to established taxonomic criteria [18].

2.3. Detection of Acanthamoeba spp. Using PCR

DNA extraction from Acanthamoeba-positive samples, confirmed via microscopy, was carried out using the Qiagen Amp DNA Micro Kit (Qiagen, Germany), following the manufacturer's protocol. The process began by placing each sample into Eppendorf tubes, where 200 µL of lysis buffer was added, followed by 20 µL of Proteinase K. The samples were incubated in a dry block at 56°C for 10 minutes. After incubation, 200 µL of absolute ethanol was added to each tube, and the mixture was centrifuged at 8,000 x g for 1 minute and 30 seconds. The supernatant was discarded, and 500 µL of RW1 buffer was introduced into each sample. After centrifugation, the supernatant was discarded again, and 500 µL of RW2 buffer was added. The final centrifugation was carried out, after which the supernatant was discarded, and 50 µL of elution buffer was added. DNA was then eluted by centrifugation and stored at -20°C.

The concentration and purity of the extracted DNA were measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). Specific primers targeting the 18S ribosomal RNA gene of *Acanthamoeba* spp. were used for PCR amplification. The forward primer, JDP1 (5'-GGCCC ATC GTTTA CCGT GAA-3'), and reverse primer, JDP2 (5'-TCTC ACAA GCTGCTAGGGGAGTCA-3'), amplified a 490 bp fragment specific to *Acanthamoeba* spp. [19].

PCR reactions were performed in a total volume of 20 µL, which included Taq DNA polymerase, 10 ng of template DNA, 10 pmol of each primer, and distilled water. Amplifications were conducted using a BIO-RAD PCR thermocycler under the following conditions: initial denaturation at 96°C for 2 minutes, followed by 35 cycles of denaturation at 96°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute. A final extension was performed at 72°C for 5 minutes [19].

The PCR products were separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide (0.5 ng/mL), and visualized under ultraviolet light to detect specific bands confirming the presence of *Acanthamoeba* spp.

2.4. Detection of Co-Infection with Pseudomonas aeruginosa

Samples that tested positive for *Acanthamoeba* spp. PCR analyses were subsequently prepared for further detection of co-infection. These samples were also examined for the presence of *P. aeruginosa* to assess the potential for co-infection between the two pathogens. The presence of *P. aeruginosa* was confirmed through a PCR assay using specific primers designed to target a unique region of the bacterium. The forward primer PA-SSF (5'-GGG GGA TCT TCG GAC CTC A-3') and the reverse primer PA-SSR (5'-TCC TTA GAG TGC CCA CCC G-3') were employed to amplify a 956 bp fragment specific to *P. aeruginosa* [20].

PCR amplification was performed in a 20 µL reaction mixture containing Taq DNA polymerase, 10 ng of DNA template, 10 pmol of each primer, and distilled water. The amplification followed a specific program using a PCR thermocycler, starting with an initial denaturation step at 95°C for 2

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minutes. This was followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes [20].

Following PCR amplification, the products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide (0.5 ng/mL). Visualization under ultraviolet light allowed for the identification of specific bands corresponding to *Acanthamoeba* spp. and *P. aeruginosa*. The detection of both bands in the same sample confirmed the co-infection of *Acanthamoeba* spp. and *P. aeruginosa*, indicating the presence of both pathogens simultaneously in the analyzed samples.

2.5. Statistical Analysis

The prevalence of Acanthamoeba spp. and co-infection with *P. aeruginosa* was calculated as proportions and expressed as percentages of the total number of dust samples analyzed. Descriptive statistics, including means and standard deviations, summarized environmental data (temperature, relative humidity, wind speed, and particulate matter concentrations). These environmental factors were correlated with *Acanthamoeba* spp. and co-infection rates using Pearson's correlation coefficient. The dependent variables included the presence of *Acanthamoeba* spp. and co-infection status, while the independent variables comprised environmental factors such as temperature, humidity, $PM_{2.5}$, and PM_{10} levels. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the strength of these associations. The chi-square (χ^2) test evaluated the relationship between the detection of *Acanthamoeba* spp. and *P. aeruginosa* and categorical variables, including dust sample location (public parks) and surface type (benches, pathways, playground equipment). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Prevalence of Acanthamoeba spp. in Different Provinces of Eastern Thailand

This study examined various environmental factors influencing the presence and distribution of pathogens in dust across seven provinces in Eastern Thailand. The temperature ranged from 29 to 37°C, averaging 34°C, while humidity levels varied from 29% to 100%, averaging 63.57%. Wind speeds averaged 13.23 km/h. Additionally, the average levels of $PM_{2.5}$ and PM_{10} were notable, at 35.09 μg/m³ and 39.29 μg/m³, respectively. Particulate matter can serve as a vehicle for microbial pathogens, with $PM_{2.5}$ posing a particular respiratory risk due to its ability to penetrate deeper into the lungs in Table S1.

From a total of 336 dust samples collected from public parks in each province, the study found an overall prevalence of *Acanthamoeba* spp. at 22.32%. PCR tests confirmed the pathogen in 43 samples (57.33%). This high confirmation rate through PCR demonstrates the method's effectiveness for detecting pathogens in environmental samples. Among the provinces, Chanthaburi reported the highest prevalence of *Acanthamoeba* spp. at 25%, followed by Trat and Chonburi at 16.67% each. Lower prevalence rates were found in Chachoengsao, Prachin Buri, and Sa Kaeo, with Rayong exhibiting the lowest prevalence at 2.08% in Figure S1.

The study highlights how industrial development and urbanization in certain provinces may contribute to a higher presence of pathogens in dust, posing a greater public health risk. These findings underscore the need for targeted public health strategies, particularly in regions with significant industrial activity, to mitigate the spread of pathogens through dust. This information can guide future public health policies and interventions to reduce exposure to potentially harmful dust-borne pathogens.

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3.2. The co-infection rate of Acanthamoeba spp. and P. aeruginosa in each province of eastern Thailand was assessed using polymerase chain reaction (PCR)

A study analyzing the co-infection of *Acanthamoeba* spp. and *P. aeruginosa* in dust samples from various provinces in eastern Thailand utilized polymerase chain reaction (PCR) to assess infection rates. The results showed significant geographical variation in co-infection rates across the regions. Prachin Buri had the highest co-infection rate at 75%, followed by Sa Kaeo at 66.67%. Chonburi and Trat recorded co-infection rates of 62.50% in Figure S2. In contrast, Rayong reported no co-infection (0.00%), highlighting notable differences in pathogen detection among the provinces. This study underscores the distribution of co-infection between *Acanthamoeba* spp. and *P. aeruginosa* in dust samples from public spaces across eastern Thailand. These findings are essential for environmental surveillance and provide critical insights for public health planning to prevent the spread of these pathogens in high-risk areas. By understanding the level of contamination in each province, proactive prevention strategies can be developed to address specific environmental conditions.

3.3. The relationship between environmental factors and co-infection between Acanthamoeba spp. and P. aeruginosa

The Table S2. displays the chi-squared test results, which examined the relationship between environmental factors and the co-infection rate of *P. aeruginosa* and *Acanthamoeba* spp. Among the factors analyzed, only temperature demonstrated a statistically significant association with co-infection rates ($\chi^2 = 7.79$, $p = 0.02$). This finding suggests that higher temperatures may lead to an increased prevalence of co-infection. In contrast, humidity, $PM_{2.5}$, and PM_{10} levels did not show statistically significant associations with co-infection rates, implying that these factors may not significantly influence the co-occurrence of these microorganisms in the environment.

3.4. Environmental factors and co-infection rates of Acanthamoeba spp. and P. aeruginosa in the provinces of eastern Thailand

According to the graph depicting co-infection rates between *Acanthamoeba* spp. and *P. aeruginosa* across various provinces and temperature ranges, Chonburi, Chanthaburi, and Chachoengsao experienced the highest temperature range (35-37°C). Chonburi and Chanthaburi reported the highest co-infection rates among these provinces at 23.81%. In contrast, in Trat, co-infection rates were distributed across different temperature ranges, with 4.76% found in the 29-31°C range and 19.05% in the 32-34°C range. This suggests that temperature may influence the co-infection rate.

When analyzing relative humidity, Prachin Buri and Chachoengsao exhibited the highest levels (86-100%), with co-infection rates of 14.29% and 4.76%, respectively. In contrast, Chonburi and Trat had the same co-infection rate of 23.81% within a relative humidity range of 50-70%. Additionally, Chonburi displayed the highest co-infection rate of 23.81% at a lower relative humidity range of 25-40%.

Regarding wind speed, the highest wind speed range (17.8-20.0 km/h) was observed in Sa Kaeo, where a co-infection rate of 9.52% was recorded. In Trat, the co-infection rate was distributed across different wind speeds, with a rate of 19.05% in the 9.0-11.2 km/h range and the highest rate of 4.76% in the 11.2-13.4 km/h range.

When examining co-infection rates between *Acanthamoeba* spp. and *P. aeruginosa* based on $PM_{2.5}$ levels (μg/m³), Chanthaburi, Chonburi, and Trat had the highest rates at 23.81%, corresponding to $PM_{2.5}$ ranges of 20-28 μg/m³ and 44 μg/m³, respectively. In contrast, Chachoengsao exhibited a lower co-infection rate of 4.76% despite having the highest PM_{10} concentration in the 78-93 μg/m³ range. It was also noted that Rayong, Chanthaburi, and Chonburi had the highest co-infection rates (23.81%) within the PM_{10} range of 30-35 μg/m³ and 46-61 μg/m³, respectively.

This study analyzed the environmental factors and co-infection rates between *Acanthamoeba* spp. and *P. aeruginosa* in various provinces of eastern Thailand. No co-infection was detected in Rayong province in Figure S3.

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4. Discussion

This study detected *Acanthamoeba* spp. and co-infection with *P. aeruginosa* in dust samples collected from public parks across provinces in eastern Thailand, indicating a potential public health risk. Notably, high rates of co-infection were observed in provinces like Prachinburi (75%), Sa Kaeo (66.67%), Chonburi, and Trat (62.50%). This finding is significant, as *Acanthamoeba* spp. is an opportunistic pathogen that can lead to severe infections, particularly in immunocompromised individuals, such as meningitis and keratitis [8, 9]. Meanwhile, *P. aeruginosa* is a known pathogen responsible for infections in the respiratory and urinary tracts, as well as wound infections [21]. Co-infection with both pathogens could complicate treatment approaches and increase disease severity, underscoring the need for effective monitoring and control of *Acanthamoeba* spp. and *P. aeruginosa* in the environment to minimize public health risks.

The results align with previous research that has identified *Acanthamoeba* spp. in various environmental samples, such as dust, soil, and water, especially in tropical and humid regions, which provide optimal conditions for its growth [7]. However, there is limited data on co-infection rates with *P. aeruginosa* in dust from Thailand, suggesting that unique environmental factors, such as temperature, humidity, and dust levels, may influence the persistence and proliferation of both pathogens in this setting.

Acanthamoeba spp. can act as both a host and a nutritional source for *P. aeruginosa*, facilitating bacterial growth within *Acanthamoeb*a spp. [22, 23]. This relationship may protect *P. aeruginosa* from adverse environmental conditions, such as nutrient scarcity and disinfectants. Furthermore, this interaction may enhance *P. aeruginosa*'s antibiotic resistance and ability to evade the human immune system [24]. Environmental factors, like elevated temperatures, could significantly promote the growth and spread of both organisms in the dust, consistent with our findings of higher co-infection rates in warmer provinces. Additionally, *Acanthamoeba* spp. may intensify the pathogenicity of *P. aeruginosa* by promoting biofilm formation and toxin production [10, 17].

A significant positive correlation was found between the co-infection rates of *Acanthamoeba* spp. and *P. aeruginosa* and average provincial temperatures, supporting the hypothesis that higher temperatures constitute a risk factor for co-infection. However, future studies should consider additional environmental factors such as humidity, dust concentration, and air pollution to understand better the variables influencing co-infection rates. The presence of *Acanthamoeba* spp. and *P. aeruginosa* in dust from public parks accessible to the general population indicates potential community infection risks, especially for immunocompromised individuals, such as those with diabetes, HIV, and the elderly. Both pathogens can cause severe infections, including respiratory illnesses, meningitis, and keratitis, which may result in severe complications and even death [4, 17].

This study has some limitations. Dust samples were collected from only one or two parks per province, which may not represent the entire provincial environment. Furthermore, the study did not examine species-specific strains of *Acanthamoeba* spp. and *P. aeruginosa*, which may vary in pathogenicity and drug response. Future studies should expand the scope of sample collection to include residential areas, schools, and hospitals to obtain more comprehensive data. Additionally, investigating other factors, such as air pollution and humidity, could provide a more thorough understanding of the environmental dynamics affecting the spread of *Acanthamoeba* spp. and *P. aeruginosa* [12].

5. Conclusions

This study examines the high prevalence and co-infection rates of *Acanthamoeba* spp. and *P. aeruginosa* in dust samples collected from public parks in Eastern Thailand. The results highlight the role of environmental dust as a reservoir for these pathogens, posing a significant public health risk, especially in industrial and urban areas with higher pollution levels. The overall prevalence of *Acanthamoeba* spp. This aligns with findings from similar studies, while co-infection rates with *P. aeruginosa* varied by province, peaking in Prachinburi. This variation suggests that environmental factors may influence the transmission and survival of these pathogens.

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Temperature emerged as a statistically significant factor linked to co-infection rates, emphasizing the importance of environmental conditions in the survival and spread of these microorganisms. This finding aligns with previous research indicating that warmer climates may promote the growth and coexistence of these pathogens. In contrast, other environmental factors, such as humidity and particulate matter ($PM_{2.5}$ and PM_{10}), did not show significant correlations with co-infection rates, indicating that temperature may be a more critical determinant of pathogen viability.

These findings have considerable implications for public health, particularly in communities with immunocompromised individuals who may be more vulnerable to infections caused by these pathogens. Targeted public health interventions focusing on environmental monitoring, pollution control, and awareness of airborne pathogens could help mitigate health risks associated with dust-borne infections in high-risk areas.

Future research should broaden its sampling scope to include various urban and rural environments to understand better the environmental factors affecting co-infection rates. Additionally, incorporating molecular typing of pathogen strains could yield further insights into the specific strains in these environments, providing valuable information for developing targeted prevention strategies.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Prevalence of *Acanthamoeba* spp. positive in different provinces of eastern Thailand; Figure S2: Co-infection rates of *Acanthamoeba* spp. and *P. aeruginosa* in public parks across different provinces in eastern Thailand.; Figure S3: Environmental factors and co-infection rate of *Acanthamoeba* spp. and *P. aeruginosa* in eastern Thailand.; Table S1: Summary of environmental conditions measured in eastern Thailand during the study period.; Table S2: Statistical analysis of environmental factors associated with co-infection rates.

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References

1. Bassetti, M., Vena, A., Croxatto, A., Righi, E., & Guery, B. How to manage *Pseudomonas aeruginosa* infections **2018**. *Drugs in Context*, 7, 212527.
2. Silby, M. W., Winstanley, C., Godfrey, S. A. C., Levy, S. B., & Jackson, R. W. *Pseudomonas genomes*: Diverse and adaptable **2011**. *FEMS Microbiology Reviews*, 35(4), 652-680.
3. Poole, K. *Pseudomonas aeruginosa*: Resistance to the max **2011**. *Frontiers in Microbiology*, 2, 65.
4. Lyczak, J. B., Cannon, C. L., & Pier, G. B. Lung infections associated with cystic fibrosis **2002**. *Clinical Microbiology Reviews*, 15(2), 194-222.
5. Costerton, J. W., Stewart, P. S., & Greenberg, E. P.. Bacterial biofilms: A common cause of persistent infections **1999**. *Science*, 284(5418), 1318-1322.
6. Breidenstein, E. B. M., de la Fuente-Núñez, C., & Hancock, R. E. W. *Pseudomonas aeruginosa*: All roads lead to resistance **2011**. *Trends in Microbiology*, 19(8), 419-426.
7. Khan, N. A. *Acanthamoeba*: Biology and increasing importance in human health **2006**. *FEMS Microbiology Reviews*, 30(4), 564-595.
8. Lorenzo-Morales, J., Khan, N. A., & Walochnik, J. An update on *Acanthamoeba* keratitis: Diagnosis, pathogenesis and treatment **2015**. *Parasite*, 22, 10.

 doi:10.20944/preprints202411.0071.v1

9. Visvesvara, G. S., Moura, H., & Schuster, F. L. Pathogenic and opportunistic free-living amoebae: A*canthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea* **2007**. *FEMS Immunology & Medical Microbiology*, 50(1), 1-26.
10. Siddiqui, R., & Khan, N. A. Biology and pathogenesis of *Acanthamoeba* **2012**. *Parasites & Vectors*, 5(1), 6. (Scopus)
11. Kim, K. H., Kabir, E., & Kabir, S. A review on the human health impact of airborne particulate matter **2015**. *Environment International*, 74, 136-143.
12. Tong, H., & Lighthart, B. The annual bacterial particle concentration and size distribution in the ambient atmosphere in a rural area of the Willamette Valley, Oregon **2000**. *Aerobiologia*, *16*(3), 393-401.
13. Buttner, M. P., & Stetzenbach, L. D. Monitoring airborne fungal spores in an experimental indoor environment to evaluate sampling methods and the effects of human activity on air sampling **2006**. *Applied and Environmental Microbiology*, *72*(12), 7884-7892.
14. Chen, C., & Zhao, B. Review of relationship between indoor and outdoor particles: I/O ratio, infiltration factor and penetration factor **2011**. *Atmospheric Environment*, *45*(2), 275-288.
15. Lee, T., Grinshpun, S. A., Martuzevicius, D., Adhikari, A., Shukla, R., & Reponen, T. Culturability and concentration of airborne fungi in six diverse indoor environments **2006**. *Environmental Health Perspectives*, *114*(5), 750-757.
16. Schuster, F. L., & Visvesvara, G. S. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals **2004**. *International Journal for Parasitology*, *34*(9), 1001-1027.
17. Marciano-Cabral, F., & Cabral, G. *Acanthamoeba* spp. as agents of disease in humans **2003**. *Clinical Microbiology Reviews*, *16*(2), 273-307.
18. Pussard, M., & Pons, R. Morphologie de la paroi kystique et taxonomie du genre *Acanthamoeba* (Protozoa, Amoebida) 1**977**. *Protistologica*, *13*(4), 557-598.
19. Schroeder, J. M., Booton, G. C., Hay, J., Niszl, I. A., Seal, D. V., Markus, M. B., ... & Fuerst, P. A. Use of subgenic 18S ribosomal DNA PCR and sequencing for genus and genotype identification of *Acanthamoeba* from humans with keratitis and from sewage sludge **2001**. *Journal of Clinical Microbiology*, *39*(5), 1903-1911.
20. Spilker, T., Coenye, T., Vandamme, P., & LiPuma, J. J. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomona*s species recovered from cystic fibrosis patients **2004**. *Journal of Clinical Microbiology*, 42(5), 2074-2079.
21. Poole, K. *Pseudomonas aeruginosa*: Resistance to the max **2011**. *Frontiers in Microbiology*, *2*, 65.
22. Greub, G., & Raoult, D. Microorganisms resistant to free-living amoebae **2004**. *Clinical Microbiology Reviews*, *17*(2), 413-433. Wang, X., & Ahearn, D. G. Effect of bacteria on survival of *Acanthamoeba castellanii* in saline solution **1997**. *Current Microbiology*, *34*(4), 212-215.
23. Matz, C., Bergfeld, T., Rice, E. W., & Kjelleberg, S. Microcolonies, quorum sensing and cytotoxicity determine the survival of *Pseudomonas aeruginosa* biofilms exposed to protozoan grazing **2004**. *Environmental Microbiology*, *6*(11), 1233-1247.