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A Short Review on Brain Eating Amoeba

Venkata Naga Kiranmayi Garlanka, Harikrishnan Narayanaswamy , Kevin C. George , Pavithra Thiruvengadam, Pavithra Velmurugan, Praveen Rajendran, Sneha Sri Ramachandran

Faculty of pharmacy, Dr. MGR Educational and Research institute, Velappanchavadi, Chennai-77.

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ABSTRACT

The first recorded instance of absurd primary amoebic meningoencephalitis (PAM) occurred in Florida, USA, in 1962, and was caused by the free-living amoeba *Naegleria fowleri*. PAM, a waterborne disease found in swimming holes, clean water, and aquatic environments, is brought on by an infection with N. fowleri. It results in the cerebral hemispheres becoming inflamed, spongy, enlarged, and blocked. *Acanthamoeba fowleri* causes it, while acanthamoeba spp. and *Balamuthia mandrillaris* develop Granulomatous Amoebic Encephalitis (GAE). PAM can also be caused in animals. Serological tests are only marginally helpful in diagnosing PAM due to the rapid death of most patients. *N. fowleri* may have grown as a successful pathogen due to its ability to evade the host immune system and stick to nasal mucosa. PAM lacks distinctive clinical features and is frequently confused with other forms of bacterial or viral meningoencephalitis, hence a comprehensive clinical history is essential for diagnosis. PAM has been treated less successfully, although "drug repurposing," or re-profiling current drugs, is a useful strategy for treating illnesses that go untreated.

*** CORRESPONDING AUTHOR:**
Dr.GVN Kiranmayi
Kiranmayi54@gmail.com

1. Introduction

A free-living, eukaryotic amoeba called *Naegleria fowleri* bears Malcolm Fowler's name. Malcolm Fowler was the first to identify the *Naegleria fowleri*-caused primary amoebic encephalitis (PAM)[1,2]. According to estimates, a 'dangerous brain-eating amoeba' is making its way up from the America' southern regions. The Centers for Disease Control and Prevention (CDC) states that cases are migrating northern. PAM develops when an amoeba enters the nose and travels to the brain. The sickness gets worse when people dive or swim in warm aquatic habitats[3]. When contaminated water travels from the nasal cavity to the brain, *Naegleria fowleri* can induce pam, a rare but dead-

ly brain infection. Infection commonly occurs while diving or swimming in contaminated water, which is frequently prevalent in warm, fresh water in southern us states. It's important to take precautions and avoid activities in warm, stagnant freshwater during hot weather to reduce the likelihood of getting sick[4]. *Naegleria fowleri*, *Acanthamoeba spp*., and *Balamuthia mandrillaris* all have been confirmed as representatives of numerous genus of free-living amoebae that exist in nature and tied to human encephalitis. The three most common genus that cause illness are Acanthamoeba, Balamuthia, and Naegleria. Acanthamoeba and Balamuthia develop granulomatous amoebic meningitis in immunodeficient and critically ill, respectively, but Naegleria causes primary amoebic meningoencephalitis in healthy young people, which is a more serious and fatal condition. Because of the lack of typical symptoms and test results, a variety of amoebic encephalitic syndromes are challenging to identify. Given the influence on future medicines, all of these brain infections cause significant fatality around the globe, with more than 90% of cases being lethal. Growing regions of human and amoebae activity in some locations, together with global climate change and population expansion, lead to increased contact, resulting in more chronic conditions and increasing public notice. This condition seems to have a low morbidity but a high mortality rate, making efficient treatment and diagnosis a massive task[5]. They may invade a host and exist as pathogens within the host organisms, therefore they named as an amphizoic amoebae. They are characterised as amphizoic amoebae due to their ability to penetrate a host and exist as parasites within the host organisms.

Among these, there are many Acanthamoeba species (including *Acanthamoeba castellanii* and *Acanthamoeba culberstoni*) and just one species from three genus: Balamuthia (*Balamuthia mandrillaris*), Naegleria (*Naegleria fowleri*), and Sappinia (*Sappiniapedata*). The first three free-living amoeba (FLA) groups are also liable for a large number of human and animal infections[6,7].

1.1. The History and Epidemiological Studies of Primary Amoebic Meningoencephalitis.

The first case of ridiculous PAM was made in Florida, USA, in 1962. PAM was named after Malcolm Fowler of Adelaide Maternity hospital in Australia and R. F. Carter, who had already identified the condition in 1965. Butt and Carter developed the name "primary amoebic meningoencephalitis" in 1966 and 1968, however, to separate it from the mysterious brain infection caused by Entamoebahistolytica[8].

1.2 The United Arab Emirates Has Brain-Eating Amoebas

Warm weather and heavy water consumption in the UAE raise the danger of waterborne illnesses such primary amoebic meningoencephalitis brought on by *Naegleria fowleri*. There aren't any known instances of this ailment in the UAE, though. The cleanliness of the water has been questioned in light of a Sharjah research that discovered coliform bacteria in household water tanks. Meningitis and primary amoebic meningoencephalitis share many symptoms, making it possible that cases of this ailment are still remaining misdiagnosed. These factors also include the absence of premortem diagnosis and the low rate of autopsy. Further investigation is required to ascertain the incidence of *N. fowleri* in the UAE and to raise awareness of this dangerous virus within the medical and public health communities[9]. This data shows that either the *N. fowleri* strain found in Pakistan is unique from strains found elsewhere in the globe or that it has evolved a resistance to saline conditions. Scientists' focus is now on climate change because to the spread of *N. fowleri*in Pakistan. Summers are becoming longer and much moister as a result of climate change, making water bodies an ideal habitat for amoebas. Examples of these unique contents might be found using a genomic method that examines the entire genome of *N. fowleri*. An exciting study opportunity exists using this technology to explain the genetic sequence of the recently identified resistant strain in Pakistan, which will benefit in early sickness detection as well as prevention[10,11].

2. An Investigation on "Brain-Eating Amoeba".

2.1. Etiology

Amoebic meningoencephalitis is mediated by *N. fowleri* infection. Fresh water with *Naegleria fowleri*

enters the nose and propagates to the Central Nervous System (CNS), causing this condition. Participating in recreational activities like swimming in hot seas can often result in this. Before showing any signs of encephalitis, this virus must incubate for one to fourteen days[3].

2.2. Epidemiology

Swimming holes, freshwater environments, and aquatic ecosystems all contain it, whereas saltwater does not. This virus has been isolated in New Zealand, Europe, Africa, Asia, USA, and Australia. Thermally toxic water may also contain the protist. Infectious diseases can be transmitted through leisure and religious activities. The reports state that between 2007 and 2008, 34 cases were reportedly seen. Many people enjoy swimming in swimming pools in the summer. Pam, which increases susceptibility to amoebic meningoencephalitis, is however little understood. Numerous untreated water sources are available, which is a significant contributor to the increase in pathogen levels in various water reservoirs[3].

2.3. Biology and Ecology

Many FLA have two stages of growth: the trophozoite, which feeds on nutrients, and the cyst, which rests. These FLA include the previously stated Acanthamoeba spp., B. mandrillaris, and S. pedata. The cyst is a dormant stage that is tolerant to the difficult conditions and has a size of less than 10m. The trophozoite is a pathogenic place that contributes in amoeboid movement. It is generally a few hundreds of microns in size[12]. When nutrients are few but water is adequate, some amoebae, most notably Naegleria spp., move from the trophozoite stage to the extra flagellate stage[13]. *N. fowleri*'s flagellate stage is typically pyriform and ranges in length from 10 to 16 m. They often transform back into trophozoites in an hour or less since they neither divide nor eat. The trophozoite, a metabolically active stage, consumes predominantly Gram-positive and Gram-negative bacteria, as well as algae, fungi, and other organisms via binary fission, the trophozoite grows and occasionally actively and continuously modifies its size and shape[12,14]. Pathogens, like Acanthamoeba and Naegleria, frequently have ectocysts and endocysts. Encystation occurs when the pH is unstable, the osmotic pressure is not enough, or the temperature is too high. It also happens when there is a nutritional shortage and when anti amoeba drugs are present[15]. Acanthamoeba and Naegleria species have been discovered in regulated water sources like potable water, tap water, coolers, private pools, hydrotherapy pools, and residential water ways[16,17]. They also affect non-traditional water sources including sewage and aquariums[18].

2.4. Immunology

Serological tests are only marginally beneficial in the diagnosis of PAM since most PAM patients pass away quickly after infection, leaving insufficient time for an immune response to develop a detectable defense. IFA has mostly been fruitless in previous attempts to identify an antibody response to *N. fowleri*[19]. Yet, a patient from California who recovered from this illness was found to have a particular antibody response to *N. fowleri*[20]. According to immunoassay studies conducted at the Centre for Disease Control and Prevention (CDC), Immunoglobulin G was the most common kind of antibody generated by this patient and three other persons who acquired PAM. The CDC analysis also discovered that IgM antibodies to *N. fowleri* were detected in the serum of many patients who had previously learned to swim mostly in freshwater lakes in the south eastern and also in California. These antibodies have been developed specifically for the antigens of *N. fowleri* that are approximately 190, 66, 30, and 14 k Da[21].

2.4.1 An Immune Response to *N. fowleri* and Pathogenesis

Automatic defence due to its potential to evade the host immune system, as well as its capability to stick to nasal mucosa, migrate more swiftly, and destroy target cells thru trogocytosis and the synthesis of cytolytic chemicals, *N. fowleri* may have grown as a successful pathogen. It has been established that Naegleria resist being eliminated by host cytolytic agents such as tumour necrosis factor (TNF)-a, interleukin (IL)-1, and the membrane attack complex (MAC) C5b-C9 of com-

plement [22,23]. Based on the present level of knowledge, innate immunity may be more important than acquired immunity in determining resistance to *N. fowleri* infection. There is evidence that the innate immune system elements complement, neutrophils, and macrophages respond to *N. fowleri* infection. Given the current state of knowledge, innate immunity rather than acquired immunity might prove to be more successful in preventing *N. fowleri* infection. There is a data that complement, neutrophils, and macrophages are immune system cells that react to *N. fowler i*nfection. It is very obvious that *N. fowleri* generates pore-forming proteins that instantly kill or inhibit the mammalian cells, secretes proteases and phospholipases that degrade mammalian tissue, and synthesises regulatory surface proteins that safeguard the amebae from complement-mediated necrosis and other cytotoxic agents. Unfortunately, a complete understanding of the pathophysiological processes is still lacking. Comparing pathogenic and non-pathogenic Naegleria, pathogenic *N. fowleri* move and divide more quickly when there are nerve cells and their byproducts present. Their capability to effectively spread within the host and evade the immune system of the host may boost their pathogenicity. In addition to being disease-causing organisms like *N. fowleri*, ameboflagellates can also serve as hosts for pathogenic bacteria and, in this position, may screen pathogens from antibiotics and environmental compounds. Therefore, their medicinal value will not be minimized. Microorganisms from environmental biocides and host-side antibiotics [24].

3. Pathogenic Mechanism of *N. fowleri* Infections.

The cerebral hemispheres are frequently inflamed, spongy, significantly expanded, and blocked. The leptomeninges are engorged, diffusely hyperemic, and opaque, with fairly modest quantities of purulent exudate in the sulci, brainstem, cerebellum, and brain base. The olfactory bulbs are bordered by hemorrhagic necrosis, which is commonly accompanied by purulent discharge. There are also several superficial haemorrhage patches seen in the cortex. The predominance of tumors are located on the bottom of the orbitofrontal and temporal lobes, the base of the brain, the hypothalamus, the midbrain, the pons, the medulla oblongata, and the upper section of the spinal cord. The subarachnoid space above the cerebral hemispheres and the cisternae around the midbrain may have been destroyed, according to CT images. When the intravenous contrast agent has been administered, these areas may exhibit substantial diffuse enhancement [25,26]. The cerebellum, brain stem, and upper portion of the spinal cord are all loaded with fibrino-purulent leptomeningeal fluid composed largely of Polymorphonuclear neutrophil [PMN] a few eosinophils, a few macrophages, and a few lymphocytes. Large numbers of amoebic trophozoites are observed throughout edematous and necrotic brain tissue, even within pockets, without the presence of PMNs. Trophic amoebae are also seen deeper within Virchow-Robin gaps, usually near blood vessels and without obvious inflammatory response. A big nucleus with a central, strongly stained massive nucleolus distinguishes amoebae between 8 and 12 mm in size, and polyclonal or monoclonal antibody staining can be utilized to detect them as *N. fowleri*. Cysts of amoeba are noticeably missing. Amoebae from *Naegleria fowleri* proliferate in meninges, brain tissue [19,20,26,27].

3.1. Primary Amoebic Meningoencephalitis:

Acanthamoeba fowleri causes it, while acanthamoeba spp. and balamuthiamandrillaris develop Granulomatous Amoebic Encephalitis (GAE). The free-living amoeba *Naegleria fowleri* induces Primary Amoebic Meningoencephalitis (PAM), a severe, fulminating brain infection in the central nervous system [28]. PAM is a short-term disease, but GAE is a chronic to subacute infection that can persist for months. Despite breakthroughs in clinical detection, diagnostic tools, and treatment modalities, amoeba-related Brain infections remain to be lethal. Neuroimaging research indicates that there are common sites for tumors, however the locations of tumours are not always stable and can probably depend on the causative factor. Further study is essential to determine the hereditary, immunology, pathogenic, and environmental factors that lead to amoebic meningoencephalitis, which is lethal. Pathogenic amoebae's capacity to serve as hyper parasites and host other microbial infections as res-

ervoirs has also increased their potential as infections of increasing concern to the well-being of humans and animals[4]. Others have suggested that the incidence of PAM may further rise as a result of worldwide changes in climate and temperature [29-31]. According to the thermophilic characteristics of *N. fowleri*, the majority of case exposures (85%) were particularly documented during warm, hot, or summer seasons [5]. In general, cases mostly affected young guys. This population might be more likely to engage in behaviours that enhance their chance of *N. fowleri* infection, or they may be predisposition due to sex-related hormones, as has been demonstrated for other infections such as Entamoeba histolytica liver abscesses [32]. Early (flu-like prodrome alone) Fever, headache, nausea/vomiting, and exhaustion/lethargy Respiratory. The later involvement of the CNS altered the mental state. Nochal rigidity Seizures, coma, photophobia, and drowsiness are all possible symptoms. Kernig and Brudzinski's logo brittleness to the extreme cranial nerve problems with hazy vision and unsteady stride abnormalities in perception [33].

3.2 Primary Amoebic Meningoencephalitis in Animals.

In addition to humans, *N. fowleri* can also cause PAM in animals. Many unprovoked occurrences of meningoencephalitis or encephalitis in carnivores, horses, ruminants, and certain wild animals have also been documented [34]. In addition to humans, *N. fowleri* can also cause PAM in animals. Many unprovoked occurrences of meningoencephalitis or encephalitis in carnivores, horses, ruminants, and certain wild animals have also been documented [35].

4. Diagnosis

A thorough and accurate clinical history is crucial for diagnosis because PAM lacks distinguishing clinical characteristics and is frequently mistaken for other bacterial or viral meningoencephalitis. Specifically in kids and young people, primary data should have any current patient contact with water and a history of allergies and disorders of the upper respiratory tract, such as rhinitis[36]. Computed tomography (CT) scans are frequently conducted in cases of early-stage *N. fowleri* infection, which later results in cerebral edoema and the obliteration of cisterns as the illness worsens. With an average-sized ventricle, the sulcus and surrounding grey matter are also highly improved [37]. When pictures from magnetic resonance imaging (MRI) are merged, they reveal that the brains of PAM patients with edoema and hydrocephalus have multifocal parenchymal lesions, pseudo tumor lesions, hemorrhagic infarcts, meningeal exudates, and necrosis [38]. For the detection of *N. fowleri* in healthcare and environmental samples, polymerase chain reaction (PCR), nested PCR, quantitative PCR, and multiplex PCR tests are much more accurate, quick, and detailed [39-43]. Additionally, it has been clearly illustrated that a PCR analysis can determine the *N. fowleri* in formalin-fixed, paraffin-embedded brain tissues. In terms of specificity, upcoming droplet digital PCR already exceeds quantitative PCR [44,45].

4.1. Treatment and Diagnosis.

Fewer patients have made it through PAM. One of these people who survived was a young Californian lady who had extensive therapy with parenteral route of Miconazole and Amphotericin B, and oral route of rifampin. She was perfectly healthy and devoid of any neurological impairments throughout a 4-year follow-up. It was believed that amphotericin B and miconazole has synergistic effect whereas rifampin had no impact on amoebae[29]. The triazole drug voriconazole proved effective against *Naegleria fowleri*; low quantities (less than 10 mg mL-1) were amoebastatic, whereas high concentrations (more than 10 mg mL-1) were amoebicidal [46].

4.2. Anti-Naegleria Drugs.

A practical method for treating such untreated disorders is known as "drug repurposing," which involves re-profiling existing medications. Examining the anti-mycotic clinically authorized medications for anti-Naegleria action was prompted by the similarity between the sterol production pathway in free-living amoebas and pathogenic fungi. Amphotericin B (Amp B), a polyene antifungal medication, was intended to

serve as the primary form of therapy for PAM. Amphotericin B kills target cells by attaching to ergosterol in the cell membrane, which changes the permeability of the membrane by generating holes. Because of its numerous negative effects, multiple studies were conducted to ascertain the effectiveness of amphotericin when combined with other medications. Due to its toxicity, including dose-related nephrotoxicity and acute infusion-related responses, amphotericin B has a restricted therapeutic usage[47-50]. The majority of individuals with amp B report fever, chills, vomiting, nausea, and headache, and Amp B may also result in anaemia. Amphotericin B's lower water solubility, which affects clearance, compartmentalization, and dissolution, may be the cause of the bulk of its complications [51,52]. The most clinical manifestations of Amp B usually involve fever, chills, nausea, headache, and vomiting. Anaemia can also be an adverse effect of Amp B. The massive majority of amphotericin B's difficulties may be due to its reduced water solubility, which has an impact on clearance, compartmentalization, and dissolution. Voriconazole, fluconazole, and azithromycin are further treatments that have shown to be effective against *N. fowleri* [53].

In order to assess the amoebicidal effects of three medications—Nystatin, Amphotericin B, and Fluconazole—silver nanoparticles alone, medications alone, and medications fused with silver nanoparticles were cultured with *N. fowleri*. This was done using Nano formulation technology to increase drug availability. Our results shown that anti-amoebic medication efficacy is greatly increased by silver nanoparticle conjugation [54]. As nanoparticles are believed to promote therapeutic efficiency, multiple therapies were combined with different nanoparticles and examined against free-living amoebas in prior studies. This approach seems effective. Conjugated medication was discovered to have substantial amoebic action against *N. fowleri*[55].

It was discovered that the anti-*N. fowleri* properties of diazepam, phenobarbitone, and phenytoin were enhanced when the medicines were conjugated with silver nanoparticles [56]. The antiamoebic properties of the investigated drugs were assessed. The compounds ZnO-CD-AMPi, ZnO-CD-CFT, ZnO-CD-Nar, ZnO-CD-AMB, and ZnO-CD-QT have been identified as having substantial amoebic effects against both *N. fowleri* and B. *mandrillaris*. This was determined using cytopathogenicity assays. ZnO-CD-AMB proved to synthesize the greatest reduction in host cell pathogenic carried by *N. fowleri.*

These Nano conjugates may be used as disinfectants against amoebae in reservoirs for water, which calls for more investigation, particularly in developing countries where water is usually short[57]. On comparison to free pharmaceuticals, NPs may be adjusted to cross the BBB very easily. Moreover, they are capable of administering drugs intravenously. This may thus prove to be helpful in the management of amoebic infections of the brain where the BBB poses a considerable barrier to medicament delivery to the brain. Reduced side effects during treatment will also result from the potential of altering NPs so that they selectively target particular cells and release the medications in response to biological or environmental stimuli. As a by-product of their biocompatibility, they able to penetrate the Blood Brain Barrier, capability of targeting certain cells, and ability to only release their load in sensitive to stimuli from outside, NPs hold great potential. NPs have a lot of potential for managing brain infections caused by harmful amoebae[58]. It has been demonstrated that metallic nanoparticles (NPs), such as AuNPs and AgNPs, improve the efficacy of medications against brain-eating amoebae [59-62]. Localized magnetic fields have been used to increase the death of injured amoebae by inducing hyperthermia or the build-up of metallic NPs in target tissue [63-65].

Furthermore, liposomes are friendly, biodegradable nanoparticles (NPs) that may be customized to primarily target specific cells and release medicaments in relation to signals from environmental factors [66]. Site-specific targeting can be another opportunity of solid lipid nanoparticles for the controlled release of both lipophilic and hydrophilic medicaments [67,68]. Solid lipid NPs have been proven to administer medicaments into the brain at a desired quantity that is significantly higher than free medicaments [69]. The versatility of modification that permits the generation of NPs with controlled sizes, shapes, external surface charges, internal morphologies, and functions makes polymeric NPs tremendous promise as delivery vehi-

cles [70]. Natural compounds derived from two medicinal plants, R. *yaundensis* and S. *triloba*, were evaluated against *N. fowleri*. The compounds under investigation have significant amoebic activity[71]. By preventing host cell toxicity caused by amoebas, these substances. When evaluated on human cells, each chemical had a very low cytotoxicity level. These findings suggest that plant-based natural drug entities may be effective in treating infections brought on by *N. fowleri*. The cost efficient of these extremely positive results will be determined by further study into different parasites and their potential as disinfectants for use in residential storage tanks for water.

Ursolic acid, betulinic acid, and betulin decreased the vitality of amoebas; betulin showed the highest reduction in amoeba viability [72]. The substances were also discovered to lessen host cell death caused by *N. fowleri*. While present therapies for *N. fowleri* have demonstrated to be toxic and capable of harming tissues, plant-derived chemicals are crucial because they have powerful anti-amoebic action while exhibiting little cytotoxicity [72]. Since that amoebae are common in the brain, the medicaments should be capable of passing the highly selective blood-brain barrier without having a negative effect. In order to establish the translational relevance of these discoveries, the pharmacodynamics, pharmacokinetics, and effectiveness of the effective drugs should be investigated [73]. Significant cidal, inhibition of encystation, and excystation effectiveness was seen with both benzodiazepines and phenytoin against amoebae that consume the brain. With a significant improvement shown at as little as 10 M concentrations, AgNP conjugation improved the bioavailabilities of these medications. Drug-conjugated silver nanoparticles displayed widespread antiamoebic action against both trophozoite and cyst stages, and remarkable improvement is shown against both amoebas. As these CNS-targeting drug-conjugated AgNPs might presumably be used as recycled therapies against brain-eating amoebae, they are worthy of further evaluation for In-vivo research. Lastly, we want to use these nanoparticles for targeted brain release to treat CNS infectious diseases caused by free-living amoebas in In-vivo models.

5. Conclusion

A devastating pathogen in warm freshwater habitats is the Brain-eating amoeba Naegleria fowleri. Enhancing survival rates necessitates early discovery, which calls for more research and public health awareness campaigns. Tissue invasion, immunological evasion, and inflammatory damage are all part of the disease's pathophysiology. The disease's fast progression and vague symptoms make diagnosis difficult. Clinical suspicion, CSF investigation, PCR, and sophisticated antigen detection techniques are necessary for an accurate diagnosis. Better management requires increased diagnostic tool accessibility and provider awareness. Rapid illness progression, challenges with early identification, and a lack of adequate treatment options are the main causes of the high death rate.

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