REVIEW ARTICLE

Occurrence of The Pathogenic Amoeba *Naegleria fowleri*, Pathogenesis, Diagnosis, and Treatment Options

Nawwar Najwa Ismail, Hartini Yusof

Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA (UiTM) Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Naegleria fowleri is the causative agent of primary amoebic meningoencephalitis (PAM) that infects the central nervous system (CNS). The amoeba is present ubiquitously. The infection is rare but has a high mortality rate. The pathogenic amoeba reaches the host through the nasal passage and migrates along the olfactory nerves to reach the human brain and cause severe destruction of the CNS. As Malaysia has a large population that practising ablutions in daily routine, the risk of infection increases. Two mechanisms associated with the infection include contact-dependent and contact-independent. Signs and symptoms vary from early stage to later stage of infection. CSF and brain biopsy are the common specimens collected used to diagnose the PAM infection and usually detect and identify by PCR method. As for treatment, the CDC of United States America has recommended the usage of miltefosine that provides promising in vitro therapy against *N. fowleri*. This review will discuss more on the occurrence of *N. fowleri*, pathogenicity, diagnostic tools, and pharmacotherapy approach against *N. fowleri* infection.

Keywords: Free-living amoeba (FLA), *Naegleria fowleri*, Primary amoebic meningoencephalitis (PAM), Central Nervous System (CNS)

Corresponding Author:
Hartini Yusof, MSc
Email: hartinieyusof@gmail.com
Tel: +603-32584431/60196635985

INTRODUCTION

Naegleria fowleri (*N. fowleri*) is a free-living amoeba (FLA) known to be pathogenic to humans. It can be found in the environment within a diverse type of habitat (1). More than thirty species have been identified, but *N. fowleri* possesses the most pathogenic characteristic and causes infection with a high risk of fatality within a short time due to rapid progression prior to diagnosis and treatment. *N. fowleri* is the primary pathogen causing primary amoebic meningoencephalitis (PAM), an infection that occurs both in the brain and the meninges. The infection occurs when the parasite's infectious form enters nasal mucosa then migrates along the olfactory neuroepithelial via cribriform plate to enter the brain (2) resulting cerebellar herniation and may lead to death if treatment is delayed. Healthy people, primarily identified as children and young adults with a history of exposure to water contaminated with amoeba, such as swimming or other water-related sports, bathing, ritual ablution, or nasal irrigation, have been documented with *N. fowleri* infection (3). Symptoms appeared similar to bacterial meningitis which includes headache, nausea, fever, back pain, and vomiting. PAM has a high mortality rate ranging between 95-99% which death approaches within 72 hours of infection. The infection usually identified only after death and a lack of treatment modalities for the specific infection become one of the contributing factors.

The earliest confirmed PAM infection in humans happened in 1965 in Australia, subsequently found in Florida and Texas in the United States (US). More than 300 cases of PAM infection have since been recorded worldwide, the majority of which occur in America, Australia, and Europe. In Asia, the disease has been found in Thailand, India, Pakistan, Iran, and China. These diseases are almost uniformly fatal, with only several survivors (1,4–6). In Thailand, *N. fowleri* has been identified as the etiological agent in 12 cases of PAM (7,8). PAM typically happens during the summer season when the ambient temperature is high (9) as people are more likely to have water-related activities, and infection almost often leads to death within a week or ten days of infection (10). While in Iran, a single case study reported on meningoencephalitis due to pathogenic *N. fowleri* in a six-month Iranian infant (11). Therefore, countries with averagely high temperatures may have an increased risk of *Naegleria* species contamination in their waters and cause infection.

In Malaysia, water-related activities have increased as the government encourages the public to spend

more time with their families (12). Thus, these family activities may increase the risk of being infected with FLA or other waterborne infections. The problem arises when *Naegleria* species able to proliferate and survive in tropical climates that have high environmental temperatures. It becomes worrisome to all people who are potentially exposed to the pathogenic species that could lead to serious disease and cause fatal. Additionally, Malaysia is known to be a Muslim country and a huge concern is not exceptional where a large population practising ablutions in daily routine causing the risk of free-living amoeba infection to increase (13). In most of the reported cases in Karachi, Pakistan, almost none of the patients infected with *N. fowleri* had a history of participation in recreational water activities but ablution with tap water was suggested as a likely risk factor for the Muslim community. Based on the obtained data, all individuals who died due to the infection belonged to the Muslim community (7,11). Besides, documentation on parasitic protozoan, especially, FLA was very limited, although many countries around the world have reported cases of deaths and threats caused by *Acanthamoeba* and *Naegleria*, in Malaysia, there is still a lack of awareness of FLA infection (15).

This review paper is generally to understand the current and updated information on the occurrence of free-living amoeba specifically to *N. fowleri*, which is associated with primary amoebic meningoencephalitis (PAM), a fatal human infection. The review will be focused on the habitat of the species, pathogenicity of the parasite, clinical diagnosis, and multiple treatment modalities applied to fight the infection. The significance of the study is to provide essential information and create awareness to the public on the risk of choosing water-related activities to spend their free time.

OCCURRENCE AND RISK FACTORS OF *Naegleria fowleri*

Studies that have been carried out were focusing on the trophozoites or cysts identification of *N. fowleri* that can be found in the most varied environments (1,16). The environmental conditions are classified into two categories which are natural habitat and urban zones (17–19). Table I shows a summary of potential habitats for the colonization of free-living amoeba (FLA). From all sites stated, any water sources or conditions with high water temperatures above 28°C are reported to be favourable for *N. fowleri* to grow and proliferate to a great number (20). This amoeba is known to be thermotolerant and thermophilic (21,22). It can tolerate and survive in high temperatures up to within 40-45°C (1,23,24).

Besides that, several parameters contribute to the presence of the *Naegleria* species. These include physical, chemical, and biological parameters which almost every sample collected was being measured

Table I: List of potential natural habitats and urban zones for FLA colonization.

Natural Habitat	Urban zones	References
Rivers	Discharge from industrial plants	(5)
Freshwater lakes	Geothermal heated water	(23)
Hot springs	Domestic and hotel swimming pools	(20)
Warm aquatic environments	Hospitals	(24)
	Tap water used for nasal flooding	(25)
	Contaminated drinking water	(22)
	Resort spas	(26)

(1,6,25). In biological parameters, water sources need to contain microorganisms such as cyanobacteria, eubacteria, and coliforms as nutrient sources for the amoeba. *Escherichia coli* is the most coliform species used to be isolated and lawn on non-nutrient agar (NNA) used for amoeba growth in the laboratory (22,23,26). As for chemical parameters, the water sources usually contain high concentrations of iron and magnesium and other chemicals that are possible to be measured, including ammonia, chlorine, nitrite, nitrate, and fluoride (25). Previously, the presence of abundant organic matter in sites is favourable for *N. fowleri* growth (27). Next, physical parameters that were measured on-site include turbidity (NTU), conductivity, dissolved oxygen (mg/L), temperature (°C), and pH (23,25,26). The temperature of the natural water bodies correlates with the proliferation of the species (22) and *N. fowleri* is found to be pathogenic due to its ability to survive in high temperatures up to 45°C. *Naegleria* species was significantly found in low turbidity of water and the level of pH does not significantly affect the presence of *Naegleria* species in the water sample. Studies by Kao et al. suggests that mildly alkaline and low turbidity may be preferable water conditions for *Naegleria* species (1). Poor quality of water disinfection and deterioration of water distribution systems (28), predation of other protozoan and invertebrates, disturbance of water surface by boating activity, and the existence of bacterial and fungal toxins are the other factors that could affect the number of these protozoa (29).

The *Naegleria* cysts are preferred to grow in watery or moist areas because of their susceptibility to heat stress. The previous report showed that the *Naegleria* species could be found not in swimming pool water samples but also in dust samples taken from the swimming pool wall. However, it is hardly found in the samples obtained from dry areas such as the swimming pool platform. (12).

The life cycle of *N. fowleri* comprises three stages which are cyst, trophozoite, and flagellate. Fig. 1 shows the stage of life and the morphology of *N. fowleri*. Under conducive environments, it reveals a reproductive-active trophozoite with a size ranging from 7-20µm that multiplies and feeds into the environment (24,30). The presence of rounded structures called lobopodia makes the trophozoite possess an elongated

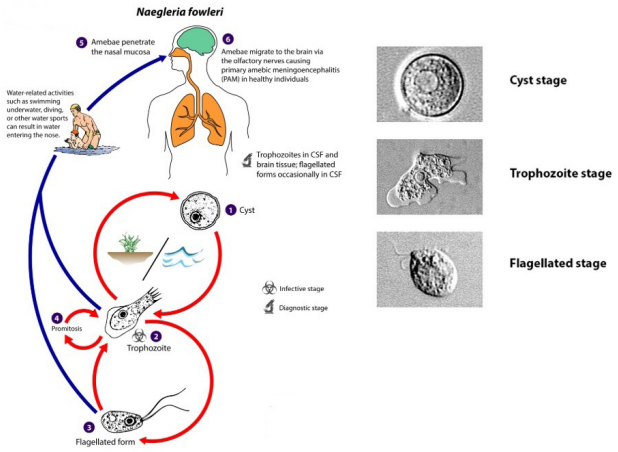

Figure 1: Life cycle and morphological form of Naegleria fowleri (52,62,63)

structure (30,31). Under poor conditions with the water existence, trophozoite switches to the transient flagellate stage (33,34). It has pear-shaped and motile. When unfavourable conditions like food deprivation, desiccation, and cold water temperature, trophozoite switch into cyst form (30,33). The cyst is a dormant spherical structure that has 7-10µm in diameter and has a smooth double-wall, refractile, and uninucleate (21,31).

The PAM cases were generally infected males compared to females with a ratio of 3:1. The risk of infection may also increase in the summertime or during the warmest season. As known, *N. fowleri* can grow optimally in warm freshwater. Therefore, the amoeba has higher chances of infecting humans, especially during summer in a country with four seasons. Another factor that may be associated with the infection is the level of water. Kids commonly play by the seaside, lakes, or rivers that have low water levels with sediment or soil underneath. This activity might accidentally expose the children to the amoeba and allow it to enter the body through the nasal passage. However, the infection will not occur if someone accidentally drinks the contaminated water (35).

The presence and prevalence of the amoeba were strongly affected by its environmental design, which suggested that pools with soil enclosures have a high FLA incidence compared to those made from a concrete and tiled surrounding pool wall or line that reduces soil contamination to the water (15).

PATHOGENESIS

The explanation of pathogenicity factors associated with *N. fowleri* infection is essential for studying of the mechanisms in charge of the damaging effects of PAM. These likely pathogenicity elements were classified based on their cellular components to identify the section that possesses the intense pathogenicity activities with the trophozoites, the infective stage of *N. fowleri* (36). Analysis of cellular components that likely possess the pathogenicity factors of *N. fowleri* is shown in Fig. 2.

N. fowleri infection-causing PAM can be divided into two mechanisms known as contact-dependent and contact-independent mechanisms leading to the death of host cells. A contact-dependent mechanism caused direct host cell destruction by *N. fowleri* trophozoites via trogocytosis that involved food-cup formation or amoebastomes in the amoeba surface. *N. fowleri* Excretory and Secretory Proteins (ESP) in contact-independent mechanism have been proposed as another possible pathogenic mechanism that indirectly damages the host cells in the central nervous system (CNS). These include acid hydrolases, phospholipases, neuraminidases, cysteine proteases, and phosphorolytic enzymes (37–40).

(Trans-) membrane domain

Adherence to human cells is a critical step for successful infection. Therefore, the membrane such as trans-membrane protein plays a part in the pathogenicity of *N. fowleri* infection. Fibronectin-binding protein is a function in the interaction of trophozoites with the extracellular matrix (ECM) glycoprotein indicates that *N. fowleri* has introduced a membrane protein related to human integrin-like receptors. It allows the attachment

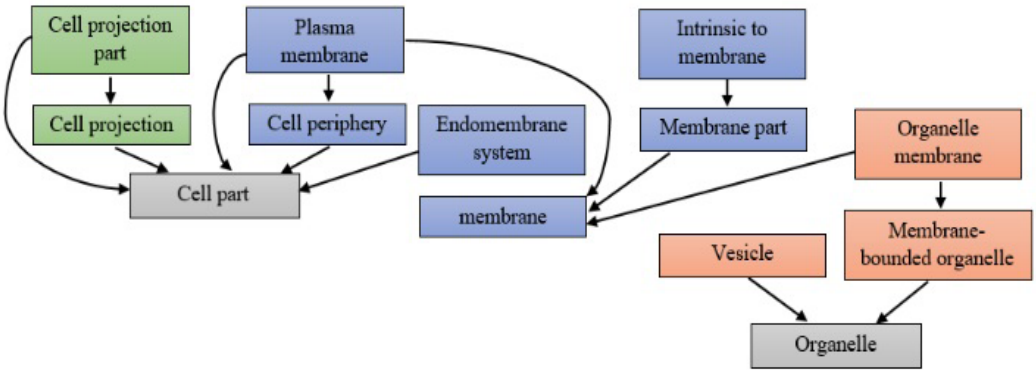

Figure 2: Cellular component analysis of potential pathogenicity factors from Naegleria fowleri (36)

of the trophozoites to the nasal epithelium of the host (36,41,42). Another membrane protein isolated from the pathogenic *N. fowleri* is Mp2Cl5 which is involved in the lytic activity of the trophozoites. A review of various membrane-bound glycoproteins associated with complement-mediated damage resistance including tumour necrosis factor-a (TNF-a), interleukin-1 (IL-1), and membrane attack complex (MAC) known as CD59-like protein, has been documented. The host's binding of complement component C9 is inhibited because membrane vesicles release CD59-like protein that causes resistance to complement lysis (36,41).

Vesicular trafficking

Membrane vesiculation is a mechanism used by *N. fowleri* to protect the amoeba from damage by the complement system. In membrane vesicles, various proteins that are likely to be pathogenic to humans are stored and released. Thus, vesicular trafficking may play a vital role in *N. fowleri* pathogenicity. Proteases are all-encompassing enzymes that contribute to the pathogenesis and physiology of parasites. Phospholipase A and B induce cytolytic activity to kill the cell membrane; neuraminidase or elastase promotes the breakdown of tissue culture cells; the target cells are lysed by a perforin-like, pore-forming protein, and the amoebic pathogenesis is caused by the cytopathic protein that induces apoptosis in the susceptible tissue culture within the *Naegleria* amoeba (39,40). Furthermore, two pore-forming glycoproteins named naegleriapore A and B were identified. They had been said to be cytotoxic in the form of membrane-permeabilizing activity to human cells. They are stored in the granular vesicles of the intracellular (36,42).

Besides, a 30 kDa secreted cysteine protease has been detected in *N. fowleri* that is involved in the deterioration of ECM proteins. Two novels cathepsin B-like cysteine proteases were identified and known as *Naegleria fowleri* cathepsin B (NfCPB) and *Naegleria fowleri* cathepsin B-like (NfCPB-L) that involves an invasion of intracellular host tissue and immune evasion by the amoeba play a crucial role, enabling skin or intestinal inflammation, tissue migration and protein depletion for nutrition. These proteases lyse the structural proteins of the host such as collagen and fibronectin, disrupt the functional proteins include haemoglobin and albumin, and also immunoglobulins. To mitigate damage to the parasite, the regulation of these proteases is strictly important. The mechanisms used to regulate the operation, however have not been clearly described and understood (38–40,43,44).

N. fowleri excretory and secretory proteins (NfESP) were said to hasten neuronal cell death through a cytolytic activity, which in concert with cellular debris as residues of brain cells lysis that attract microglial cells to release proinflammatory cytokines thus lead to activation on the inflammatory cascade and accumulate at the site of infection. Overproduction of inflammatory cytokines causing unfavourable inflammatory response thus induce hyper inflammation, breakage of the blood-brain barrier, and a massive influx of peripheral immune cells hence contribute to disease progression which may lead to death (37,38).

Cell projection

Trophozoites of *Naegleria* were forming food-cups which are pseudopodial projections. This formation involved the attachment of amoeba to substrates also the ingestion of bacteria, yeast, and cellular debris through the phagocytic process that employs to destroy the human cells. The phagocytosis process is depending on the cytoskeleton protein actin (Nfa1 and Nf-actin) that is associated with the food-cup formation (37,39). The protein is strictly regulated by actin-binding proteins known as formins. Formin D is a potential pathogenic factor that regulates actin filament formation. Another potential protein is severin that acts as an actin-modifying protein that is classified as actin-fragmenting and –capping proteins. Next, cofilin is an actin-filament-severing protein that forms free barbed ends and finally is a villin that possesses multifunctional cytoskeleton actin regulating protein that performs all the actin-modifying functions listed above.

Last but not least, a protein identified as heat shock protein 70 (hsp70) that localizes within the food-cup formation plays in the adaptive survival of the amoeba and is also involved in the proliferation of the trophozoites that cause the infection. This protein helps the amoeba withstand the high temperature of the surrounding, making this parasite known as a thermotolerant and thermophilic organism (36,38,39).

DIAGNOSIS OF PRIMARY AMOEBIC MENINGOENCEPHALITIS (PAM)

After *N. fowleri* manages to enter the host from exposure or direct contact to the contaminated source with the pathogen, the incubation period takes place from within 3 to 7 days before the clinical onset of symptoms. Once symptomatic, rapid progression of PAM can be observed and often fatal (19,31).

Clinical Diagnosis

Primary amoebic meningoencephalitis (PAM) can be considered when patients present with symptoms such as headache, nausea, fever, vomiting, back pain, exhaustion, lethargy, neck stiffness, and change in smell and taste at the early stage of infection (45). As the infection starts to progress, symptoms include confusion, hallucinations, lack of focus, and seizures are developed (46). All these symptoms were considered when patients present together with a history of contaminated water exposure with pathogenic amoeba, hence PAM infection can be considered for prognosis (47–49). The clinical sign and symptoms of PAM are generally identical

within meningitis infection. Therefore, a detailed clinical history of a patient is crucial for physicians to come with a good prognosis (48,50). Physicians need to retrieve information from patients regarding any recent activities that involve contact with freshwater, including hot springs, and data related to rhinitis, an allergic reaction, and other upper respiratory tract diseases.

PAM infection occurs in the brain, and therefore, patients need to undergo computed tomographic (CT) scans or magnetic resonance imaging (MRI) studies of the brain to highlight any central nervous system (CNS) involvement (47,48). PAM is characterized by increased intracranial pressure and brain oedema (48,50). A variety of CNS alterations includes multifocal parenchymal lesions, pseudotumoural lesions, meningeal exudates, haemorrhagic infarction, and brain necrosis can be observed. These conditions will develop, rapidly deteriorating into more advanced stages, and ultimately leading to death. As PAM is considered a rare disease, their high mortality rate, and short incubation period highlighted as the critical factor of the infection (27) which death usually occurs between the third and seventh days after the onset of symptoms (47,49,50). Due to rapid progression, it requires highly sensitive and rapid diagnostic tools for timely treatment (51). These diagnostic tools are commonly time-consuming and technically challenging causing PAM cases often diagnosed only after death during post-mortem through brain biopsies (27).

Clinical specimens

For PAM, the most useful specimen type for diagnosis remains with cerebrospinal fluid (CSF) and brain biopsy. Such samples should be aseptically collected and stored at room temperature (~25°C) before any laboratory test. This condition allows the viability of thermophilic amoeba in the specimen, enables immediate observation of live amoeba through microscopic examination, and allows the growth of amoeba in suitable culture media. For staff who is handling these specimens, adequate personal protective equipment (PPE) such as gloves, surgical masks, and laboratory coats is a must. Containers that contain specimens can only be opened within the biological safety cabinet to minimize the possibility of contamination (52).

Cerebrospinal fluid (CSF) examination

Early infection of PAM is hard to diagnose and differentiate from other meningitis. Therefore, CSF examination is required to distinguish naegleriasis from other meningitis. Lumbar puncture is performed to collect CSF for analysis (27). Normal CSF has yellowish-white in colour but infected CSF samples have a turbid appearance. The presence of red blood cells (RBCs) in the sample may indicate an early infection stage. CSF pressure appears high, protein level increases, glucose concentration may appear lower, and high cell count mainly neutrophils in the CSF can be observed under a microscope using a high power field (HPF) (9,47,50,51).

Laboratory Diagnosis

a) Microscopic identification of amoeba in CSF

Patients suspect of PAM infection; a wet mount of CSF is performed after sample collection and immediately observed under a microscope to detect motile *Naegleria* trophozoites (49). Whenever the procedure was unable to perform immediately, the CSF may be kept at room temperature (~25°C) before visualization. To prepare a slide for a wet mount, smooth agitation of the container holding CSF can help to dislodge possible amoeba that adheres to the container. Then, the obtained sample is proceeding with quick centrifugation at 5000 X g for 5 minutes allowing amoeba to concentrate at the bottom of the container. The supernatant is discarded carefully leaving about 200-300 µl of residual liquid without dislodging any noticeable pellet. A small volume is taken and place on the slide. After that, the microscope slide with CSF is incubated within temperature 35-37°C inducing a warm environment to facilitate any *N. fowleri* amoeba movement. The CSF smear is stained with Wright-Giemsa or Trichrome stains to visualize the *Naegleria* trophozoite (48–51). It is easy to differentiate amoeba from host cells in which the nucleus is centrally located with prominent nucleolus appeared clearly. The size of the trophozoite is ranging from 10-25 µm. Several vacuoles are visible around the nucleus and pseudopodia can be observed (49,51). Gram staining is generally not useful to visualize amoeba in the CSF samples as it mostly appears negative (9,47). The microscopic examination provides a simple and rapid procedure with the low cost needed makes it favourable for detection. However, a major disadvantage is that an expert microscopist and pathologist are needed to accurately identify the amoeba and this method is also known to be highly dependent on the integrity of amoeba morphology on specimens tested (53).

b) Culture method and enflagellation test

N. fowleri can also be detected by the culture method. CSF or brain tissue is cultured onto non-nutrient agar (NNA) lawn with heat-killed *Escherichia coli* American Type Culture Collection 29522 (ATCC 29522) in Page amoeba saline (PAS) (9). *N. fowleri* utilizes bacteria as a food source (12). Besides, the culture methods also allow possibilities to obtain all three forms of *N. fowleri*, trophozoite, flagellate, and cyst. All stages are characterized by prominent nucleolus and halo in the nucleus (48,54). *Naegleria* can revert formation from the trophozoite form into a flagellate form (33,34) through the flagellation test (9,12). To perform a flagellation test, the specimen is incubated at 37°C in an isotonic saline solution or distilled water. Observations are made every 30 minutes time interval within 2 hours until two flagella are expressed to detect *Naegleria*. The time taken for the expression of flagella can be varied up to 6 hours (12). Then from flagellate form, it can transform into a cyst when grown onto NNA with absence or low nutrients.

Therefore, encystation and flagellation are processes used to identify *N. fowleri*. The appearance of food cups and flagellates that can be observed microscopically are the key morphological feature of *N. fowleri* (22,54).

c) Molecular detection

Polymerase chain reaction (PCR) assays are always considered the gold standard to identify the parasite. Complete DNA is most commonly extracted from the cells found in samples (CSF, brain tissue) and PCR is performed using *Naegleria* specific primers (27). This method offers a sensitive and specific identification procedure; hence, several primers sets have been developed to identify *N. fowleri*. PCR type that has been widely used to test clinical samples collected from PAM patients is known as the TaqMan real-time PCR assay. This type can detect single amoeba in the patient samples because the replication can make hundreds of copies of targeted DNA per amoeba (51). These molecular tools are useful for rapid differentiation of species and genotyping by analysis of PCR-amplified *N. fowleri* 18S rRNA, internal transcribed spacer 1 (ITS1), 5.8s ribosomal(r) RNA gene, internal transcribed spacer 2 (ITS2), and 28S rRNA (49). The value of using rDNA as a PCR target is due to its internal variable regions that allow individual identification of genotypes (20). Sequencing together with bioinformatics software is performed to identify the organisms once the amplicons are collected (18,24). Different modalities of PCR which is a Nested-PCR assay have been created to detect *N. fowleri* amoeba in environmental samples. This method is based on amplifying a portion of the Mp2Cl5 gene, a protein produced by *N. fowleri* which is related to the virulence factor of the amoeba (55).

Apart from that, an invented assay does not require sophisticated PCR instruments to visualize the amplification product using the naked eye. This modern approach is known as loop-mediated isothermal amplification (LAMP) assay developed to detect *N. fowleri*.LAMP assay can also be applied to both environment water samples. LAMP assay could detect the presence of a single amoeba-spiked specific to *N. fowleri* for each of the reactions with minimal equipment for both DNA extraction and analysis. This method is used for its simplicity, rapidity, high specificity, and reproducible assay in amplifying the DNA (30).

TREATMENT OPTIONS

PAM is an infection that occurs when trophozoite, an infective stage of *N. fowleri* able to invade the brain which is associated with warm water-related activities. PAM is more susceptible to healthy individuals who are immunocompetent. PAM is an immediate cause of disease in which death may occur within days after symptoms onset (56–58). Therefore, a good prognosis on presented signs and symptoms with a detailed history of patient routine or activities is crucial to get an early diagnosis thus allowing an appropriate and suitable treatment to be started (17,51).

N. fowleri has been measured against a wide variety of antiparasitic, antimicrobial, and other pharmacologic agents. Despite the study, the activity of the tested drugs remains limited against the protozoan. Currently, the treatments available for PAM infection is inadequate and provides no guarantee for survival. Administration of intravenous amphotericin B (AmB), an antifungal, is a drug of choice to treat PAM patients that provide the most scientific proof of successful treatment in humans with PAM (17,44,59). Amphotericin B is usually administered alone or in combination with other potential drugs that include rifampin, fluconazole, and other clinical tested drugs to treat PAM (8,43,56). Administration of this drug is limited and restricted because it can give side effects to the recipient including fever, shaking chills, vomiting, headache, hypotension, nausea, tachypnoea, dyspnoea to severe systemic adverse effects which is acute kidney damage due to nephrotoxicity. AmB is also highly toxic towards renal function manifested as azotemia and hypokalemia (44,59). The pharmacodynamics of PAM found in the CNS is disrupted by the fact that it takes a longer time for systemic administration to enter and penetrate the target organ. Also, the presentation of the blood-brain barrier makes it difficult for the administered drug to effectively kill the parasite due to high selectivity causing low drug penetration to the target infected site in the CNS (56).

At present, the Centers for Disease Control and Prevention (CDC) of the United States America has found and recommends using miltefosine to treat PAM. Miltefosine is an anti-cancer drug manufactured to treat breast cancer and an antileishmanial drug that also provides promising in vitro therapy against *N. fowleri* and fortunately, miltefosine has been supplied greatly and widely by CDC as treatment of fulminant *Naegleria* infections (16,61). During the summer of 2013 in the United States, two children with *N. fowleri* infection survived. A 12-year-old girl was diagnosed approximately 30 hours after becoming ill and starts the recommended treatment within 36 hours. Miltefosine is prescribed and her brain swelling was highly responding with the drug and gradually reduced the body temperature below normal. This patient made a full neurologic recovery and returned to school. The other patient is also considered a survivor although he has suffered with likely to be permanent brain damage. He was also treated with miltefosine but was diagnosed later after the onset of the symptom. In summer 2016, a 16-year-old boy survived the PAM infection. He was diagnosed within hours after visiting the hospital and treated with the same protocol as the 12-year-old girl who survived the infection in 2013 with full neurologic recovery and back to school (62). However, the administration of high dose miltefosine may lead to increased side effects including nausea, vomiting, or diarrhoea. This drug is

mildly nephrotoxic and therefore suitable adjustment on the dosage for impaired kidney function patients is made (63). Table II displays recommended drugs used for the treatment of PAM infections.

Other potent drugs that could be used are agents from the azoles group. These include fluconazole, clotrimazole, voriconazole, itraconazole, chlorpromazine, and ketoconazole. These compounds have a broad spectrum of antimicrobial properties and are clinically used as antifungal and anti-parasitic agents with strong antiamoebic activity against *N. fowleri* (50). Conjugation of azole compounds with silver nanoparticles further improved the compounds' ability to fight these amoebae. Even with a variation of remedies used to treat the infection, the fatality rates remain highly worrisome, suggesting a lack of adequate and effective treatments. Thus, essential modification in treatment is required (60,64).

Posaconazole contains an essential key to the pharmacodynamics properties needed in the development of new drugs for PAM. PAM is known for rapid disease progression and therefore rapidity of drug action is important. Posaconazole and ketoconazole were demonstrated as the fastest-acting compounds that have been identified for inhibiting the growth of the amoeba. A combination with azithromycin gives an effective outcome when they manage to prolong the survival rate and increase the cure rate of the infected hosts (65).

Next, previous research has shown that corifungin effectively kills both non-pathogenic *N. gruberi* and pathogenic *N. fowleri* in vitro. The analysis of ultrastructural showed that corifungin manage to cause damage toward both *N. fowleri* internal and surface membranes. Corifungin also targets the mitochondria of *N. fowleri* more effectively than AmB, and corifungin is well tolerated in animals causing minimal toxicity (59). Besides, azithromycin, another potential drug used in treatment against amoeba infection had previously shown an active effect on *N. fowleri* in vitro (50).

Recently, a new approach to treatment for PAM infections has been studied. Conjugation of drugs used with nanoparticles-based materials significantly enhanced the antiamoebic effects against *N. fowleri*. For instance, conjugation with silver nanoparticles has proven that the effects of nystatin, amphotericin B, and fluconazole increased the antiamoebic actions. Nanoparticles have gained great attention in recent years related to the biotechnology and biomedical fields. Generally, the pharmacokinetics and pharmacodynamics of drugs were improved with nanomaterials-based drug delivery systems. The materials consist of metals and semiconductors because of their ability to load maximum drugs due to high surface area (60,64).

In the meantime, a few non-pharmacological procedures were done to treat cerebral oedema resulting from elevated intracranial pressure. Dexamethasone, an anti-inflammatory drug is used to counter elevated pressure. Other procedures to perform include drainage of CNS through an external ventricular drain, hyperosmolar therapy by the admission of mannitol and 3% saline, moderate hyperventilation and lastly inducing hypothermia with temperature ranging between 32 – 34 ^{0}C (16,46).

Due to adverse effects caused by many of the drugs used to treat PAM, some researchers suggest a traditional approach. Diosgenin, a purified extract found in *Momordica charantia* has been identified to have antimicrobial, antiviral, antifungal, antihelminth, antiamoebic, and antimalarial activity. This compound also can be extracted from *Dioscorea alata, Smilax china*, and *Trigonella foenum graecum* plants. Diosgenin had a 100% inhibitory effect on *N. fowleri* trophozoites as the compound can down-regulate the expression of the Nf cysteine protease gene and decrease the number of sucker-like apparatuses and inhibit food cup formation. Diosgenin also has anti-inflammatory activity in human monocyte U937-derived macrophages by blocking the synthesis of TNF-alpha (α). It has minimal toxicity against mammalian cells at therapeutic levels thus making it highly recommended to use in treatment for *N. fowleri* infection (56,58,66).

CONCLUSION

In conclusion, an increase in the number of reported PAM

Table II: Recommended drug treatment for primary amoebic meningoencephalitis (PAM) caused by *Naegleria fowleri* (63)

Drug	Dose	Route	Duration
Amphotericin B	1.5 mg/kg/day in two divided doses.	IV	3 days
(followed by)	1.0 mg/kg/day once daily.		11 days
Amphotericin B	1.5 mg once daily.	Intrathecal	2 days
(followed by)	1.0 mg every two days.		8 days
Azithromycin	10 mg/kg/day once daily.	IV/PO	28 days
Fluconazole	10 mg/kg/day once daily.	IV/PO	28 days
Rifampin	10 mg/kg/day once daily.	IV/PO	28 days
Miltefosine	Weight < 45 kg - 50 mg BID Weight > 45 kg - 50 mg TID	PO	28 days
Dexamethasone	0.6 mg/kg/day in four divided doses.	IV	4 days

cases can be influenced by a few potential risk factors ranging from conditions with poor treatment of water supply, recreational activities on warm water bodies to nasal irrigations with contaminated water, and other unlikely factors that can cause harm to the public. Up to date, issues on global warming were accounted for as a new factor causing the number of reported PAM cases to increase. To prevent problems with *N. fowleri* infection, it is suggested to minimize and avoid potential exposure towards the pathogenic amoeba. Habitats known with *N. fowleri* presence should be prohibited regardless of their concentration. Moreover, the prevention of infection is highly dependent on two pillars. Firstly, rapid and accurate diagnosis is done to start appropriate treatment to ensure their effectiveness. Secondly, the prevention of environmental contamination focusing on water resources by regular water treatment that needs cooperation between responsible parties such as the Department of Environment (DOE) and the public by protecting these sources. As described, the occurrence of *N. fowleri* has a worldwide distribution; thus surveys and research on the presence of the amoeba in the environment can increase people's awareness and provide knowledge on the potential habitat of free-living amoeba localization.

As a result, many good diagnostic tools can be applied to detect this pathogenic amoeba on various types of samples, including clinical and environmental. One of the popular identification methods is using molecular biology techniques that are more practical to use in research and clinical investigations. In conjunction with advanced diagnostic tools, many different treatment options have been studied and developed to fight against this fatal infection.

Therefore, good technical skills of a health professional are needed in diagnosing, and continued research should be done to better understand the pathogenicity of the infectious agent and develop strategies on prevention. Finally, educational efforts are needed to improve knowledge and gain access to the information of this parasitic infection for health practitioners and officials, the government, and the public.

ACKNOWLEDGEMENT

This work is supported by the Ministry of Higher Education (MOHE) under the Fundamental Research Grant Scheme (FRGS) (600-IRMI/FRGS 5/3 (018/2019). The authors would like to thank the Faculty of Health Sciences, Universiti Teknologi MARA, Selangor, Malaysia for providing facilities to conduct the present study.

REFERENCES

1. Kao PM, Tung MC, Hsu BM, Hsueh CJ, Chiu YC, Chen NH, et al. Occurrence and Distribution of *Naegleria* species from Thermal Spring Environments in Taiwan. Lett Appl Microbiol. 2013;56(1):1–7.
2. El-Maaty DAA, Hamza RS. Primary Amoebic Meningoencephalitis Caused By *Naegleria fowleri*. Parasitologists United J. 2012;5(2):93-104.
3. Grace E, Asbill S & Virga K. *Naegleria fowleri*: Pathogenesis, Diagnosis, and Treatment Options. Antimicrob Agents Chemother. American Society For Microbiology. 2015;59(11):6677–6681.
4. Latifi AR, Niyyati M, Lorenzo-Morales J, Haghighi A, Tabaei SJS, Lasjerdi Z, et al. Occurrence of *Naegleria* species in Therapeutic Geothermal Water Sources, Northern Iran. Acta Parasitol. 2017;62(1):104–109.
5. Wang W, Wei F, Li J, Li N, Liu Q. Isolation and Identification of *Naegleria* species from Environmental Water in Changchun, Northeastern China. Iran J Parasitol. 2014;9(2):254–259.
6. Tung MC, Hsu BM, Tao CW, Lin WC, Tsai HF, Ji D Der, et al. Identification and Significance of *Naegleria fowleri* Isolated from The Hot Spring Which Related to The First Primary Amebic Meningoencephalitis (PAM) Patient in Taiwan. Int J Parasitol [Internet]. 2013;43(9):691–696. Available from: http://dx.doi.org/10.1016/j.ijpara.2013.01.012
7. BunjongpukS.PrimaryAmebicMeningoencephalitis (PAM). Thai J Ped. 2000;39:38–49.
8. Sithinamsuwan P, Sangruchi T, Chiewvit P, Poungvarin N. Free Living Ameba Infections of The Central Nervous System in Thailand. Report of Two Patients. Int Med J Thai. 2000;17:350–360.
9. Shakoor S, Beg MA, Mahmood SF, Bandea R, Sriram R, Noman F, et al. Primary Amebic Meningoencephalitis Caused By *Naegleria fowleri*, Karachi, Pakistan. Emerg Infect Dis. 2011;17(2):258-261.
10. Visvesvara GS. Infections With Free-Living Amebae. Handb Clin Neurol. 2013;114(July 2013):153–168.
11. Movahedi Z, Shokrollahi MR, Aghaali M & Heydari H. Primary Amoebic Meningoencephalitis in An Iranian Infant. Case Rep Med. 2012;2012.
12. Ithoi I, Lau YL, Fadzlun AA, Foead AI, Neilson RS, Nissapatorn V. Detection of Free Living Amoebae, *Acanthamoeba* and *Naegleria*, in Swimming Pools, Malaysia. Trop Biomed. 2010;27(3):566–577.
13. Gabriel S, Khan NA, Siddiqui R. Occurrence of Free-Living Amoebae (*Acanthamoeba, Balamuthia, Naegleria*) in Water Samples in Peninsular Malaysia. J Water Health. 2019;17(1):160–171.
14. Naqvi A, Yazdani N, Ahmad R, Zehra F, Ahmad N. Epidemiology of Primary Amoebic Meningoencephalitis-Related Deaths Due to *Naegleria fowleri* Infections from Freshwater in Pakistan: An Analysis of 8-Year Dataset. Arch Pharm Pract. 2016;7(4):119.
15. Latiff NSA, Jali A, Azmi NA, Ithoi I, Sulaiman

WYW, Yusuf N. The Occurence of *Acanthamoeba* and *Naegleria* from Recreational Water of Selected Hot Springs in Selangor, Malaysia. Int J Trop Med. 2018;13(3):21–24.
16. Heggie TW & Кърper T. Surviving *Naegleria fowleri* Infections: A Successful Case Report and Novel Therapeutic Approach. Travel Med Infect Dis [Internet]. 2017;16(July 2013):49–51. Available from: http://dx.doi.org/10.1016/j.tmaid.2016.12.005
17. Chomba M, Mucheleng'anga LA, Fwoloshi S, Ngulube J, Mutengo MM. A Case Report: Primary Amoebic Meningoencephalitis in A Young Zambian Adult. BMC Infect Dis. 2017;17(1):1–5.
18. Gianinazzi C, Schild M, Zumkehr B, Wьthrich F, Nьesch I, Ryter R, et al. Screening of Swiss Hot Spring Resorts for Potentially Pathogenic Free-Living Amoebae. Exp Parasitol [Internet]. 2010;126(1):45–53. Available from: http://dx.doi.org/10.1016/j.exppara.2009.12.008
19. Shakeel S, Iffat W, Khan M. Pharmacy Students' Knowledge Assessment of *Naegleria fowleri* Infection. Scientifica (Cairo). 2016;2016:5–10.
20. Bellini NK, Santos TM, da Silva MTA, Thiemann OH. The Therapeutic Strategies Against *Naegleria fowleri*. Exp Parasitol [Internet]. 2018;187:1–11. Available from: https://doi.org/10.1016/j.exppara.2018.02.010
21. Bellini NK, da Fonseca ALM, Reyes-Batlle M, Lorenzo-Morales J, Rocha O, Thiemann OH. Isolation of *Naegleria* spp. From A Brazilian Water Source. Pathogens. 2020;9(2):1–13.
22. Lares-Villa F, Hern6ndez-Peca C. Concentration of *Naegleria fowleri* in Natural Waters Used for Recreational Purposes in Sonora, Mexico (November 2007-October 2008). Exp Parasitol [Internet]. 2010;126(1):33–36. Available from: http://dx.doi.org/10.1016/j.exppara.2010.04.011
23. Panda A, Khalil S, Mirdha BR, Singh Y, Kaushik S. Prevalence of *Naegleria fowleri* in Environmental Samples from Northern Part of India. PLoS One. 2015;10(10):1–14.
24. Al-Herrawy AZ, Gad MA. Isolation and Molecular Identification of *Naegleria fowleri* from Nile River, Egypt. J Egypt Public Health Assoc. 2015;90(4):161–165.
25. Majid MAA, Mahboob T, Mong BGJ, Jaturas N, Richard RL, Tian-Chye T, et al. Pathogenic Waterborne Free-Living Amoebae: An Update from Selected Southeast Asian Countries. PLoS One. 2017;12(2):1–17.
26. Muchesa P, Barnard TG, Bartie C. The Prevalence of Free-Living Amoebae in A South African Hospital Water Distribution System. S Afr J Sci. 2015;111(1–2):3–5.
27. Farra A, Bekondi C, Tricou V, Mbecko JR, Talarmin A. Free-Living Amoebae Isolated in The Central African Republic: Epidemiological and Molecular Aspects. Pan Afr Med J. 2017;26:1–10.
28. Mukhtar F, Wazir MS. Special Communication *Naeglaria fowleri* : The Brain Eating Amoeba or An Enigma ? J Ayub Med Coll Abbottabad. 2015;27(3):735–736.
29. Mushtaq S, Shinwari ZK. *Naegleria fowleri*, The Causative Agent of Primary Amoebic Meningoencephalitis. Proc Pakistan Acad Sci Part B. 2016;53(4B):299–308.
30. Mahittikorn A, Mori H, Popruk S, Roobthaisong A, Sutthikornchai C, Koompapong K, et al. Development of A Rapid, Simple Method for Detecting *Naegleria fowleri* Vsually in Water Samples by Loop-Mediated Isothermal Amplification (LAMP). PLoS One. 2015;10(3):1–15.
31. Fero K. *Naegleria fowleri*. ParaSite. 2010:1-5.
32. Eldeek HE, Attia RA, Nageeb MM, Sakla AA. Comparative Evaluation of Multiple Staining Techniques for Identification of Different Developmental Stages of *Acanthamoeba* and *Naegleria*. J Egypt Soc Parasitol. 2019;49(2):409-422.
33. Al-Herrawy A. Z., Mohamed S. H., Ali M., Mohammed A. H., Gad M. A. Distribution of *Naegleria* in Water Resources in Egypt. Egypt J Environ Reseacrh. 2014;2:1–14.
34. Ithoi I, Ahmad AF, Mak JW, Nissapatorn V, Lau YL, Mahmud R. Morphological Characteristics of Developmental Stages of *Acanthamoeba* and *Naegleria* species Before and After Staining by Various Techniques. Southeast Asian J Trop Med Public Health. 2011;42(6):1327–1338.
35. Sutiono DR & Aisyah S. Primary Amebic Meningoencephalitis (PAM). CDK-259. 2017;44(12):852-854
36. Zysset-Burri DC, Mьller N, Beuret C, Heller M, Schьrch N, Gottstein B, et al. Genome-wide Identification of Pathogenicity Factors of The Free-Living Amoeba *Naegleria fowleri*. BMC Genomics. 2014;15(1):1–15.
37. Lee J, Kang J-M, Kim TI, Kim J-H, Sohn H-J, Na B-K, et al. Excretory and Secretory Proteins of *Naegleria fowleri* Induce Inflammatory Responses in BV-2 Microglial Cells. Journal of Eukaryotic Microbiology. 2016;64(2).
38. Thaн TL, Kang JM, Lк HG, Lee J, Yoo WG, Shin HJ, et al. Fowlerstefin, A Cysteine Protease Inhibitor of *Naegleria fowleri*, Induces Inflammatory Responses in BV-2 Microglial Cells In Vitro. Parasites and Vectors. 2020;13(41).
39. Lee J, Kim JH, Sohn HJ, Yang HJ, Na BK, Chwae YJ, et al. Novel Cathepsin B and Cathepsin B-like Cysteine Protease of *Naegleria fowleri* Excretory-Secretory Proteins and Their Biochemical Properties. Parasitol Res. 2014;113(8):2765–2776.
40. Seong GS, Sohn HJ, Kang H, Seo GE, Kim JH, Shin HJ. Production and Characterization of Monoclonal Antibodies Against Cathepsin B and Cathepsin B-Like Proteins of *Naegleria fowleri*. Exp Parasitol

[Internet]. 2017;183:171–177. Available from: http://dx.doi.org/10.1016/j.exppara.2017.09.004
41. Lam C, Jamerson M, Cabral G, Carlesso AM, Marciano-Cabral F. Expression of Matrix Metalloproteinases in *Naegleria fowleri* and Their Role in Invasion of The Central Nervous System. Microbiol (United Kingdom). 2017;163(10):1436–1444.
42. Burri DC, Gottstein B, Zumkehr B, Hemphill A, Schьrch N, Wittwer M, et al. Development of A High- Versus Low-Pathogenicity Model of The Free-Living Amoeba *Naegleria fowleri*. Microbiol (United Kingdom). 2012;158(10):2652–2660.
43. Herman EK, Greninger AL, Visvesvara GS, Marciano-Cabral F, Dacks JB, Chiu CY. MT Genome of *Naegleria*. J Eukaryot Microbiol. 2014;60(2):179–191.
44. Zyserman I, Mondal D, Sarabia F, McKerrow JH, Roush WR, Debnath A. Identification of Cysteine Protease Inhibitors As New Drug Leads Against *Naegleria fowleri*. Exp Parasitol. 2018;188:36–41.
45. Ma P, Visvesvara GS, Martinez AJ, Theodore FH, Daggett P-M, Sawyer TK. *Naegleria* and *Acanthamoeba* Infections: Review. Rev Infect Dis. 1990;12(3):490–513.44
46. Dunn AL, Reed T, Stewart C, Levy RA. *Naegleria fowleri* That Induces Primary Amoebic Meningoencephalitis: Rapid Diagnosis and Rare Case of Survival in A 12-Year-Old Caucasian Girl. Lab Med. 2016;47(2):149–154.
47. Azam N & Hayat A. Primary Amoebic Meningoencephalitis - A Case Study. Pak Armed Forces Med J. 2013;63(2):282–284.
48. Stubhaug TT, Reiakvam OM, Stensvold CR, Hermansen NO, Holberg-Petersen M, Antal EA, et al. Fatal Primary Amoebic Meningoencephalitis in A Norwegian Tourist Returning from Thailand. JMM Case Reports. 2016;3(3):1–5.
49. Zhang L ling, Wu M, Hu B chuan, Chen H liang, Pan JR, Ruan W, et al. Identification and Molecular Typing of *Naegleria fowleri* from A Patient With Primary Amebic Meningoencephalitis in China. Int J Infect Dis [Internet]. 2018;72:28–33. Available from: https://doi.org/10.1016/j.ijid.2018.05.001
50. Linam WM, Ahmed M, Cope JR, Chu C, Visvesvara GS, Silva AJ da, et al. Successful Treatment of An Adolescent With *Naegleria fowleri* Primary Amebic Meningoencephalitis. Pediatrics. 2015;135(3):744-748.
51. Su MY, Lee MS, Shyu LY, Lin WC, Hsiao PC, Wang CP, et al. A Fatal Case of *Naegleria fowleri* Meningoencephalitis in Taiwan. Korean J Parasitol. 2013;51(2):203–206.
52. CDC. Chapter 5: Collection and Transport of Clinical Specimens [Internet]. 2016. Available from: https://www.cdc.gov/meningitis/lab-manual/chpt05-collect-transport-specimens.html
53. Cope JR, Ali IK. Primary Amebic Meningoencephalitis: What Have We Learned in The Last 5 Years? Curr Infect Dis Rep [Internet]. 2016;18(10):1–7. Available from: http://dx.doi.org/10.1007/s11908-016-0539-4
54. Abrahams-sandн E, Retana-Moreira L, Castro-Castillo A, Reyes-Batlle M, Lorenzo-Morales J. Fatal Meningoencephalitis in Child and Isolation of *Naegleria fowleri* from Hot Springs in Costa Rica. 2015;21(2):382–384.
55. Biyela PT, Hodon R, Brown A, Alum A, Abbaszadegan M, Rittmann BE. Distribution Systems As Reservoirs of *Naegleria fowleri* and Other Amoebae. J Am Water Works Assoc. 2012;104(1):49–50.
56. Rabablert J, Kaewchiangwang S, Tiewcharoen S, Auewarakul P, Roytrakul S, Rungreung T, et al. Anti-Tumor Necrosis Factor-α Activity of Diosgenin in *Naegleria fowleri* Lysate-Induced Human Monocyte U937- Derived Macrophages. 2016;5:1-15.
57. Luangboribun T, Tiewcharoen S, Rabablert J, Auewarakul P, Lumlerdkij N, Junnu V, et al. Screening of Anti-Amoebic Activity on *Naegleria fowleri* in Extracts of Thai Medicinal Plants. JITMM Proceedings. 2014;3:16-22.
58. Tiewcharoen S, Rabablert J, Atithep T, Katzenmeier G, Chetanachan P, Junnu V. Ultra Structure Changes of Diosgenin-Treated Human Monocyte U937-Derived Macrophages Induced by *Naegleria fowleri* Lysate. Southeast Asian J Trop Med Public Health. 2017;48(5):945–954.
59. Debnath A, Tunac JB, Galindo-Gymez S, Silva-Olivares A, Shibayama M, McKerrow JH. Corifungin, A New Drug Lead Against *Naegleria*, Identified From A High-Throughput Screen. Antimicrob Agents Chemother. 2012;56(11):5450–5457.
60. Rajendran K, Anwar A, Khan NA, Siddiqui R. Brain-Eating Amoebae: Silver Nanoparticle Conjugation Enhanced Efficacy of Anti-Amoebic Drugs against *Naegleria fowleri*. ACS Chem Neurosci. 2017;8(12):2626–2630.
61. Cope JR, Conrad DA, Cohen N, Cotilla M, DaSilva A, Jackson J, et al. Use of The Novel Therapeutic Agent Miltefosine for The Treatment of Primary Amebic Meningoencephalitis: Report of One Fatal and One Surviving Case. Clin Infect Dis. 2016;62(6):774-776.
62. Centers for Disease Control and Prevention. Parasites-*Naegleria fowleri*-Primary Amebic Meningoencephalitis (PAM)-Amebic Encephalitis [Internet]. 2020. Available from: https://www.cdc.gov/parasites/naegleria/treatment.html
63. CDC. Treatment [Internet]. 2019. Available from: https://www.cdc.gov/parasites/naegleria/treatment.html
64. Anwar A, Mungroo MR, Khan S, Fatima I, Rafique R, Kanwal, et al. Novel Azoles as Antiparasitic Remedies Against Brain-Eating Amoebae. Antibiotics. 2020;9(4):1–13.

65. Colon BL, Rice CA, Guy RK, Kyle DE. Phenotypic Screens Reveal Posaconazole as A Rapidly Acting Amebicidal Combination Partner for Treatment of Primary Amoebic Meningoencephalitis. J Infect Dis. 2019;219(7):1095–1103.

66. Rabablert J, Tiewcharoen S, Auewarakul P, Atithep T, Lumlerdkij N, Vejaratpimol R, et al. Anti-Amebic Activity of Diosgenin on *Naegleria fowleri* Trophozoites. Southeast Asian J Trop Med Public Health. 2015;46(5):827–834.