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Naegleria Fowleri : A. Comprehensive Review of Pathogenesis, Diagnosis, and Treatment Strategies

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Abstract: *Naegleria fowleri is a heat-loving amoeba often called the "brain-eating amoeba." It causes a very dangerous water-related disease called primary amoebic meningoencephalitis (PAM) in both humans and animals. These amoebae live widely in nature, especially in soil and warm freshwater, where they feed on bacteria. Although infections with N. fowleri are extremely rare, the disease it causes is very severe and almost always fatal. Because so few people get infected, doctors have not been able to conduct clinical trials to find out which treatment works best. This review will cover the key information about N. fowleri, including its spread in different regions (epidemiology), its life cycle, how it enters the body, what causes the disease, how it damages the body, how it is diagnosed, the medicines used for treatment, and ways to prevent infection.*

Keywords: Naegleria fowleri ,Primary Ameobic maningoncephalitis, Brain Eating Ameaba, Olfactorynuroepitheliun, Trophozoites, Amphotericin B

I. INTRODUCTION

Naegleria fowleri is the main organism that causes primary amoebic meningoencephalitis (PAM). It is called the "brain-eating amoeba" because it causes severe brain infection, and very few people (about 5%) survive it. This amoeba is usually found in water or wet soil and can grow easily in lab conditions on cell cultures or artificial media.This is the only type of amoeba that has three different forms: trophozoite, flagellate, and cyst.

Naegleria species are tiny organisms (protists) that belong to the family Vahlkampfiidae and the class Heterolobosea. Like others in this class, they live freely in the environment and mainly feed on bacteria. These organisms are called amoeboflagellates because they can change from an amoeba form into a flagellate form. They can also turn into a cyst to survive harsh conditions. Different types of Naegleria species have been identified.During the warmer months, Naegleria fowleri grows rapidly because it can survive in temperatures up to 45°C. It mainly feeds on bacteria and is often found in natural warm freshwater sources.Naegleria fowleri is a free-living organism that mainly feeds on bacteria (both Grampositive and Gram-negative), as well as algae and yeast. It reacts to bacteria by forming by food cups and moving toward them using chemical signals. Out of more than 40 known Naegleria species, Naegleria fowleri is the only one that causes a deadly brain infection called primary amoebic meningoencephalitis (PAM). Although PAM is rare, it is very dangerous, with a death rate of 95–97%. The disease usually leads to death within about seven days because it causes severe and rapid brain damage and bleeding.

Death usually happens within about seven days because PAM is a very serious and deadly brain infection that causes severe tissue damage and bleeding. PAM can affect people with strong immune systems, including healthy children and young adults who recently came in contact with freshwater. Since PAM spreads through water, most cases occur after swimming or diving in poorly chlorinated pools, polluted canals, lakes, or hot springs. It can also happen during water sports like water skiing in contaminated water or from using neti pots for nasal cleaning if the water is not clean.The disease usually progresses very fast.

After being infected, symptoms start within 2 to 15 days. The illness begins suddenly and gets worse quickly, often leading to death.From 1962 to 2018, there were 381 reported cases of this infection in 33 countries. Even though medical science has advanced, the number of cases has slowly increased over the years. Between 1965 and 2016, the total number of reported cases increased by about 1.6% each year. and the number of confirmed cases increased by

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about 4.5% each year.A study from 2020 found that Naegleria fowleri was present in about 26.42% of water sources tested around the world.

The highest number of cases was in America (about 33.18%), likely because there are many places where infection can happen and because many studies have been conducted there.

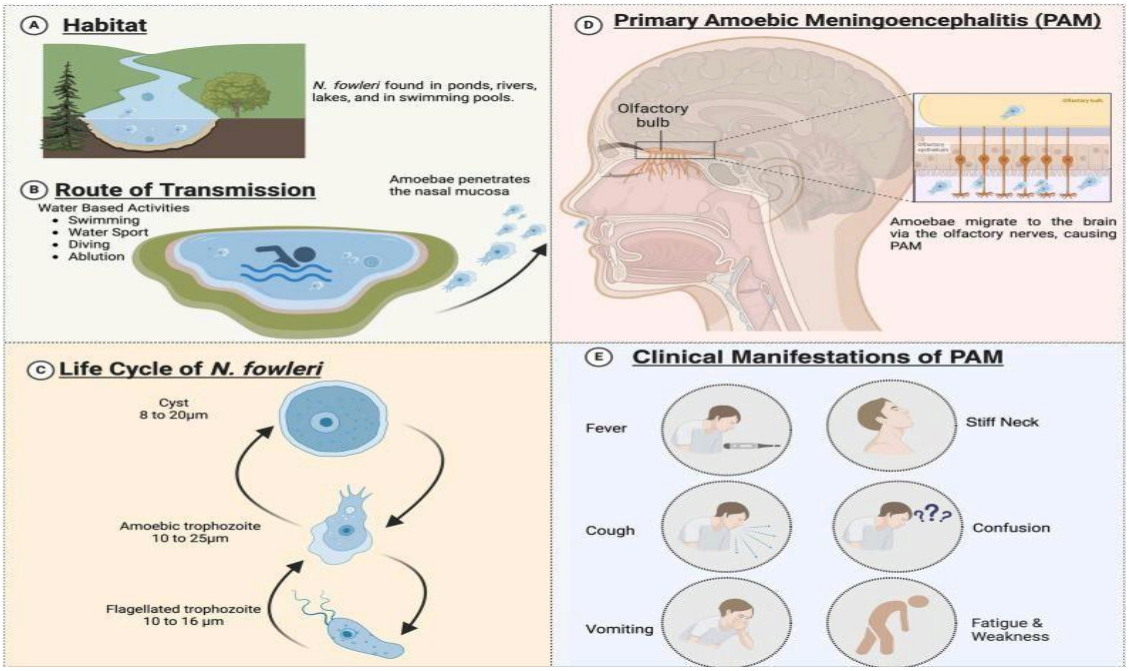

Fig 1: Cycle of Brain Eating Ameaba

Primary amoebic meningoencephalitis (PAM) causes symptoms very similar to viral or bacterial meningitis. These include fever, headache, stiff neck, vomiting, loss of appetite, and seizures. The disease gets worse very fast, and death usually happens within 3 to 7 days after symptoms begin.How quickly symptoms appear depend on how dangerous the amoeba strain is and how many amoeba enter the body. Symptoms can start as early as 2 to 3 days after exposure, but in some cases they may take up to 7 to 15 days to appear.

Naegleria fowleri is called an amphizoic amoeba because it can live freely in water or soil, or act as a pathogen (disease-causing organism) in humans and animals. Other free-living amoebae (FLA) found in the environment — like Balamuthia mandrillaris, Sappinia diploidea, and Acanthamoeba species — can also cause brain infections in people. Balamuthia and Acanthamoeba cause a deadly brain disease called granulomatous amebic encephalitis (GAE). Sappinia diploidea can cause a nonfatal brain infection. GAE usually affects people with weak immune systems, while PAM (primary amebic meningoencephalitis) — caused by Naegleria fowleri — occurs in healthy people and progresses much faster. Infection with Naegleria fowleri happens when water containing the amoeba enters the nose. [1,2,3,4].

Epidemiology

Naegleria fowleri is found almost everywhere and mostly lives in warm freshwater, such as lakes, hot springs, poorly cleaned swimming pools, and warm polluted water. It has never been found in seawater.Human infections have happened in many parts of the world, including Australia (where it was first discovered), New Zealand, Europe, Africa, Asia, and Latin America. In the United States, most cases occur in the southern states.This virus has been found in New Zealand, Europe, Africa, Asia, the USA, and Australia. It can also live in very warm water. People can get infected during activities like swimming or even certain religious practices that involve water.Between 2007 and 2008, 34 cases were reported. Many people swim in pools during the summer, but PAM is still not well understood. PAM makes people more likely to get a dangerous brain infection caused by amoebas.

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There are many water sources that are not treated properly, and this allows the organism to grow and spread in different water bodies.Naegleria fowleri usually survives in warm or temperate regions. In China, out of 11 reported cases, 10 were linked to outdoor swimming during the hot summer, showing that swimming in warm water during summer is a major risk and should be a main focus for prevention. Researchers collected data on N. fowleri infections reported in different continents from 1937 to 2024 using PubMed and CNKI, and they summarized how many people were infected and how many died in each region. According to recent data, 39 countries have reported cases of Naegleria fowleri infection. The countries most affected are the United States, Pakistan, Mexico, Australia, the Czech Republic, and India. These places are more likely to have infections because they have warm climates all year and more exposure to contaminated water. In the United States, most cases occur in the southern states, where the weather is hotter. [5,6,7,].

Etiology

A very severe brain infection called amebic meningoencephalitis is caused by a tiny living thing called the Naegleria fowleri amoeba (a parasite).This amoeba can sometimes be found in public drinking water and private well water, which means there's a risk of exposure.However, even though people come into contact with water containing the amoeba pretty often in the United States, it's very rare for anyone to actually get the disease.When this infection does occur, the Naegleria amoeba mainly attacks the nervous system, which includes the brain and spinal cord.[8 ,9].

Diagnosis

Symptoms of Naegleria fowleri infection usually appear 2 to 8 days after exposure, but in some cases, they can start within 24 hours. The early symptoms are not unique to this infection, so it can be hard to diagnose at first. The most common signs and symptoms include Severe headache , Fever and chills ,Neck stiffness (positive Brudzinski and Kernig signs) ,Sensitivity to light (photophobia), Confusion or disorientation ,Seizures ,Possible coma ,In some patients, heart rhythm problems and damage to the heart muscle have also been reported. A major danger of this disease is the increase in pressure inside the skull (intracranial pressure) and spinal fluid (CSF) pressure, which can lead to death.

In some cases, CSF pressure has reached 600 mm H_2O, which is extremely high. Tests of the cerebrospinal fluid often show changes in color — gray in the early stages and turning red later due to bleeding in the brain. There is also a high number of white blood cells (up to 26,000/mm^3) and sometimes trophozoites (the active form of the amoeba) can be seen under a microscope when special stains like trichrome or Giemsa are used. MRI scans of the brain may show damage or inflammation in different areas, including the midbrain and spaces around the brain (subarachnoid space).

In patients with Naegleria fowleri infection, the pressure of the cerebrospinal fluid (CSF) can become very high — up to 600 mm H_2O. When doctors test the CSF, they notice changes in its color: it may look gray in the early stage of infection and turn red in the later stage because of bleeding caused by many red blood cells. There is also a big increase in white blood cells (especially a type called polymorphonuclear cells), sometimes reaching 26,000 cells per mm^3. In addition, the trophozoite form of the amoeba can be seen in the CSF when special stains like trichrome or Giemsa are used under a microscope. MRI scans of the brain usually show abnormalities or damage in several parts, especially in the midbrain and the spaces around the brain (subarachnoid space).

In Naegleria fowleri infection, the spinal fluid shows signs of inflammation, with high numbers of white blood cells and red blood cells. A regular Gram stain test cannot detect the amoeba. However, it may sometimes be seen when using a Wright-Giemsa stain, which is normally done to count cells. To find the amoeba, doctors must quickly check a fresh sample of spinal fluid under a microscope using a wet mount. The amoeba can be seen moving in the sample, but they only move if the fluid is kept warm.

They may also start moving if a drop of distilled water is added to the slide. Sometimes this test doesn't work well because too many white blood cells can make it hard to spot the amoeba. Also, the amoeba and white blood cells can look very similar, which makes it difficult for technicians who are not experienced in identifying Naegleria fowleri. Although solid non-nutrient agar is the usual method to grow Naegleria fowleri, it has some problems — such as bacterial contamination and producing fewer cysts. Recent research suggests using a liquid medium (a modified version of Page's amoeba saline) instead.

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In this new method: The amoeba is first grown in Nelson's growth medium with 10% fetal bovine serum and kept at 37 °C. After the trophozoites (active forms) develop, about 2×10^6 cells are washed twice with PBS (pH 7.4). They are then placed in 24-well plates with 5 mL of the encystment medium at 37 °C. The encystment medium contains: 120 mM NaCl, 0.03 mM $MgCl_2$, 1 mM $NaHPO_4$, 1 mM KH_2PO_4, 0.03 mM $CaCl_2$, and 0.02 mM $FeCl_2$ (pH 6.8). Using this method, the trophozoites turn into cysts after about 48 hours, making it a good alternative for producing N. fowleri cysts.

Because this disease is very rare, not well known, and has symptoms similar to bacterial meningitis, doctors often don't suspect PAM at first.

Doctors usually start to suspect PAM when bacterial tests on the cerebrospinal fluid (CSF) are negative and the patient has a history of swimming or nasal rinsing. Since PAM patients have very high pressure inside the skull, taking CSF with a spinal tap can be dangerous and may cause brain herniation..Therefore, the main focus is on lowering the brain pressure safely. [10,11,12,13,14]

Life cycle

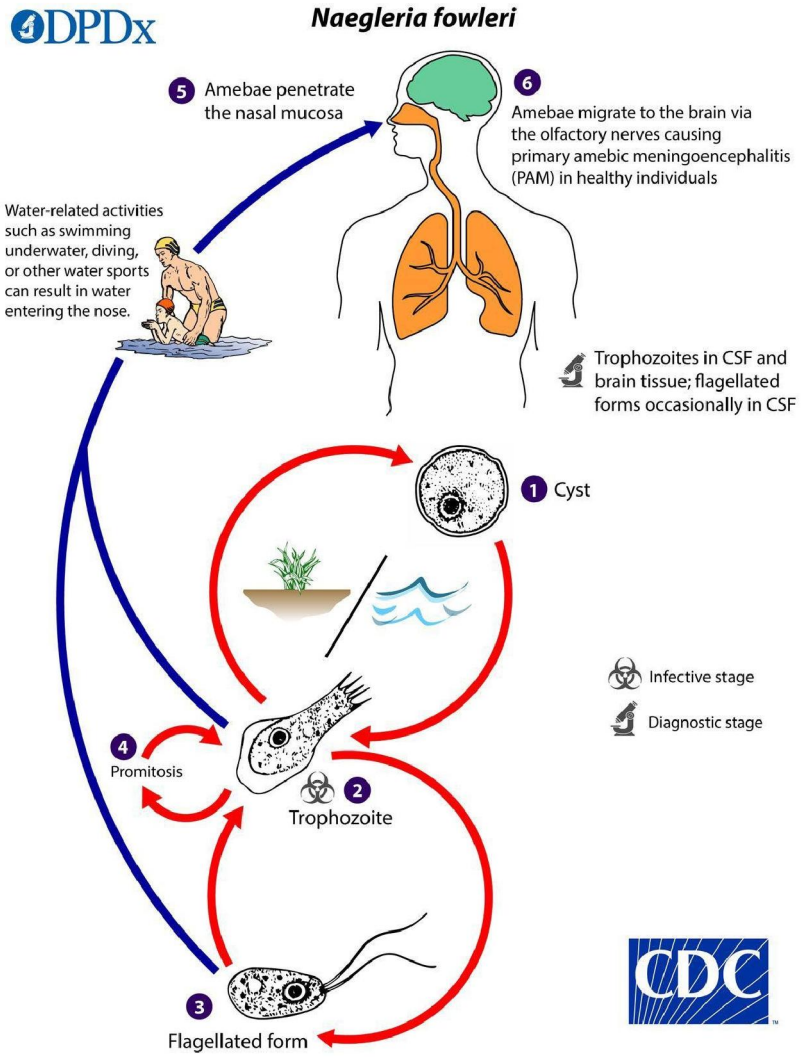

Fig 2. : Life cycle of Naegleria Fowleri

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Pathogenesis

Naegleria fowleri is called an amphizoic amoeba because it can live freely in water or soil, or inside a host, such as the human brain and nervous system. Infections usually happen in healthy children and adults after doing water activities like swimming, diving, or water skiing in contaminated water. Naegleria fowleri releases different harmful substances (called virulence factors) that make it more dangerous. These help the amoeba enter the brain and damage brain tissue. Naegleria fowleri can cause more damage to the host by triggering a strong immune reaction. Studies in animals show that the amoeba reaches the olfactory bulb (part of the brain) within about 72 hours after infection, and tissue damage and inflammation start around 96 hours later. Research has found that N. fowleri causes cells to make reactive oxygen species (ROS) — harmful molecules that lead to several changes: They activate the EGFR pathway, causing the cells to produce mucus (MUC5AC) and the inflammatory chemical IL-8, which attracts immune cells (especially neutrophils).

ROS also increase the production of another inflammatory molecule, IL-1β, through a different pathway. ROS help form the NLRP3 inflammasome, a structure that turns on enzymes (like caspase-1) and releases active IL-1β, adding to inflammation. Another study showed that N. fowleri can cause programmed cell death (called necroptosis) in certain immune cells. This might be the body's way of trying to defend itself, but it also leads to cell destruction and more tissue damage. Naegleria fowleri causes serious damage to the brain and nervous system because of its harmful nature and the strong immune reaction it triggers.

There is still limited knowledge about the exact factors that make it dangerous, but scientists have created different laboratory models (in animals, cells, and outside living systems) to study how it causes Primary Amebic Meningoencephalitis (PAM). So far, researchers have found two main ways the amoeba causes damage. Contact-dependent mechanisms when the amoeba sticks to host cells and eats them using structures called food cups , Contact-independent mechanisms – when the amoeba releases toxic molecules that can destroy cells from a distance. Besides destroying tissue using its food cup, Naegleria fowleri also releases harmful chemicals like acid hydrolases, phospholipases, neuraminidases, and other enzymes. These substances help the amoeba break down and destroy nerve and brain cells.

The combined effect of the amoeba's attack and the strong immune reaction it causes leads to severe nerve and brain damage, which often results in death. In vivo models use live animals, such as mice.In these studies, mice are infected through the nose with N. fowleri, which usually causes a high death rate. Researchers study N. fowleri using live animals, tissues, or cells. In live animal studies, mice are infected through their noses with the amoeba, and most of them die from the infection. During the middle and later stages of primary amoebic meningoencephalitis (PAM), Naegleria fowleri releases certain substances—like cysteine proteases, VCAM-1, and ICAM-1—that damage and help it cross the blood-brain barrier. The breakdown of blood vessel linings in the brain triggers the production of nitric oxide (NO) by enzymes called nitric oxide synthases (NOS), which further increase the brain's barrier permeability. As the infection continues, immune cells such as macrophages and microglia are activated. They release inflammatory molecules like TNFα, IL-1, and NO to try to kill the amoeba. In lab studies, N. fowleri has been shown to activate microglial cells, leading them to produce more inflammatory molecules (IL-1β, IL6, TNFα) and harmful compounds called reactive oxygen and nitrogen species (ROS and RNS). However, the amoeba can survive even in the presence of nitric oxide, which means the infection keeps damaging brain tissue without being eliminated.[15,16,17,18,19,20].

Treatment

Treating a brain-eating amoeba infection is difficult because it is very rare and usually found only after the symptoms get serious. But getting fast and strong medical treatment can increase the chances of survival. Most of what we know about possible treatments comes from a few patient case reports and research done in labs and animals. Sadly, because the disease is extremely rare and gets worse very quickly, it is not practical to do clinical trials to test how well these treatments work or how safe they are.

Most of what we know about how well these medicines work comes from case reports or lab studies. The drug doctors most commonly agree on for treating N. fowleri infection is amphotericin B. It has been tested in the lab and used in several patient cases. Other medicines that have been tried in case reports include fluconazole, miconazole, miltefosine,

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azithromycin, and rifampin. Some additional drugs have only been tested in the lab or in animals, such as hygromycin, rokitamycin, clarithromycin, erythromycin, roxithromycin, and zeocin.

Amphotericin

Amphotericin B can kill Naegleria fowleri at very small amounts (as low as 0.01 µg/ml). But lab tests show that you need. 0.1 µg/ml to stop more than 90% of the amoebae from growing, 0.39 µg/ml to completely stop them from multiplying, and 0.78 µg/ml to kill all of them. Because of this, and because a few patients have survived after using it, amphotericin B is the main medicine used to treat N. fowleri infections. It can be given through a vein (IV) or directly into the spinal fluid. Amphotericin B is the main medicine used to treat primary amoebic meningoencephalitis (PAM), but it can cause many side effects, especially kidney damage, which often limits how much of the drug can be safely used. These problems mostly come from the fact that amphotericin B does not dissolve well in water, which affects how it spreads and clears from the body. A newer drug called corifungin has recently been given orphan drug status for treating PAM. Early studies in mice show that corifungin works better than amphotericin B when both are given at the same dose. Corifungin is basically the sodium salt form of amphotericin B, but it dissolves extremely well in water (more than 100 mg/ml). This better solubility is likely the reason it seems to work more effectively. However, there are no human studies yet, so we don't know if this improved solubility will actually give real benefits in people, such as better ability to reach the brain or fewer kidney side effects.

Miltefosine

Miltefosine was first made as a cancer drug, but now it is used to treat several infections, including visceral and cutaneous leishmaniasis, Chagas disease, cryptococcosis, and Trichomonas vaginalis. Studies have shown that miltefosine can kill free-living amoebae in the lab, including Naegleria fowleri, Acanthamoeba, and Balamuthia. Recently, miltefosine was used in a patient who had a severe Acanthamoeba brain infection along with miliary tuberculosis, tuberculous meningitis, and Acanthamoeba skin lesions. The patient did not get better with several other medicines that are normally used against Acanthamoeba, including streptomycin, fluconazole, TMP-SMX, amphotericin B, flucytosine, and sulfadiazine. But the patient finally recovered when treated with oral and topical miltefosine, plus intrathecal and intravenous amikacin. In our study, miltefosine was able to kill N. fowleri in lab tests and helped increase survival in mice with experimental PAM.

Some N. fowleri survived after being treated with miltefosine (explained later in more detail). Even though the data are limited, these results suggest that miltefosine may be helpful when PAM is diagnosed early. Miltefosine is a phospholipid-like molecule: it has a polar (water-attracting) head and a non-polar (waterrepelling) tail, so it is amphiphilic. It also carries both a positive and a negative charge, making it zwitterionic. Miltefosine is thought to work by blocking protein kinase B (PKB or Akt), a protein involved in cell growth and survival. This makes sense because miltefosine was originally studied as a cancer drug, and cancer cells rely heavily on the PI3K–Akt pathway.

Fluconazole

Fluconazole is an antifungal medicine that has been used along with amphotericin B to treat some cases of N. fowleri infection. Adding fluconazole seems to give extra benefit when used with amphotericin B. One reason fluconazole may help is that it enters the brain and spinal fluid well, which is important for treating brain infections. When fluconazole and amphotericin B are used together, they work better as a team (synergistically), partly because they help recruit neutrophils, a type of white blood cell that fights infection. Because of these findings, fluconazole is recommended as an additional medicine (add-on therapy) for patients with suspected N. fowleri infection. The CDC recommends giving fluconazole through a vein at 10 mg/kg/day once daily, with a maximum dose of 600 mg/day, for 28 days. Another antifungal, voriconazole, has also been shown in lab studies to kill N. fowleri at concentrations of 1 µg/ml or higher.

Azithromycin

Azithromycin, a macrolide antibiotic, has been tested in the lab against N. fowleri. It was able to stop more than 90% of the amoeba from growing at concentrations of 10–100 µg/ml. Its MIC (the amount needed to kill all the organisms in

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lab tests) is 10 µg/ml. In mouse studies, a dose of 75 mg/kg/day was needed to prevent death from N. fowleri infection. The CDC recommends giving azithromycin through a vein at 10 mg/kg/day once daily (up to a maximum of 500 mg/day) for 28 days. Several other drugs—such as miconazole, hygromycin, rokitamycin, clarithromycin, erythromycin, roxithromycin, chlorpromazine, and rifampin—have also been tested against N. fowleri in lab studies. However, these drugs were either not effective or gave inconsistent results, so they are not reliable treatments.

Rifampicin

Rifampin has been used in every PAM survivor case reported in the U.S. and Mexico, but we still do not know if it actually works. The main concern is whether rifampin can reach high enough levels in the brain and spinal fluid (CNS) to kill N. fowleri. Some studies have shown that rifampin can enter the CNS and appear in the spinal fluid. But another study found that rifampin levels in different parts of the brain were very low—around 0.3 µg/ml. This amount is good enough to kill many bacteria, but it is likely too low to kill N. fowleri. Rifampin is commonly added to the treatment and may help other medicines work better.[21,22,23,24,25,26]

Prevention

People who swim or play in water in warm climates can reduce their risk of N. fowleri infection by following these steps:

Avoid swimming in warm freshwater (like lakes, rivers, and ponds), especially in the summer when the water is hot.

Chlorinated pools and saltwater (like the ocean) are much safer because N. fowleri cannot survive well in these environments.

If you must enter freshwater:

Try not to jump, splash hard, or put your head underwater, because this can push water up the nose, which is how the amoeba enters the body. If you can't avoid these activities, use nose clips to help keep water out of your nose.

Some people suggest rinsing the nose with clean water after swimming, but we do not know if this actually helps.

For sinus rinsing (like using a neti pot):

Use distilled or purified bottled water.

If those are not available, the CDC recommends boiling the water or using a filter with pore size 1 micron or smaller to make it safe. [27,28,29,30].

II. CONCLUSION

Naegleria fowleri is a heat-loving, flagellated amoeba often referred to as the "brain-eating amoeba." It causes a rare but highly fatal infection called primary amoebic meningoencephalitis (PAM) in both humans and animals, with a death rate of over 95%. PAM occurs more often in developed countries but is now increasingly reported in developing countries with warm climates.The amoeba thrives in warm, contaminated water. Its infectious form, the trophozoite, enters the body through the nose and travels along the olfactory nerves through the cribriform plate to reach the brain. Once there, it rapidly destroys brain tissue, causing bleeding and severe damage. Without immediate diagnosis and treatment, death usually happens within 3–7 days. Late detection and limited effective treatment options make this infection extremely deadly. Even though amphotericin B is used in combination therapies, the survival rate remains only about 5%. Therefore, improving diagnostic tools, developing new antiparasitic drugs, and working toward possible vaccines are important goals for researchers and pharmaceutical companies. Achieving this will require extensive human and animal studies, along with significant technical and financial resources. Additionally, strengthening immune defenses and following preventive measures are crucial for controlling infections caused by this pathogenic amoeba

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