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PERSPECTIVE

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Emerging therapies against *Naegleria fowleri*

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ABSTRACT

Introduction: *Naegleria fowleri* is a free-living protist pathogen. Given the opportunity, it can produce infection of the central nervous system. It is distressing that the brain-eating amoebae, *Naegleria fowleri* remains one of the lethal parasites resulting almost always in death, despite advances in antimicrobial chemotherapy and supportive care.

Areas covered: The overall aim is to present a timely review of our current understanding of emerging therapies and priorities. By searching bibliographic databases (PubMed) for the available peer-reviewed research literature, herein, we discuss current advances, challenges and opportunities pertinent in the development of therapeutic interventions.

Expert opinion: The prospect of exploring repurposed drugs in combination with nanotechnology and a theranostic approach to concurrently achieve diagnosis and drug delivery will offer promise in the rational development of effective therapies to counter *N. folweri*-associated fatal brain infections.

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Naegleria fowleri; brain-eating amoeba; CNS infections; free-living amoebae; emerging therapies

1. Introduction

Naegleria fowleri, are free-living pathogenic amoebae, that are ubiquitously present in the environment globally [1]. These amoebae are known to cause the 'rare' disease, primary amoebic encephalitis (PAM), a typically fatal infection of the central nervous system (CNS) [2]. The Centers for Disease Control (CDC) reports 157 cases caused by *Naegleria fowleri* in the United States since 1962–2022 [3]. However, recently there have been several reports of cases in countries such as Bangladesh, India, Taiwan, Pakistan, Turkey, Zambia and China [4,5], this is an indication that cases are increasing in occurrence, perhaps exacerbated due to the impact of climate change as well as other factors, or perhaps there is more awareness of the parasite [1]. Of note, the actual magnitude of the disease and impact attributed to infections resulting from *Naegleria* are likely underreported [1]. One reason is because up to 60% of encephalitis instances may be overlooked or inaccurately diagnosed, often mistaken for cases of bacterial meningitis [6,7], and in developing countries often the cause of death may not even be determined due to various reasons, such as religious beliefs [8].

Naegleria fowleri, in its trophozoite state, causes infections in humans by entering through the nasal cavity during water-related activities (swimming/nasal cleansing or ablution with contaminated water) [8,9]. Once inside, it attaches to the nasal mucosa, penetrates the olfactory neuroepithelium, migrates through the olfactory nerve, and reaches the olfactory bulb [10]. Upon entering the brain, it induces inflammation, damaging nerves and the CNS, ultimately resulting in mortality [11]. The clinical symptoms progress in two stages: Stage 1 includes a severe frontal headache, nausea, vomiting, and fever, while Stage 2 presents more severe symptoms like a stiff neck, altered mental status, seizures, cerebral edema, cerebellar herniation, leading to coma, and ultimately death within about a week [9]. These symptoms resemble bacterial or viral meningitis, causing delays in the diagnosis of PAM [7,11]. Currently, there are no effective drugs for treating PAM caused by *Naegleria fowleri* and of concern, PAM has a mortality rate exceeding 97% [9]. For example, the most reported cases of PAM caused by *N. fowleri* infection in the United States have been fatal (153/157 in the United States). There have been five documented survivors in North America: one in the United States in 1978, one in Mexico in 2003, two additional survivors from the United States in 2013, and one from the United States in 2016 [3]. It has been suggested that the original U.S. survivor's strain of *N. fowleri* was less virulent, which contributed to the patient's recovery. In laboratory experiments, the original U.S. survivor's strain did not cause damage to cells as rapidly as other strains, suggesting that it is less virulent than strains recovered from other fatal infections [3]. Regardless, the recommended treatment by CDC involves a combination of drugs, including amphotericin B, fluconazole, rifampin, miltefosine, azithromycin, and dexamethasone [12]. However, the use of amphotericin B is limited due to its toxicity, especially kidney-related issues. These drugs, administered intravenously, have systemic side effects and are hindered by the slow passage through the blood–brain barrier, resulting in insufficient distribution within the central nervous system to effectively eliminate *N. fowleri* [12]. Therefore, there is an urgent need to develop more effective and less toxic therapeutic interventions, but this has been hampered due to the

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Article highlights

- *Naegleria fowleri* invasion of the central nervous system is a deadly infection.
- The diagnosis is often delayed due to similarities with bacterial meningitis.
- In the absence of effective available drugs, treatment is challenging resulting in extremely poor prognosis.
- Current understanding of the advances made in this field is discussed as well as future research priorities.
- Theranostics combined with nanotechnology offer a promising approach in the development of therapeutic interventions.

lack of clinical trials for this infection as well as little interest from the pharmaceutical industry due to this being a rare disease [9,12].

Further exacerbating the issue is the rise of worldwide water crises, exemplified by the complete drying of the Aral Sea's eastern basin after 600 years and California's unprecedented three-year drought [13]. Demographic changes and unsustainable economic practices, rapid urbanization may be further stressing water resources and infrastructure, with lasting consequences for human health and urban environments [13]. Recently, the parasite was detected in Grand Teton National Park, in the U.S.A. [14]. In developing countries, the situation is even more dire, and the population often rely on water storage tanks, which are often teeming with microorganisms such as amoebae, bacteria and other pathogens [15]. Herein, we review the current and emerging therapies versus *N. fowleri*, both *in vivo* and *in vitro,* as well as discuss novel nasal inhalers and the plausibility of utilizing the nasal route to treat this infection. Furthermore, opportunities and challenges in future research are deliberated upon.

2. Methods

A PubMed search using '*Naegleria fowleri*' combined with 'Primary amoebic meningoencephalitis,' 'pathogenesis,' 'treatment,' 'drugs' and 'nanotechnology' as keywords was carried out. In addition, we consulted conference proceedings, original unpublished research undertaken in our laboratories, and discussions in the Free-Living Amoebae Meetings.

3. Pathogenesis

Naegleria migrates along the olfactory nerves to the brain, causing severe damage like cerebral edema and eventually death. Two primary pathogenic mechanisms have been identified: contact-dependent and contact-independent. The contact-dependent mechanism involves adhesion and the formation of specialized structures called phagocytic food-cups, which facilitate the amoeba's direct interaction with target cells [16]. In contrast, the contact-independent mechanism relies on the secretion of cytolytic molecules, allowing the amoeba to cause damage without direct contact [16]. *In vitro* studies, *N. fowleri* has been observed to traverse the nasal epithelium, adhering to various basement membrane components such as collagen I, fibronectin and laminin-1 [17]. This adhesion process is thought to be facilitated by integrin-like proteins that co-localize with actin filaments (specifically, 53 and 70 kDa) and by fibronectin-binding proteins (60 kDa). Furthermore, protein kinase C has been shown to enhance the amoeba's adhesion and cytotoxicity toward host cells [17–19].

Naegleria fowleri adhesion to components of the extracellular matrix (ECM) may initiate signal transduction pathways that lead to the expression of proteins and proteases, facilitating amoebal entry into the central nervous system (CNS). *In vivo* studies have shown that axenically maintained *N. fowleri* amoebae exhibit mild pathogenicity. However, after being passed through a mouse brain, they became nearly 100 times virulent. This heightened virulence is associated with a group of proteins linked to cytoskeletal stability and reorganization. Among these proteins, one similar to Rho guanine nucleotide exchange factor 28 was present in the highly virulent *N. fowleri*, indicating that proteins involved in cytoskeletal restructuring and stability play a significant role in pathogenic characteristics [17–19]. Amoebae elicit severe tissue damage through contact-dependent phagocytosis, wherein they utilize specialized structures known as amoebastomes to progressively engulf neuronal cells [20]. The Nfa1 gene within *N. fowleri*, encoding the Nfa1 protein (13.1 kDa), was implicated in amoebastome formation and amoeba locomotion [21]. Studies utilizing anti-Nfa1 antibodies have demonstrated a reduction in ameobal cytotoxicity, indicative of the critical role of this protein. Moreover, the transfection of *N. fowleri's* Nfa1 gene into nonpathogenic *N. gruberi* has been shown to elevate the parasite's cytotoxicity toward Chinese hamster ovary cells (CHO) compared to untreated *N. gruberi* strains. Additionally, the Nf-actin protein (50.1 kDa), encoded by the Nf-actin gene, has been identified in various amoebal cellular compartments, including amoebastomes, cytoplasm, pseudopodia and is associated with augmented cell adhesion, phagocytosis, and cytotoxicity in *N. fowleri*. Furthermore, a protein of the membrane, termed Mp2CL5 (17 kDa) has been identified in pathogenic *Naegleria* species, suggesting a potential role in cellular recognition and adhesion processes of the amoeba [22,23]. Various contact independent mechanisms have also been identified. Matrix metalloproteinases (MMPs) are a class of endopeptidases known to facilitate the infiltration of various parasites and leukocytes into the central nervous system (CNS). Recent research has identified three enzymes: MMP-2, MMP-9, and MMP-14 in *Naegleria fowleri* trophozoites. While MMP-2 and MMP-9 are adept at breaking down gelatin and type IV collagen, they require activation by MMP-14 to become functional. The study's findings indicate that *N. fowleri* utilizes these MMPs to degrade the extracellular matrix, thereby enabling the amoeba to traverse the nasal cavity and enter the olfactory bulb with greater ease. This mechanism likely plays a crucial role in the amoeba's ability to reach and infect the CNS [24]. It is speculated that *Naegleria fowleri* can breach the blood–brain barrier (BBB) by degrading key tight-junction proteins (TJPs). An *in vitro* study demonstrated that the amoeba secretes cysteine proteases that disrupt and break down TJPs, including claudins-1 and occludins (ZO-1), while also altering the actin cytoskeleton [25]. Moreover, a specific 30 kDa cysteine protease secreted by *N. fowleri* has been observed to cause cytopathic effects in baby hamster

kidney (BHK) cells, while another cysteine protease, weighing 37 kDa, demonstrates mucinolytic activity, suggesting a role in mucin degradation and immune evasion [26]. Other cysteine proteases of 58 kDa, 128 kDa, and 170 kDa were identified in *N. fowleri* [20].

Additionally, cathepsin B (NfCPB) and cathepsin B-like (NfCPB-L) cysteine proteases (molecular weights of 38.4 kDa and 34 kDa) are believed to contribute to the proteolytic degradation of collagen, albumin, immunoglobulins, fibronectin, and hemoglobin. These findings indicate a diverse range of proteolytic mechanisms utilized *by N. fowleri*, contributing to its pathogenicity and ability to circumvent host defenses [27]. Other studies have identified various enzymes and molecules in *Naegleria fowleri* that likely contribute to its ability to lyse host cells and cause tissue damage. Phospholipases (A, A2, C), sphingomyelinases, neuraminidases, elastases, and other proteolytic enzymes are linked to cell membrane damage and demyelination in primary amoebic meningoencephalitis (PAM) patients, suggesting their involvement in neurodegeneration. Additionally, a range of other enzymes, including N-acetylglucosaminidase, acid phosphatase, and beta-glucosidase, along with electron-dense granules, thrombin receptors and peroxiredoxin, have been identified in *N. fowleri*, indicating a complex mechanism of pathogenesis. Despite these insights, the complete pathogenic mechanism of *N. fowleri* remains unclear, highlighting the need for further research [23,28].

4. Efficacious therapies *in vitro*

The majority of research efforts aimed at developing therapeutic interventions against *Naegleria* have primarily been conducted *in vitro* [9]. This trend largely stems from the limited interest shown by the pharmaceutical industry toward this parasite, owing to the rarity of the associated disease and the formidable challenges associated with conducting clinical trials due to its rapid disease progression and the rarity of the disease [9]. Various investigations have predominantly involved repurposing existing compounds, alongside the exploration of novel approaches such as conjugation with various nanoparticles to enhance their efficacy [29–33].

5. Repurposing of existing compounds and other *in vitro* studies

In view of the limited participation of pharmaceutical companies in the exploration of novel drugs for infections caused by amoebae, the concept of drug repurposing is a promising method to develop and utilize drugs already available in the market [34]. In a recent study, the ReFRAME drug repurposing library that was developed by the California Institute for Biomedical Research (comprises around 12,000 small molecules, including FDA-approved drugs, clinical trial candidates, lead compounds, and preclinical agents) was utilised. This drug library is accessible via https://reframedb.org and is a valuable resource for drug repurposing. The study aimed to discover clinically approved compounds with previously no reported activity against pathogenic amoebae, potentially repurposing them for treating various amoebic diseases. Ninety micro-molar inhibitors were identified against *N. fowleri*. Importantly, 19 compounds exhibited *N. fowleri* inhibition within 24 h, surpassing the speed of the standard CDC-recommended drug regimen for PAM treatment. As most of the ReFRAME library has already undergone preclinical safety profiling or clinical development, this study revealed several new therapeutic applications, against *Naegleria* however future work is needed to determine *in vivo* efficacy of these drugs [31].

At the mucosal level, proteins such as lactoferrin (Lf) and lysozyme (Lz) are integral components of the innate immune response, effectively targeting a variety of microorganisms. A recent study uncovered that *N. fowleri*, despite exposure, can resist both bovine milk lactoferrin (bLf) and chicken egg lysozyme (cLz) either individually or in combination [34]. Remarkably, the presence of these proteins did not impede the proliferation of the amoeba even after 24 h of co-incubation. Transmission electron microscopy showed that trophozoite ultrastructure remained largely unaltered despite exposure to bLf and cLz. Analysis using gelatin-zymograms indicated that the secretion of *N. fowleri's* proteases responded differently to bLf and cLz over varying time intervals. Combining both bLf and cLz was able to inhibit the activity of *N. fowleri's* secreted proteases. Furthermore, protease inhibitors used on bLf-zymograms revealed the involvement of cysteine proteases in bLf degradation. Despite this, co-incubation with bLf and/or cLz mitigated the cytopathic effects of *N. fowleri* on the Madin-Darby canine kidney cell line by inhibiting secreted proteases. These findings suggested that bLf and cLz, whether used alone or in combination, can effectively inhibit secreted proteases and alleviate the cytopathic effects caused by *N. fowleri*, albeit without impacting trophozoite viability and proliferation [34].

It is thought that cytolytic molecules may aid *N. fowleri* infection progression, possibly packaged into extracellular vesicles [35]. In a recent study, the immunomodulatory effects of extracellular vesicles from two *N. fowleri* clinical isolates were investigated using *in vitro* models. The extracellular vesicles induced increased expression of nitric oxide synthase (NOS), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-23 (IL-23), and interleukin-10 (IL-10) in primary microglia cultures, and NOS and interleukin-13 (IL-13) in BV-2 cells [36]. They also prompted morphological changes in microglia toward a more amoeboid shape. *N. fowleri* DNA was confirmed in vitro in extracellular vesicle fractions [36]. These findings suggest extracellular vesicles as potential biomarkers for primary acute meningoencephalitis.

6. Nanotechnology/theranostics

Recent studies show that when Amphotericin B, or Fluconazole, or Nystatin are combined with silver nanoparticles, show more effectiveness, especially at micromolar levels, compared with using the compounds alone. For example, during a 24-h period, Amphotericin B at a concentration of 2.5 µm showed significant improvements [32]. Another study from our group revealed that oleic acid combined with silver nanoparticles had a considerable impact on both the viability of *N. fowleri* and increased ability to cause host cell damage

[33]. In recent investigations conducted in our group, 34 novel compounds were synthesized and assessed for their efficacy against *N. fowleri*, both alone and in conjunction with silver nanoparticles [37]. This study revealed that the azole derivatives demonstrated significant amoebicidal and amoebistatic activities against trophozoites [37]. Similarly, another investigation focused on four bisindole and thiazole derivatives, with two of them demonstrating significant activity [38].

Both silver and gold nanoparticles have been shown to enhance drug bioavailability and antimicrobial activity. Guanabenz acetate (GA) conjugated with both types of nanoparticles significantly increased amoebicidal effects at concentrations as low as 2.5 µM [39]. Additionally, green nanoparticles stabilized with plant-derived polysaccharides and conjugated with flavonoids such as hesperidin and naringin demonstrated significant amoebicidal activity against *N. fowleri*, outperforming conventional antiamoebic agents like Amphotericin B [40]. These findings highlight the potential of using nanoparticles to enhance the effectiveness of both existing and new compounds against *N. fowleri*. Therefore, using nanotechnology to enhance compounds to make them more effective against amoebic infections is very promising. Furthermore, the convergence of therapeutics and diagnostics into a unified system, known as 'theranostics,' may offer potential for simultaneous disease diagnosis and treatment, representing a significant breakthrough versus brain eating amoebae [41]. Nanomaterials can provide sensitive diagnostic capabilities and effective treatment within a single platform. While strides have been made in developing efficient theranostic systems for various tumors [42], the application of nanomaterials in combating infectious diseases, especially parasitic infections, remains relatively novel and significant further studies involving *in vivo* experiments are urgently needed, in order to bring these emerging therapies to patients.

7. Omics in the development of therapeutic approach

By employing omics techniques such as genomics, transcriptomics, and proteomics, researchers can delve into the sequenced genomes of pathogens such as *N. fowleri*, leading to potential significant discoveries about its pathogenicity and genetic makeup. Current efforts involve comparative genomics analyses and investigations into differential gene and protein expression in free-living amoebae [43].

In an important study, de novo RNA transcriptome analysis using RNA-Seq and differential gene expression analysis with the Trinity software (Holland Computing Center, Schorr Center, Lincoln, NE, U.S.A.) was conducted. The analysis revealed that over 2000 genes exhibited differential expression in response to *N. fowleri* treatment with Hesperidin-conjugated silver nanoparticles. Among these genes, some were associated with oxidative stress response, DNA repair, cell division, cell signaling, and protein synthesis. The down-regulated genes were found to be associated with processes such as protein modification and the synthesis of aromatic amino acids when compared with untreated *N. fowleri*. These findings indicate that further transcriptome studies in unraveling the genetic mechanisms underlying the biology and pathogenesis of *N. fowleri*, as well as in identifying potential drug candidates are necessary [44].

Recently, a study was conducted whereby, two new strains of *N. fowleri* were sequenced, and transcriptomic analysis of low pathogenicity versus high-pathogenicity *N. fowleri* cultivated in the mouse infection *in vivo* model was accomplished [45]. This comparative analysis was able to provide an in-depth assessment of the encoded protein complement between the two strains, and it revealed high conservation. Notably, transcriptomic analysis can identify genes that are differentially expressed between low and high pathogenicity *N. fowleri* strains, but it does not establish causative relationships between gene expression changes and pathogenicity. Molecular evolutionary examination of diverse cellular systems demonstrated that the *N. fowleri* genome encoded a similarly complete cellular system as in *N. gruberi*. From transcriptomic studies, it was revealed that characteristics acquired via lateral gene transfer or stress responses were not essential for pathogenicity. Conversely, lysosomal machinery, proteases and motility, metabolic reprogramming and novel *N. fowleri* proteins, were all thought to be associated with pathogenicity within the host. The upregulation of genes in mouse-passaged *N. fowleri* correlated with the metabolism of glutamate and ammonia transport suggest adjustments to available carbon sources in the CNS [45]. Future studies that aim to identify unique genes in *N. fowleri*, assess genes with active transcriptional activity, and explore their differential expression under conditions of modified virulence are warranted. Integrating data from diverse omic sciences may offer a comprehensive approach to unraveling the molecular complexity underlying virulence factors and understanding the associated molecular mechanisms. These breakthroughs hold promise for advancing therapy design and medical diagnosis in the near future.

8. Research conducted *in vivo*

In comparison to *in vitro* studies, relatively fewer *in vivo* studies have been accomplished against *Naegleria fowleri*, despite *in vivo* demonstration of promising compounds being a critical stage for future clinical use [9]. In an interesting previous *in vivo* study, the immunoprotective effect of two vaccine antigens against *Naegleria* infection were identified, given that there is no effective vaccine against this disease [46]. Researchers developed two vaccine contenders: one consisting of a 19 kDa polypeptide band and another containing an anticipated immunogenic peptide sourced from the membrane protein, MP2CL5 (Smp145). These vaccines were administered either individually or simultaneously with cholera toxin acting as an enhancer, via intranasal immunization in BALB/c mice using a model that mimics meningitis induced by *Naegleria fowleri*. Both the tested vaccines were able to prompt a robust immune response versus challenge with *N. fowleri* trophozoites in the immunized mice. The response was examined in the naso-pharynx-associated lymphoid tissue and nasal passages of mice using flow cytometry. Moreover, the antibody reaction in both serum and nasal washes was determined, and a comparative assessment of the suggested vaccines was done. The antigens assessed in the study

revealed the ability to provide protection, suggesting their potential as candidates for vaccines targeting meningitis caused by *N. fowleri* [46]. Moreover, the potential of intranasal immunization was also assessed in this study, and was found to be of value. However, the duration of immunity and its immunomodulatory mechanisms conferred by these vaccines and their ability to provide long-term protection remain unclear. Long-term studies are needed to assess the durability, safety and persistence of vaccine-induced immunity. Additionally, the diversity of *N. fowleri* strains and its antigenic variability may affect the effectiveness of vaccine-induced immunity across different strains and these aspects need further investigations. Another interesting study focused on evaluating the capacity of *N. fowleri* cysts to attach, undergo excystation into the trophozoite stage, and induce damage in cell culture, alongside evaluating the potential of the cysts to instigate PAM in a murine model [47]. Interestingly, within the murine model, it was observed that *N. fowleri* cysts did not manifest infectivity *in vivo*. Furthermore, the study delved into the possible role of the intracellular concentration of (cyclic Adenosine MonoPhosphate) cAMP in mediating the encystment process. To investigate this, trophozoites were subjected to dipyridamole, a compound that inhibits cAMP-specific phosphodiesterases. The administration of dipyridamole led to an approximately two-fold acceleration in the rate of encystment, accompanied by a nearly sixfold increase in the intracellular concentration of cAMP within the cysts over the course of the experimental period. The data indicated that cAMP may serve as a mediator for the encystment process in *N. fowleri* [47].

The infectious lifecycle stage of *N. fowleri* involves glycolysis, which is a metabolic pathway essential for the parasite's survival, a recent study was conducted to examine if inhibitors targeting glucose metabolism demonstrate toxicity to the pathogen [48]. Although the study has not yet been peer reviewed, the authors developed a class of compounds known as human enolase 2 (ENO2) phosphonate inhibitors as potential agents for treating glioblastoma multiforme (GBM). The compounds revealed efficacy in curing glioblastoma (GBM) in rodent models and exhibited good tolerability in mammals due to the predominant usage of enolase 1 (ENO1) isoform systemically. The study demonstrated that these ENO2 phosphonate inhibitors effectively inhibited the *Naegleria fowleri* ENO (NfENO) enzyme and proved lethal to the amoebae. Notably, (1-hydroxy-2-oxopiperidin-3-yl) phosphonic acid (HEX) emerged as a potent enzyme inhibitor and demonstrated toxicity to trophozoites. Further, the authors conducted molecular docking simulations which further confirmed the strong binding affinity of HEX to the active site of NfENO. The authors also conducted metabolomic studies of parasites treated with HEX which revealed a significant accumulation of glycolytic intermediates upstream of NfENO, indicating disruption of the glycolytic pathway. Additionally, nasal instillation of HEX in rodents infected with amoebae resulted in increased longevity. Specifically, rodents treated with 3 mg/kg HEX for 10 days followed by 1 week of observation exhibited enhanced survival rates compared to vehicle-treated controls. While brain samples from survivors still tested positive for amoebae, suggesting suppression rather than elimination of infection, the results suggest that HEX may hold promise as a leading compound for the treatment of PAM as long as the risk of off-target effects associated with pharmacological inhibition of metabolic pathways is kept in view.

9. Nasal inhalers, and intranasal route as potential therapies

As already discussed, *N. fowleri* typically localizes in the frontal lobe postmortem, as it enters through the nose [49]. As indicated earlier in laboratory settings, Amphotericin B, which disrupts ergosterol production, demonstrates significant effectiveness *in vitro* [50]. However, its ability to target the parasite within the central nervous system is limited in clinical practice, primarily due to challenges crossing the blood–brain barrier and achieving sufficient drug concentrations within cysts or abscesses [51]. To overcome the limited efficacy of CNS-targeting drugs, high intravenous doses are often administered to achieve adequate brain concentrations for parasite elimination. Unfortunately, this strategy leads to drug distribution to unintended tissues, resulting in notable side effects such as liver and kidney toxicity [51]. Given the nasal entry point and migration along the olfactory neuroepithelium, intranasal delivery of solubilized Amphotericin B deoxycholate appears rational and may help mitigate adverse effects like hepatotoxicity and nephrotoxicity. Recently, we conducted a study comparing intranasal and intravenous administration of Amphotericin B, assessing differences in adverse effects through blood biochemistry and histopathological examination of liver, kidney, and brain tissues post-administration [50,52]. The findings strongly support the use of the intranasal route for Amphotericin B administration as a means to reduce adverse side effects.

In a recent study, we have proposed the potential exploration of vaporized anti-*N. fowleri* drugs to be delivered intranasally for the treatment of PAM, via nasal inhalers [53]. This alternative route of drug delivery alongside the conventional intravenous method warrants investigation in future clinical research. Should the efficacy of intranasal vaporized drug administration against *N. fowleri* induced PAM be established, it could potentially supplement or replace intravenous administration in mitigating neurological complications. The proposed method offers the advantage of administering treatment at regular intervals, which could aid in preventing brain damage. Nasal inhalers containing specific anti-*N. fowleri* drugs, possibly combined with steroids like dexamethasone, hold promise for targeting pathogenic amoebae while also alleviating increased intracranial pressure. Crucially, the determination of an optimal exposure time for achieving complete eradication is necessary for effective application. The simplicity of nasal administration and the rapid delivery of vaporized drugs to the brain render this approach particularly advantageous, especially in the primary healthcare setting where consideration of the ease of administration is paramount. Moreover, the potential for self-administration of such a device may make it a viable option in scenarios where access to tertiary healthcare facilities is limited or delayed, such as in developing countries. Additionally, nasal administration could serve as both prophylactic and postexposure purposes, thereby offering a versatile strategy for designing preventive and therapeutic interventions against PAM [53].

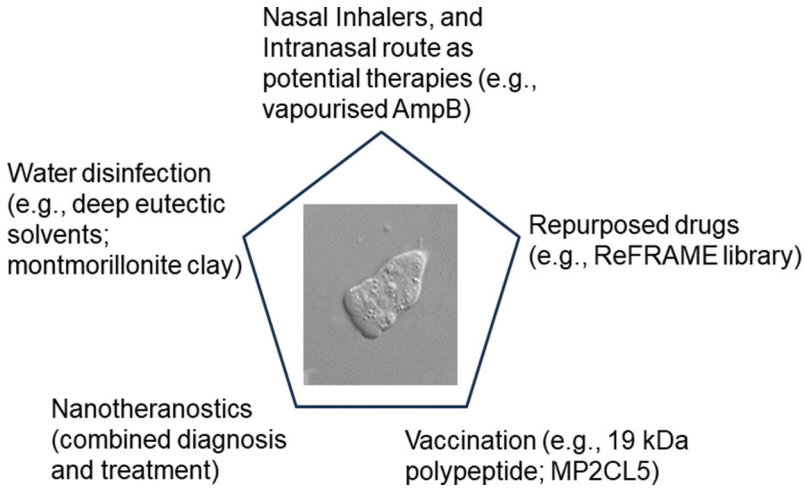

Figure 1. Emerging therapies in the management and eradication of brain-eating amoebae.

However, it is necessary to determine the optimal dosage and exposure time of intranasal time of intranasal spray drug delivery for achieving complete eradication of *N. fowleri* while minimizing adverse effects is crucial. Variability in individual nasal anatomy and physiology may also affect drug deposition and adsorption. *In vivo* studies to determine the clinical efficacy of this device are warranted (Figure 1).

10. Augmenting and monitoring water quality against *N. fowleri*

Despite growing awareness, it is perplexing that none of the global water quality monitoring programs currently incorporate surveillance for brain-eating amoebae in public water supplies. Presently, water quality assessments primarily focus on bacterial contamination (such as elevated levels of *E. coli*), along with monitoring for norovirus, *Shigella*, *Cryptosporidium*, and *Giardia* [54,55]. While it is impractical to monitor all pathogens in water, considering the free-living nature of *N. fowleri* and being a waterborne pathogen, coupled with practices involving nasal cleansing, the severe consequences for affected individuals, and its potential to host other pathogenic microbes, there is a pressing need to include *N. fowleri* in the roster of pathogens monitored in water quality surveillance programs.

Recently, we have suggested the use of innovative adsorbents, specifically micelle clay complexes containing montmorillonite clay combined with activated carbon, as a viable solution for effectively removing neuropathogenic microbes like *N. fowleri* from water sources used for ablution and nasal irrigation [56]. These adsorbents could be conveniently integrated into water collection devices such as taps and water bottles within the domestic setting. Offering a cost-effective and easy-to-install option, these filters could serve as ideal disinfection systems. These innovative approaches are particularly promising for communities facing challenges related to limited access to safe water, dependence on water storage tanks, or inadequate water sanitation facilities which are prevalent in developing regions [56].

Of interest, recently, it has been demonstrated that deep eutectic solvents (binary or ternary mixtures of compounds that are able to associate mainly via hydrogen resulting a melting point significantly lower compared to the starting materials) exhibit significant and effective antimicrobial characteristics against a wide array of pathogens, encompassing multidrug-resistant bacteria, fungi, amoebae, and certain viruses [52]. Moreover, deep eutectic solvents are environmentally friendly, and economical antimicrobial agents. Deep eutectic solvents could also be utilized to target waterborne pathogens such as *Naegleria fowleri* present in storage tanks, however future research to understand potential toxicity and safety should be accomplished.

11. Concluding remarks

PAM, though deadly, is a rare infection, making pharmaceutical companies hesitant to invest in new drug development. However, with the rise in global warming and the thermophilic nature of *N. fowleri*, infectious diseases like PAM will likely become more prevalent [57]. Currently, there are no specific drugs for treating PAM, leading medical practitioners to rely on a combination of antifungal, antibiotic, anti-cancer, and anti-inflammatory compounds. However, numerous investigations have delved into adapting current compounds and combining them with nanoparticles, along with some newly discovered compounds showing promise against *N. fowleri*. However, there are very few patents focused on treating PAM. These nanoparticle-conjugated compounds and novel drug leads are potential candidates for further *in vivo* studies, transcriptome analysis, and clinical testing, particularly through intranasal administration and should be developed further for clinical use. Comprehensive research is needed in the coming years to assess the therapeutic value of these treatments. It is imperative to foster collaboration among pharmaceutical and water industries/companies and academia to accelerate the development of strategies against this lethal CNS amoebae. Given the gravity of the infection and its profound implications, raising public awareness and providing education to clinical practitioners about PAM infection is crucial, as patients frequently face misdiagnosis, particularly in developing nations.

12. Expert opinion

Drug development is time-consuming and expensive, taking about $2.6 billion to bring a single medicine to the market [58]. Hence, it is logical to explore clinically used drugs for repurposing against rare disorders. In this context, repurposing medications for the effective treatment of PAM due to *N. fowleri* may be made easier by using clinically developed compounds, which can also expedite the development process and save time and money. Investigating drug repurposing prospects and integrating nanotechnology and a targeted theranostic approach (combining therapeutics and diagnostics to concurrently or consecutively diagnose and treat disorders) to improve drug delivery and diagnostic techniques is a highly promising approach in our efforts in the development of effective therapeutic interventions against PAM due to *N. fowleri*. Despite the availability of molecular biology and genomic tools, research in the area of free-living amoebae is still in its early stages, largely due to the rarity of the number of cases resulting in limited interest of the pharmaceutical industry. Recent advances using omics techniques such as genomics, transcriptomics, proteomics, or metabolomics can provide potential for a mechanism-based approach to discover new anti- *N. fowleri* compounds, which could involve combining phenotypic screening with the identification of target-based inhibitors.

Given the high mortality associated with PAM due to *N. fowleri*, conducting a phase II clinical trial for this infection is deemed unfeasible. Therefore, it becomes crucial to demonstrate *in vivo* efficacy to expedite the path for regulatory processes and approvals to clinical application. The rarity of PAM presents unique opportunities, such as obtaining Food and Drug Administration (FDA) orphan drug designation, rare pediatric disease designation, and expanded access via treatment protocols or Investigational New Drug programs. Utilizing novel strategies for treatment such as nasal inhalers or nasal route instead of the intravenous route for treatment should be considered as an important way forward to develop effective countermeasures.

Moving forward, it will be useful to focus on several critical areas. These include enhancing international and multidisciplinary collaborations to accelerate drug discovery research, making collections of approved and investigational products accessible through various public and private sector initiatives, engaging or introducing in programs that promote drug repurposing especially against rare disorders, and fostering stronger partnerships among academic institutions, industry, patient advocacy groups, foundations, and government regulatory agencies. These collaborative efforts are vital for advancing treatments and enhancing better outcomes for PAM patients. Given the rise in global warming associated with increased outdoor activities especially in developing countries will result in a likely a surge in infections due to brain eating amoebae, strategies to promote awareness about these amoebae and the development of ways to target these pathogens in water storage tanks such as the use of Deep eutectic solvents as an additive and/or alternative to chlorine, or developing regular water monitoring strategies against these rare but deadly parasites should be accomplished. In addition, there is a clear and urgent need for increased awareness with the use of unsafe water for ritual bathing and/or nasal irrigation. This is particularly important for developing countries with poor compliance of water disinfection and/or purifying programs and/or water supplies due to poor infrastructure.

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Authors' contributions

R Siddiqui and NA Khan conceptualized the study amid discussions with D Lloyd. R Siddiqui and NA Khan reviewed available literature and prepared the first draft of the manuscript. AM Alharbi further reviewed the literature, revised and corrected the manuscript under the supervision of R Siddiqui, NA Khan and D Lloyd. All authors approved the final manuscript.

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