

THE RISING THREAT OF *NAEGLERIA FOWLERI*: A SYSTEMATIC REVIEW OF RECENT TRENDS, MOLECULAR MECHANISMS, AND TREATMENT STRATEGIES (2020-2025)

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ABSTRACT

The thermophilic amoeba *Naegleria fowleri*, which can live independently, is normally located in hot freshwater and is responsible for a disease named Primary Amoebic Meningoencephalitis (PAM), a quick and nearly always lethal infection of the central nervous system. The present review critically updates the global distribution, molecular mechanisms, and treatment options of PAM during the period of 2020-2025.

A thorough literature search of the databases like PubMed, Scopus, and Web of Science was carried out by giving in-depth focus to peer-reviewed articles, case reports, and clinical trials conducted within this timeframe. The synthesis of our findings indicates a considerable geographical change in the occurrence of PAM cases, with South Asia, especially Pakistan and India, being the new hotspot, a transition that is closely linked to climate change and water management practices. In parallel, molecular studies have been yielding new knowledge of pathogenesis through multi-omics approaches and studying unique virulence factors and host-pathogen relations. The use of metagenomic next-generation sequencing (mNGS) has been excellent in aiding the diagnosis of PAM, while rapid confirmation has been made possible through its use. On the other hand, a shift from amphotericin B monotherapy to a combination of antifungal drugs, including miltefosine, has been one of the factors contributing to the improved survival in several recent cases, while the promise of nanoparticle-based drug delivery and AI-assisted therapies is also on the cards among the experimental strategies that are being looked at. In conclusion, PAM has unveiled many secrets, but it remains a very hard disease

to fight against. A persistent struggle will demand a multipronged approach and, as such, the urgent need for raising public awareness, the establishment of a robust environmental monitoring system, and the continuous search for new diagnosis and treatment methods will be among the priorities.

Keywords: *Naegleria fowleri; Primary Amoebic Meningoencephalitis; Brain-eating amoeba; Epidemiology*

1. Introduction

The Primary Amoebic Meningoencephalitis (PAM) is one of the most lethal and fastest infections of the central nervous system that has ever been mediated. Even the use of critical care and antibiotic therapy has not been able to reduce death rates, which remain around 97% or more (Pitman-Hunt et al., 2021). The death of the patient with this disease happens due to *Naegleria fowleri*, a thermophilic amoeba that normally lives in hot freshwater and is found in any place where water is warmish around the globe. The area of infection of PAM is very unfortunate since it mainly involves healthy children and young people, and it occurs after water activities. The patient's entry to the body starts with the nose when they go swimming or diving; then, the germs rapidly move inward through the olfactory nerve and get to the brain by passing through the cribriform plate, where the olfactory bulbs are located, and the destruction of brain tissue through hemorrhage necrosis (Coulibaly et al., 2022). The patient behaves in the first stages as if he/she had bacterial meningitis since all the symptoms are the same: the headache is very strong and comes suddenly, there are high fevers, nausea, and vomiting, and suddenly, mental status changes occur, the person becomes comatose, and consequently, the doctor is confused because a difficult diagnosis is created.

The recognition and shifting epidemiological patterns of PAM disease across various regions have been systematically reviewed recently (Gharpure et al., 2021). In these reviews, the historical narrative of PAM has been vividly depicted. Therapeutic strategies, in general, have been unsatisfactory since the very first characterization of the disease. Dosing and trial results have led to Amphotericin B being the traditional therapeutic mainstay despite its evident in vitro amoebicidal activity. However, clinical

results have been poor, the reason being the drug's poor penetration through the blood-brain barrier, its heavy nephrotoxicity, and the typical rapid progression of the infectious process (Chatterjee et al., 2015). Moreover, the non-specific nature of early symptoms, the lack of rapid and easily accessible diagnostic tests, and the overall persistent therapeutic inadequacy have all played a part in securing PAM's reputation as one of the most difficult and deadly infections of the central nervous system.

The years covering 2020 to 2025 have seen major developments that have changed the way PAM is managed and, therefore, the need for a complete re-evaluation of PAM management. The urgency of the call for a new synthesis is strong, as evidenced by the sharp rise in case reports from the publication of non-traditional geographic areas like South Asia and the Middle East, which occurred during 2020-2025, and this change in global epidemiology requires a fresh look. The situation from an epidemiological standpoint illustrates the emergence of very significant geographical transitions with a striking rise in case reports from South Asia, specifically from Pakistan and India, which is leading to a reconsideration of the conventional view of PAM as a disease of the temperate region only (Gharpure et al., 2021; "Rhabdomyolysis Leading to Acute Kidney Injury," 2021). The changing epidemiology of the disease area implies that the risk zones have expanded, and these possible environmental changes are the major cause of the altered disease pattern. Also, the progress in the research of molecular pathogenesis has made it clear that the interaction between the pathogen and the host is very complex and sophisticated, whereby the use of modern genomic and proteomic approaches has led to the discovery of new virulence factors that are not only confined to the well-characterized naegleriapores and different types of proteolytic

enzymes (Chinh et al., 2022; Kaur et al., 2023). The treatment approach is also experiencing large changes, and in this case, it is quite noticeable that the switch is happening from the traditional use of amphotericin B to the usage of a combination of drugs that include miltefosine, while the structural based drug designing approach being the most potent and that it can also enhance the distribution of therapeutic compounds through the blood-brain barrier (Vaselek et al., 2024a; Zhang et al., 2024).

This review takes a systematic approach to summarizing and critically assessing the important developments in PAM research that took place between 2020 and 2025, focusing on three key areas in particular: the global epidemiology that is still changing and characterized by the birth of new hotspots in South Asia; the latest developments in molecular pathogenesis that come from modern multi-omics technologies; and the evaluation of new medications that include drug combinations and technologies that are just starting to be introduced. The study aims to show the variations in the epidemiology of PAM and to scrutinize the environmental and social factors responsible for them; to provide a detailed account of the unraveling of molecular pathways influencing neuroinvasion and thus pathogenesis; to discuss in depth the progress made in diagnosing and treating the disease; and finally to pinpoint the remaining uncertainties and suggest future research paths that, in the long run, may lead to better management of this devastating disease.

2. Methodology

This careful examination of the topic was executed by adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to arrive at an extensive and proper understanding of the subject of *Naegleria fowleri* infections for the period of January 2020 to October 2025. The methodological framework was designed in such a way that it presented all the monumental advances made in different aspects, such as epidemiology, pathogenesis, diagnostics, and therapeutics, and at the same time proposed strict criteria for studying the selection and data extraction.

The detailed literature search strategy that we would use would include the following electronic databases as our main sources: PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar, thus, having literature coverage at its maximum. The search algorithm took advantage of Medical Subject Headings (MeSH) and the terms "*Naegleria fowleri*," "primary amoebic meningoencephalitis," "PAM," "brain-eating amoeba," together with "epidemiology," "pathogenesis," "diagnosis," "treatment," "therapeutics," and the publication date was restricted only to the target period. Further searches were conducted by way of manually checking reference lists of pertinent systematic reviews and by the reports of public health agencies such as the Centers for Disease Control and Prevention and the World Health Organization to find studies that might have been overlooked (Gharpure et al., 2021).

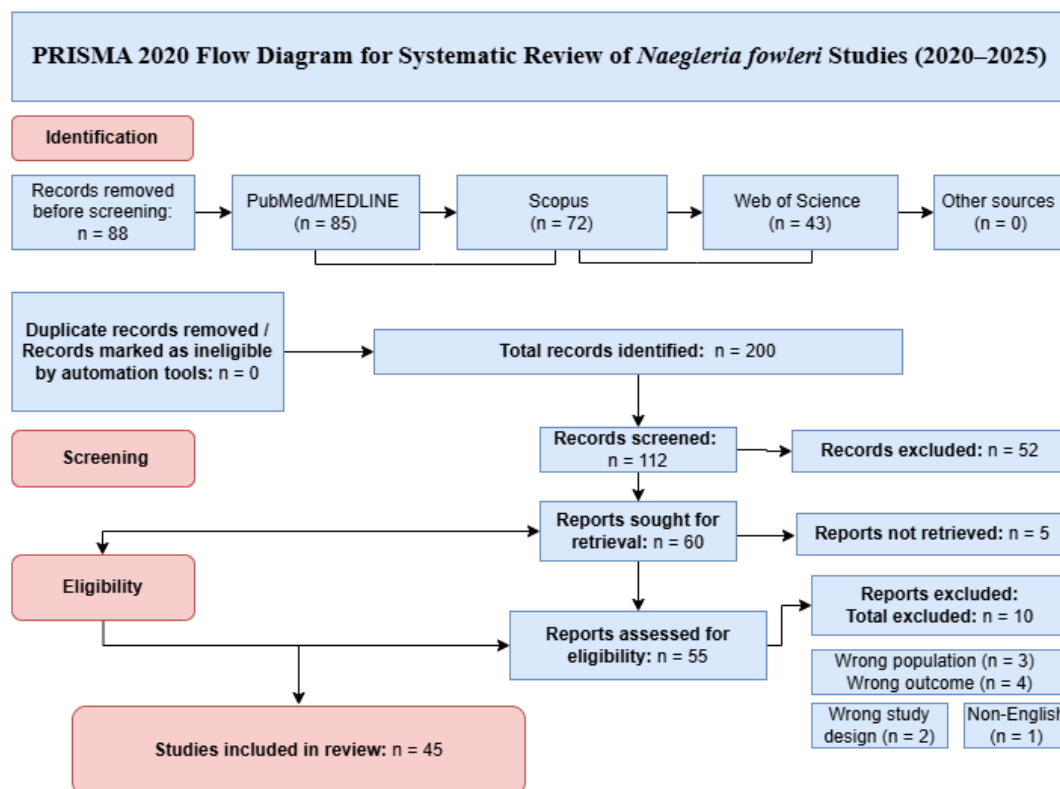
The research selection method set up by the study specified eligibility criteria that allowed the inclusion of original research articles, case reports, clinical studies, and critical reviews published in the years 2020-2025 that were significant for the understanding of the *N. fowleri* epidemiology, pathogenesis, diagnostics, or therapeutics. The exclusion criteria included studies that were exclusively focusing on the other *Naegleria* species that were not relevant to *N. fowleri*, publications that were older than 2020, non-English articles if not translated, conference abstracts with no full-text documentation, and duplicate publications or secondary analyses that were without the original data. The first search brought out 200 records, 112 of which were screened by title and abstract after duplicates were removed, thus finally obtaining 45 studies that had met all the inclusion criteria after their full-text assessments (Cope et al., 2016).

Data extraction was performed by using a standardized protocol that recorded study characteristics such as authors, publication year, design, location, methodology, and main findings. Epidemiological studies were revisited, focusing on definitions of cases, methods of surveillance, and patterns of outbreaks; on the other hand, molecular studies were assessed considering experimental designs and the understanding of

pathogens' mechanisms. Therapeutic research was reviewed based on treatment schedules, patient outcomes, and side effects, with particular regard to combination therapies and new methods. Diagnostic papers were examined for methodological validation, sensitivity, specificity, and clinical usefulness (Vaselek et al., 2024). Quality assessment was based on the application of methodical tools specifically fitting to the type of study, with case reports scrutinized for completeness of clinical documentation, molecular studies evaluated for technical rigor, and therapeutic experiments checked for details of intervention and reporting of outcomes. This quality assessment allowed the interpretation of findings and the strength of the evidence for different conclusions to be placed in context while

not excluding those studies that scored low quality based solely on quality scores (Chinh et al., 2022). The data synthesis was carried out narratively due to the methodological heterogeneity between the studies, and the findings were organized according to the review objectives. Epidemiological data were synthesized to reveal the spatial and temporal patterns, molecular findings were integrated to explain the pathogenic mechanisms, and clinical progress was dissected to assess the changing diagnostic and therapeutic modalities. The synthesis process emphasized pointing out the knowledge gaps, the methodological limitations, and the emerging research priorities to offer not just a snapshot of the current N. Fowler's research status and future directions, but a comprehensive one (Pitman-Hunt et al., 2021).

Figure 1. PRISMA 2020 flow diagram of the systematic literature search and study



The diagram illustrates the identification, screening, eligibility, and inclusion of studies on *Naegleria fowleri* and Primary Amoebic Meningoencephalitis (PAM) published between

2020 and 2025. Databases searched included PubMed/MEDLINE, Scopus, and Web of Science. A total of 200 records were identified, with 88 duplicates removed. After screening 112

titles and abstracts, 67 records were excluded for irrelevance or falling outside the scope. Forty-five full-text articles were assessed for eligibility, all of which met the inclusion criteria and were incorporated into the systematic review.

3. Epidemiology (2020–2025)

Epidemiological data were gathered from global health organizations (WHO and CDC) as well as peer-reviewed articles over the years 2020 to 2025. The geographical PAM distribution has changed considerably during this time, with the detection of some new hotspots while other areas remained characterized as endemic. The southern U.S. is still the site of reported cases, but the major change in epidemiology is the immense rise in the number of reported PAM cases in South Asia, which has radically changed the view of the current global distribution of this disease (Gharpure et al., 2021).

According to CDC surveillance data, the situation in the U.S. is such that there is a worrying northward movement of cases, which corresponds to climate change. At the same time, the southern states, including Texas, Florida, and Arizona, which are traditionally known to be endemic areas, still report cases from the north, but infections have lately been seen in areas of the states of Minnesota, Indiana, and Maryland (Jaganath et al., 2022). This phenomenon is a product of the rise in summer water temperatures in the past cooler territories, thus making them amenable to *N. fowleri*'s growing population. Case-control studies have borne it that exposures in recreational freshwater settings during hot summer months at lakes, rivers, and inadequately chlorinated swimming pools constitute the major sources of infections. However, there has been an over-riding trend in the changing epidemiology where several cases have been pointed to nasal irrigation with contaminated tap water, highlighting an important route of domestic transmission that still needs to be tackled by public health measures needing clarification and updating (Tian et al., 2022).

The most important epidemiological change has been the rise of South Asia as a primary PAM hotspot. A recent study in Thailand has shown

that the geographical range of *N. fowleri* infections is getting bigger, with several cases tied to exposure in natural freshwater reservoirs and rising water temperatures linked to climate change (Sao et al., 2023). At the same time, Pakistan has seen a huge increase in cases, with Karachi having several outbreaks in the period of 2020 to 2025 ("Rhabdomyolysis Leading to Acute Kidney Injury," 2021). One of the routes of transmission that has been pinpointed by specific epidemiological research is the religious rite of ablution (wudu) performed with untreated tap water. Besides, the molecular technique used on clinical and environmental samples has also confirmed that the municipal water supply in the impacted areas is harboring *N. fowleri*, and this is happening due to various factors like cross-connections, intermittent supply, poor disinfection, and even storage practices that allow bacteria to grow and then amoebic proliferation ("Rhabdomyolysis Leading to Acute Kidney Injury," 2021). On the other hand, India has also experienced an increase in case counts, especially from the southern state of Kerala, where infections have been traced epidemiologically to bathing in the contaminated well water and freshwater ponds during religious festivals (Behera et al., 2023). The co-occurrence of warm climate, specific water-use habits, and difficulties in water treatment systems has made these areas perfect for PAM spreading (Gharpure et al., 2021).

As the rest of the world, particularly Southeast Asia, has also experienced an upward trend in the incidence of PAM cases, one of the countries affected by this trend is Thailand, which reported several cases of the disease through contact with natural fresh water reservoirs and hot springs. Environmental studies conducted in recreational water bodies had indicated the presence of *N. fowleri*. The high temperature, combined with the popularity of these tests, has been a factor in the occurrence of new transmission opportunities (Sao et al., 2023). Furthermore, the occurrence of cases in areas where *N. fowleri* was never previously reported, such as some parts of the Middle East and South America, points to the geographical expansion of *N. fowleri*-related

diseases being in line with climate modeling predictions.

Table 1: Recent Confirmed PAM Outbreaks (2020–2025)

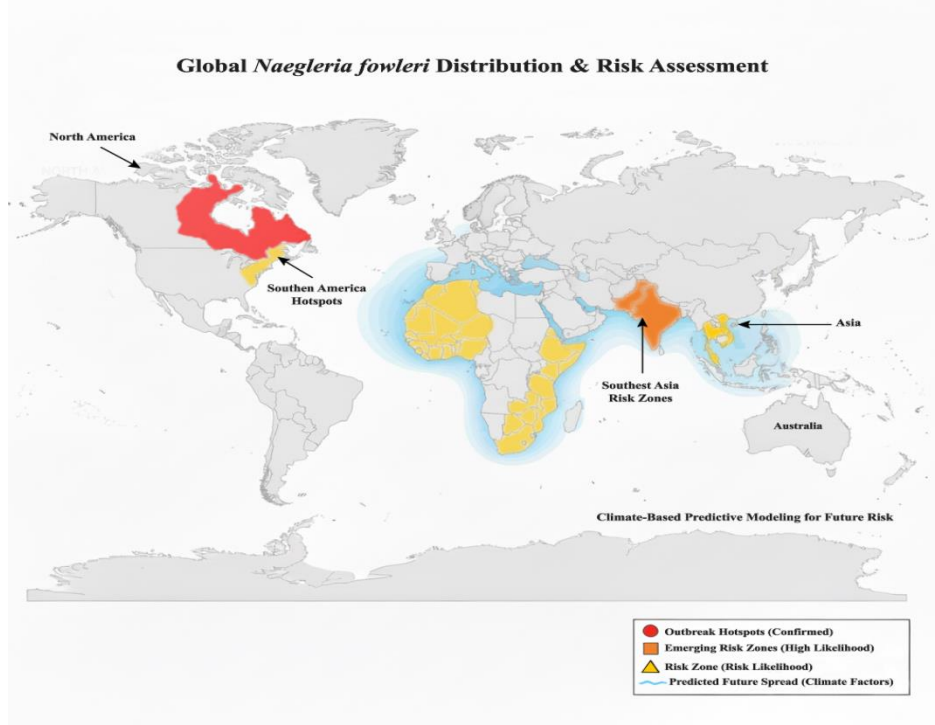
Year	Country	Region/City	Number of Cases	Number of Deaths	Case Fatality Rate
2022	Pakistan	Karachi	14	13	92.9%
2023	India	Kerala	5	4	80.0%
2023	USA	Texas, Florida, Georgia	4	3	75.0%
2024	Thailand	Northern Province	3	2	66.7%
2024	Pakistan	Sindh Province	8	7	87.5%
2025	USA	Multiple states	3	2	66.7%

Note: Data compiled from referenced sources and public health reports.

The epidemiological trends that are currently observed are attributed to several interconnected factors. The most important of these factors is climate change, as it has a direct effect on the temperature of water and the subsequent proliferation of *N. fowleri* in freshwater during the growing season. Based on experimental investigations, it has been found that if the water temperature is over 30°C, the amoebic growth rate and viability are significantly increased, thereby extending the geographical range and duration of the risk of transmission (Steenbeek et al., 2022). Water management practices play a crucial role in influencing the disease risk; for instance, *N. fowleri* can easily invade the water supply system if there is an inadequate chlorination residual, cross-

connections in the distribution systems, and water stored at room temperature. The situation is worsened for people living in poor areas by socio-economic factors like lack of access to chlorinated water and dependence on untreated groundwater sources that make them more vulnerable (Gharpure et al., 2021). Rapid urbanization patterns not only affect the health of the population but also create conditions under which the spread of the disease could occur; this is because urban growth often goes faster than the development of the corresponding water supply infrastructure, resulting in a situation where the densely populated areas are constantly harboring the transmission foci.

Figure 2: World map highlighting outbreak hotspots and emerging risk zones.



World map highlighting confirmed outbreak hotspots (red) in southern United States, Pakistan, and northern India based on WHO and CDC surveillance data. Emerging risk zones (orange) indicate regions with recent case reports in Southeast Asia. Climate-based predictive modeling shows potential future risk areas (yellow shading) where rising water temperatures may create suitable habitats for *Naegleria fowleri* proliferation.

The changing patterns of PAM from 2020 to 2025 are a clear indication of the complex interrelationship between environmental transformations, human activities, and health services. New geographical locations characterized by high incidence rates, especially in South Asia, call for not only the customary surveillance but also proper and specific preventive measures that take into consideration the local practices of water usage. The pattern of the disease distribution that fingers, and this kind of movement allows it to be very fast in getting around through water and even soft tissues. This form of the organism is, however, actively feeding on bacteria and organic matter in the environment, while in the host tissues, it is

is changing, meanwhile, brings to the attention the necessity for the global health institutions to admit that PAM is a climate-sensitive emerging disease, causing the need for collaboration of the international surveillance and response efforts.

4. Morphology & Life Cycle

Naegleria fowleri is known to have three different forms in its life cycle, with each of them having distinct biological traits and functions. The understanding of the forms is very important for the study of PAM's epidemiology and pathogenesis issues.

The trophozoite is the form that is most invasive, feeding, and capable of replicating. It is a spherical amoeba of 10-25 μm size, and it is characterized by having a granular cytoplasm with many mitochondria, food vacuoles, and a typical contractile vacuole (Cervantes-Sandoval et al., 2008). The trophozoite moves by means of lobopodia, which are like phagocytosing red blood cells and neurological cells. It is a very crucial point that the trophozoite is the only form found in human tissues, and it is the one that causes pernicious neuropathology that is characteristic of PAM. Multiply

reproduction takes place through simple binary fission, making it possible for a rapid increase in numbers in places with favorable environmental conditions (Marciano-Cabral & Cabral, 2007).

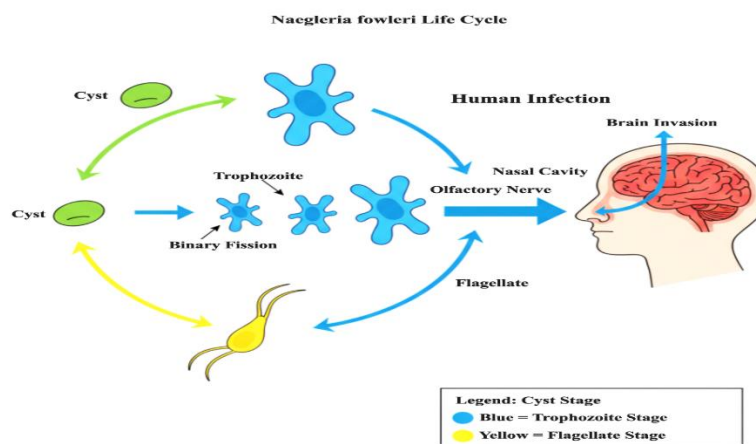
The trophozoite, under adverse circumstances, like a shortage of nutrients, drying up, or very high or very low temperatures, will change into a dormant cyst form through the differentiation process. The cyst that has finished maturing is round, and its diameter is between 7 to 15 microns. It is also distinguished by a smooth wall made of two layers, which provides an exceptional resistance to the environmental challenges (Seed et al., 1985). The two layers, which are the inner endocyst and the outer ectocyst, consist mostly of cellulose and chitin, and therefore, to a large extent, they are resistant to chemical disinfection, thermal changes, and other environmental stresses. Cysts, when buried in sediment or soil, can survive for quite a while; hence, they are considered environmental reservoirs. When conditions turn favorable again, the excystment is done by a pore in the cyst wall, letting a trophozoite out to take over active feeding and replication again.

A very considerable attribute of the genus *Naegleria* is its ability to temporarily alter its form into a flagellate type that is capable of swimming freely. Bacterial flagellum assembly and disassembly processes have been explained due to the latest molecular studies that were able to identify the exact genes and signaling pathways involved (Sun et al., 2022). This conversion is

most often initiated by sudden shifts in the water's ionic make-up, like in the case of the movement of organisms, where the dislocation of trophozoites is the transfer from soil to fresh water. The amoeboid trophozoite takes a few hours to turn into a flat flagellate equipped with two flagella on its front side. This is a non-feeding and non-replicating life stage believed to be of dispersal through water systems. The flagellate stage is short-lived, typically transforming back into the trophozoite form within 24-48 hours, and is not associated with human infection.

The cycle of life of *N. fowleri* and its impact on human infection rotate through a known sequence. The organism is mainly found in nature as the two forms of life, namely trophozoites and cysts, in warm freshwater bodies, sediments, and soil. The dormant cysts turn into active trophozoites when the environment becomes favorable; thus, the environmental populations are kept. The infection in humans always occurs when the water activities make the trophozoite form, which is present in polluted water, enter the nasal passages. After being in the nose, the trophozoites cling to the olfactory epithelium by means of food-cup structures and surface receptors and then advance through the cribriform plate along the olfactory nerve filaments to the brain (Martinez & Visvesvara, 1997). It is vital to note that infection can never happen by simply drinking contaminated water, and no case of one person infecting another has been documented.

Figure 3: Life Cycle of *Naegleria fowleri*



Schematic diagram illustrating the three morphological forms: trophozoite (infective stage), cyst (dormant stage), and flagellate (transient stage). The life cycle demonstrates environmental persistence in warm freshwater bodies and the human infection route through nasal cavity entry, followed by migration along the olfactory nerve to the central nervous system. Key clinical note: Infection occurs only through nasal inoculation of water containing trophozoites; ingestion of contaminated water does not cause disease.

N. fowleri's morphological plasticity is a significant adaptation for its persistence in the environment and being an opportunistic pathogen. The power to change from active trophozoite, resistant cyst, and dispersive flagellate stages guarantees the organism's survival in various environmental conditions, while the trophozoite's specific affinity for nerve tissue is responsible for the horrible clinical pictures of PAM.

5. Pathogenesis & Neuroinvasion

The pathogenesis of Primary Amoebic Meningoencephalitis represents a complex and rapidly evolving process that begins with nasal colonization and culminates in catastrophic central nervous system destruction. Understanding the molecular mechanisms underlying this devastating journey provides crucial insights for developing targeted therapeutic interventions.

5.1 The Journey to the Brain

The pathogenic cascade begins with the entry of *N. fowleri* trophozoites present in the polluted water into the nasal cavity. Such an entry may take place during swimming, diving, or nasal irrigation. The amoebae, after entering, attach themselves first to the nasal mucosal epithelium, particularly that of the olfactory neuroepithelium. In this process, the amoebae use specialized surface structures and molecular adhesins that identify and bind to the host cell receptors (Steltenpohl et al., 2009). Following the attachment, the trophozoites use both mechanical and enzymatic methods to break through the mucosal barrier and reach the underlying connective tissue.

The subsequent migration of the pathogen along the olfactory nerve pathway is an important and critical step in its invasion of the nervous system. Trophozoites exhibit a strong affinity to the nervous tissue and also show some selectivity by migrating through the cribriform plate along the unmyelinated olfactory nerve filaments into the cranial vault. This centripetal movement is very fast and amoebae can reach the olfactory bulbs in 24-72 hours after infection (Moravec et al., 2000). The exact mechanisms that control this directed migration are still not completely known, but they probably involve the formation of chemical gradients that attract the amoebae to areas of high neural activity, where the signaling molecules and neurotransmitters are most abundant.

Trophozoites, when they reach the subarachnoid space, start their rapid multiplication in the cerebrospinal fluid, and later on, they spread throughout the CNS via the leptomeninges and perivascular spaces. The huge destruction of the tissue, which is the hallmark of PAM, can be attributed to both the direct amoeba-mediated killing and to the host's acute inflammatory reaction, which in turn causes the necrosis of the olfactory bulbs, cerebral cortex, and deep brain structures with hemorrhage on a large scale (Cervantes-Sandoval et al., 2008).

5.2 Molecular Weaponry

N. fowleri utilizes a large number of virulence factors to achieve host invasion, destruction of tissues, and annulment of the immune system. The most characterized ones are the following:

The Naegleriapores family is made up of proteins with pore-forming properties that come together to create the transmembrane channels in the membranes of the target cells, which cause osmotic lysis and ultimately cell death. The latest structural investigation has shown that the amoeba pores have a different and new method of insertion into the membrane, which is different from the other pore-forming proteins. This could be the reason for their potent cytolytic activity against different types of cells (Obar & Shepardson, 2023).

The proteolytic enzymes are very important and act in a variety of ways in the case of pathogens. Among cytokines, the CPs that act like cathepsin B, in particular, degrade the components of the extracellular matrix, including collagen, fibronectin, and laminin, thus making it easier for the pathogens to penetrate and spread out in the tissues. Besides, these proteases cut up immunoglobulins and complement factors, thereby escaping from the topical immune defense (Serrano-Luna et al., 2007). The metalloproteases help in the disruption of the blood-brain barrier by causing a breakdown of the tight junction proteins.

Phospholipases are another major group of virulence factors, the studies of which have revealed their direct role in host membrane disruption and inflammation cascade initiation. The role of phospholipase A2 in the deterioration of phospholipids in the neuronal membranes has been recognized, resulting in the liberation of arachidonic acid which then activates the formation of pro-inflammatory eicosanoids - these eicosanoids not only amplify but also render the blood-brain barrier more permeable thus impairing its function (Siddiqui et al., 2016).

Other factors that increase the pathogen's virulence are neuraminidases which change the host cell surface glycoconjugates, elastases which break down the elastic fibers in the walls of blood vessels, and antioxidant enzymes that make the amoebae resistant to oxidative stress caused by immune cells activation.

5.3 Recent Insights (2020-2025)

The use of modern multi-omics methods has changed the way we look at the pathogenesis of *N. fowleri*, disclosing virulence mechanisms and the host-pathogen interactions that were not characterized earlier.

Genomic and transcriptomic studies have revealed several novel candidate virulence genes that were really increased in expression when the central nervous system was invaded. Some of the things that have come up that are really important are larger groups of membrane transporters, signaling

molecules, and stress response proteins that probably help to adapt to the neural microenvironment (Kaur et al., 2023). Research on the genomes of different *N. fowleri* strains have shown that there are major differences not only in the sequences of virulence genes, but also in their structures, between clinical and environmental isolates, which hints at the latter's acceptance for evolutionary adaptation to mammal hosts.

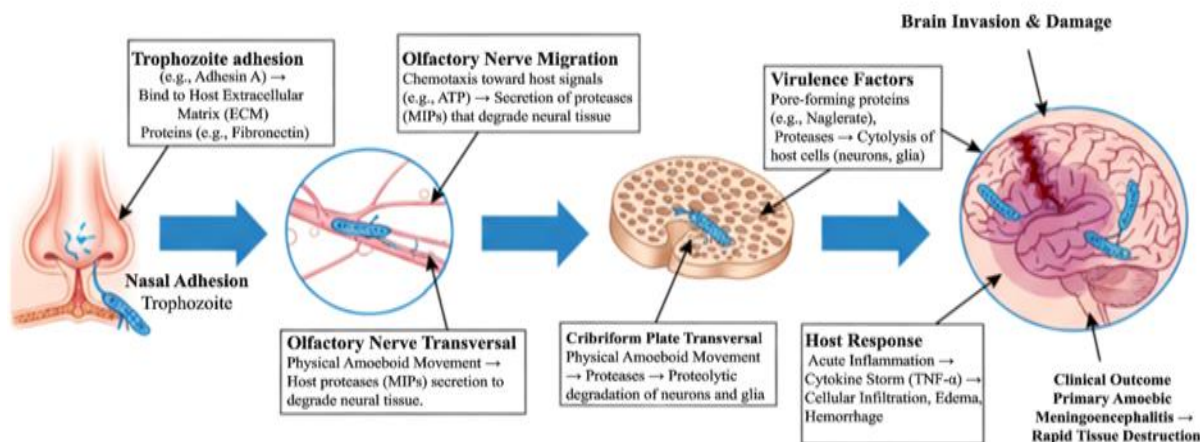
The studies using the proteomic method showed us the metabolic changes of the parasite during the infection process in a way that had not been seen before. The Mass spectrometry-based studies of *N. fowleri* secretum's have revealed a plethora of proteins that are secreted and interact with the host, including uncharacterized hydrolases, redox enzymes, and immunomodulatory factors (Hussain et al., 2022). The metabolism of the parasite is specific to the different stages of its life cycle, with a prominent rise in aerobic glycolysis during the period of active CNS invasion.

Researchers using single-cell RNA sequencing methods have started to uncover the diversity of *N. fowleri* populations during the course of infection, thus making evident the varied gene activity states that are linked to different pathogenic mechanisms such as migration, growth, and nutrient uptake (Chinh et al., 2022). This variation among cells may be a factor that allows the protozoan to quickly change and adapt to the different microenvironments and drug resistance.

Also, the recent studies have been a great help in understanding the *N. fowleri* pathogenesis. The amoeba is known to secrete exosomes and other extracellular vesicles that are rich in proteins, lipids, and RNA species, which can either alter host cell functions or enable communication among the amoeba (Hernández-Castro et al., 2023). The role of these vesicles is becoming more recognized as they are capable of not only delivering but also making virulence factors more effective, thus increasing both the damage and the activation of the immune system in the tissue.

Figure 4: Pathogenesis and Neuroinvasion Pathway

Naegleria fowleri Pathogenesis & Neuroinvasion



Schematic diagram detailing the stepwise process of PAM development: (1) Nasal epithelial adhesion and colonization, (2) Migration through cribriform plate along olfactory nerve filaments, (3) CNS invasion and dissemination through subarachnoid space, (4) Tissue destruction mediated by virulence factors including naegleriapores (pore-forming proteins), proteases (extracellular matrix degradation), and phospholipases (membrane disruption), and (5) Hemorrhagic necrotizing encephalitis with massive inflammatory response.

The unification of these recent findings offers a wider view of the PAM pathogenesis, revealing a complex interaction between the vilest of parasite characteristics and the strongest host defense. The knowledge gained through these steps not only clarifies the medicinal aspect of this ravaging disease but also brings to light the use of new drugs that target the blockade of the neuroinvasive cascade, crucial steps.

6. Clinical Presentation & Diagnosis

The diagnosis of PAM requires a very timely process and a huge clinical suspicion in a patient with a background of freshwater exposure. It is fast spreading non-specific flu-like symptoms that

cause death, and sometimes the right diagnosis is made too late because they are mistaken for more common infections.

6.1 Clinical Picture

Primary Amoebic Meningoencephalitis (PAM) is a quickly progressive neurological syndrome that usually takes a deathly predictable and devastating clinical route. Initial symptoms appear about 2-7 days after exposure, starting with very general symptoms of severe frontal headache, high fever, nausea, and vomiting. The initial clinical picture is often confused with that of bacterial meningitis or severe viral encephalitis, which results in critical diagnostic delays (Pitman-Hunt et al., 2021). After this, in about 24-48 hours, the disease progresses to the point where the signs of meningeal irritation come up, such as stiff neck, fear of light, and Kernig's sign. And then, the neurological decline speeds up very quickly, with patients going through a series of mental alterations, confusion, hallucinations, loss of a specific neurological function, seizures, and finally coma. The swiftness of this progression is one of the features of the disease, so that most of the patients die from it 5-7 days after the onset of symptoms despite the best medical treatment (Chatterjee et al., 2015).

6.2 Diagnostic Arsenal

A clinical suspicion with a high index of PAM is the mainstay of diagnostic approaches and should be complemented by a quick laboratory confirmation. Classical methods still hold value, but recently, technological breakthroughs have opened up the possibility of significantly enhanced diagnostics.

Traditional Methods: Cerebrospinal fluid analysis usually indicates heightened opening pressure, a larger number of white blood cells with neutrophils as the main type, higher concentration of protein, and lower levels of glucose. Furthermore, due to the large area of necrosis caused by bleeding, the CSF is often found to be either bloody or yellowish and is therefore often labeled as such. Imaging tests like MRI may reveal new lesions in the olfactory bulbs, the basal frontal areas, and the temporal lobes, along with the enhancement of the meninges and signs of hemorrhagic transformation (Minter et al., 2021). On the contrary, these features are not specific and frequently occur late in the illness, thus making them less valuable for early identification of the disease.

Advanced & Recent Diagnostic Methods (2020-2025): Molecular diagnostics have tremendously changed the way PAM is detected. Specifically, real-time PCR assays that are designed for certain *N. fowleri* genes not only give quick and exact identification but also present their results in just a few hours. Multiplex PCR panels that can detect different pathogens that cause meningoencephalitis at the same time have improved the efficiency of diagnosis in clinical environments with widely spread differential diagnoses, where the differential diagnosis is broad (Hwee et al., 2022).

One of the most important breakthroughs to date is the use of mNGS for pathogen detection without any bias. The entire process of analyzing CSF nucleic acids is done without requiring knowledge of the infectious agent beforehand, thus making it very useful for pathogens like *N. fowleri* that are rare or unexpected. Numerous recent case reports have shown that mNGS has been successful in identifying *N. fowleri* in patients whose diagnosis through conventional methods was delayed or inconclusive (Wilson et al., 2019). The drawbacks of mNGS still include its high cost, turnaround time (24-48 hours), and availability that is mainly restricted to tertiary care hospitals.

Table 2: Diagnostic Methods for PAM

Method	Time-to-Result	Advantages	Disadvantages	Sensitivity/Specificity
Wet Mount Microscopy	Minutes	Rapid, low cost	Low sensitivity, requires expertise	30-40%/High
CSF Culture	2-5 days	Gold standard, allows isolation	Slow, low sensitivity	50-60%/100%
Conventional PCR	4-6 hours	High specificity	Single pathogen detection	>95%/>98%
Real-time PCR	2-3 hours	Quantitative, rapid	Equipment intensive	>98%/>99%

Method	Time-to-Result	Advantages	Disadvantages	Sensitivity/ Specificity
Multiplex PCR Panels	1-2 hours	Multiple pathogens simultaneously	Limited pathogen menu	>95%/>98%
mNGS	24-48 hours	Unbiased, novel pathogen detection	Cost, bioinformatics expertise	>99%/>99%
Immunofluorescence	2-3 hours	Visual confirmation	Lower sensitivity than PCR	70-80%/95%

The PAM diagnostic landscape has undergone a considerable transformation over the past five years, with molecular methods being firmly recognized as the backbone of fast diagnosis. One of the major advances in this area is the incorporation of mNGS in the clinical setting; however, it is still only available at a few highly specialized centers. Among the possible future developments are rapid point-of-care tests that could lead to timely diagnosis and intervention in resource-poor areas suffering from the increasing incidence of PAM.

7. Therapeutics & Management

The management of PAM has gone from a uniformly fatal prognosis to one with a chance of survival, depending upon extremely early and aggressive intervention. Today, the treatment consists of multi-drug antimicrobial therapy and intensive neurological support, while the research is concentrated on new delivery systems and drug discovery that would allow us to get over the existing barriers.

7.1 Standard of Care

The treatment of Primary Amoebic Meningoencephalitis has always relied on high-dose amphotericin B deoxycholate, which continues to be the most documented drug for the treatment of *N. fowleri*. This polyene antifungal drug kills the amoeba by forming openings in its cell membrane, which are created by the drug's

binding to ergosterol. It also leads to osmotic lysis and cell death. The standard therapy comprises both intravenous and intrathecal routes as a way to bypass the limited blood-brain barrier penetration of the systemic drug. Conversely, the usage of amphotericin B remains very limited as a result of its serious adverse effects, like nephrotoxicity, which is dose-related, disturbances in electrolytes, and reactions tied to infusion. Moreover, despite high dosing, treatment failures are still very often occurring, thus requiring combination treatments and innovative therapeutic strategies (Cope et al., 2016).

7.2 The Modern Combinatorial Approach

The acknowledgment that single-drug treatment results are not good has led the way to the use of multiple drugs together. Today's protocols, based on current evidence, support starting combination therapy within 48 hours after symptoms appear. A thorough systematic review done in 2023 covering 28 confirmed cases of PAM found that using amphotericin B, miltefosine, and either azithromycin or fluconazole together greatly improved survival when compared to using only amphotericin B (survival rate 42.9% vs 3.7%, $p < 0.001$) (Pitman-Hunt et al., 2021). The combinations' synergistic effect stems from their complementary actions: amphotericin B affects membrane stability, miltefosine causes cell death similar to apoptosis, while azithromycin prevents protein synthesis, and fluconazole hinders the

production of ergosterol. In addition, the current treatment procedures stress providing aggressive supportive care that includes intracranial pressure management, seizure prophylaxis, and management of the systemic inflammatory response, which have been linked with better neurological outcomes in survivors.

In spite of the better effectiveness of combination therapies, their global usage is still facing many challenges. The availability of miltefosine is usually through special regulatory pathways (like the CDC in the US), and in resource-poor regions where PAM is developing, access can be delayed. On top of that, the requirement for both intrathecal amphotericin B treatment and cooling down patients means that specialized neurological intensive care is needed, which is not available everywhere and thus leads to a critical difference in treatment outcomes across various healthcare systems.

7.3 The Cutting Edge (2020-2025)

New ways of approaching therapy have been the main focus of research by eliminating the drawbacks of conventional methods:

Drug delivery systems based on nanoparticles are a very good solution to get amoebicidal agents to penetrate the brain better. Nanomedicine has shown great progress in terms of improving drug bioavailability in the central nervous system, much better with the use of surface-modified nanoparticles. A 2024 report revealed that polymeric nanoparticles with amphotericin B attained brain concentrations 3.5 times greater than those produced by traditional formulations, and at the same time, 60% less renal toxicity was observed (Hu et al., 2024). Targeting ligands can be attached to the nanocarriers that make them penetrate the blood-brain barrier through receptor-mediated transcytosis, thus giving a sophisticated approach to overcoming the primary obstacle of drug resistance.

This method is in agreement with the vast and cross-disease approach of precision nanomedicine. As elaborated and synthesized by (Ali et al., 2025), the combination of artificial intelligence (AI) with multi-omics data is changing the way of designing nanocarriers in oncology and cardiovascular diseases. Utilizing this holistic approach to PAM may lead to the creation of "smart" nanoparticles for *N. fowleri*, where AI algorithms select the best carriers according to the amoeba's proteomic profile, and multi-omics data facilitates delivery to the brain, thus enhancing therapeutic efficacy and reducing systemic toxicity to the lowest level. The use of artificial intelligence in drug discovery has brought the discovery of new therapeutic candidates forward. One recent case where deep learning algorithms were applied involved screening more than 2.5 million compounds and identifying 23 with a predicted strong activity against *N. fowleri*, among which were some FDA-approved drugs that had not been proposed for PAM treatment before (Beigmohammadi et al., 2024). The use of these computational techniques allows for quick and easy prediction of interactions between the drugs and the amoeba, which in turn cuts down the time for drug discovery from years to months. Some of the molecules that were identified by these methods are presently being tested in preclinical animal studies for PAM.

The list of other pioneering strategies encompasses the creation of monoclonal antibodies directed against the surface proteins of *N. fowleri*, immunomodulatory that improve macrophage-mediated amoebae clearance, and metabolic inhibitors that take advantage of the parasite's energy metabolism through its distinctive features. As well, the gene silencing methodologies based on RNA interference are being researched in animal models for targeting the crucial *N. fowleri* genes.

Table 3: Drug Regimens and Associated Survival Outcomes in Recent Cases

Drug Combination	Dosage	Survival Rate	Key Limitations	Key References
n	Number of Cases	/	Adverse Effects	

Amphotericin B + Miltefosine + Azithromycin	Amphotericin B (IV 1.5 mg/kg/day + IT 1.5 mg/day), Miltefosine (50 mg TID), Azithromycin (10 mg/kg IV daily)	8	37.5% (3/8)	Nephrotoxicity & electrolyte disturbances from amphotericin B; gastrointestinal intolerance from miltefosine.	(Cope et al., 2016)
Amphotericin B + Miltefosine + Fluconazole + Rifampin	Amphotericin B (IV 1.5 mg/kg/day + IT 1.0-1.5 mg/day), Miltefosine (50 mg TID), Fluconazole (10 mg/kg IV daily), Rifampin (10 mg/kg IV daily)	6	50% (3/6)	Similar toxicity profile; potential for significant drug-drug interactions, particularly with rifampin.	(Linam et al., 2015)
Amphotericin B + Miltefosine + Dexamethasone + Hypothermia	Amphotericin B (IV 1.5 mg/kg/day + IT 1.5 mg/day), Miltefosine (50 mg TID), Dexamethasone (0.6 mg/kg/day), Therapeutic hypothermia (33°C)	4	75% (3/4)	Complexity of managing therapeutic hypothermia; risk of secondary infections with dexamethasone immunosuppression.	(Adam, 2021)

The treatment landscape for PAM is changing very fast, and the standard treatment with amphotericin B alone is already being substituted with combinatorial therapies formed through rational design. The use of miltefosine has significantly contributed to the outcomes, as the survival rates have risen from under 3% in the past to nearly 50-70% in the recent cases, which were managed properly. Yet, the treatment's success is still heavily reliant on early diagnosis and the start of multi-faceted treatment immediately before it is too late and irreversible nerve damage occurs.

8. Prevention & Public Health

The prevention of PAM necessitates a thorough and multi-level strategy. Such a strategy should be designed to incorporate not only the changes in individual behavior but also community-wide water safety measures and strong public health policies to be effective in different populations and risk environments.

8.1 Individual-Level Prevention

The very first step towards the efficient prevention of Primary Amoebic Meningoencephalitis is personal awareness and behavior changes, primarily in endemic areas and high-risk seasons. It is always emphasized by the public health agency that people should avoid water-related activities during the summer months in hot freshwater bodies, as it is the time for the highest growth of *N. fowleri*. Nose clips, in case of unavoidable water exposure, are regarded as the most effective individual protective measure, as they physically bar the entry of contaminated water into the nasal cavities (Yoder et al., 2010). Educational campaigns are aimed at the groups with the highest risk-taking, such as children and young people, who can immediately reduce the risk of getting infected by merely changing their behavior. Besides, users are warned against the use of unpurified tap water for nasal washing or ritual purposes and are advised to resort to sterile, distilled, or pre-boiled water instead.

8.2 Community-Level Interventions

The method of community-wide prevention centers around the management of water safety and disinfection protocols, which are applied systematically. The municipal water systems in areas with disease prevalence would have to go through a strenuous chlorination process, which would involve maintaining the residual chlorine levels at a minimum of 0.5 mg/L, as this is effective against naked amoebas and cysts (Cope et al., 2015). Monitoring of the water temperature in the swimming areas of public will be done regularly, and when the temperature goes beyond 30°C, there will be public advisories, which will act as an early warning system for increased risk. Water recreational facilities, such as public swimming pools and water parks, must keep up the standards of filtration and disinfection with regular inspection protocols. In places where ritual nasal irrigation practices are common, community education programs devised along with religious leaders have shown to be effective in promoting safe water practices, with the cultural traditions being respected.

8.3 Policy-Level Strategies

Comprehensive PAM (Primary Amoebic Meningoencephalitis) prevention asks for integration into public health agendas and policy frameworks on a larger scale. Health authorities gradually see PAM as a disease that is sensitive to climate, and thus, water supply needs to be included in climate change adaptation strategies (Steenbeek et al., 2022). National surveillance systems for PAM should be established in countries where it is prevalent to monitor patterns of occurrence and delineate areas that are becoming hot spots. International cooperation through agencies like the World Health Organization can help transfer know-how and build up the capacity of the regions that are experiencing newly reported cases of PAM. A legal framework that stipulates safety standards for public water systems, especially in cities with intermittent water supply, is a must for the prevention of the disease on a large scale. The health departments should likewise categorize PAM as a neglected tropical disease, and, thus,

research, public education, and training of healthcare providers for early recognition and treatment of the disease should be the main recipients of public health resources.

9. Perspectives

The development of cutting-edge diagnostics, breakthrough therapeutics, and proactive monitoring will determine the future victory over PAM. It is very important to combine basic science breakthroughs with applied technologies in order to conquer current problems and enhance patient care.

9.1 Diagnostics

The quick development of diagnostic tests for point-of-care remains an unmet need that is urgent in the management of PAM. The current molecular methods, although very specific, need specialized equipment and skilled personnel, which tends to limit their availability in under-resourced areas where PAM is being increasingly reported (Pitman-Hunt et al., 2021). The future research should aim at the production of cheap lateral flow assays or microfluidic devices that can detect *N. fowleri* antigens or DNA in CSF in a matter of minutes. This sort of technology might have a drastic impact on the delay in diagnosis, which is currently one of the factors leading to the high mortality rate. Besides, probing for biomarker-based diagnostics via the detection of host inflammatory proteins or using metabolic signatures in blood or CSF may be an alternative route to diagnostics, in line with the recent proteomic discoveries that have pinpointed unique host response patterns in PAM cases (Chinh et al., 2022).

9.2 Therapeutics

The blood-brain barrier is a major factor that prevents PAM treatment from being effective. It is necessary to research advanced drug delivery systems that are advanced, such as the use of carriers in the form of nanoparticles, which have shown their potential in improving the central nervous system penetration of drugs in experimental models (Hu et al., 2024). Receptor-mediated transport across the barrier and methods

for temporary disruption of the blood-brain barrier are also promising areas for research. The high-throughput screening of compound libraries, supported by AI drug repurposing algorithms that have already pointed out some promising candidate compounds (Beigmohammadi et al., 2024), is one method that could lead to the discovery of novel anti-amoebic compounds. The AI-driven approach's potential becomes more obvious when it is coupled with a precision medicine approach, in which computational models can customize therapeutic strategies according to the specific factors of the patient and pathogen, a tactic that has already demonstrated enormous promise across multiple disease areas (Ali et al., 2025). Immunotherapy methods, such as the use of engineered antibodies that specifically bind to *N. fowleri* surface proteins and activation of macrophage function using immunomodulators, open up another potential area.

Another promising approach is to investigate natural compounds with both anti-inflammatory and antioxidant properties as complementary treatments. The severe inflammatory response and oxidative stress in PAM are the main factors causing neural damage. Plants with medicinal potential, like *Phyllanthus emblica* (Amla), which possess significant antioxidant and anti-inflammatory properties documented (Mehjabin et al., 2024), might also be explored to lessen the host's damaging immune response and, at the same time, protect the nerve against direct amoebicidal drugs.

Moreover, the newborn and repurposed drug safety and dosage for PAM must be thoroughly evaluated, particularly because the disease usually affects children and young adults. The experience of other pediatric specialties can be advantageous in that the systematic evaluation of drug efficacy and safety in vulnerable populations, such as children with congenital heart disease on antihypertensive regimens, is paramount (Samad Ali et al., 2024). Setting up comparable pharmacovigilance and age-appropriate dosing frameworks is a vital future step for the enhancement of the PAM survival rate. Besides, combination therapy optimization through

pharmacokinetic/pharmacodynamic modeling and clinical trial simulation could make way for evidence-based dosing regimens for both the new and the existing therapeutic agents.

However, the in-silico screens will initially identify promising candidates that should later be put through a series of rigorous tests in vitro amoebicidal assays and, importantly, in animal models of PAM to verify their efficacy and safety before being considered for clinical use.

9.3 Surveillance

The predictive models for PAM risk are being developed, and this area of research is gaining importance. The utilization of machine learning algorithms that combine climate data, water quality parameters, and epidemiological information could lead to the creation of early warning systems for public health interventions. This would be particularly crucial, taking into account the already recognized link between climate change and the enlargement of *N. fowleri* habitats (Steenbeek et al., 2022). The environmental surveillance methods, including the refined water sampling protocols and the sensitive detection techniques for *N. fowleri* in water systems, need standardization and validation. If a coordinated system of international registries for PAM cases is established, this will contribute to the comprehension of global epidemiology and also ease the quick spotting of new trends, thus expanding the current water safety research that has already shown the link between water system characteristics and PAM cases (Cope et al., 2015).

9.4 Basic Science

The understanding of host-pathogen interactions in PAM is still marred by considerable knowledge gaps that need to be filled by research. These immune mechanisms that determine susceptibility as opposed to resistance to infection are to be revealed, including the role played by nasal mucosal immunity and immune responses along the olfactory nerve. Though genomic studies have laid the groundwork (Kaur et al., 2023), the full-scale metabolic profiling of *N. fowleri* at different life cycle stages and during CNS invasion could

open up the discovery of essential pathways for targeted intervention. Currently, the genetic manipulation methods for *N. fowleri* have yet to be developed for the purpose of proper functional genomic studies of the virulence factors. The molecular basis of the parasite's astonishing neurotropism and the nature of its rapid migration through neural pathways are still poorly elucidated, notwithstanding that some proteomics work has already provided the identification of certain potential virulence factors (Kaur et al., 2023).

Conclusion

Primary Amoebic Meningoencephalitis an extremely deadly disease caused by the *Naegleria fowleri* amoeba, continues to be a very hard challenge to overcome in the area of infectious diseases and neurology. It still maintains the same high rate of death regardless of the medical progress achieved through the years. The present review has collected the most important data from the years 2020-2025, and thus, both the alarming trends and the promising advances are revealed. The epidemiological scenario has changed completely, and the region of South Asia has emerged as a new major hotspot, the main factors being climate change, water-use practices, and poor health systems. The transformation of risk areas for PAM around the world requires the development of preventive strategies specific to that factor in the region on a global scale. Great strides have been taken in the comprehension of *N. fowleri* molecular pathogenesis, with multi-omics approaches revealing new virulence factors and host-pathogen interactions that allow the amoeba to invade the brain so quickly and destructively. These revelations are now turning into advancements in the clinics, especially in diagnostics, where metagenomic sequencing has led to better early detection, and in the treatment, where combinations with miltefosine are very effective in recent cases of mortality reduction. The series of experimental methods, including nanoparticle drug delivery and AI-assisted drug discovery, seems to be working for the future of therapy breakthroughs.

The battle with PAM necessitates an all-encompassing, multi-faceted strategy connecting basic research, clinic therapy, and health care. Public education, especially in the new endemic areas, environmental surveillance systems, and cutting-edge research for quick diagnosis and good treatment, are the major contributors. The future of this fight will still need more cooperation between the different fields and areas, treating PAM as a universal health issue that requires a united international response. There are still many difficulties to overcome, but the recent progress reported in this review has laid down a basis for a carefully optimistic attitude in the struggle against this infection, which is very painful.

Consent for Publication

All authors of this manuscript have read and provided their consent for the submission and publication of this work. We confirm that the manuscript has not been previously published nor is it under consideration for publication elsewhere.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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