

MINI REVIEW

PRIMARY MENINGOENCEPHALITIS CAUSED BY *NAEGLERIA FOWLERI*: A MINI REVIEW

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ABSTRACT

The recent outbreak of primary meningoencephalitis caused by free-living amoebae (FLA), *Naegleria fowleri*, has gained increasing attention due to their confirmed fatality. It is caused by the entrance of contaminated water into nasal passage mainly by ablution practices. The symptoms include severe headache, nausea, vomiting along with fever finally leading to death. FLA other than *N. fowleri* such as *Acanthamoeba* and *Balamuthia species* are also harmful because they are vectors of many bacterial pathogens including *Vibrio*, *Pseudomonas*, *Legionella*, *Enterobacter* and *Mycobacterium species* which help them to feed and colonize in environments, thus contributing to their pathogenesis and transferability to their hosts. Pakistan, being an underdeveloped country, faces long-term shortfalls of electricity resulting in serious water scarcity leading to public dependence on stored water resources, which are breeding hubs for FLA. The rationale of the present review is to highlight the importance of *N. fowleri* and primary meningoencephalitis and to investigate the recent outbreak in Pakistan.

Keywords: Free-living amoeba, *Naegleria fowleri*, primary meningoencephalitis.

1. INTRODUCTION

Free-living amoeba (FLA) are widely distributed in nature such as soil, sea water, dust, sewage, swimming pools, eye wash solutions, contact lenses, dialysis and dental treatment units¹⁻⁶. Except for *Entamoeba histolytica*, pathogenic FLA such as *Acanthamoeba species*, *Balamuthia mandrillaris*, and *Naegleria fowleri* cause CNS infections which are fatal and life threatening⁷. *Acanthamoeba* is the most common opportunistic amphizoic protozoan, which can tolerate a wide range of temperature, pH, and osmolarity. *B. mandrillaris* is the only species of *Balamuthia* which cause infection in humans and animals. Unlike *Acanthamoeba* and *N. fowleri*; *B. mandrillaris* is present in soil and is difficult to isolate. According to the new taxonomic classification proposed by International Society of Protozoologists, the three pathogenic FLA have been classified under two 'Supergroups'. *Acanthamoeba* and *Balamuthia* are in supergroup Amoebozoa: Acanthamoebidae while *N. fowleri* under supergroup Excavata:

Heterolobosea: Vahlkampfia⁸. The main features of these three FLA are presented in Table 1.

FLA belonging to kingdom Protista is ubiquitous as well as opportunistic in nature. Among FLA, only four genera are known to cause infections in humans and animals. Some of the infections caused by these FLA were opportunistic (mainly occur in the immunocompromised host) while other are non-opportunistic (including *Naegleria PAM*, *Acanthamoeba keratitis*)⁸. Even though many advances have been made in the field of diagnostics, antibiotic resistance, antimicrobial chemotherapy and supportive care, the rate of fatality due to *N. fowleri* related meningoencephalitis remained over 95%. The reason behind is the lack of awareness in public, lack of proper sanitization and sterilization procedures for stored water used in different recreational, religious and cultural practices and lack of baseline data about the genotypes and genome structure of this pathogen from Pakistan. Moreover,

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Table 1. Characteristic features of free-living amoeba (FLA)

Properties	<i>Acanthamoeba species</i>	<i>Balamuthia mandrillaris</i>	<i>Naegleria fowleri</i>
First isolated / reported	1931 by Volkonsky	1986 from the brain of a baboon	1970 by Malcolm Fowler from human brain tissues
Distribution in nature	Soil and water	Soil and dust	Soil and water
Infections	Acanthamoeba granulomatous amoebic encephalitis (AGAE), acanthamoeba keratitis (AK), nasopharyngeal and cutaneous acanthamoeba	Granulomatous amoebic encephalitis (GAE), cutaneous, sinus	Primary amoebic meningoencephalitis (PAM)
Host	Immunocompromised patients with AIDS	Immunocompetent and compromised host	Children and young adults
Growth conditions	Grow optimally at 37°C on non-nutrient (NN) agar covered with bacteria	Grows well in tissue culture cells including monkey kidney, human lung fibroblasts, rat glial cells, human brain microvascular cells	Grows up to 45°C on NN agar covered with bacteria
Species	>24 (based on size and morphology of trophozoites and cysts)	Only known species to harm humans and animals	>40
Differentiation	Sequencing of 18S rDNA	–	Sequencing of 5.8S rRNA/internal transcribed spacers (ITS1 and ITS2)
Pathogenic host	<i>Legionella spp.</i> , <i>M. avium</i> , <i>L. monocytogenes</i> , <i>V. cholera</i>	<i>Legionella species</i>	<i>Legionella pneumophila</i>
Mode of action	Phagocytosis, chemotactic response. Produce serine and cysteine proteinases, metalloproteinases, plasminogen activators.	Release of pleiotropic cytokine IL6, produce metalloprotease.	Produce sucker like appendages, cytolytic factors, elastase activity, produce phospholipase A and B.
Antimicrobial therapy	No antimicrobial agent is successful against AGAE. Chlorhexidine gluconate, polyhexamethylene biguanide (PHMB), propamidine isethionate for AK	Combination of pentamidine isethionate, sulfadiazine, clarithromycin, fluconazole, and flucytosine	Antifungal polyene antibiotic amphotericin B (AMB)
Lifecycle/stages	Vegetative or trophozoites and cysts (dormant) stage	Trophozoites and cysts stage	Trophozoites, cyst and flagellate stages

frequent sewage spills, lack of rainfall plus favorable temperatures (mostly hot weather and heat wave in Karachi) also contribute to the breeding and survival of these pathogens.

2. NAEGLERIA FOWLERI

FLA such as *Acanthamoeba*, *Balamuthia*, and *Naegleria* are established causative agents of central nervous system (CNS) infections. FLA which causes primary amoebic meningoencephalitis (PAM), a rapidly progressive and highly fatal disease. *N. fowleri* is a thermo-tolerant, it enters the nasal cavity, invades through the olfactory neuroepithelium and affects CNS causing a fatal infection that clinically resembles acute bacterial meningitis¹⁻⁶.

2.1. Habitat

N. fowleri is widely distributed worldwide in soil and water but its distribution is less than *Acanthamoeba*. Because of its thermophilic nature and survival capability at 45°C, it proliferates in summer months when the temperature rises. It has been isolated from soil, warm and freshwater lakes, streams, heated swimming pools, spas, hydrotherapy and remedial pools, sewage, hot

springs, aquaria, and even from nasal passages and throats of healthy individuals⁹. Most of the PAM cases were reported in summers when people are engaged in recreational activities like diving, swimming, and water skiing in lakes, ponds, and non-chlorinated swimming pools.

2.2. Mechanism of Pathogenesis

N. fowleri encounters its prey by producing appendages similar to suckers or amebostomes and nibbles away at the tissues and cells. Other ways include the production of neuraminidase or elastase which facilitates tissue culture cells, phospholipase A and B for cell membrane destruction, the presence of a cytopathic protein that triggers the apoptosis pathway in susceptible tissue culture cells and the presence of performing-like pore-forming proteins that lyses target cells¹⁰⁻¹².

2.3. Stages of Life

N. fowleri exists in three forms of life (Fig. 1):

- Cyst (environmentally stable)
- Trophozoites (motile amoeboid form)
- Flagellate

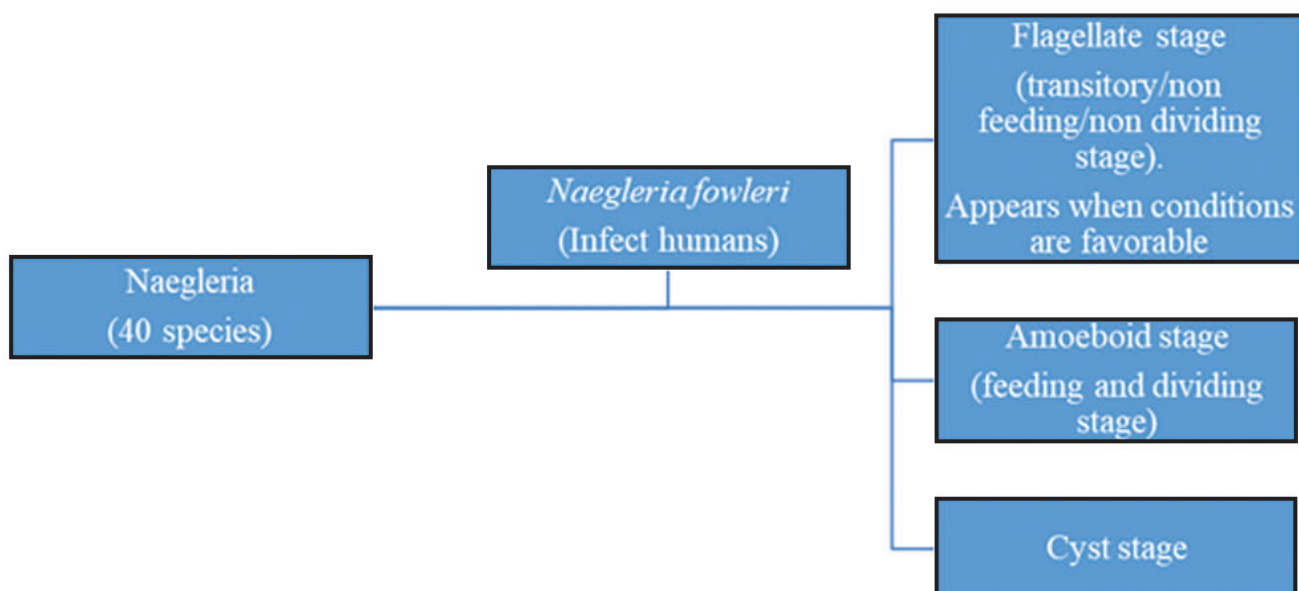


Fig. 1. Lifecycle/stages of *N. fowleri*.

3. PRIMARY AMOEBIC MENINGOENCEPHALITIS

Primary amoebic meningoencephalitis (PAM) is a fatal disease caused by amoeba *N. fowleri*, which is commonly known as ‘brain-eating amoeba’, discovered in 1899¹³. PAM is characterized as a necrotizing, acute, fulminant and hemorrhagic meningoencephalitis with nuchal rigidity, severe headache, altered mental status, behavioral and cardiac rhythm abnormalities, fever (41°C), seizures and coma leading to the death. It occurs 1 to 2 days

after exposure to contaminated water¹⁴. Since 2008, when the first case of PAM was reported from Pakistan¹⁵, an increasing number of fatalities have been observed continuously (Fig. 2). According to seasonal awareness and alert letter (SAAL) issued by National Health Services, Government of Pakistan, 39 fatal cases of PAM have been reported from different hospitals of Karachi including 12 fatal cases in 2015¹⁶. Recently, two cases of *N. fowleri* infection have also been reported^{17,18}

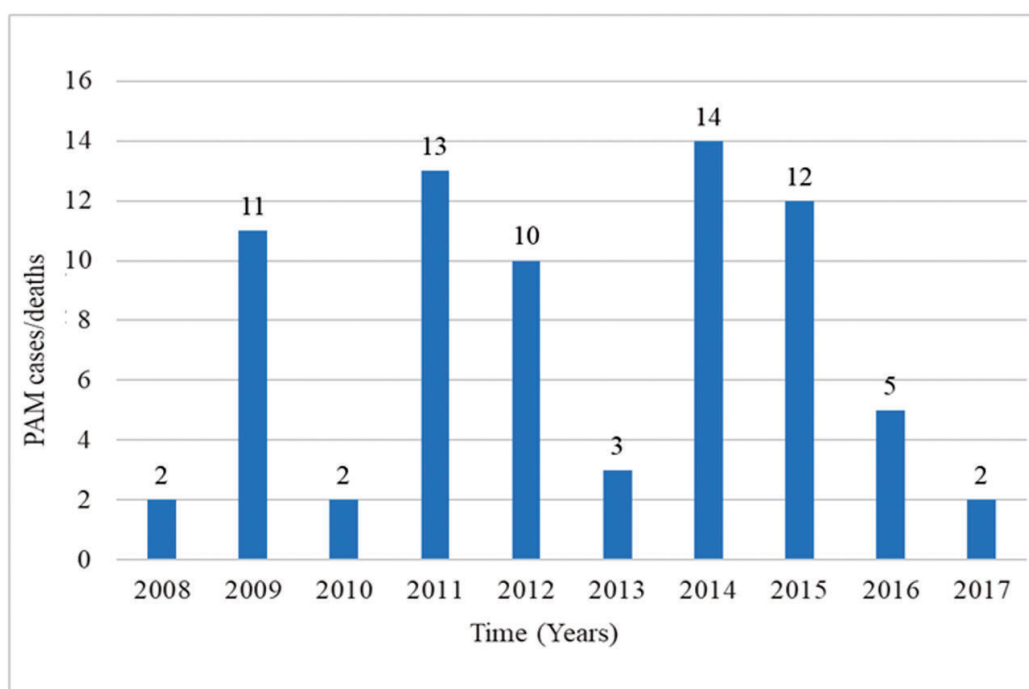


Fig. 2. Graphical representation of deaths caused by *N. fowleri* since 2008 from Pakistan.

3.1. Animal Model

Laboratory mouse is an ideal model for studying PAM. *N. fowleri* suspension can be administered intra-nasally or intra-cerebrally into mice. It is also reported that pathogenic isolates from PAM can lose their virulence characters with repeated sub-culturing which can be enhanced by animal passage or culturing amoeba on tissue culture monolayers^{12,14,19}.

3.2. Diagnosis

Immediate diagnosis of PAM is attained by examining motile *N. fowleri* in freshly drawn cerebrospinal fluid (CSF). Trophozoites can be seen under the microscope by Giemsa or Wright stains

of CSF smears along with enflagellation test²⁰. Due to lack of distinctive symptoms and clinical features, PAM can sometimes be mistaken for viral, bacterial or pyogenic meningitis. More accurate diagnosis can be achieved in less than 5 hours starting from the time of arrival of the specimen into the laboratory by multiplex RT-PCR assay which can identify *N. fowleri* along with *Acanthamoeba* and *Balamuthia mandrillaris*. Furthermore, sequencing of 5.8S rRNA gene and ITS1 and ITS2 of *N. fowleri* can differentiate between different genotypes¹². Other diagnostic parameters of infection caused by *N. fowleri* are shown in Fig. 3.

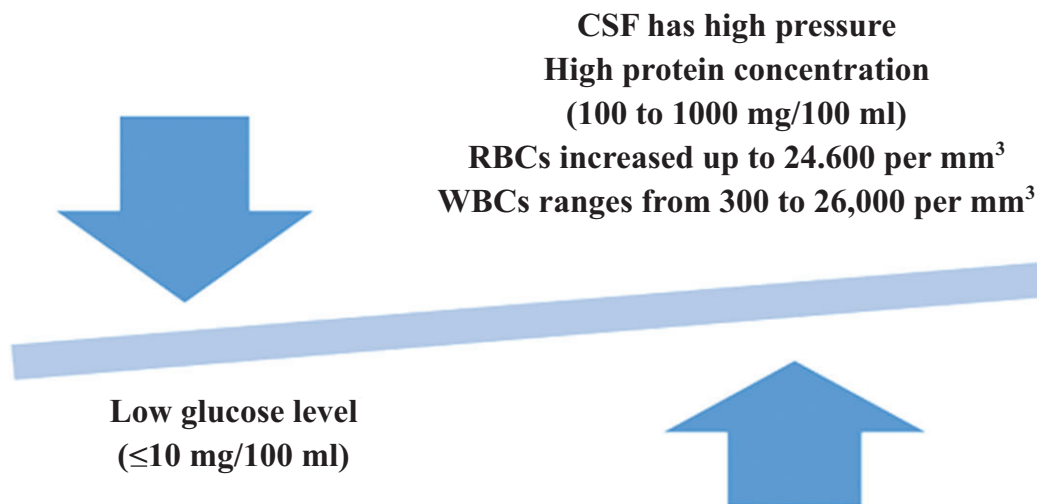


Fig. 3. Diagnostic parameter for primary meningoencephalitis.

3.3. Antimicrobial Treatment

The drug of choice for the disease is antifungal polyene and amphotericin B. Despite this, the fatality rate is still very high (~98%). Among all the reported cases of PAM, only one well-documented case of a 9-year-old female was reported to be successful. The kid was given amphotericin B, miconazole, and oral rifampin. Based on in vitro studies, the authors suggested that both amphotericin B and miconazole acts synergistically²¹. It has also been reported that amphotericin B is more effective than its methyl ester derivative (a water-soluble form). Some other compounds like phenothiazine (chlorpromazine and trifluoperazine) and azithromycin (macrolide) have been observed to be effective in growth inhibition^{12,22}.

3.4. Risk Factors and Precautions

Being an underdeveloped country, Pakistan is facing many problems especially long hours of power shutdown which force the public to visit different freshwater canals, ponds, and standing waters for picnic purposes. *N. fowleri* is a free-living amoeba which feeds on organic debris and bacteria in the fresh water. One of the most important risk factors of getting an infection is the recreational activities, like swimming in fresh water during hot summers and diving in contaminated water unescorted by a

nose clip which forces amoeba in water to enter the nostrils. A second important risk factor is the ablution practice followed by all Muslims five times a day. If water is contaminated with *N. fowleri* and goes into the nostrils, the amoeba approaches nasal mucosa and occupy the brain to cause PAM. Recently many deaths were recorded from *N. fowleri* in one of the leading hospitals of Karachi. The history of patients did not include any recreational activity but they all performed ablution (for prayers) regularly. Even though ablution has many advantages but they can only be achieved when the water is free from contaminants. Another important ritual bathing festival is Kumbh Mela that takes place in Allahabad city of India. Thousands of Hindus gathered in river Ganges and Yamuna for this holy bathing festival. Although no case of PAM associated with *N. fowleri* is reported from this practice, however, this could also be a hotspot for the dissemination of infectious microbes²⁰.

3.5. Host Factors

As PAM cases are mainly associated with youth especially young men who are more involved in outdoor activities and ablutions in mosques, there are many factors along with the virulence of *N. fowleri* which contributes to the pathogenesis of PAM infection. As far as the progression and rapid onset of PAM in humans is concerned, little chances are there to develop an effective humoral response

against *N. fowleri*. Humans are exposed to *Naegleria* species (whether pathogenic or non-pathogenic) through swimming, inhalation of dusts and aerosols due to which they generate antibodies against them. Since different studies have reported the presence of IgM antibodies in patient's sera against *N. fowleri*, it is still unclear that whether these antibodies have some protective role or not in the prevention of this infection^{11,12,14,19}.

4. CONCLUSION

Pakistan is one of the most thickly populated countries of the world having an estimated population of 180.8 million which is expected to reach 208 million by 2020. It is basically an agricultural country with a blend of all four seasons. Karachi is a subtropical region with mostly hot weather (March–October). Because of rapid urbanization in Pakistan, Karachi emerged as a metropolitan city habituating people from almost all ethnic groups. The warm climate of Karachi along with an increase in population and long hours' power shut down are some of the serious concerns which result in storage of water. Most of the population relies on stored water resources which are most of the time not up to the quality of use. Thus, these water bodies become the breeding point of FLA (especially *N. fowleri*). Since 2008, when the first case of *N. fowleri* was reported from Pakistan, an increasing wave of death cases due to this pathogen has been reported from the country. Most of the patients from Karachi acquired this disease from ablution and not from recreational activities. There is an immediate need for extensive awareness about this pathogen in general public as this can tolerate warmer temperatures and can proliferate in potable as well as non-potable water. Moreover, due to increase in population, urbanization, and decrease resources, people of Pakistan get affected by different diseases including Chikungunya and dengue. Chikungunya is an arboviral disease caused by chikungunya virus which is transmitted by *Aedes* mosquito (*Aedes aegypti* and *Aedes albopictus*). According to Ministry of National Health Services, Regulation and Coordination of Pakistan 1018 cases of chikungunya have also been reported from Karachi between

December 2016 to March 2017. Similarly, in October 2017, Khyber Pakhtunkhwa province of Pakistan experienced 12,430 suspected cases of dengue fever with 4 deaths. The district Peshawar has reported the highest number of cases, with a current total of 78,844 suspected cases, including 16,991 confirmed cases and 53 deaths. Keeping in view the aforementioned, there is an immediate need for:

- (i) Continuous surveillance,
- (ii) In-depth knowledge related to the prevalence,
- (iii) Viral genotyping,
- (iv) Variations in proteomes and genomes of these viruses and/or parasites to cut down diseases burden.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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