

Understanding *Naegleria fowleri*: diagnostic challenges, geographic distribution, and emerging threats

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Abstract

Primary Amoebic Meningoencephalitis (PAM), a rare and often fatal brain infection caused by the free-living amoeba *Naegleria fowleri* (also known as brain eating amoeba), presents significant clinical challenges due to its rapid progression and limited therapeutic options. The infection is primarily contacted through warm, inadequately chlorinated freshwater environments, leading to severe neurological damage and high mortality rates. Treatment typically involves Amphotericin B (AmB), an antifungal that targets ergosterol in the amoeba's cell membrane, but its water insolubility and nephrotoxicity limit its effectiveness. Miltefosine (MLT), initially developed for leishmaniasis and breast cancer, shows promise, particularly in combination with AmB and other drugs like Fluconazole (FCZ), Azithromycin (AZM), and Rifampin (RIF), though its variable efficacy highlights the need for further research. In vitro studies have explored drug repurposing, identifying several agents with potential anti-amoebic activity, such as azole antifungals (e.g., Itraconazole and Posaconazole) and other drugs like Auranofin and Pitavastatin. Nanoparticle-based drug delivery systems, including silver (AgNp) and gold nanoparticles (AuNp), have enhanced the efficacy of various drugs by improving CNS penetration and targeting. In vivo studies have demonstrated that Chlorpromazine, an antipsychotic, offers superior survival rates compared to AmB and MLT, while Corifungin shows exceptional promise with a 100% survival rate in preclinical models. To decrease the risk of PAM, one needs to avoid water chlorination and practice other forms of water chlorination, get more information about the illness, and avoid wrong nasal rinsing techniques. Finally, the enhancement in the drug repurposing, nanoparticle conjugation, and prevention has a positive implication, further research is crucial for optimizing the therapeutic management of this severe infection.

Introduction

Naegleria fowleri, often called the “brain-eating amoeba” is a free-living ameboflagellate that causes infections in humans and is a much-studied and feared pathogen in rare diseases. Together with a number of closely related species, *N. fowleri* is classified in the genus *Naegleria*, which comprises more than 30 species (Khurshid &

Khurshid, 2024). This pathogen is more commonly found in warm and flowing water in freshwater, reaching the surface in warm water bodies like lakes, rivers, and hot springs and in soil in which the temperature of the atmosphere is high (Andrea Güémez & Elisa García, 2021). From the neurotropic perspective, it is an organism of special interest due to its link with primary amoebic meningoencephalitis (PAM) a rapidly progressive nearly always fatal infection of

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the central nervous system (CNS) (Shaukat et al., 2024). The virulence of *N. fowleri* is mainly due to its ability to penetrate the human cranial cavity via the nasal passage when people engage in swimming, diving, or using water contaminated with the *N. fowleri* organism for that which is called nasal neti or religious purification (Nadeem, Malik, Afridi, & Shariq, 2023). The amoeba moves along the olfactory nerve into the brain where it leads to significant inflammation, swelling of the brain, and finally herniation (Gillen, Augusta, & Schneckenburger, 2019).

The infection becomes worse in a few days and the fatality rate is estimated to have a range of between 95% and 99% (Shaukat et al., 2024). Although PAM is still quite rare it is being identified in parts of Pakistan, Africa, and some regions of the United States more so because early diagnosis is another major challenge that needs to be acknowledged and addressed alongside improved therapeutic approaches. There are several issues associated with the enhanced risk of *N. fowleri* infection; listed as follows below Climate change, Global warming, and increased usage of recreational water activities (Sheybani, Haddad, & Shirazinia, 2023). The improved weather has fueled the growth of the conditions that boost the proliferation of *N. fowleri*, causing people to worry about its further expansion as a powerful disease vector (Bilal et al., 2023). However, the direct diagnosis of Primary amoebic meningoencephalitis (PAM) is still difficult because early symptoms associated with the sickness include; headache, fever, nausea, and changes in mental status which resemble signs of bacterial or viral meningoencephalitis (Ahmad Zamzuri et al., 2023). In addition, there is no unique biomarker and poor coverage among physicians that causes missing diagnoses and underestimation, which deepens the identification of true prevalence (Paul, 2024).

At the molecular level, *N. fowleri* shows a remarkable setup that allows it to change its form according to its environment, namely the trophozoite, flagellate as well as cysts form. The trophozoite stage is the pathogenicity determinative form for brain invasion and injury because of a minimum of cytolytic enzymes such as neuraminidases, phospholipases, and hydrolases (Samba-Louaka, Delafont, Rodier, Cateau, & Hécard, 2019). These enzymes enhance host tissue destruction and enable the amoeba to survive in the CNS (Datta, 2024). Fourthly, *N. fowleri* is able to activate the massive immune response in the host's body directed at the invading organism; however, these efforts are

counterproductive as they lead to even greater lesions and inflammation of affected tissues – which is characteristic of Primary amoebic meningoencephalitis (PAM) (Sarraz et al., 2023).

In addition, new diagnostic procedures like PCR assays and CSF analysis have enhanced the chances of diagnosing *N. fowleri*-infected patients (Nadeem et al., 2023). But this makes the disease highly fatal because it progresses faster and is only diagnosable at this stage with little time for the doctor to treat (Wu, Chen, Li, & Lian, 2023). Available treatment still remains a significant challenge, with the traditional clinical antifungal agent, Amphotericin B, and the new agents like Miltefosine if given in the early stage of the disease have been reported to improve the disease condition. However, they are only moderately effective and are associated with severe side effects demanding further studies to establish better therapies (Altamura, Rajesh, Catta-Preta, Moretti, & Cestari, 2022; Hoenigl et al., 2024).

The purpose of the current review is to offer an extensive description of *N. fowleri* with emphasis on the ecology, pathogenicity, clinical manifestations, and recent diagnostic and therapeutic modalities. It is with this in mind that, using the scientific literature published in the last decade, this article aims to underline key knowledge gaps regarding this pathogen and, at the same time, stress the need to devise new approaches for dealing with Primary amoebic meningoencephalitis (PAM).

Overview of *Naegleria fowleri*

Free-living ameboflagellate are present in the *Naegleria* genus and consist of a great number of different forms (Martínez-Castillo et al., 2024). To go further, this genus includes more than thirty species, of which *Naegleria fowleri* (*N. fowleri*) is the most dangerous for humans (Shaukat et al., 2024). The “brain-eating amoeba” is a notorious species, involved in primary amoebic meningoencephalitis (PAM), a fatally progressive disease caused by the invasion of cranial tissues and subsequent inflammation (Borkens, 2024).

Geographic Distribution and Incidence

PAM is an acute and usually fatal disease in men with mortality ranging from 95% to 99%; the disease preferentially affects young hypoechoic adults with apparently healthy physiques. While PAM cases have previously been described mainly in developed countries,

recent data suggests that it is increasingly being identified in developing countries as well (Tunali & Korkmaz, 2023). Currently it is present in some Asian countries like Pakistan and several African countries; however, it is most predominant in the southern parts of the United States, in freshwater in stratified states (Iqbal et al., 2023).

Diagnostic Challenges

Rule out its diagnosis is quite difficult always being associated with other kinds of meningoencephalitis which may be caused by viruses or bacteria. It masks itself often in similar symptoms with other diseases, often causing misdiagnosis, which probably explains why there are few reports of PAM cases. In addition, the limited accessibility of most of the specific diagnostic tests as well as a general ignorance concerning the disease by most of healthcare professions only worsens the situation (Gharpure et al., 2021; Khurana et al., 2022).

Impact of Climate Change

Exacerbating these problems is the fact that global climate change, particularly the increase in average global temperature, is expected to affect the distribution and frequency of *N. fowleri*, and, therefore, PAM. (Nadeem et al., 2023; Shaukat et al., 2024).

Need for Enhanced Strategies

Given the high fatality rate and the emerging nature of *N. fowleri* as a significant pathogen, it is crucial to enhance our diagnostic capabilities, preventive strategies, and therapeutic interventions. Effective measures are essential to manage and mitigate the impact of this lethal pathogen, necessitating ongoing research and public health efforts (Shaukat et al., 2024). Addressing these challenges requires a multifaceted approach that integrates improved diagnostic techniques, preventive public health measures, and novel therapeutic strategies to combat the threats posed by *N. fowleri* (Upadhyay, Singh, & Sharma, 2024).

Pathogenicity

Most of the cases of *Naegleria fowleri* infections are caused by soaking, swimming or splashing water at warmer sources that allow the Amoeba's trophozoites to enter through the nasal opening. Though swimming is the main reason people get infected with the parasite, other activities like washing with water infested with the parasite, or using a

net-gong or similar apparatus to rinse out their nasal passages also lead to an infection (Chen, Zhang, Wen, Chen, & Zhou, 2023). Further, although relatively rare, primary amoebic meningoencephalitis may occur if one breathes in aerosolized cysts in dust; this approach constitutes approximately 6.5% of PAM infections. Having invaded the nasal cavity, *N. fowleri* trophozoites together with the associated salivary sheath attach to the mucosa; they then penetrate the tissue and cross the cribriform plate to gain access to the olfactory bulb (Jahangeer et al., 2020). It then moves along the olfactory nerve bundle into the brains to reproduce and results into major complications such as cerebral edema, herniation and death. *N. fowleri* pathogenicity involves a markedly effective immune response that actively damages the central nervous system (Andrea Güémez & Elisa García, 2021). They cause tissue damage via the production of cytolytic molecules such as nutraceuticals and phospholipase successfully enhancing the destructive capability of the amoeba. Two primary pathogenic mechanisms have been identified: direct host cell recognition and attachment as well as ingestion of host cells and more indirect cytotoxic processes via the secretion of toxic agents. This two-pronged process thus establishes the dual virulence of *N. fowleri*, and the ability of the parasite to evade host surveillance mechanisms, and cause significant neurological manifestations (Aykur & Dagci, 2021; Malinowski et al., 2024).

Stages of *Naegleria fowleri*

Naegleria fowleri exhibits a complex life cycle comprising three distinct stages: These include cyst, trophozoite and flagellate.

Cyst Stage

During the cyst stage, *N. fowleri* develops a spherical, double-barrier structure that allows it to be encased and sealed in an unfavorable environment. In most cases, cysts have 2-8 ostia, but this may differ with the species of the fish. The cyst is a nonculturable form and becomes encapsulated in a cyst when a stressed condition prevails. Subsequently, the cyst reverts back to an active amoeboid mode movement when conditions are favorable for the process (Evdokiou, Marciano-Cabral, & Jamerson, 2022).

Trophozoite Stage

Trophozoite is the feeding stage in the life cycle of *N. fowleri* and is a pathognomonic feature. Trophozoites are

irregular in shape, mainly cylindrical because of the blunt pseudopodia or lobopodian arising from the cell membrane. They usually range from about 10–20 μm in size. Pseudopodia are actin-rich structures responsible for both, locomotion, as well as engulfing prey such as bacteria (Jahangeer et al., 2020). Trophozoites sexually reproduce through a process known as binary fission while the asexual replication cycle takes place about every 1.6 hours provided the parasite feeds on bacteria. This process is the pro-meiosis or intracytoplasmic mitotic division, NO sexual reproduction occurred, however, meiotic genes were found in the genome. They are motile but may only change into flagellated forms which quickly restore to the trophozoite form under favourable conditions. Trophozoites of these species are located in the tissue and the cerebrospinal fluid of their respective host (Sohn, Kim, Kim, Park, & Shin, 2023).

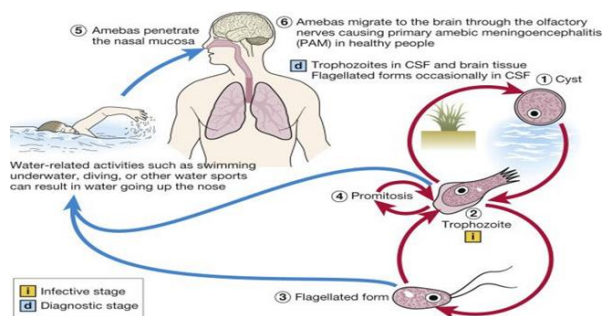


Figure 1. Life cycle of *Naegleria fowleri* (CDC - DPDx - Free Living Amebic Infections)

Flagellate Stage

In the flagellate stage in *N. fowleri*, two flagella are formed, pro generic of which originates from the reorganization of the microtubule cytoskeleton replacing the earlier actin-based cytoskeleton. This stage comprises microtubules and basal bodies including 200 proteins in the construction of flagellum (Nguyen, 2024). One nucleus is placed adjacent to the flagellar root. This flagellate stage is non-sporocystic and temporary or appears when the organism is in the process of moving due to unfavorable conditions in their habitat. The flagellated form quickly transforms into the amoeboid stage within the first one hour depending on the change in the ionic profile within the surrounding aquatic medium (Mohapatra, Rawat, Sah, & Satapathy, 2024).

Diagnosis

Onset of *Naegleria fowleri* Infection

The time taken for signs of *Naegleria fowleri* to show may take between 2 to 3 days and up to 7 to 15 days after coming into contact with the parasite. This variability is influenced by factors such as the virulence of the amoebic strain and the size of the inoculum involved (Ahmad Zamzuri et al., 2023). Early symptoms of the infection typically include fever, headache, nausea, vomiting, and alterations in the senses of smell and taste. As the disease progresses, the manifestations become more severe, leading to symptoms such as stiff neck, fatigue, hemorrhage, confusion, changes in personality, hallucinations, seizures, and ultimately, coma (Milanes et al., 2024).

Table 1. Inception Manifestation of *Naegleria fowleri* Infection.

Stages	Symptoms
Early	Fever, Headache, Nausea, Vomiting, Smell and Taste alternation.
Late	Stiff neck, Fatigue, Hemorrhage, Confusion, Changes in personality, Hallucinations, Seizures, Coma.

Clinical Diagnosis of Primary Amoebic Meningoencephalitis (PAM)

The clinical presentation of primary amoebic meningoencephalitis (PAM) often resembles that of bacterial meningitis, which complicates the diagnostic process. Due to the similarity in symptoms, obtaining a detailed epidemiological history is crucial for distinguishing PAM from other conditions (Rehman et al., 2023). Differential diagnoses must consider a range of conditions including bacterial brain abscesses, tuberculosis, Nocardiosis, central nervous system (CNS) aspergillosis, cryptococcosis, histoplasmosis, toxoplasmosis, cysticercosis, and CNS lymphoma (Puthanpurayil et al., 2024). Diagnostic imaging techniques such as computed tomography (CT) scans and magnetic resonance imaging (MRI) are essential for evaluating CNS damage. Another feature that may be characteristic of PAM refers to intracranial hypertension and brain edema. Expect to find multiple parenchymal space involvement, pseudo-tumoral lesions, meningeal deposits,

hemorrhagic infarction, and brain necrosis in neuroimaging. These findings support the severe pathology characteristic of PAM and the frequently deadly course of the disease (McCormick-Baw, Pence, & Filkins, 2023).

Clinical specimens for diagnosing the disease

Thus, in the correct diagnosis of PAM, brain biopsy together with CSF samples are mainly employed. These specimens must be collected aseptically and stored at 25°C before getting into the laboratory analysis in order to ensure that the amoeba is viable (Przybylski, Bukowski, Kowalska, Pilaczyńska-Cemel, & Krawiecka, 2023). Appropriate handling of specimens guarantees the live amoebae to be captured on the microscope as well as cultured for further analysis. Proper and early identification of this serious but very infrequent disease is crucial to its appropriate control (Boldi et al., 2023).

Investigation of Cerebrospinal Fluid (CSF)

In the diagnostic process of differentiating PAM by *Naegleria fowleri* from other forms of meningitis, test for cerebrospinal fluid is very essential. An LP is performed to collect CSF samples which are tested for certain features indicative of PAM. Pyogenic abscess mater (PAM)-infected CSF samples are cloudy compared to clear fluid from other causes of meningitis (John et al., 2023). This turbidity is due to the over-proliferation of inflammatory cells as well as debris in the infected tissue. Erythrocytes are present in CSF during the early stages of parasitic infestation and are commonly observed during the initial stages of the infection (Datta, 2024).

Additionally, CSF analysis often reveals several other alterations: In the assessment of lipid profile, low glucose level is detected, increase in protein levels and high cell count with neutrophil predominance easily visualized under the HPF (Russell, Dainis, Alexander, Ali, & Kyle). The lower glucose level is attributed to enhanced metabolic turnover by the amoebae and the host's inflammation, high protein level is an index of breach in the blood-brain barrier and the high cell count is suggestive of an intense inflammation. These data are important to identify PAM in distinguishing from bacterial and viral meningitis in patients with low glucose, increased protein, and initially determined a predominance of lymphocytes compared to neutrophils (Rojo, Rajendran, & Salazar, 2023).

Laboratory Diagnosis

The identification of the amoeba through a Microscopic evaluation of Cerebrospinal Fluid. Telescopic assessment is one of the main diagnostic approaches used in the diagnosis of PAM. *Naegleria fowleri* trophozoites appear as motile structures, so a wet mount of the CSF is made (Tadke). The preparation procedure starts with a swaying of a CSF container to bring off the amoebae that might be stuck on its walls. This is done by centrifuging the sample at 5000 x g for 5 minutes after which the amoebae will be pelleted at the base of the container. The supernatant is then carefully drained off, and a small volume of liquid with the pellet is left behind. If there is residue then it is put on a microscope slide for viewing. The slide is warmed by incubating at 35-37 °C so as to ensure that the amoeba movement is comparable to an organism's body temperature. The patient's CSF smear is then stained with Wright-Giemsa or Trichrome stain to increase the visibility of amoebae (Nadeem et al., 2023).

Naegleria fowleri trophozoites are in the size of 10-25 µm (Shaukat et al., 2024). They are easily identified microscopically due to the clearance surrounding their nuclei, along with vacuoles and pseudopodias. There are two steps in the identification process; these amoebae must be distinguished from host cells; this is done bearing in mind the large nucleolus and the morphological characteristics of the trophozoites.

Culture Method and Flagellation Test

However, cultural techniques used are employed for further and accurate identification of *Naegleria fowleri*. The performance of CSF and brain tissue samples is done on a non-nutrient agar (NNA) with heat-killed *Escherichia coli* in Page's amoeba saline (PAS). This culture medium supports the growth of all three developmental stages of *N. fowleri*: has erythrocytic stage- trophozoite, tissue stage- schizont, exo erythrocytic stage- gametocyte and flagellate form and last one is cyst (Rajendran et al., 2024). To establish the type of bacteria or *E. coli* for that matter the amoeba feeds on it and this makes identification easier. The culture method affords a wider picture of the life cycle of the parasite as well as being essential for the identification (Rajendran et al., 2023).

Furthermore, a flagellation test can be used to study the conversion of trophozoite forms into flagellate forms. In this test, the specimen is put into an incubator and kept at 37°C in isotonic saline solution or distilled water. Some recordings

are taken at shrinks of 30 minutes over a period of up to 6 hours in order to check for the development of flagella (Nadeem et al., 2023). Formation of flagella is evident during the later stages of development which is also useful in identifying *N. fowleri*. Another value diagnostic information is obtained from encystation, where amoebae form cysts under conditions of poor nutrients. The observation of cyst forms in NNA is evidence that the amoeba can withstand difficult conditions (Mehnaz, Ashfaq, & Sajjad).

Molecular Detection

Tissue invasion and encystation of the host brain: Molecular identification of *Naegleria fowleri* is best done by PCR assays. A sample of the PCR assays present utilizes parasite-specific primers to open and replicate DNA from the CSF and brain tissue samples (Aykur & Dagci, 2023). Members of the TaqMan real-time PCR are especially useful for clinical samples, and it is capable of detecting as low as one amoeba through amplification of the target DNA sequence. This method is also very sensitive and specific, a characteristic that is very important in the early diagnosis of diseases (Elseadawy, Abbas, Al-Araby, & Abu-Elwafa, 2023).

Different PCR primers amplify DNA sequences in *N. fowleri*: 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, and the 28S rRNA (Zurita-Artaloitia, Rivera, & Vinuesa, 2023). These primers help in initial detection and genotyping of the amoeba that is useful in the study of epidemiology and genetic variation of the parasite. Furthermore, LAMP, a newly developed molecular diagnostic tool, offers a sensitive, selective, and time-effective method to identify *N. fowleri* from environmental water samples and clinical materials. This assay is attractive due to the rapid and highly reproducible PCR for DNA. It has shown proof of efficacy as well as being easy to use thus making it desirable for both clinical and environmental assessment of *Naegleria fowleri* (Olie, Andersen, van de Beek, & Brouwer, 2024).

Treatment

Management of Primary Amoebic meningoencephalitis (PAM)

Naegleria fowleri-induced primary amoebic meningoencephalitis is an exotic, rapidly progressive and almost invariably fatal disease (Paul, 2024). This paper will therefore bring out the impacts and complexities inherent in

the treatment of PAM from its nature and rarity. This has conspired to force physicians into prescribing treatments based on case reports and in vitro investigations because large-scale trials have been rendered impossible by the existence and nature of the disease.

Pharmacological, Psychological, and Complementary Medicine Treatment Strategies

Amphotericin B (AmB)

Therefore, antimony compounds at high doses and poor penetration into the brain parenchyma, are now almost exclusively used for the treatment of *Naegleria fowleri* infection together with the broad-spectrum polyene antifungal, Amphotericin B (AmB). This drug utilized the perturbation of ergosterol in the cell membranes of the amoebas thereby triggering cell death pathways similar to apoptosis. Nevertheless, AmB has been proved to be rather effective and still, it includes some cons as follows; It is insoluble in water and as a result has poor CNS uptake, a vital requirement for handling infections in the brain. Further, AmB has nephrotoxicity and hematotoxicity therefore its long-term use and efficiency are hampered. To pump up the therapeutic effect, AmB is often given together with other anti-fungal agents including Fluconazole (FCZ), Azithromycin (AZM), and Rifampin (RIF). These combinations have revealed the variable effects which again depend on the time of administration and the state of the particular infection (Siddiqui et al., 2023).

Miltefosine (MLT)

Miltefosine (MLT) belongs to the group of lipophilic phospholipid ether analogs which were initially synthesized for the treatment of leishmaniasis and breast cancer, but it can be used to treat PAM. The effectiveness of MLT has been proved in a number of case reports, in particular, one describing a 12-year-old girl, who fully recovered her neurological state after using MLT combined with AmB, FCZ, AZM, RIF, and Dexamethasone. MLT targets and interferes with lipid metabolism essential to the survival of *Naegleria fowleri* amoeba. Other than medications, induced hypothermia has been used in the management of ICP in patients with PAM and in moderation of brain inflammation mediated by the severe inflammation leading to brain damage. Nonetheless, the efficacy of MLT differs, and additional investigations to enhance its application and to

ascertain the correct dose of drugs with the best therapeutic outcomes are required (Nadeem et al., 2023).

While there are many signs of progress, we have yet to identify effective treatments for COVID-19, but in vitro studies and drug repurposing puts us on the right track. Specifically, identification of more effective treatments naturally requires research that can only be conducted in vitro for PAM. Drug repurposing represents a promising approach for targeting this rare infection because, like few other diseases, the development of novel drugs is unprofitable and expensive. Azole antifungals and all their categories have some common features because they owe their functions to the disruption of either the synthesis or the function of ergosterol.

Azole antifungals inhibit the biosynthesis of sterols in fungi and may be beneficial against *N. fowleri* because the 2 parasites have shared membrane characteristics (Sharma et al., 2023). They have antifungal properties because they block the action of the enzyme sterol 14-demethylase (CYP51) that forms ergosterol, which is an essential component of the cell membrane of the amoeba. All the azoles Itraconazole, Posaconazole, Ketoconazole, Isavuconazole, Miconazole, Voriconazole, Clotrimazole, and Fluconazole have been found to have a variable measure of anti-amoebic activity in vitro. Of the three, itraconazole and posaconazole seemed to have the highest yield and concomitantly the lowest EC50 value ranging from $\leq 0.01 \mu\text{M}$. However, Posaconazole's CNS bioavailability is a drawback and it is not efficient in the CNS affected cases, so the technique of CNS drug delivery needs to be enhanced (Nes, Chaudhuri, & Leaver, 2024).

Alternative Diagnostic Methods: LAMP Assay

Loop-mediated Isothermal Amplification (LAMP) assay performed through loop-mediated isothermal amplification has emerged as a highly sensitive and specific technique for the identification of *N. fowleri* in environmental and clinical samples. LAMP is highly specific, very sensitive, and rapid compared to other molecular methods; its applications in diagnosis and the monitoring of the therapeutic response make it quite valuable (Shaukat et al., 2024).

Drug Screening of Different Drugs in vitro

Recent in vitro evaluations have identified several drugs with significant anti-amoebic potential:

Antifungal Agents: This activity was significantly higher than the previously reported GI50 values for resveratrol and paclitaxel. The values of EC50 for Posaconazole and Itraconazole were in the range of $\leq 0.01 \mu\text{M}$. A water-soluble combination of Isavuconazole and Epiminolanosterol at low concentrations in combination with Tamoxifen also produced 95% growth inhibition. The growth inhibitory activities of Ebselen and its analogs BAY 11-7082 and BAY 11-7085 were also assessed and complete growth inhibition was achieved with all three compounds at significantly lower concentrations than MLT. Auranofin separately at $40 \mu\text{M}$ was much less effective than AmB but it killed 97% of the trophozoite with a concentration of $12.5 \mu\text{M}$ and did not exert a toxic effect on human astrocyte cells. The most active compounds in this study were Pitavastatin and Cerivastatin the IC 50 values of which ranged from $0.038 \mu\text{M}$ to $1.68 \mu\text{M}$. The most potential drug was identified as pitavastatin because of its capacity to cross the barrier of the central nervous system and cause apoptosis in the amoeba. Farnesyltransferase Inhibitors: Pitavastatin's potency was positive while combining it with Lonaferin yielded a growth inhibition rate of 95% (Madia et al., 2023).

Combined Drug Therapy and Synergy

This sterol profile has been recently the focus of investigations, which demonstrated that *N. fowleri* sterol pathway could be effectively targeted by using drugs in combination. This was treated individually with Isavuconazole which is an inhibitor of CYP51, Epiminolanosterol which inhibits SMT, and Tamoxifen which inhibits ERG2, and when combined, the mixture gave 95% growth inhibition. Furthermore, Ebselen and its derivatives demonstrated high effectiveness, and penetration and, thus, might be potentially valuable for the treatment of neurological diseases (Chao-Pellicer et al., 2023).

Additional Promising Agents

Staurosporine (STS): Showed high activity against *N. fowleri* trophozoites, indicating that it and its derivatives might be useful for treating PAM. Etomoxir (ETO), Perhexiline (PHX), Valproic acid (VPA), and Thioridazine (TDZ). All demonstrated some inhibition for amoebae, with the IC50 value of TDZ being the lowest. Combinations were shown to be additive or synergistic with mixtures of MLT to ETO (Siddiqui, Lloyd, Alharbi, & Khan, 2024).

Immunomodulatory in-vivo efficacy of drugs against *N. fowleri*

Primary amoebic meningoencephalitis(PAM) caused by the free-living *Naegleria fowleri* continues to be a difficult problem to manage because the disease usually presents itself abruptly and is often fatal. Unfortunately, up to now the disease is still highly fatal, and concerned therapeutic opportunities are rare and not completely efficient; the usual treatments are Amphotericin B (AmB) and Miltefosine (MLT) (Ahmad Zamzuri et al., 2023). Recent studies on the new molecular entities showed several promising compounds with excellent amoebicidal effects in experimental animals. These results present newer therapeutic approaches for PAM and suggest that there is still much to learn about repurposing drugs for this deadly disease. As for the in-vivo application of these drugs, the data has indicated their efficacy with significant variations in their safety in practice settings.

Chlorpromazine (CPZ)

Formerly classified as an antipsychotic drug, Chlorpromazine (CPZ) is one of the potent candidates for PAM therapy. In previous research works, it has been established that CPZ has a promising treatment index for infected mice, which amounts to 75% of survival, whereas AmB has only 35% of rats' survival, and MLT – 55% of rats' survival. In the in vitro study, AmB and MLT were effective in inhibiting *N. fowleri* at concentrations of 0.78 µg/mL and 12.5 µg/mL. Each of these drugs' in vivo efficacy, however, could be considered relatively low; the relevant survival rate equal to 40% and 55%. However, CPZ which possesses an in vitro inhibition concentration of 12.5 µg/mL fared better in vivo and had a survival rate of 75%. Further, no hepatic or nephrotoxicity was observed with doses of either 10 or 20 mg/kg; this has also provided some evidence for the safety of the current treatment as opposed to CPZ (Andrea Güémez & Elisa García, 2021; A Güémez & E García, 2021).

Hygromycin B (Hy) and Rokitamycin (RKM)

Hygromycin B (Hy) and Rokitamycin (RKM); antibiotics with possible amoebicidal activity have also been tested against *N. fowleri*. Fatma Wati and her colleagues also established in vitro actions of Hy and RKM as the most potent antibiotics with 100% inhibition at 12.5 µg/mL and 6.25 µg/mL, respectively. Thus, while they appeared very effective when tested on tissue culture, their in vivo performance was less consistent. In the case of infected mice,

the efficacy of the drugs RKM and Hy was markedly different, with 80% of the mice treated with RKM surviving the infection, while all the mice treated with Hy died from the disease. Another macrolide antibiotic, Roxithromycin (ROX), sustained growth inhibition at 25 µg/mL while that of survival rate was only 25%. Most important of all, no liver or renal toxicity was observed with RKM when administered at a dose of 20 mg/kg; therefore may be safer, at least in comparison with other compounds to which Krebs and Gonzalez et al assigned the same use classification (Andrea Güémez & Elisa García, 2021; A Güémez & E García, 2021).

Corifungin

Corifungin a water-soluble polyene macrolide is highly effective against *N. fowleri*. This compound causes oncosis resembling generally understood apoptotic cell death in amoeba trophozoites and demonstrates high amoebicidal efficacy. Earlier in vitro experiments demonstrated that Corifungin reduced the viability of amoebae by 20-100% with compound concentrations of 3.12-25 µM. Consistent in vivo assessments confirmed no mortality in infected mice administrated with Corifungin at 9 mg/kg/day for ten consecutive days. This is far better than Amphotericin B (AmB) which was able to record a survival rate of 60% in another study while Corifungin therefore ushers its articulation as a better treatment (Debnath, 2021).

Posaconazole (PCZ)

Recently, posaconazole (PCZ), an inhibitor of fungal enzymes, is often preferred in the treatment of PAM because of its quick kill rate of the amoebas. Out of the compounds, high-throughput phenotypic screening *S. cerevisiae* showed that PCZ is more effective, in growth inhibition of amoeba within 12 hours with a lower IC₅₀ value of 0.24µM. The compound also displayed synergism with Azithromycin (AZM), Amphotericin B (AmB), and Miltefosine (MLT) when the compounds were used jointly. However, many of those positive outcomes were achieved with only a 33 percent survival rate of the mice treated with PCZ at a dose of 20 mg/kg. However, the concomitant use of PCZ with AZM improved the survival rate indicating that even though PCZ has a moderate anti-cancer effect, the overall therapeutic effect may be boosted by combining it with other drugs (Nadeem et al., 2023).

Nanoparticle Conjugation

There is an excellent recent work on nanoparticles that work as drug delivery systems for the treatment of CNS infection including PAM. Conjugation of nanoparticles improves drug effectiveness and allows for the delivery of molecules, suggesting a potential for increasing therapeutic yield. This section discusses the developments in the field of nanoparticle conjugation in relation to AgNp, AuNp, and green nanoparticles for the management of PAM. Silver nanoparticles (AgNp) have been extensively investigated for their ability to enhance drug delivery and therapeutic efficacy (Debnath, 2021).

Antifungal Agents

The incorporation of AmB and NYS with AgNp has shown higher efficacy against *N. fowleri* compared to the free drugs. Interestingly, among all formulations, OA conjugated with AgNp was found to be significantly more active against the amoebae than the standard drug AmB (Debnath, 2021).

Anticonvulsants

Currently available FDA-approved anticonvulsants including Diazepam, Phenobarbitone, and Phenytoin when conjugated with AgNp offer improved amoebicidal properties. These results indicate that AgNp has the ability to augment the efficacy of currently employed drugs. A large number of new azole and bisindole derivatives conjugated with AgNp have also revealed good amoebicidal activity, which suggests that AgNp has the potential to potentiate a lot of therapeutic agents (Song, Zheng, & Zhao, 2024).

Gold Nanoparticle Conjugation

Gold nanoparticles (AuNp) have been explored for their ability to deliver and enhance the activity of various drugs:

Curcumin

D conjugation of curcumin with AuNp has enabled the enhancement of the amoebicidal efficacy against *Naegleria fowleri*. This implies that the AuNp has the potential to enhance the curcumin therapeutic outcomes. Trans-Cinnamic Acid: Alkylated trans-cinnamic acid (CA) linked to AuNp showed higher amoebicidal effectiveness also supporting the ability of AuNp to boost the efficiency of drugs (Debnath, 2021).

Green Nanoparticle Conjugation

Green nanoparticles, synthesized using environmentally friendly methods, have shown promising results:

Flavonoids

The present study showed that the (Andrea Güémez & Elisa García, 2021) conjugation of Hesperidin (HDN)/Naringin (NRG) with green nanoparticles displayed potent amoebicidal activity. Further, these conjugates also had lower cytotoxicity making it evident that green nanoparticles could be used to reduce system toxicity to optimize therapeutic gain. The process of conjugation of nanoparticles with different drugs has demonstrated certain potential in increasing the impact of drugs and targeting CNS infections like PAM. Each of these, silver, gold, and green nanoparticles has been found to have benefits in enhancing the activity of conjugated therapeutics. Nonetheless, subsequent in vivo approves are indispensable to pertain to these findings and to assess the preciseness of these nanoparticle-mediated drug delivery systems in the clinical setting (Andrea Güémez & Elisa García, 2021; Lê et al., 2023).

Prevention of Primary Amoebic Meningoencephalitis (PAM)

Primary Amoebic Meningoencephalitis (PAM) is a devastating infection of the central nervous system that occurs in humans due to the thermophilic amoeba *Naegleria fowleri*. This pathogen thrives in warm, inadequately chlorinated fresh water, for instance, causing serious consequences on anyone who contracts this disease. Measures that may help prevent PAM are most important in areas with high rates of this amoeba, which may help avoid the development of this complication. The information for this section includes major selective preventive strategies in relation to water quality, raising public awareness, and safe practices.

Water Quality Management

Chlorination and Water Treatment: The major preventable approach related to PAM is aimed towards making water that is used in recreational and therapeutic activities efficiently treated to get rid of *Naegleria fowleri*. This is particularly so in chlorination, which is a very important process in the organization. The World Health Organization WHO encourages water systems residuals with free chlorine concentrations of 0.5 mg/L and above. This

level of chlorine should not be more than 2.0 and should be effective at a temperature not exceeding 20°C and a pH of not more than 8.0 and water should be disinfected for a minimum of thirty minutes. To minimize the growth of the amoeba it is important that the levels of chlorination in water supply networks remain constant (Towne & Polivka, 2023). As earlier stated if chlorinated water is not readily available people should avoid using untreated freshwater or tap water where its contact with Nasal passage is required. It is therefore recommended that only distilled or sterile or filtered water with a purify level of 1 µm be used to rinse the nose. Tap water has undergone processes that make it harbour certain impurities that may be dangerous for nasal rinsing and thus distilled water is preferable. Another way is to heat water before using it – by doing this, most of the contaminants may be successfully removed. Users must also ensure they clean and drain all irrigation devices after each use; preferably with water that has been boiled, filtered or distilled or is sterile (Hall, Kumar, Golba, Luckett, & Bryant, 2024).

Citizens' Awareness and Enlightenment

Since PAM is a preventable disease, the mainstay of management is to educate the public on ways through which they can avoid contracting the disease. Specifically, in the highly risky regions including Pakistan, there is a need to conduct focused and targeted primary educative campaigns aimed at explaining the consequences of water chlorination deficiencies, and the measures that individuals can take to guard against the deadly diseases. Posters and informative writings should be assembled at schools, colleges, Masjids, and recreation centers to cover the many people possible. Community health education thus calls for treated water to be used and activities that could predispose one to amoebic infection avoided (Pook, 2020).

Safe use of Nasal Irrigation

Use of Purified Water: In nasal irrigation, water ought to be either filtered or purified. According to the CDC, the water treatment solution should be commercially available, distilled bottled water. In case of a lack of distilled water, clean water filtered through a filter not larger than one micron should be used. Another preventive measure that should be taken is to heat water before use (Hall et al., 2024).

Nasal irrigation solutions

Nasal irrigation solutions may be prepared using a saltwater solution of less than 0.9% non-iodized sodium chloride and a buffer of sodium bicarbonate. To help avoid PAM, proper sanitation of nasal irrigation devices is incredibly crucial. These devices should be washed, as in rinsing the mouth, with water that has been boiled, filtered, or distilled at the time that they are being used. Most germs spread in an environment should be removed through proper washing so that they do not pile up and cause diseases (Towne & Polivka, 2023).

Conclusion

Primary Amoebic Meningoencephalitis (PAM) is a rare and frequently fatal disease induced by *Naegleria fowleri* for which the main therapeutic difficulties are for a short period and the relatively low efficacy of available treatments. Again, the primary therapeutic effort is based on Amphotericin B (AmB), a fungicide that acts on ergosterol in amoebic cell membrane. Although AmB is effective, it is associated with low solubility, which affects its access to CNS; also, nephrotoxicity and hematotoxicity limit the use of the drug. Previous antimonial compound Miltefosine (MLT) was synthesised for Leish-manniasis and breast cancer which have also been found useful when administered in synergy with AmB and other drugs including Fluconazole (FCZ), Azithromycin (AZM), and Rifampin (RIF). MLT interferes with lipid metabolism in *N. fowleri* but has moderate therapeutic effects; its effectiveness can thus be enhanced and a better understanding pursued. These drugs exhibit outstanding in vitro effectiveness in combating *Naegleria fowleri*, but their ability to cross the blood-brain barrier is still an issue. Combination therapy which targets sterol synthesis has also demonstrated therapeutic potential against *Naegleria fowleri*.

Nanoparticles to deliver drugs offer one of the most possible and innovative methods of extending the chances of new treatment techniques. As for now, in the case of antifungal agents, AgNp boosts the Amphotericin B and Nystatin action, whereas AuNp, green nanoparticles amplify the curcumin and flavonoids action. The efficiency of current and future treatments is emphasized by in vivo investigations. An antipsychotic chlorpromazine gave an improved survival profile over AmB and MLT in experimental models, and Corifungin a polyene macrolide gave 100 % survival reflecting its better efficacy than amphotericin. Like other azoles posaconazole also has a potential for the treatment of

invasive molds but it is more effective when combined with other drugs to increase the survival of animal models. Elimination of PAM continues to be of most concern while stressing proper water chlorination, public health, and proper use of nasal rinsing among individuals. Chlorination and use of purified water while performing nasal activities cannot be over-emphasized, better still creating public awareness and regular maintenance of the devices. Altogether, one can conclude that drug repurposing, nanoparticle technology, and preventive strategies are potential new directions to manage PAM, but more studies are required to improve these perspectives and to adapt them for a clinic. The research findings may help inform efforts aimed at enhancing patient outcomes and reducing the grave effects of this emotionally devastating infection.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Conceptualization, Iqra Tabasum; Writing—original draft preparation, Amal Shaheen, Syeda Habiba Badar, Iqra Tabassum, Arfa Zubair Hussain, Areeb, Tayyaba Tahir; Writing—review and editing, Amal Shaheen, Syeda Habiba Badar, Iqra Tabassum, Arfa Zubair Hussain, Areeb, Tayyaba Tahir; supervision, Zunair Ahsan Syeda Habiba Badar; All authors have read and agreed to the published version of the manuscript.

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