Emerging patents versus brain eating amoebae, *Naegleria fowleri*

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Highlights Box

- *Naegleria fowleri* infection exhibits >90% mortality, despite advances in drug discovery
- Recent patents along with research and innovations are reviewed and categorized into therapeutic agents, water treatment technologies, and diagnostic methods
- Water treatment strategies are explored in the rationale development of preventative strategies
- Current challenges and opportunities for further research and development, emphasizing the need for targeted strategies are discussed

Abstract

Primary Amoebic Meningoencephalitis (PAM) is a severe and often fatal infection caused by the free-living amoeba *Naegleria fowleri*. This condition typically results from exposure to contaminated warm freshwater/inadequately treated recreational water/or ablution/nasal irrigation with contaminated water. The management of PAM is hindered by the absence of effective treatment coupled with challenges in early diagnosis. This review explores emerging patents that could be utilized for the treatment, diagnosis of PAM, as well as water treatment. Recent patents from the past five years, along with research and innovations are reviewed and categorized into therapeutic agents, water treatment technologies, and diagnostic methods. It is hoped that collaboration and awareness between pharmaceutical companies, water industries, and academic institutions is essential for advancing effective strategies against this severe central nervous system pathogen.

Key words: *Naegleria fowleri*; Brain eating amoebae; Patents; Primary Amebic Meningoencephalitis; Central nervous system infections.

1. Introduction

Naegleria fowleri are free-living pathogenic amoebae with a global distribution [1-3]. These microorganisms also known as “brain eating amoebae” are implicated in the rare but typically fatal condition known as primary amoebic meningoencephalitis (PAM), a severe infection of the central nervous system (CNS) [4-5]. It's important to note that the true extent of the disease and the impact of infection caused by *Naegleria* is likely underestimated, and this is of great concern given the high mortality rate of more than 95% [5-8]. *Naegleria fowleri* in its trophozoite form, infects humans by entering through the nasal cavity during activities involving water, such as swimming or nasal cleansing and/or ablution with contaminated water [1]. Once inside, it adheres to the nasal mucosa, penetrates the olfactory neuroepithelium, travels along the olfactory nerve, and reaches the olfactory bulb [1]. Upon reaching the brain, there is inflammation, which damages nerves and the central nervous system, ultimately leading to death [1]. The progression of symptoms involves severe frontal headache, nausea, vomiting, and fever, leading to stiff neck, altered mental status, seizures, cerebral edema, and cerebellar herniation, eventually leading to coma and death within about a week [1,4].

Currently, no effective treatments exist for PAM caused by *Naegleria fowleri* [4]. The Centers for Disease Control and Prevention (CDC) recommends a combination therapy that includes amphotericin B, fluconazole, rifampin, miltefosine, azithromycin, and dexamethasone [9]. However, amphotericin B's use is constrained by its significant toxicity, particularly nephrotoxicity [10]. These drugs, administered intravenously, can cause systemic side effects and face challenges in crossing the blood-brain barrier, leading to inadequate concentrations in the central nervous system to effectively target *N. fowleri* [9-10].

Progress in developing novel treatments against *Naegleria* has been hindered by the absence of clinical trials, limited patents and inventions, and a general lack of interest from the pharmaceutical industry, primarily because PAM is considered a rare disease [4]. However, recent data reveal an increasing number of PAM cases since 2000, which is concerning [5]. Compounding this issue is the global shortage of water supplies, particularly in developing countries, leading to the widespread use of water storage tanks. These tanks frequently harbour microorganisms, including free-living amoebae, heightening the need for more effective and less toxic therapies to combat this devastating infection [8-10]. Because of the rarity and fatality of the disease, conducting phase II clinical trials for safe and effective treatment candidates is not feasible. As a result, determining the efficacy of one clinically safe compound over another is dependent on *in vitro* assays and animal testing [4]. In this review, we analyze recent advancements by exploring bibliographic databases such as PubMed, Google Scholar, and Google Patents. We focus on patents and inventions developed over the past five years that hold potential in combating *Naegleria fowleri*, the causative agent of Primary Amebic Meningoencephalitis (PAM). These innovations are categorized into three key areas: therapeutic agents and devices, water treatment technologies, and diagnostic methods. The review also identifies current challenges and uncovers opportunities for further research and development, emphasizing the need for targeted strategies to address the growing threat posed by this lethal pathogen.

2. Therapeutic agents and devices

Most research directed toward developing therapeutic strategies against *Naegleria* has largely been performed through *in vitro* studies [4]. Many of these studies have utilized existing compounds and repurposed them or utilized existing or novel drugs modified with

nanotechnology [11-15], however very few of these have studies have led to patents or inventions or translation to the clinic. A recent patent was filed consisting of a mesenchymal stem cell preparation combined with one or more biomolecules [16]. The compositions in the patent are intended for the treatment of a variety of microbial infections, as well as the symptoms or secondary pathological conditions related to such infections. These include viral, bacterial, fungal, or parasitic infections, including *Naegleria fowleri* [16]. Currently this application has been filed and is pending approval and could be applied for treatment of PAM caused by *Naegleria fowleri*. Another recent patent is of interest, which outlines methods and compositions for modulating the response of microorganisms to antimicrobial agents [17]. Specifically, one embodiment in the invention involves treating the microorganism with both an antimicrobial agent and bicarbonate. Another embodiment of the invention provides methods for treating microbial infections by administering an effective dose of (i) bicarbonate and (ii) an antimicrobial agent to a subject in need. Additionally, the patent includes methodologies for screening and identifying antimicrobial compounds, and could be applied for the treatment of PAM, however both these inventions should be verified *in vitro/in vivo* studies specifically exploring the activities against *Naegleria fowleri* [17].

Another invention pertaining to therapeutic agents comprising 1,2,4-oxadiazole and 1,2,4-thiadiazole compounds, that are designed to inhibit the programmed cell death 1 (PD-1) signaling pathway, may be applied against *Naegleria fowleri* [18]. This invention also includes derivatives of these therapeutic agents. Furthermore, it covers the application of these agents and their derivatives in the treatment of disorders through immunopotentiation, specifically by inhibiting immunosuppressive signals associated with programmed cell death. The invention also encompasses therapeutic strategies utilizing these agents. The inventors claim that this could be

utilized against *Naegleria fowleri* [18]. Given that previous studies *in vitro* show activities of azole compounds against these free-living amoebae, this invention is promising [19-22].

Another patent of interest, which seems particularly promising has been filed recently [23] and focuses on using ultrasound to enhance the delivery of antimicrobial agents directly to infection sites, with particular emphasis on treating infections, with the inventors referring to *Naegleria fowleri*, brain-eating amoebae in particular [23]. The invention offers both a cluster composition and a pharmaceutical formulation designed to facilitate the targeted delivery and administration of antimicrobial agents, aiming to improve infection management [23]. The inventors have found that Acoustic Cluster Therapy (ACT®) can specifically target infection sites and improve the uptake of antimicrobial agents. This method enhances the treatment's effectiveness and reduces toxicity by increasing the localized concentration of the agents [23]. This approach could be particularly relevant against *Naegleria fowleri,* given the high toxicity of current treatments available against PAM [10].

Another interesting invention describes a chimeric antigen receptor (CAR) designed for targeting a variety of microbial pathogens and could be utilized against *Naegleria fowleri* [24]. This CAR consists of: (a) an extracellular domain with one or more antigen-binding regions specific to pathogen-related antigens; (b) a transmembrane domain; and (c) one or more intracellular signaling domains from an 'eat-me' signal receptor, enabling the receptor to specifically bind to pathogen antigens and trigger an endogenous, silent phagocytic clearance signal. Additionally, the invention includes a polynucleotide encoding the receptor, a gene vector, a recombinant cell expressing the CAR, and a composition. Notably, the CAR directs the recombinant cell to clear pathogens via phagocytosis without significant cytokine release,

resulting in a 'silent' pathogen clearance mechanism [24]. However, how this particular invention can be used against PAM is not detailed and needs to be investigated further.

Although several novel patents have been filed in the last five years, of which the pertinent ones are indicated herein, most of these inventions, if not all; have not directly been tested against *Naegleria fowleri* and it remains to be elucidated whether these are effective for the treatment of PAM. To overcome the challenge of drug transport to the brain, particularly for treating PAM caused by *Naegleria fowleri*, an intranasal drug delivery route using nasal inhalers has been proposed by our group [25]. This approach takes advantage of the glymphatic system associated with the trigeminal and olfactory pathways, allowing for efficient and noninvasive delivery of therapeutics directly to the CNS, bypassing the blood-brain barrier. Intranasal administration reduces the risk of systemic side effects, such as hepatotoxicity and nephrotoxicity, that are common with higher drug concentrations. Additionally, this method may improve drug efficacy and reduce mortality by ensuring targeted delivery to the brain with minimal damage to other tissues [25]. Although this approach has been shared with the scientific community and not been patented, such an invention should be evaluated and developed as a much-needed treatment against PAM.

Another pertinent invention relates to the use of miltefosine, alone or in combination with other agents, to treat infections caused by free-living amoebae, including *Naegleria fowleri*, *Balamuthia mandrillaris*, *Sappinia diploidea*, and *Acanthamoeba* species, targeting both trophozoite and cyst forms. The inventors state that miltefosine can be administered systemically (e.g., orally or intravenously) or locally (e.g., topically). Treatment may last from one month to a year, with dosing adjustments over time. The method may include a second agent, such as an antifungal or antibiotic, to enhance treatment efficacy [26].

3. Water treatment technologies

Despite growing awareness, it is concerning that current global water quality monitoring programs do not account for brain-eating amoebae in public water supplies [27]. Although monitoring all waterborne pathogens may not be feasible, the unique characteristics of *Naegleria fowleri,* including its free-living nature, severe health risks, potential association with nasal cleansing practices, and its role as a host for other pathogenic microorganisms, warrant its inclusion in water quality surveillance protocols [27]. A method/invention for regenerating ion exchange materials used in water softening or conditioning systems involves treating the ion exchange material with an aqueous process fluid to restore its function. This process effectively removes at least one type of target material from the resin, which may include metal ions (typically extracted from hard water sources), ionically soluble organic compounds, or active waterborne pathogens [28]. The inventors claim that method could be utilized against protozoa including one or more of the following: *Naegleria fowleri, Acanthamoeba polyphaga, Acanthamoeba castellanii, Entamoeba histolytica, Cryptosporidium parvum, Cyclospora cayetanensis, Giardia lamblia, Microsporidia,* and *Encephalitozoon intestinalis* [28]. However, the status of this invention is now stated as abandoned.

Conventional pool disinfection methods are not always effective against certain microorganisms, such as *Naegleria fowleri* and *Acanthamoeba,* which can cause severe. Studies have shown that these amoebas can survive in chlorinated pools, even those maintained according to standard safety regulations. For example, a study in Chile found that several public swimming pools contained *Naegleria fowleri* and *Acanthamoeba* [29]**.** Another recent patent of interest details a method for treating large water bodies to make them suitable for recreational use [30]. The invention details the method which states the water body is divided into a

sedimentation zone and a dissipation zone. In the sedimentation zone, a disinfection method based on the concentration-time index (CT index) and a flocculant composition are used to help settle microorganisms and contaminants, with minimal disturbance to the water to aid this process. In the dissipation zone, a chlorine disinfectant is added to maintain at least 0.5 mg/L of free chlorine, ensuring a lasting chlorine presence. Water is introduced through inlet nozzles, creating a dissipation pattern that, along with natural currents, promotes movement from the dissipation zone to the sedimentation zone [30]. This invention is very timely and topical, given the free-living nature of *Naegleria fowleri* and its presence worldwide, and in abundance in swimming pools [31].

Another recent noteworthy patent describes systems and methods for eliminating and transforming pollutants in water [32]. The system features electrodes, with at least one electrode incorporating a catalyst material. The invention can operate in two modes: first, an electrodialysis mode where pollutants are separated from the incoming water stream, and second, an electrolysis mode where the separated pollutants are converted into harmless substances using the catalyst material on the electrode. Thus, both electrodialysis and electrolysis are performed within the same system [32].

We have recently proposed the use of novel adsorbents, particularly micelle clay complexes that combine montmorillonite clay with activated carbon, as an effective method for removing neuropathogenic microbes like *Naegleria fowleri* from water sources used in ablution and nasal irrigation [33]. These adsorbents can be seamlessly integrated into household water collection devices, such as taps and water bottles, providing an affordable and easy-to-install disinfection solution [33]. These innovative methods are especially promising for communities in developing regions where access to safe water is limited, reliance on water storage tanks is

common, and sanitation facilities are often inadequate, however, further research is required to translate this invention for communal use [33]. Other pertinent recent from our group research has highlighted the potential of deep eutectic solvents; these are binary or ternary mixtures of compounds that form through predominantly hydrogen-bonding interactions, leading to a significantly lower melting point compared to the individual substances and are highly effective antimicrobial agents [34]. These solvents have demonstrated strong antimicrobial activity against a diverse range of pathogens, including multidrug-resistant bacteria, fungi, amoebae, and some viruses [34]. Additionally, deep eutectic solvents offer the advantages of being both environmentally friendly and cost-effective. They present a promising option for targeting waterborne pathogens like *Naegleria fowleri* in storage tanks. However, further studies are essential to assess their potential toxicity and safety comprehensively [34].

4. Potential Diagnostic methods

Diagnosis involves obtaining cerebrospinal fluid through a lumbar puncture, which typically shows an increased number of white blood cells and the presence of *Naegleria fowleri* trophozoites [35-36]. These can be identified using Giemsa-Wright or trichrome stains. The amoebae can be cultured on non-nutrient agar with live bacteria, ideally in Nelson's growth medium. Trophozoites usually develop within three days and encyst after seven to ten days. The conversion of trophozoites into flagellates can help confirm the diagnosis. Additional tests, such as microscopy, enzyme-linked immunosorbent assay, and reverse transcription polymerase chain reaction, further aid in identifying the amoebae [35-36]. Increased pressure in the cerebrospinal fluid, along with a rise in red and white blood cell counts, indicates an advanced infection. Prompt medical treatment is critical, particularly if there has been recent exposure to water [35].

A recent patent disclosure presents a method and kit for biomarker detection using light scattering microscopy, which proves particularly effective for identifying low-abundance biomarkers within complex samples [37]. The inventors state that the approach is designed for use in point-of-care settings but may also be adapted for various other applications, including quality control, environmental monitoring, and bio-analytical techniques. Specifically, the method utilizes mass photometry to detect biomarkers. According to the patent documents, the technology can be applied to diagnose a wide range of infections, including bacterial pathogens such as *Bordetella*, *Chlamydia*, *Mycoplasma*, *Legionella*, as well as conditions like bacterial meningitis, pneumonia, bronchitis, and sepsis. It also addresses viral infections, including but not limited to *Coronavirus*, HIV, Hepatitis B (HBV), Hepatitis C (HCV), HSV, CMV, Rhinovirus, Influenza A and B, Parainfluenza, and RSV. Additionally, the method covers fungal infections like *Aspergillus*, *Candida*, *Penicillium*, *Histoplasma*, and various other fungi such as *C albicans*, *C glabrata*, and *Saccaromyces cerevisiae*. It also includes parasitic diseases like Malaria, Toxoplasmosis, Leishmaniasis, and African Trypanosomiasis, as well as amoebic infections, including *Naegleria fowleri* and *Entamoeba histolytica* [37].

A recent patent for an invention describes a method for analyzing metagenomic next-generation sequencing (mNGS) data [38]. This includes: 1) extracting nucleic acids from samples, creating a library, and sequencing; 2) processing the data; 3) calculating various metrics such as RPM (Micro), RPM (Micro)Ratio of Coverage, microbial abundance, and filtering out negative controls; 4) performing significance analysis to distinguish background microorganisms from actual pathogens; and 5) assessing pathogen confidence based on metrics like read numbers, species abundance, and coverage to identify potential pathogens. The inventors state that this mNGS method is particularly useful for identifying infectious disease pathogens. Unlike

traditional methods, which focus on culturing, morphological, biochemical, immunological, or PCR-based detection of specific pathogens, mNGS enables high-throughput sequencing of all nucleic acids in a sample [38]. It then compares the sequences to a database to identify pathogens without prior assumptions, offering broad coverage and the ability to detect unexpected pathogens. The application of mNGS has shown significant promise in diagnosing central nervous system infections. For instance, in 2018, mNGS was instrumental in diagnosing a rare case of PAM caused by *Naegleria fowleri* in Shenzhen [39]. This highlights the advantage of mNGS in identifying difficult-to-diagnose infections that present with symptoms similar to other conditions like autoimmune encephalitis.

5. Conclusion

Despite its rarity, PAM caused by *Naegleria fowleri* remains a severe and often fatal condition. This rarity has contributed to pharmaceutical companies' reluctance to invest in the development of specific treatments. However, with global warming potentially increasing the prevalence of this thermophilic pathogen, the urgency for effective treatments is growing. Currently, there are no specific drugs approved for PAM; treatment regimens typically involve a combination of antifungal, antibiotic, anti-cancer, and anti-inflammatory agents.

In conclusion, research is actively exploring the adaptation of existing drugs and their combination with nanoparticles, alongside testing new compounds that show promise against *N. fowleri*. Despite these efforts, there is a notable deficiency in patents specifically targeting PAM treatment. Nanoparticle-conjugated drugs and novel therapeutic candidates require further investigation through rigorous *in vivo* studies, transcriptome analyses, and clinical trials, particularly with intranasal delivery methods.

In addition to treatment, effective diagnostics and water treatment strategies are critical. Current diagnostic approaches for *N. fowleri* and other free-living amoebae are limited, which complicates early detection and intervention. Enhanced diagnostic tools are needed to identify infections promptly and accurately. Moreover, conventional water treatment methods have proven insufficient in eliminating *Naegleria fowleri* from recreational water bodies. As such, there is a pressing need for improved water treatment solutions that can more effectively address these pathogens and reduce the risk of infection.

6. Future perspectives

At present, there is a substantial gap in patent literature concerning targeted therapies for PAM and *Naegleria fowleri*. Most current inventions are directed towards broader pathogen categories or other infectious diseases, highlighting a critical need for more focused research and development. Addressing this gap will require intensified research efforts, enhanced diagnostic capabilities, and innovative water treatment technologies. Collaboration among pharmaceutical companies, water industry stakeholders, and academic institutions is essential to develop comprehensive strategies for combating this dangerous central nervous system pathogen and improving public health safety. It is important to mention that the use of patented materials in managing rare diseases like *Naegleria fowleri* may present unique opportunities and challenges. Patents incentivize research into novel therapeutic agents for conditions with limited commercial interest due to their rarity. This is particularly crucial for *Naegleria fowleri*, where the current treatment options are few and often ineffective. However, the high cost associated with patented innovations may pose accessibility challenges, especially in low-resource settings. To address this, mechanisms such as collaborative licensing agreements, public-private partnerships, and global health initiatives may be considered to balance innovation with equitable access. While

the rarity of the disease complicates commercial viability, fostering strategic collaborations can ensure that breakthroughs benefit those in need without exacerbating healthcare disparities. The use of patents in treatment must be carefully regulated to balance innovation with patient accessibility and safety, ensuring that any patented materials undergo the necessary approvals from regulatory bodies before being utilized as viable treatments.

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Author's contributions

Naveed Ahmed Khan and Ruqaiyyah Siddiqui conceived the study amid critical discussions with David Lloyd. Ruqaiyyah Siddiqui reviewed the literature together with Naveed Ahmed Khan. Ruqaiyyah Siddiqui prepared the first draft of the manuscript together with David Lloyd and Naveed Ahmed Khan. David Lloyd and Naveed Ahmed Khan corrected the manuscript. All authors approved the final manuscript.

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