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Review

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Naegleria fowleri and Primary Amoebic Meningoencephalitis: From Environmental Reservoirs to Fatal CNS Infections

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Abstract

Naegleriafowleri, a thermophilic, free-living amoeba, is the causative agent of *primary amoebic meningoencephalitis (PAM)*, a rapidly progressing and often fatal infection of the central nervous system. During exposure to contaminated warm freshwater, the parasite enters the human host via the nasal cavity, migrates along the olfactory nerves, and induces severe inflammation in the brain. Despite its high fatality rate (> 95 %) , effective treatment remains elusive due to delayed diagnosis and limited therapeutic efficacy. Current diagnostic approaches include microscopy, PCR, and imaging techniques, but these are seldom applied promptly to alter outcomes. Therapeutic regimens typically involve amphotericin B, miltefosine , and combination antimicrobials, although survival is rare. Recent advances in drug repurposing, nanotechnology-based delivery systems, and immunotherapeutic strategies show potential but require further validation and assessment of effectiveness. Preventive measures, including public awareness initiatives and improved water treatment, are currently the most effective means of reducing the risk of *PAM*. This review provides an updated synthesis of the literature focusing on the causative agent, pathogenesis, diagnostic modalities, treatment options, and prevention strategies for *N. fowleri* infection. Background: *Naegleriafowleri* is a free-living, thermophilic amoeba that causes *primary amoebic meningoencephalitis (PAM)*, a rare but fatal CNS infection. It inhabits warm freshwater environments such as lakes and hot springs. Infection occurs when contaminated water enters the nasal cavity, allowing the amoeba to reach the brain via the olfactory nerves. With a mortality rate above 95%, early diagnosis and improved preventive strategies are critical to reduce its devastating impact.

Keywords: Naegleriafowleri, Primary amoebic meningoencephalitis, PAM, Public health.

1. INTRODUCTION

1.1. Definition

Brain-eating amoeba is a heat-adapted, unicellular amoeba found in the *Percolozoa* phylum of protozoa. It's typically free-living, growing in freshwater and soil environments, feeding on organic matter and bacteria.

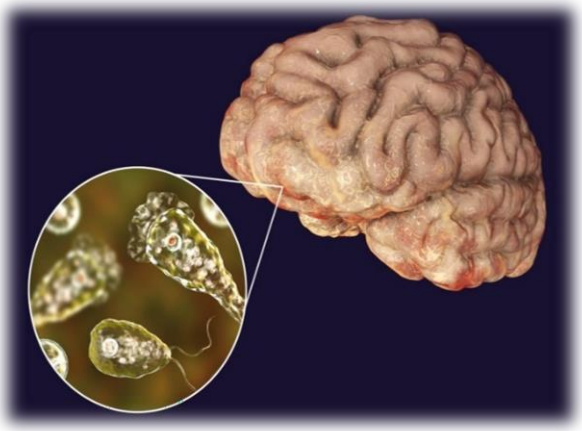

Fig 1: Brain Eating Amoeba

1.2 Overview

Naegleriafowleri is a free-living, eukaryotic amoeba belonging to the genus *Naegleria* and is commonly known as the *"brain-eating amoeba".* Unicellular organism classified under the phylum *Percolozoa*[1]. It is commonly found in warm freshwater environments, such as lakes, rivers, hot springs, and poorly chlorinated swimming pools.This amoeba is the causative agent of *primary amoebic meningoencephalitis (PAM)*, a central nervous system (CNS) infection with a case fatality rate exceeding 95 %.*N fowleri* is a facultative parasite, meaning it does not require a host to complete its life cycle and reproduces by mitosis.[2] The *4* free-living amoebae—*N fowleri*, *Balamuthia mandrillaris*, *Sappiniaspp*, *and Acanthamoebaspp*—areresponsible for human amoebic meningoencephalitis, which are severe and often fatal infections of the central nervous system (CNS).[2][3] Of the 47 species of *Naegleria*, only *Nfowleri* causes *primary amoebicmeningoencephalitis (PAM)*, which is a rare but rapidly progressing and almost always fatal CNS infection, leading to the death of patients within 3 to 7 days [4]. *Naegleriafowleri* is one of the few *FLA* capable of causing fatal infections in humans. This parasite causes *primary amoebic meningoencephalitis (PAM)*, an acute and fulminating infection that can lead to death 7 to 10 days after the amoeba enters the body.Studies have suggested that an early diagnosis is critical for a patient to survive *PAM. N. fowleri*is also known as *amphibolicamoeba* and there are three morphological stages of *Naegleria* species life cycle have been identified: *trophozoite (10–25mm), pear-shaped temporary flagellate stage(10–16mm) and cyst stage (8–20mm).*

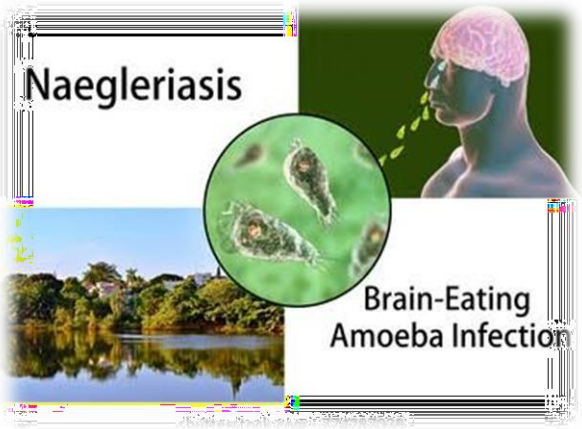

Fig 2: Overview of Naegleria fowleri

2. ETIOLOGYAND MORPHOLOGY

2.1 Taxonomy And Classification

Domain: Eukaryota
Kingdom: Protista
Phylum: Percolozoa
Class: Heterolobosea
Order: Schizopyrenida
Family: Vahlkampfiidae
Genus: Naegleria
Species: N. fowleri

2.2 Morphology and Lifecycle

The life cycle of *N.fowleri* consists of 3 stages *trophozoites*, *flagellates,and cyst.* The trophozoite stage is the reproductive and invasive form responsible for human infection. As a *thermophilic organism*, it is most active during the warm summer when human exposure is more likely. *Trophozoites* are long and slender, approximately 22 µm long, equipped with pseudopodia for movement and bacterial ingestion, and capable of forming large colonies.[11]

Under poor nutritive conditions, *trophozoites* transition into flagellates by developing flagella.[11] If unfavorable conditions persist, they further transform into metabolically inactive cysts, measuring 7 to 12 µm. These *cysts* are highly resistant, capable of withstanding low temperatures, and can survive during winter.[11] *Trophozoites* feed on bacteria and fungi in warm waters and can encyst and settle into sediments when water temperatures drop during winter.[12]

Trophozoite stage: Considered as the active, infective, feeding, reproductive stage (10–to 25 mm) with one nucleus that multiplies by mitosis in optimum environmental conditions.

Flagellate stage: Pear - shaped, mobile, non-reproductive and non-feeding stages of 10 – 16 mm.

Cyst stage: Non-reproductive and non-feeding stages of about 8 - 20 mm.

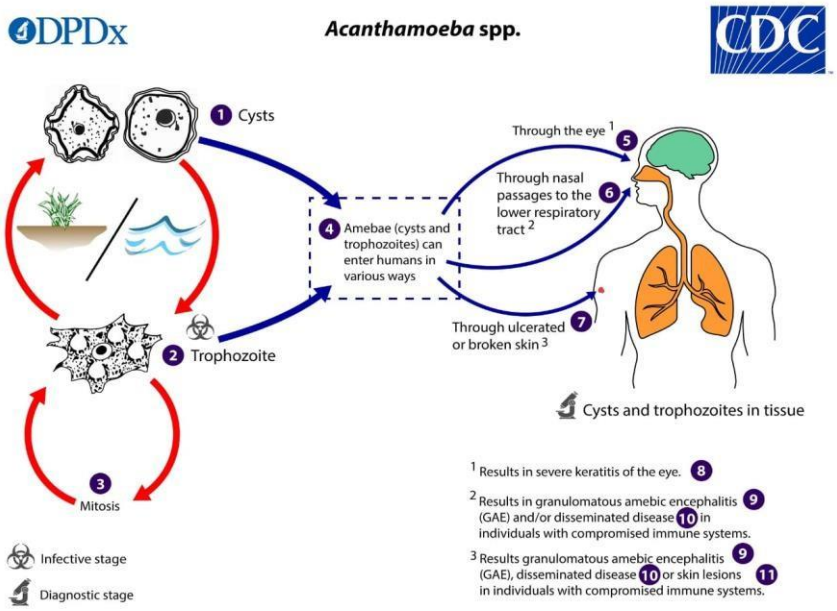

Fig 3: Life cycle of N.fowleri

3. EPIDEMIOLOGY

PAM is a hemorrhagic-necrotizing meningoencephalitis caused by *N. fowleri* and is seen mainly in immunocompetent children and young adults [12].*Fowler and Carter* were the first to describe *PAM* in *1965* after four people died in *Australia's Adelaide Children's Hospital*. The cause of death was attributed to an amoeba invading their meninges, which unleashed severe damage and inflammation in the brain [14,17,18]. According to the most recent data, a total of 39 countries have reported cases of *N. fowleri* infections. However, the United States of America (USA), Pakistan, Mexico, Australia, the Czech Republic, and India have been the most affected.

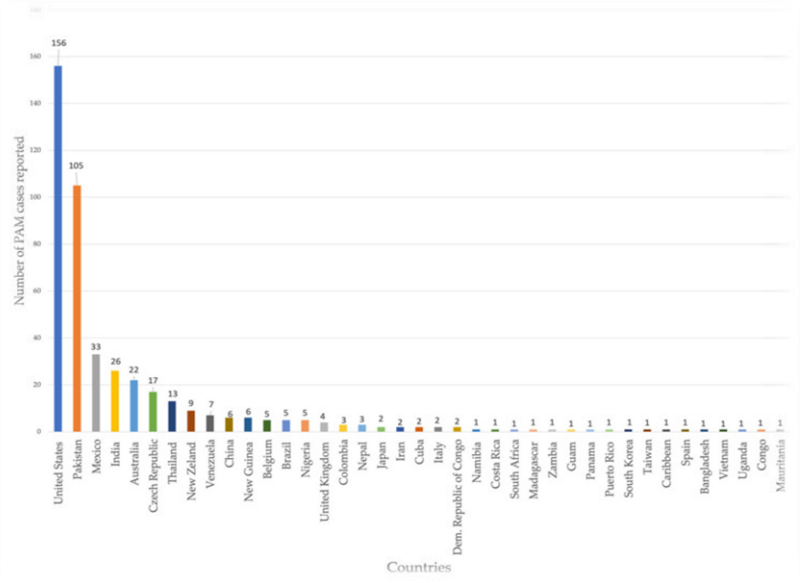

Fig 4: Cases Percentage

3.1 Global Dynamics Of *PAM*

- Since the discovery of *N. fowleri*, 0–8 cases of infection by this pathogen have been reported annually, with 260 cases documented worldwide between 1962 and 2014 and only 11 patients surviving.
- These cases are primarily linked to contaminated swimming pools, ponds, and other freshwater bodies and poor water management practices [28].

- The United States has historically recorded the highest number of *PAM* cases, particularly in its southern states, due to their warm climates [20].
- However, northern states, such as *Minnesota* in 2010 and *Kansas* and *Indiana* in 2011 and 2012, have also documented cases, likely due to warming trends associated with climate change [29].
- Between *1962 and 2022*, 157 cases were reported in the United States, making it the most affected country globally.
- By 2023, Pakistan had emerged as the largest hotspot for *N. fowleri* infections, with 143 cases reported, most of which were from Karachi.
- The increase in infections is driven by high temperatures (above 50 °C) in some areas, monsoon rainfall, and poorly maintained municipal water supplies [3], [30]
- The initial cases of amoebic *PAM* in the country involved people who had been in contact with contaminated water sources.
- Later findings indicated that a significant proportion of infections were attributed to vigorous nasal irrigation during ablution and showering [11].
- From *2008 to 2017, Pakistan had 105 cases of PAM in Karachi,* with the peak occurring in *2012* when *22 cases* were reported [31].
- *Aga Khan University in Karachi* reports *20* average PAM-related deaths each year.
- The transmission of *PAM* has been significantly influenced by forcefully inhaling tap water through the nose during ritual practices rather than swimming in the contaminated water [11].
- This distinctive method of transmission enables the amoebae to penetrate the nasal passages, travel to the brain, and inflict significant tissue damage.
- The high prevalence of *N. fowleri* infections in Pakistan underscores a serious public health challenge, such as inadequate water sanitation, limited public knowledge, and climate change.
- Globally, *Australia has reported 22 cases*, while *Mexico*, *India,* and the *Czech Republic* have documented *33, 26, and 17 cases*, respectively.
- Additional cases have been identified in *New Zealand (9), Nigeria (4),Venezuela (7),* and a range of other countries, including *South Africa*, *China, and Thailand* [28], [35].
- In total, *488 PAM* cases have been reported worldwide as of *2023*, based on compiled data from research articles, case reports, and surveillance data published in the literature between *1962 and 2023*.
- However, the true burden likely underestimates due to inadequate surveillance and diagnostic systems, especially in resource-limited settings [36].
- Alarmingly, the disease carries a *97 % fatality rate*, with only a few survivors documented between *1962 and 2022.*

Fig 5: Global dynamics of PAM

4. TRANSMISSION AND PATHOGENESIS

4.1 Transmission

- Natural Habitat: Found in warm fresh water like lakes, ponds, rivers.It can also survive in poorly chlorinated or untreated water systems like swimming pools, water tanks, tap water.
- Mode Of Entry: Infection occurs when contaminated water enters the nose.
- Initial Infection Site: Amoeba attach to the nasal mucosa and migrate along the Olfactory Nerve.

- Spread to CNS: Travel through the Cribriform plate > enter the Olfactory Bulb> disseminate into the brain.

4.2.Pathogenesis

- *N.fowleri* infection occurs when contaminated freshwater enters the nasal passages, typically during swimming in freshwater bodies, using poorly chlorinated pools, or performing nasal rinses with nonsterile water.[9]
- After entering through the nose, *N fowleri* migrates via the olfactory nerves, crosses the cribriform plate, and invades the CNS, thereby causing PAM.
- This infection is characterized by cerebral edema, hemorrhagic necrosis, herniation, and, in most cases, death.[4]
- The most severely affected regions of the brain include the olfactory bulbs, the basilar portion of the frontal cerebrum, and the cerebellum.
- Once *N. fowleri* reaches the olfactory bulbs, it elicits a significant immune response through activation of the innate immune system, including macrophages and neutrophils (3, 4).
- Structures on the surface of *trophozoites* known as food cups enable the organism to ingest bacteria, fungi, and human tissue (3).
- The pathogenicity of *N. fowleri* is dependent upon the release of cytolytic molecules, including acid hydrolases, phospholipases, neuraminidases, and phospholipolytic enzymes that play a role in host cell and nerve destruction (1).
- The combination of the pathogenicity of *N. fowleri* and the intense immune response resulting from its presence results in significant nerve damage and subsequent CNS tissue damage, which often result in death.

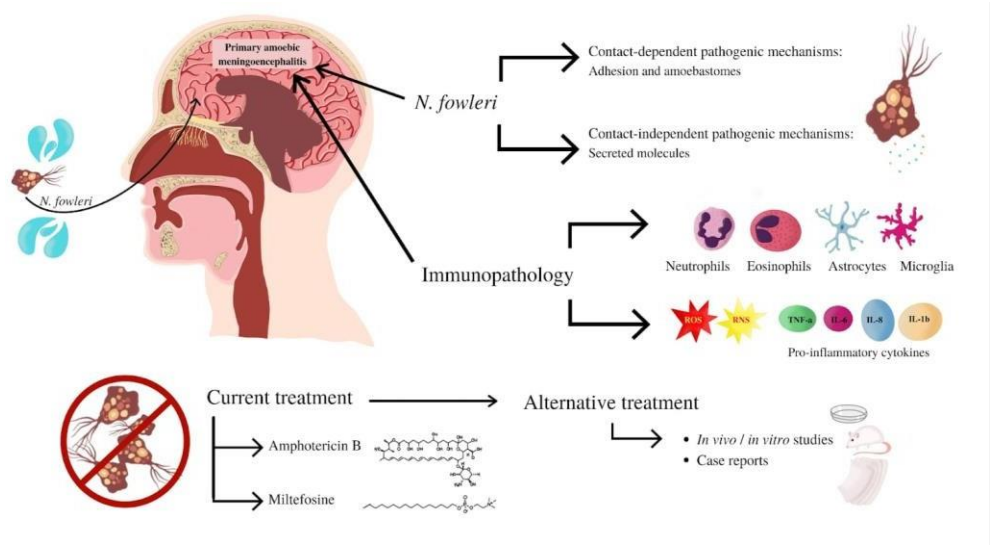

Fig 6: Pathogenesis of N.fowleri

5. CLINICAL MANIFESTATION

- Clinical symptoms and signs of infection with *N. fowleri* usually present within *2 to 8 days* of infectivity, though some have been reported within *24 h* (5, 6).
- *Early Symptoms :N. fowleri* infection, the most common symptoms include severe headache, fever, chills, nausea, vomiting, stiff neck, loss of appetite and fatigue, altered smell or taste.
- *Progressive Symptoms :* positive Brudzinski sign, positive Kernig sign, photophobia, confusion, seizures, coma and death, hallucinations, loss of balance.
- In addition, *cardiac rhythm abnormalities* and *myocardial necrosis* have been observed in some cases (7).

5.1. DIFFERNTIAL DIAGNOSIS WITH BACTERIA AND VIRAL MENINGITIS

Feature	Primary Amoebic Meningoencephalitis (PAM)	Bacterial Meningitis	Viral Meningitis

Causative Agent	Naegleriafowleri (free-living amoeba)	Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae, etc.	Enteroviruses (Coxsackie, Echovirus), HSV, VZV, etc.
Source of Infection	Fresh warm water (lakes, pools, neti pot use, hot springs)	Respiratory droplets, ear/sinus infection, skull fracture	Fecal-oral, respiratory secretions
Route of Entry	Nasal cavity → Olfactory nerve → Brain	Hematogenous (bloodstream) spread	Hematogenous (bloodstream) spread
Incubation Period	1–9 days after exposure	2–10 days	3–10 days
Onset	Sudden and fulminant	Acute, rapid	Subacute, milder
Early Symptoms	Headache, fever, nausea, vomiting, loss of smell/taste, stiff neck	Headache, fever, photophobia, vomiting, altered sensorium	Headache, mild fever, photophobia, irritability
Neurological Signs	coma Seizures, confusion, positive Kernig's&Brudzinski's	Same (meningeal signs + cranial nerve palsy possible)	Usually mild meningeal signs, rarely coma
CSF Appearance	Purulent or hemorrhagic, often red-tinged	Turbid / purulent	Clear or slightly turbid
CSF WBC Count	500–26,000/mm³ (mostly neutrophils)	1,000–5,000/mm³ (mostly neutrophils)	100–1,000/mm³ (mostly lymphocytes)
CSF Glucose	Very low or absent	Low (<40 mg/dL)	Normal or slightly decreased
CSF Protein	Markedly elevated	High (100–500 mg/dL)	Mildly elevated (50–100 mg/dL)
CSF Gram Stain / Culture	Negative for bacteria; motile amoebae visible in fresh wet mount	Positive for specific bacteria	Negative (requires PCR or viral culture)
Special Diagnostic Test	Wet mount or PCR for Naegleriafowleri DNA	Gram stain, culture	Viral PCR (HSV, Enterovirus, etc.)
Response to Antibiotics	No improvement	Improves antibiotics	Usually self-limiting
Mortality Rate	>95% (highly fatal)	10–30% (with treatment)	<1%(usually recover fully)with antibiotics

6. DIAGNOSIS

Diagnosis of *PAM* is often performed postmortem following histopathological microscopic examination of brain tissue revealing *N. fowleritrophozoites* and confirmation by immunohistochemistry (IHC), indirect immunofluorescence (IIF), or polymerase chain reaction (PCR). Cases that were diagnosed before the death of the patient have been primarily done by direct visualization of the amoebas in cerebrospinal fluid (CSF) and confirmation by IHF, IIF, or PCR.1 Successful diagnosis of *PAM* relies on prompt identification and awareness of available testing methodologies. To this end, the purpose of this review is to summarize the current clinical laboratory methods used in the diagnosis of *PAM.*

6.1. LABORATORY METHODS

Methods	Technique	Notes
Microscopy	Wet mount	Observation of motile trophozoites
	Cytospin	Marked neutrophilia Wright-Giemsa, H&E, or modified trichrome
	Tissue biopsy	Observation of trophozoites

		Wright-Giemsa, H&E, trichrome stain, or PAS
Antigen detection	IIF	Uses FITC-conjugated secondary antibodies
	IHC	Uses HRP-conjugated secondary antibodies
	Flow cytometry	Uses N3B monoclonal antibody Research setting only
Nucleic acid detection	Triplex real-time PCR	Tests for Acanthamoeba, B. mandrillaris, and N. fowleri Targets 18S rRNA gene sequences
	NGS	Used in clinical and research settings
Culture	Monoxenic	Uses non-nutrient agar supplemented with E. coli
	Axenic	Nelson, SCGYEM, or PYNFH media
Serology	ELISA	Detection of antibody titers Not used for diagnosis of PAM
	Immunofluorescence	Used for detection of N. fowleri-binding antibodies Not used for diagnosis of PAM

6.2. Direct Visualization

Initial CSF findings usually reveal elevated opening pressure, increased red blood cell (RBC) and white blood cell (WBC) counts with marked neutrophilia. The CSF chemistry analysis, as in cases of bacterial meningitis, is characterized by cloudy CSF with increased protein and decreased glucose levels.

Direct wet mount microscopic examination of fresh unrefrigerated CSF specimens can reveal numerous motile *N. fowleri* amoebas.However, amoebas may be missed at first examination and repeat CSF analysis may be required for successful observation by wet mount preparations. Amoebas most often appear as actively moving cells in the *trophozoite* stage measuring 15 to 30 μm in diameter.

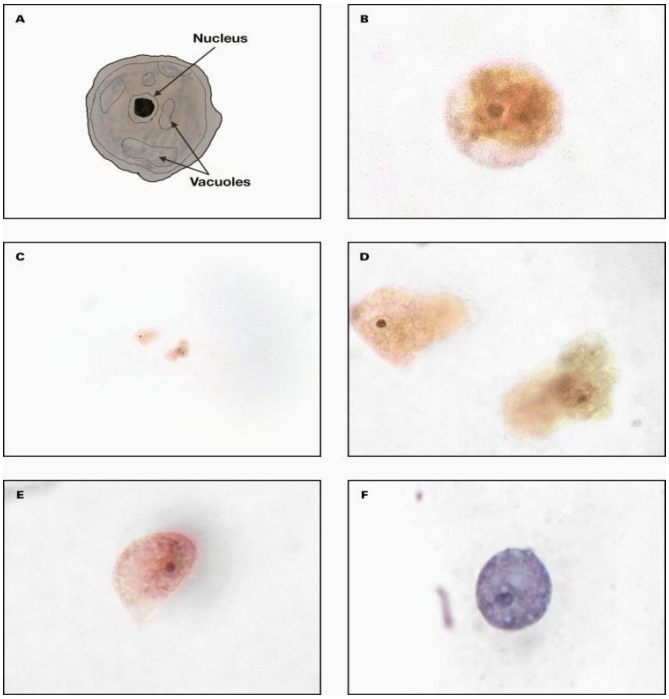

Fig 7: Microscopy Method

Alternatively, *cytospin* preparations of CSF at 5000 g for 5 min can be used to concentrate amoebas followed by staining with *Wright-Giemsa*, hematoxylin and eosin (H&E), or modified trichrome stains to increase contrast and ease visualization.

To maximize viability and observation of N. fowleri *trophozoites*, CSF preparations must be evaluated as soon as possible after lumbar puncture. Oil-immersion examination of CSF cytospin preparations stained with Wright-Giemsa reveals prominent presence of neutrophils and observation of *N. fowleri* cells, which are distinguishedby vacuolated *trophozoites* with a single large pale nucleus.

Postmorten diagnosis of *N.fowleri* is usually performed on examination of brain tissue biopsy. Ideally, fresh unfixed brain tissue is recommended for histological examination. Tissue sections can then be stained with H&E, trichrome stain, periodic acid-Schiff(PAS), or Wright-Giemsa Care must be taken when examining tissue biopsies as other amoebas such as *Balamuthia mandrillaris* and *Acanthamoeba* spp. resemble *N. fowleri*, making it difficult differentiate them based on morphology alone.23 Therefore, confirmatory tests are required for proper identification.

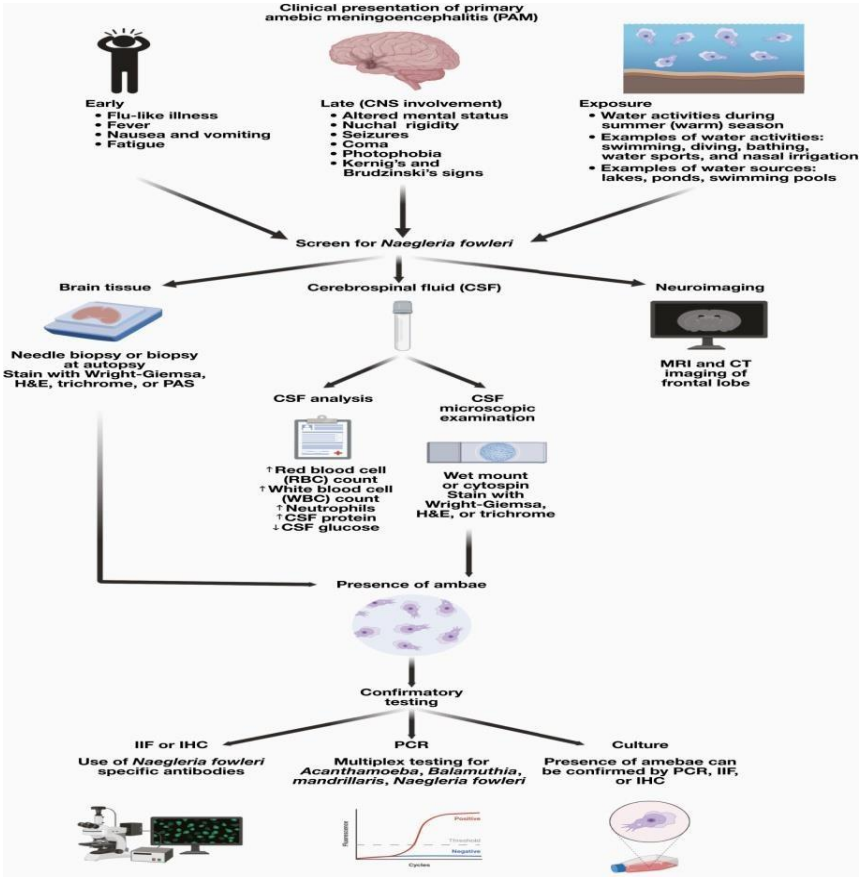

Fig 8: Diagnosis Methods of N.fowleri

6.3. Antigen Detection Methods

Identification of *N. fowleri* from brain tissue samples and CSF can be accomplished by the use of immunohistochemical staining techniques. The two main methods used are IIF staining and IHC using antibodies specific to *N. fowleri*. These methodologies employ polyclonal or monoclonal antibodies that can identify amoebas in tissue sections and CSF.The IIF protocols typically use rabbit immunoglobulin (Ig)G incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG as secondary antibody.In this IIF technique, the antibodies are labeled with fluorescent markers, such as fluorophores, that emit distinctive fluorescence when exposed to specific wavelengths of light. Under fluorescent microscopy, fluorescence within the samples indicates the binding of the labeled antibodies to *N. fowleri*. This method provides a visual and highly sensitive means of detecting the pathogen within the specimen30

IHC and IIF staining techniques enhance the diagnostic capabilities for *N. fowleri* infection, especially when dealing with formalin-fixed tissues or CSF samples. Their high specificity and ability to target specific antigens make them invaluable tools in confirming the presence of this pathogenic amoeba.

6.4. CULTURE

The culture procedure is a method employed to detect free-living amoebae in clinical and environmental samples. When growing on an E. coli lawn, the sample is placed on a growth plate covered with bacteria that can serve as a food source for *N. fowleri*. The initial screening step is carried out by incubating the plate at a higher temperature (108°F/42°C), which is lethal to most free-living amoebae but favors the growth of thermophilic amoebae like *N. fowleri*.During this initial screening, tracks created by an amoeba as it moves across the plate while consuming bacteria become visible. If no amoebae are observed on the plate incubated at a higher temperature, it indicates the absence of *N. fowleri*.

Naegleria can be definitively identified in cultures from clinical specimens using various staining methods such as *H&E, PAS, trichrome, Giemsa, or Wright–Giemsa stains.* Stained cultures may reveal trophozoites with morphological characteristics typical of *N. fowleri*, as previously described. In culture, these trophozoites may measure more than 40 μm. It is important to note that a negative culture result does not conclusively rule out the presence of *free-living amoebae*, and additional testing should be considered.

6.5. PCR AS A MOLECULAR DIAGNOSTIC TOOL FOR *N.FOWLERI*

PCR diagnostic methods offer significant advantages by overcoming many of these issues. They are valuable for diagnosing both clinical and environmental samples. PCR allows DNA isolation from samples without prior cultivation, streamlining the diagnostic process. Moreover, PCR not only detects N. *fowleri* but also facilitates the differentiation of other *Naegleria*species within the genus. Specific PCR tests for *N. fowleri* are highly recommended to confirm suspected infectious agents in clinical samples. They offer improved sensitivity, quicker results, and the ability to identify various *Naegleria* species, making them a valuable tool in diagnosing *PAM* and related conditions39.

Presently, the laboratory detection of *PAM* using PCR encompasses conventional PCR techniques40–43, nested PCR approaches39,44,45, multiplex PCR procedures46, and real-time PCR techniques47–52.

Real-time PCR-based diagnostic methods are advantageous in the rapidity of results and the possibility of monitoring the amplification process in real-time. The goal of our study was to develop a real-time PCR-based diagnostic method for detection of *Naegleriafowleri*, using amplification with a LightCycler instrument and primers and probe based on the Mp2Cl5 gene. Such an effective laboratory diagnostic method is needed for the confirmation of *N. fowleri* in both clinical and environmental samples.

6.6. IMAGINGTECHNIQUES

Computed tomography (CT) scanning and magnetic resonance imaging (MRI)

In cases where clinical signs suggest focal CNS involvement or elevated ICP, it is advisable to perform head CT scanning or MRI before conducting a lumbar puncture. In *PAM*, these imaging studies may reveal nonspecific findings, such as the obliteration of the cisterns around the midbrain and subarachnoid space.

6.7. LIMITATIONS

CT and MRI scans are typically utilized to visualize structural anomalies within the brain, such as tumors, vascular problems, or injuries. They are not intended to detect microscopic pathogens like *N. fowleri* directly. In advanced PAM, where neurological complications have developed, these imaging modalities can be supplementary diagnostic tools to assess brain damage and guide treatment decisions. However, it is crucial to emphasize that CT and MRI scans are not the primary methods for diagnosing *N. fowleri* infections.

7. TREATMENT AND MANAGEMENT

Due to the rarity of *N. fowleri* infections in humans, there are no clinical trials to date that assess the efficacy of one treatment regimen over another. Most of the information regarding medication efficacy is based on either case reports or in vitro studies. Perhaps the most-agreed-upon medication for the treatment of *N. fowleri* infection is *amphotericin B,* which has been studied in vitro and also used in several case reports. Other anti-infectives which have been used in case reports include *fluconazole, miconazole, miltefosine, azithromycin,* and *rifampin.* Various other agents have been studied in vitro and/or in vivo, including *hygromycin, rokitamycin, clarithromycin, erythromycin, roxithromycin,* and *zeocin* (10).

7.1. AMPHOTERICIN B

However, in vitro studies have shown that an amphotericin B concentration of at least 0.1 μg/ml was needed to suppress greater than 90% of growth, while 0.39 μg/ml was needed to completely suppress amoeba proliferation (11). The associated MIC of amphotericin B to kill 100% of the organisms in the in vitro studies was 0.78 μg/ml (12).

The *Centers for Disease Control and Prevention(CDC)* recommends intravenous conventional amphotericin B at doses of 1.5 mg/kg/day in 2 divided doses for 3 days followed by 1 mg/kg/day once daily for an additional 11 days (total of 14 days of therapy) (13). Intrathecal amphotericin B should also utilize the

conventional amphotericin B formulation. The CDC-recommended dose of conventional amphotericin B intrathecally is 1.5 mg/day for 2 days followed by 1 mg/day for an additional 8 days (total of 10 days of therapy) (13).

Although amphotericin B has become the primary drug of choice for treating *primary amoebic meningoencephalitis (PAM),* its use is associated with multiple side effects, including use-limiting renal toxicity (17).

Many of the problems with amphotericin B can be linked to its lack of aqueous solubility, which affects dissolution, compartmental concentration, and clearance. Recently, corifungin, which is described as a new drug entity, was granted orphan drug status for the treatment of *PAM* (18). Initial reports for the in vivo efficacy of corifungin in a mouse model of *PAM* showed activity superior to that of amphotericin B at equivalent dosing (18). Chemically,corifungin is the sodium salt of amphotericin B with excellent aqueous solubility (>100 mg/ml) (18).

7.2. MILTEFOSINE

Chemically, miltefosine is a phospholipid with an attached choline, an alkylphosphocholine. The overall molecule is amphiphilic, with a polar phosphocholine head region and an aliphatic tail. It also exists in the zwitterionic form, resulting from the permanently charged quaternary ammonium ion, as well as the anionic phosphate. The reported mechanism of action for miltefosine is inhibitory action against protein kinase B (PKB or Akt).

Miltefosine demonstrates a high level of plasma protein binding (>95%) and also has wide tissue distribution in rodent models, with the highest concentration of drug being found in organ tissues: lung, adrenal glands, spleen, and liver. It is difficult to rationalize the CNS penetration of miltefosine, as a permanently charged species, without an active transport mechanism. Specific studies of miltefosine concentrations in brain have not been described, but its effective use in N. fowleri infection demonstrates some level of penetration. It is not clearly understood if the blood-brain barrier of these patients has been in any way compromised due to the infection. Metabolism studies on human subjects have not been performed. However, miltefosine has been measured for oxidative metabolism against a panel of cytochrome P-450 (CYP450) isoforms, as well as the ability to induce isoforms of CYP3A. None of those evaluations predicted a significant degree of metabolism or potential for induction, suggesting that the risk for drug-drug interactions would be low (21). The primary metabolites produced in animal studies have been choline, phosphocholine, and cetyl alcohol.

Miltefosine has shown in vitro efficacy against N. fowleri, suggesting a possible benefit in treatment (22). In vitro studies have shown that N. fowleri amoebas have survived in miltefosine concentrations of 40 μg but not 80 μg (22). The CDC has made miltefosine available on a need basis through an *investigational new drug (IND)* protocol for the treatment of infections caused by free-living amoebas, which include *N. fowleri,Balamuthiamandrillaris, and Acanthamoeba* species (23). The CDC recommends miltefosine at doses of 50 mg orally two to three times daily (based on body weight) with a maximum dose of 1.5 mg/kg/day for a total of 28 days (23).

7.3. ADJUNCTIVE THERAPIES

FLUCONAZOLE

Fluconazole, an azole antifungal agent, has been used in conjunction with amphotericin B in the treatment of some cases of *N. fowleri* infection (24). Fluconazole and amphotericin B in combination show synergistic effects on eradicating *N. fowleri* infection through recruitment of neutrophils (28). Based on these findings, fluconazole can be considered an add-on therapy to amphotericin B in patients with suspected *N. fowleri* infections. The CDC recommends intravenous fluconazole at a dose of 10 mg/kg/day once daily (maximum dose of 600 mg/day) for a total of 28 days (13). Voriconazole, a broader-spectrum azole antifungal agent, has been shown in vitro to effectively kill *N. fowleri* at concentrations of ≥1 μg/ml (22).

AZITHROMYCIN

Azithromycin, a macrolide antibiotic, has been tested in vitro against *N. fowleri* and has been shown to suppress greater than 90% of organism growth at concentrations of 10 and 100 μg/ml (21). Azithromycin has been shown to have a MIC of 10 μg/ml against N. fowleri (11). In vivo studies in mice have shown that azithromycin administered at a dose of 75 mg/kg/day was needed to prevent death in mice infected with *N. fowleri*. The CDC recommends intravenous azithromycin at doses of 10 mg/kg/day once daily (maximum 500-mg daily dose) for 28 days (13).

Other agents such as miconazole, hygromycin (aminoglycoside antibiotic; not available in the United States), rokitamycin (macrolide antibiotic; not available in the United States), clarithromycin (macrolide antibiotic), erythromycin (macrolide antibiotic), roxithromycin (macrolide antibiotic), chlorpromazine

(antipsychotic), and rifampin (RNA polymerase inhibitor antibiotic) have been tested in vitro against *N. fowleri*, showing either a lack of efficacy or inconsistency with efficacy in vitro (10, 12).

8. PREVENTION

Steps which can be taken by individuals who participate in water-related sports in warmer climates include avoidance of exposure to freshwater bodies such as lakes, rivers, and ponds, especially during the summer months when the water temperature is higher. Both chlorinated and salt water significantly decrease the risk of *N. fowleri* infection due to the inability of *N. fowleri* to survive in such environments. If freshwater activities cannot be avoided, it is recommended that individuals avoid jumping into the body of water, splashing, or submerging their heads under the water in order to avoid *N.fowleri* entering the nasal passages. If such activities cannot be avoided, individuals should use nose clips to decrease the chance of contaminated water entering the nose. Some advocate rinsing the nose and nasal passages with clean water after swimming in fresh bodies of water; however, the effectiveness of this method is hypothetical and unknown at this time. If water is going to be used for sinus rinsing, the CDC recommends commercially available distilled or purified bottled water. In the absence of the abovementioned options, the CDC recommends treating water for sinus rinsing by either boiling or filtering the water using a filter with pores of 1 μm or smaller.

8.1. FUTURE DIRECTIONS

Early detection of the pathogen is crucial for effective management and combat of future N. *fowleri* infections. There is a strong need to address research gaps, for example, in understanding *N. fowleri* transmission and disease mechanism. Delayed detection of *Naegleria* infections has led to the loss of many lives over the past decades. The highest numbers of *PAM* cases are in the US and Pakistan, spotlighting the meticulous need for surveillance and a deeper understanding of the disease mechanism to track the phylogenetics of cases and limit infections. Strong efforts are required to advance the healthcare system capacity, particularly in developing countries, to facilitate early diagnosis and appropriate treatment to enhance the prognosis of *PAM* cases. More in-depth research on the genetics and pathogenic proteins of *N. fowleri* is essential to understand antigens, which could lead to the development of an effective vaccine. Recent studies have highlighted promising advancements in vaccine development, including the identification of monoclonal antibodies with an anti-amoebastatic activity that enhances immune responses and prolongs survival during infection [71]. Immunization approaches using antigens like Nfa1 protein and cholera toxin-based adjuvants have shown significant protective immunity in animal models, with survival rates reaching up to 100 % [68], [72]. Additionally, several antigenic proteins, including membrane-associated targets and polypeptides identified via proteomics, have demonstrated potential for vaccine development [73], [74]. Furthermore, *N. fowleri's* immune evasion strategies, including resistance to complement-mediated lysis, underscore the challenges of developing effective immunological interventions and highlight the critical need for further exploration of immune-pathogen interactions [17]. These findings signify the potential for targeted immunological strategies against this deadly pathogen. Until an appropriate vaccine is available, various treatment combinations should be employed to manage the cases.

Future outbreaks of *N. fowleri* can also be averted by adhering to cleaning regulations for freshwater sources, conducting routine testing, and repairing water pipelines, especially in the warmer months. We recommend broader surveillance worldwide, the establishment of a genetic data bank, development of prompt diagnostic techniques, exploration of potential therapeutic options, and development of vaccines to prevent *N. fowleri* outbreaks.

9. CONCLUSION

N. fowleri infection is a significant menace due to its endangerment of human lives and high fatality rate and is a public health concern globally. A substantial number of *PAM* cases have been reported from the US, Pakistan, and India and are associated with swimming in contaminated water or inhalation of unsafe water into the nostrils during ablution. Various factors contribute to the transmission and spread of *N. fowleri* to different regions. Poor maintenance and cleaning of water tanks, along with global warming and increased industrial activities, create favorable conditions for the pathogen's survival and proliferation. Phylogenetically, *N. fowleri* genotypes II and III contribute more to the dissemination of the pathogen's global impact, emphasizing the need for extensive close monitoring and novel diagnostic strategies. The current therapies, such as amphotericin B, azithromycin, and miltefosine, have presented limited success. Widespread chlorination of water, avoiding swimming in contaminated water, using nasal plugs, and avoiding deep inhalation of water during ablution are some preventive methods that can reduce the risk of *N. fowleri* infection. The development of vaccines, improved surveillance to address current research gaps, and enhancing public awareness of preventive measures require collaboration between government and public organizations.

Abbreviations
PAM: Primary Amoebic Meningoencephalitis
PCR: Polymerase Chain Reaction
CNS: Central Nervous Systems
FLA: Free-Living Amoeba
USA: United State Of America
HSV : Herpes Simplex Virus
VZV: Varicella-Zoster Virus
CSF: Cerebrospinal Fluid
IHC: ImmunoHisto Chemistry
IIF: Indirect Immuno Fluorescence
NGS: Next-Generation Sequencing
ELISA: Enzyme-Linked Immunosorbent Assay
RBC: Red Blood Cells
WBC: White Blood Cells
H&E: Hematoxylin and Eosin
PAS: Periodic Acid Schiff
FITC: Fluorescein Isothiocynate
DNA: De-oxyribo Nucleic Acid
CT: Computed Tomography Scanning
MRI: Magnetic Resonance Imaging
ICP: Intra Cranial Pressure
CDC: CentersFor Disease Control and Prevention
IND: Investigational New Drug

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