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EXPLORING THE MOLECULAR INTRICACIES OF IMMUNE REACTIONS TO *NAEGLERIA FOWLERI* IN CONJUNCTION WITH GUT MICROBIOTA FOR NOVEL THERAPEUTIC ADVANCEMENTS

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Abstract

Introduction: *Naegleria fowleri*, a pathogenic amoeba found on all continents except Antarctica. It causes are Primary Amoebic Meningoencephalitis (PAM) with 95% fatality rate, predominantly affecting healthy people from all age group. Research on *Naegleria*'s biology and behavior intensified the efforts which helps to develop prevention and treatment strategies. **Objectives:** To investigate the mechanisms of humoral and innate immunity with molecular characters in arguing for *Naegleria fowleri* infections in gut microbiota and explore their potential benefits for therapeutic development.

Materials and Methods: Different data bases were used to collect the data for article in systematics pattern. The globally epidemiologically surgery was performed to analyze the risk factor, death rate and consequence of viral load in community especially in gut disease and corresponding infections.

Results: Risk factors which also add the water resources and cultural practice are expressing the main cause of disease spreading the defense mechanism of the human body against viral load involves the participation of both humoral and innate immunity intimate with gut bacterial colonization. The humoral immunity is responsible for producing antibodies target and neutralize pathogenic organism. The innate immunity provides a rapid response to viral infections through the activation of various cells and molecules. This collaboration between the humoral and innate immunity ensures a comprehensive defense against viral load in gut microbiota. The social collaboration among different communities is found an effective agenda to reduce the risk of

disease and death rate as well by developing gut infection.
Conclusion: To conclude, the *Naegleria Fowleri* infections highlights the pressing need for continued vigilance, collaboration and innovation to combat this rare yet lethal disease. Further immunity role and health precaution are necessary element to reduce the effects.

Keywords: Gut Microbiota, humoral immunity, *Naegleria fowleri*, Outbreaks, Pathogenic Amoeba

Introduction

The pathogenic species *Naegleria fowleri* has been detected on all continents in nasal cavity and gut canal, except Antarctica. The number of reported primary amoebic meningoencephalitis cases is relatively low, with approximately 250 cases worldwide. It remains a significant concern due to its high fatality rate. Only about 5% of patients affected by Pathogenic Amoeba Meningitis (PAM) survive, which is an alarming aspect, especially considering that the disease primarily affects healthy children through gut infection and throat diseases (Jahangeer *et al.*, 2020). Over time, the intensification of research on the genus *Naegleria* has followed the discovery of *N. fowleri*'s pathogenicity (Martínez-Castillo *et al.*, 2016). Scientists have been making efforts to comprehend the biology, behavior, and factors influencing the transformation of *Naegleria* amoebae into the dangerous flagellated form responsible factors to causing PAM (Dos Santos *et al.*, 2022). Moreover, they have been trying to develop effective prevention and treatment strategies to mitigate the impact of this rare but deadly disease by developing infection in gut canal. Despite the rarity of PAM cases, public health authorities worldwide have recognized the significance of addressing this issue. They are conducting education and awareness campaigns to inform communities, especially those in regions where *Naegleria fowleri* has been found, about the potential risks and preventive measures towards the gut infection (Reyes-López *et al.*, 2022). Additionally, advancements in medical technology and research continue to contribute in understanding this pathogen, which may lead to improved diagnostics and treatment options in the future. Although the gut disease's occurrence in Africa and South America is relatively low, it is vital to monitor and study its prevalence to ensure early detection and appropriate response if cases arise (Lupi *et al.*, 2009). Therefore, public health efforts must persist, even in regions with seemingly low incidence rates, as proactive measures can help safeguard communities and prevent potential outbreaks. In conclusion, the discovery of *Naegleria fowleri* as a pathogen causing primary amoebic meningoencephalitis marked and gut infection in common cases, a pivotal moment in the study of this genus (Maciver *et al.*, 2020). Increased knowledge and awareness have shed light on the disease, prompting dedicated research aimed at finding ways to combat and manage this fatal infection. Continued vigilance and collaboration among the scientific community and public health organizations remain crucial in minimizing the impact of this rare but life-threatening condition on vulnerable populations worldwide.

Materials and Methods

This study was conducted by using electronic databases (ERIC, PubMed, Google Scholar and Science Direct) which are particularly relevant to study. The scoping review protocols were followed to conduct this research work. The selected articles underwent a rigors screening and filtering process to ensure alignment with study objectives. Multiple databases were utilized in order to gather the necessary information for the article pertaining to the study of patterns within systematics. To comprehensively analyze the risk factor, mortality rate, and repercussions of viral load within the community in gut microbiota, a globally conducted epidemiological surgery was performed. This surgical procedure aimed to delve into the intricate details of these factors and their implications on a global scale. Additionally, the risk factors that contribute to the spread of protozoan, such as water resources and cultural practices, were identified as the primary causes of transmission in gut, brain and other parts of body. These risk factors, which play a significant role in the dissemination of pathogenic organism, were also found to be instrumental in the continuation

of this viral epidemic. A Boolean formula was applied to search the article according to topic.

Eligibility criteria

Inclusion criteria: The qualitative, quantitative mix methodology of study was used in-between 2010 to 2022 time period. The major object of this study to investigate the mechanisms of humoral and innate immunity in defending against *Naegleria fowleri* infections and explore their potential for therapeutic development in gut microbiota

Exclusion Criteria: Books, conference reports, citations, lectures, opinions, abstracts only, and handouts will not be included in the study.

Results

The investigation into the molecular immune responses to *Naegleria fowleri* has unveiled a complex defense mechanism that effectively combats this pathogenic amoeba in gut microbiota. Particularly notable is the humoral immunity's pivotal role in this response, as it generates specific antibodies through gut flora that are custom-tailored to neutralize the amoeba. These antibodies selectively target crucial elements of *Naegleria fowleri*, thereby impeding its ability to infect and harm host cells in gut. The innate immunity, acting as a non-specific defense, exhibits a rapid and immediate reaction upon encountering *Naegleria fowleri.* This response involves the activation of diverse immune cells and molecules, which contribute to the containment and eradication of this protozoan. These discoveries emphasize the intricate coordination between humoral and innate immunity in gut part of body, collectively creating a formidable barrier against *Naegleria fowleri* infections. The search engine was utilized for this comprehensive study are listed in Table 1.

Table 1: Search details on different data base by applying Boolean formula.

Sr. No	Words used on PubMed databases after Boolean search operators (AND) (OR) (NOT) were used.	Number of articles on databases after Boolean search operators (AND) (OR) (NOT) were found.	Number of articles selected after inclusion and exclusion criteria.
1.	Exploring the Molecular Intricacies of Immune Reactions to *Naegleria fowleri* in Conjunction with Gut Microbiota for Novel Therapeutic Advancements.	74	09
2.	Exploring the Molecular Intricacies of Immune Reactions to *Naegleria fowleri* in Conjunction with Gut Microbiota for Novel **OR** Therapeutic Advancements.	49	08
3	Exploring the Molecular Intricacies of "Immune Reactions to *Naegleria fowleri* in Conjunction with Gut Microbiota" for Novel Therapeutic Advancements.	36	6

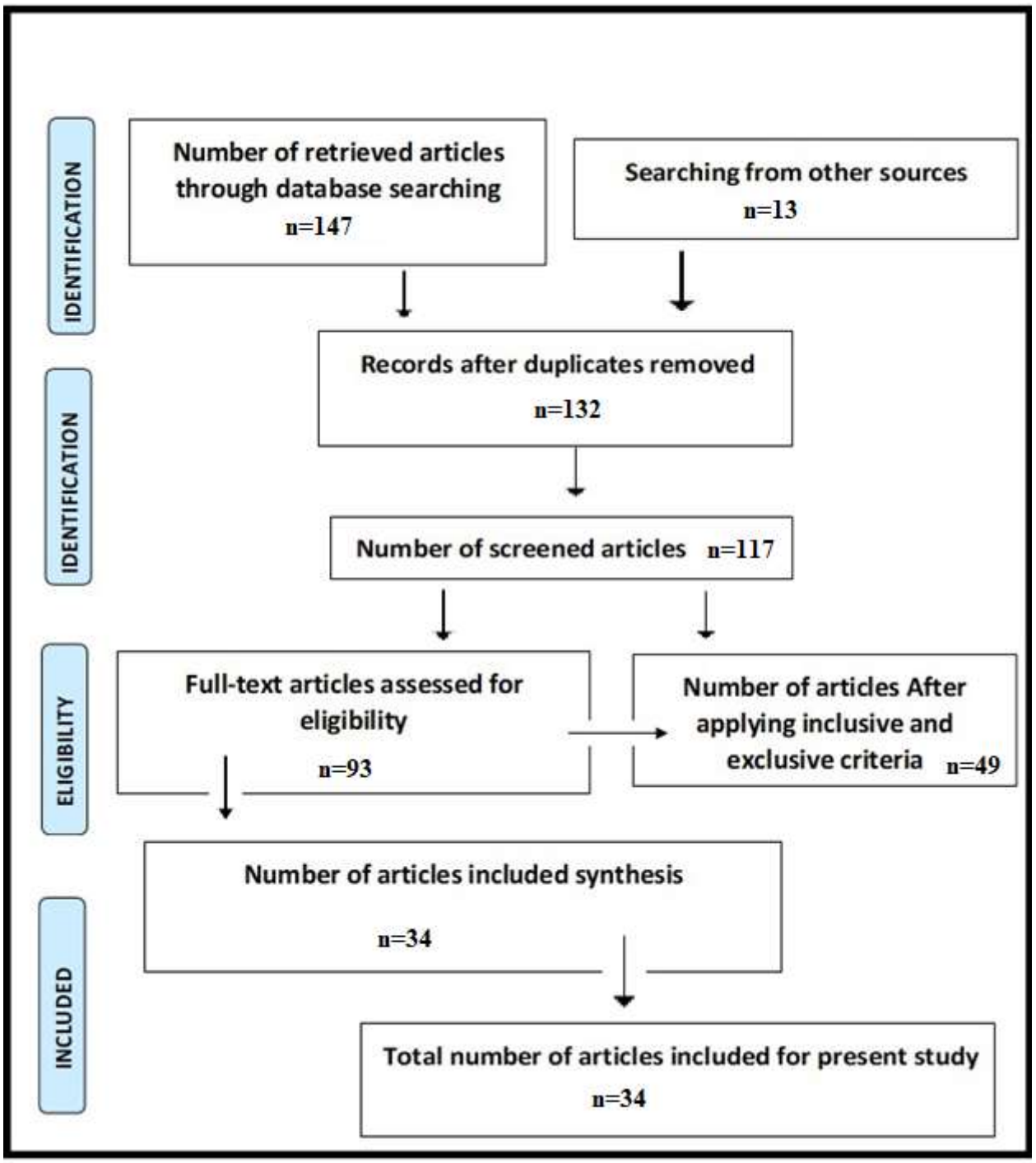
Search terms on Google Scholar with Boolean Commands			
Sr. No	**Words used on PubMed databases after Boolean search operators (AND) (OR) (NOT) were used.**	**Number of articles on PubMed databases after Boolean search operators (AND) (OR) (NOT) were used.**	**Number of articles selected after inclusion and exclusion criteria.**
1.	Exploring the Molecular Intricacies **AND** Immune Reactions to *Naegleria*	54	13

	fowleri in Conjunction with Gut Microbiota for Novel Therapeutic Advancements		
2.	Exploring the Molecular Intricacies of Immune Reactions to *Naegleria fowleri* in Conjunction with "Gut Microbiota for Novel Therapeutic Advancements"	60	8

ERIC Search details on ERIC data base

Sr. No	**Search terms on ERIC**	**Number of results found**	**Number of articles selected after removing duplicates and applying exclusion and inclusion criteria.**
1.	"Immune Reactions to *Naegleria fowleri* in Conjunction with Gut Microbiota"	81	17
3.	Exploring the Molecular Intricacies **NOT** Immune Reactions to *Naegleria fowleri* in Conjunction with Gut Microbiota for Novel Therapeutic Advancements	12	14
4.	Exploring the Molecular Intricacies of Immune Reactions to *Naegleria fowleri* in Conjunction **AND** Gut Microbiota for Novel Therapeutic Advancements	30	06

The expressed flow sheet provides a comprehensive depiction of the intricate and systematic process. The selection of data basis and past study is conducted. To evaluate the analysis of the devastating and life-threatening *Naegleria fleweri* infection in gut critical analysis was performed.



This list includes 14 survivors of gut infection from various regions, which includes the Hong Kong, Australia, USA, Thailand, India, UK, Mexico, Iran. This indicate that gut infection can take place in different parts of the world, despite the fact that, it is usually common in warm freshwater bodies i.e., river and lakes.

Combination of survivors in this database offer motivation to those influence by gut infection or who may be at risk to contracting the disease. The sex and age group are shown in Table 2. However, it is analytical to keep in mind that prevention is the most effective approach to avoid gut infection. Abstaining from warm freshwater sources or engaging with nasal clips while performing swimming activities can substantially reduce the likelihood of vulnerability to the *Naegleria fowleri* amoeba.

Table 2: Survivors of gastrointestinal infection, or gastroenteritis or (gastro) infection linked with *Naegleria fowleri*

SR. NO	AGE	GENDER	GEOGRAPHICAL REGION
1	6	F	UK
2	4	F	UK
3	14	M	Australia
4	9	F	California (USA)
5	18	F	Thailand
6	61	M	Thailand
7	30	M	Italy
8	38	M	Hong Kong
9	26	F	India
10	10	M	Mexico
11	0.8	M	India
12	0.5	M	Iran
143	73	M	India
14	0.1	M	India

*GI stands for gastrointestinal infection, or gastroenteritis or (gastro), which is a rare and deadly brain infection caused by the amoeba *Naegleria fowleri*

Numerous investigations have been conducted to determine the existence of *N. fowleri* in different cooling waters, particularly in industrial environments such as electricity power plants (Ladki and Samad, 2017). The management of this amoeba's growth in cooling waters is a difficult task, which led the French public health ministry to establish an upper threshold of 100 *N. fowleri* per liter in bodies of water where human exposure could occur. This same limit has been enforced in swimming areas, like the geothermal baths in Guadeloupe, a tropical overseas territory of France (De Jonckheere, 2014).

Following an incident of *N. fowleri* infection at a geothermal bath in Guadeloupe, a thorough investigation was carried out to determine the presence of *N. fowleri* in geothermal waters in the region. Authorities have placed a sign at the infection site to indicate that the concentration of *N. fowleri* has never surpassed 5 per liter, which is well below the legally mandated limit of 100 per liter. Despite this, the authorities have recommended the use of nose clips for added safety. This approach has raised concerns since the upper acceptable limit was not proven to have been reached in the water at the time of infection, thereby necessitating a norm of complete absence of *N. fowleri* in 1 liter of water to ensure swimmers' safety (Yoder *et al.*, 2012).

Australia is the only other country that has implemented a legal standard for *Naegleria* in surface water (De Jonckheere, 2012). As early as 1980, the Western Australian government implemented an amoeba alert causing gut infections and severe diseases when the water temperature reaches 28°C. In 2000, they introduced a guideline level of five thermophilic *Naegleria* per liter and an action level of two thermophilic *Naegleria* per liter in the state. Interestingly, the Australian standard does not specify the identification of the pathogenic *N. fowleri*, considering any *Naegleria* species growing at 42°C in this context. This cautious approach contrasts with the French system (Wang *et al.*, 2017). *Naegleria Fowleri*, an amoeba thriving in temperature as high as 46C is notorious for causing PAM, a devasting condition that impacts the central nervous system. PAM leads to significant damage to brain tissue, resulting in inflammation and swelling.

The *Naegleria Fowleri* is found globally, it is more company reported in tropical and subtropical regions across the globe. Cases of PAM tend to be sporadic and rare, with a higher incidence in certain areas where fresh water habitats are prevalent. The majority of reported cases occurred in the United States, particularly in southern States like Florida and Taxes. The second most infected

country by this is documented as Pakistan, especially the province of Baluchistan which has highest rate of patients, while Lahore city is also reported most effective city by *N. fowleri*. Now it has been already infected the people in many countries of globe which are listed in Tale 3. The data collected according to year 2024. The infected countries with number are shown in Figure 1.

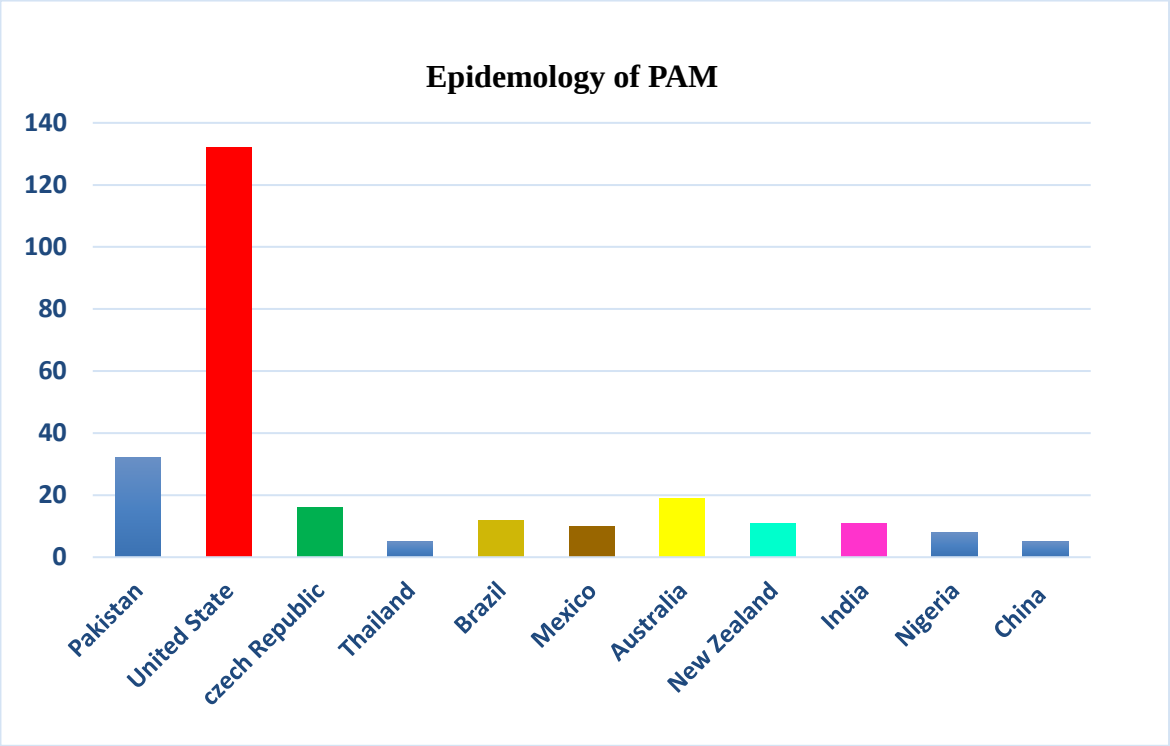


Figure 1: Epidemiological view of *N.fowleri* infection in different countries

Molecular character of *Naegleria*

In the genus *Naegleria*, including all other members of the Vahlkampfiid family, the ribosomal DNA (rDNA) is situated on a circular plasmid, with approximately 4000 copies present in each cell. This is in-contrast to the majority of other eukaryotes, wherein the rDNA exists in tandem repeats on the chromosomes. The particular interest in the study of *Naegleria* are the internal transcribed spacers (ITS) and 5.8S rDNA sequences, which lie between the SSU and LSU rDNA (Fritz-Laylin and Fulton, 2016).

Through the analysis of length differences in ITS1 and the identification of a one-base pair variation in the 5.8S rDNA sequence, researchers have discerned eight distinct types of *N. fowleri*. However, among these eight types, only four have been detected in gut microbiota of patients thus far. These eight types of *N. fowleri* exhibit an uneven global distribution, although more data is required to further substantiate this observation (Otero-Ruiz *et al.*, 2022).

Among the eight types, seven have been found in Europe, indicating a relatively high presence of *N. fowleri* diversity in this region. Additionally, two of the three types identified in the Americas have also been identified in Europe. Only type 1 has not been detected in Europe, being restricted to occurrences in the USA (Boucher *et al.*, 2017). The Table 2 showing the characteristic of *Nagleria.*

Table 3: Species within the genus *Naegleria* have been documented.

Species	Maximum Temperature Tolerance (°C)	Flagellates
N. gruberi	39	Present (+)
N. fowleri	45	Present (+)
N. jadini	35	Present (+)
N. lovaniensis	45	Present (+)
N. australiensis	42	Present (+)
N. italica	42	Present (+)
N. andersoni	40	Present (+)
N. jamiesoni	42	Present (+)
N. clarki	37	Present (+)
N. galeacystis	35	Present (+)
N. minor	38	Division
N. pussardi	41	Present (+)
N. carteri	45	Present (+)
N. morganensis	44	Present (+)
N. niuginensis	45	Present (+)
N. sturti	44	Present (+)
N. robinsoni	38	Division
N. fultoni	35	Present (+)
N. chilensis	30	Absent (-)
N. indonesiensis	38	Absent (-)
N. tihangensis	42	Present (+)
N. pringsheimi	37	Present (+)
N. pagei	37	Present (+)
N. philippinensis	40	Present (+)
N. gallica	33	Present (+)
N. americana	35	Present (+)
N. schusteri	37	Present (+)
N. dobsoni	<35	Present (+)
N. byersi	42	Present (+)
N. endoi	45	Present (+)
N. laresi	42	Present (+)
N. martinezi	45	Present (+)
N. johanseni	45	Present (+)
N. antarctica	28	Present (+)
N. dunnebackei	37	Present (+)
N. angularis	40	Present (+)
N. tenerifensis	42	Present (+)
N. canariensis	37	Present (+)
N. polaris	<30	Present (+)
N. neopolaris	<30	Present (+)
N. arctica	<30	Absent (-)
N. spitzbergenensis	<30	Present (+)
N. neodobsoni	<30	Present (+)
N. neoantarctica	<30	Present (+)
N. neochilensis	<30	Absent (-)
N. paradobsoni	<30	Absent

* Table lists various species within the genus *Naegleria* and their maximum temperature tolerance.

The list comprises 36 distinct species of *Naegleria*, each exhibiting maximum temperature tolerances that vary from 28°C to 45°C. The list also denotes the presence or absence of flagellates in each species, which are whip-like structures employed for movement by certain *Naegleria* species. This showing that *Naegleria* can survive even at high temperature and pH in gut cell wall.
N. chilensis and *N. neochilensis* are two different species that particularly highest temperature tolerance of less than 30°C and have no flagellates, which could be adapted because of cooler environments. In contrast, other species, such as *N. fowleri* and *N. carteri*, exhibit a maximum temperature tolerance of 45°C and possess flagellates, signifying a potentially superior adaptation to warmer habitats.

Risk factor

N. fowleri is a free-living amoeba that can be located in various bodies of water, such as rivers, freshwater lakes, canals, geothermal springs, untreated, under-treated domestic water supplies, and poorly maintained or untreated swimming pools. The majority of cases of gut infections arise in young individuals who have recently been exposed or had contact with contaminated water and engulf water drops intentionally and unintentionally. Unfortunately, in developing countries where numerous other infections are prevalent, gut infection cases often go unnoticed (Matanock *et al.*, 2018).
For example, in countries such as Pakistan with temperatures that can reach up to 50°C, people seek relief in freshwater canals, ponds, and standing water due to persistent power cuts, engaging in "recreational activities" daily for months. The presence of *N. fowleri* in these waters, combined with a lack of awareness, control measures, poor healthcare infrastructure might be dangerous. The limited access to effective drugs, and already suffering from minor gut infection or casual gut diseases, poses a significant health (Apfelbaum *et al.*, 2013).
GIs (Gastrointestinal infections) has also been linked to ablution practices among religious groups. For instance, Muslims perform ablution before every prayer, which involves washing various body parts, including the nose, throat and oral body part. Although oral rinsing is not mandatory, some individuals forcefully drive water up their deep oral parts during ablution. Increasing awareness about water safety during ablution practices is crucial (Siddiqui and Khan, 2014).
Furthermore, in many developing countries, water scarcity necessitates storing water in tanks for extended periods, which presents another major risk factor. Public awareness is essential regarding the risks associated with the use of water storage tanks at home and at prayer places. It emphasizing routine cleaning and disinfection of tanks, especially in mosques where ablution is commonly performed.
In addition, large religious festivals, such as the Kumbh Mela in India, where millions of Hindus participate in ceremonial baths in the Ganges River, can also pose a threat to public health due to potential transmission of infectious agents in oral, gut and digestive parts of body. Moreover, oral cleansing practices like "jala neti," involving the use of neti pots or similar devices, are widespread globally to relieve sinusitis. However, these practices can lead to GIs (Gastrointestinal infections) if not done using disinfected, filtered, or boiled water. Patients presenting with symptoms of Gastrointestinal infections and a history of swimming or exposure to contaminated water for oral cleansing should be suspected of having Gastrointestinal infections (GIs).

Clinical and laboratory assessment

The diagnosis of Gastrointestinal infections (GIs) is definitive upon examination of fluid for the presence of amoebae (Oncel *et al.*, 2022). Wet mount observation of motile trophozoites, in addition to brief centrifugation, aids in concentrating amoebae. Diagnostic tools such as Immunofluorescence assay (IF), enzyme-linked immunosorbent assay (ELISA), flow cytometry, and PCR-based assays have been developed and should be performed on samples (Mungroo *et al.*, 2019). The findings in Gastrointestinal infections (GIs) are similar to bacterial meningitis, with elevated white and red blood cell counts, as well as increased pressure. Protein concentration and

glucose levels may also present abnormalities. To attempt culturing amoebae, it can be transferred to a non-nutrient agar plate seeded with bacteria, and the growth of amoebae can be observed daily for up to seven days. To differentiate *N. fowleri* from other pathogenic amoebae, a flagellation experiment can be conducted, but molecular methods are preferred. Indirect immunofluorescence assay (IIF) is utilized to recognize *N. fowleri* antigen in gastrointestinal cavity tissue slides. Additionally, PCR-based assays have been established for the sensitive and rapid identification of *N. fowleri* in clinical samples (Aykur *et al.*, 2022).

Disease etiology

Numerous in vivo, ex vivo, and in vitro models have been established to explore the molecular mechanisms implicated in the pathogenesis of *Naegleria fowleri.* In vivo investigations entail intranasal inoculation of mice with *N. fowleri*, resulting in a considerable mortality rate. The susceptibility of mice is influenced by their weight and age, with younger and lighter mice being more vulnerable to infection. Following infection, *N. fowleri* can be detected in the mucous layer of the olfactory epithelium within 8 hours, and within 24 hours, infected mice display focal inflammation with the presence of *N. fowleri*. At 96 hours post-infection, a severe inflammatory response, mainly composed of polymorphs is observed, accompanied by tissue damage. Numerous amoebae are found interspersed with degenerating neurons, glial processes, with significant concentrations in gut regions and within the lumina of blood vessels running with gut body part (Coronado *et al.*, 2018).

In vitro investigations, where *N. fowleri* is incubated with host cells, demonstrate cell shrinkage, cell damage, and invasion, leading to destruction through phagocytic processes in oral flora. Organotypic slice cultures from rat mouth, esophagus, stomach, small intestine, large intestine, infected with amoebae exhibit similarities to in vivo infection, indicating their potential usefulness in studying *N. fowleri* pathogenesis. The pathogenicity of *N. fowleri* is categorized into contact-mediated and contact-independent mechanisms for simplicity.

Innate immunity

In addition to its capacity to adhere to nasal mucosa and oral cavity moves to gut flora, display enhanced motility, and eliminate target cells via trogocytosis. The release of cytolytic molecules, *N. fowleri* potentially attained success as a pathogen by devising methods to circumvent the host's immune system. Research has indicated that *Naegleria* exhibits resistance to destruction by host cytolytic molecules, such as tumor necrosis factor (TNF)-α, IL-1, and the complement membrane attack complex (Humphrey *et al.*) C5b-C9. Current data implies that innate immunity may play a more significant part in resisting *N. fowleri* infection than acquired immunity. Elements of the innate immune system, like complement, neutrophils, and macrophages, have demonstrated the ability to react to *N. fowleri* infection (Rajendran *et al.*, 2023).

Polymorph nuclear leukocytes (PMNs)

The examination of human gut lesions that contain amebic trophozoites reveals the presence of an inflammatory infiltrate composed of neutrophils, eosinophils, and macrophages. It is believed that activated neutrophils have a significant role in the early phase of *Naegleria* infections. Notably, the white blood cell counts of individuals infected with *Naegleria* show a remarkable increase in neutrophils. Similarly, experimental infections in mice demonstrate that neutrophils are abundant in tissues early in the infection. Research has indicated that neutrophils are involved in immunity against *Naegleria*, as lymphokine-activated neutrophils are capable of killing *Naegleria* in vitro. The myeloperoxidase H_2O_2 halide system of neutrophils has been suggested to express anti-amebic activity. The immunological reactions are represented in Figure 2. TNF-α was shown to enhance the neutrophil response in digestive tract to *N. fowleri*, and activated neutrophils adhered to *N. fowleri* and subsequently destroyed the amebae in vitro. Although TNF-α may not have a direct effect on *N. fowleri*, the destruction of amebae by neutrophils occurs in the presence of this pro inflammatory

cytokine (Ferrari M *et al.,* 2024).

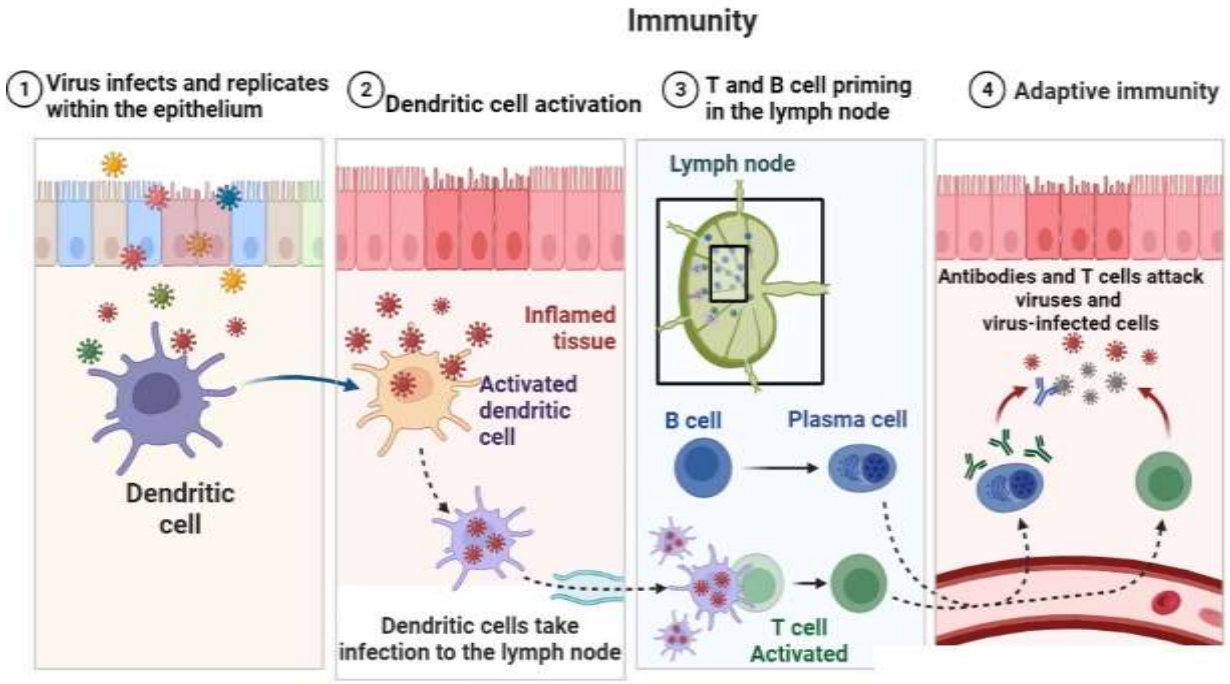


Figure 2: The immunological mechanism of infection by *N. fowleri.*

* In this figure, we illustrate the anti-amebic activity of neutrophils. It's worth noting that TNF-α enhances neutrophil responses to *N. fowleri* within the digestive tract, resulting in the adhesion of activated neutrophils to *N. fowleri* and subsequent amebic destruction in vitro. It's worth noting that TNF-α enhances neutrophil responses in the digestive tract to *N. fowleri,* resulting in the adhesion of activated neutrophils, b cells, t cells, and dendritic cells, to *N. fowleri* and subsequent amebic destruction in vitro. While TNF-α may not directly affect *N. fowleri,* the presence of this pro-inflammatory cytokine plays a crucial role in the destruction of amebae by neutrophils, contributing to adaptive immunity through the production of antibodies.

Studies involving mice have shown that the depletion of circulating neutrophils or the inhibition of neutrophil function through the use of a monoclonal antibody resulted in extensive amebic invasion of the gut and increased mortality when challenged with *N. fowleri*. The complement activation products are believed to trigger a strong chemotactic response leading to the accumulation of neutrophils around amebae, which ultimately leads to their destruction. The precise mechanism by which neutrophils exert anti-*Naeglerial* activities is not yet fully understood, but it is evident that they play a crucial role in the destruction of amebae. The aggregation of neutrophils at sites containing amebae is mediated by various soluble factors, including cytokines and complement components.

Humoral immunity

The humoral immune response to *Naegleria* has been the subject of research in both humans and experimental animals. Studies conducted on healthy individuals from the United States and the Czech Republic have demonstrated that *N. fowleri* antibodies are present in nearly all human sera, indicating widespread exposure to the amoeba. There is also evidence linking *Naegleria* to "humidifier fever," a nonfatal hypersensitivity reaction caused by inhaled antigenic material in humidifier systems into the digestive tract. Antigenic material from *Naegleria* has been found to react with sera from individuals with "humidifier fever. "Numerous investigations the digestive tract to have explored the role of antibodies in host resistance to *Naegleria* infection. In regions

where amebiasis is endemic, higher levels of *IgA* antibody to *N. fowleri* in serum and saliva have been associated with individuals who have upper gut tract infections. This suggests that *IgA* and *IgM* present in mucosal secretions may help prevent amebic infection by blocking trophozoite adhesion to mucosal epithelium. Regarding the correlation between susceptibility to Gastrointestinal infections (GIs) and humoral immune status, findings have been inconsistent. While some studies have hinted at a possible connection between *IgA* deficiency and heightened susceptibility to infection, others have not found a significant association. In animal models, various immunization protocols have elicited a humoral response, but protection against *N. fowleri* challenge infection has been limited. Active acquired immunity to *N. fowleri* has not been achieved in mice, and passive immune therapy with antibodies produced only a minor protective effect. Taken together, the data from both human and animal studies suggest that humoral immunity may not be a major factor in defending against *Nagleria* infection in digestive gut tract. Other aspects of the immune response, such as innate immunity, are likely to play a more significant role in combating the pathogen (Gutiérrez *et al.,* 2023).

Acanthamoeba, amongst free-living amoebae, has undergone extensive scrutiny due to its interactions with various bacteria, such as *E. coli, S. aureus*, and *Pseudomonas aeruginosa*. It has been noted that Acanthamoeba can bind and interact with bacterial cell walls to ingest bacteria. However, research on the specific impacts of monosaccharides or polysaccharides of free-living amoebae is limited. A single study identified that *L. pneumophila*, a bacterium, displayed a high affinity towards specific domains of *A. castellanii*, implying plausible binding interactions between bacteria and amoebae. Recent studies have demonstrated that mannose and fructose are related to host cell adhesion and cytotoxicity in the case of *N. fowleri*. This has led to the proposal of using lectins, sugar binding proteins, for potential vaccine and diagnostic purposes. To delve deeper into bacterial interactions, a study was conducted to investigate the association, invasion, and survival of clinically significant bacteria such as MRSA, *E. faecalis*, and *S. typhi* with *N. fowleri* trophozoites and cysts treated with mannose. The study disclosed that *S. typhi* displayed the highest percentage of interaction with *N. fowleri* trophozoites and cysts, but none of the bacteria survived within them. The hypothesis is that bacteria entering the amoebic cytoplasm may be killed by various proteolytic enzymes during the 24-hour incubation period for survival evaluation. Previous reports on Acanthamoeba suggest that secreted factors from the amoebae, rather than direct contact, can foster the survival of specific bacteria. Chemotaxis, the process of directional movement towards a chemical gradient, may play a role in the interaction between *N. fowleri* and target cells, stimulating the mobility and proliferation of the amoebae. Furthermore, various lectin binding assays demonstrated variations in surface glycol-conjugates of pathogenic and non-pathogenic *Naegleria*. Alpha-D-mannose, Alpha-D-glucose, and terminal Alpha-L-fucose residues were higher in pathogenic *N. fowleri* than in non-pathogenic *N. gruberi* (Son DH and Jung SY 2021*)*.

Treatment:

Recent advancement in nano formulation technology have been utilized to improve drug availability targeting them with silver nanoparticles. A recent study explored the effectiveness of three drugs Nystatin, amphotericin B, and Fluconazole against Naegleria Fowler. This investigation compared the impact of silver nanoparticles alone individual drug and drug conjugated silver nano particles when exposed to *N. fowler*. The results highlighted a significant enhancement in the efficacy of anti-amoebic drugs upon their conjugation with silver nano particles. The CDCs current treatment guidelines for PAM patients recommended as combination therapy approach. this treatment regimen comprises miltefosine an investigational anti-cancer agent, along with antibiotics fluconazole and amphotericin B are administrated. Additionally, dexamethasone an inflammatory drug, is prescribed to alleviate cerebral edema. Table produce further details on PAM survivors and their respective treatment regimens (Güémez A, García *et al.,* 2021).

Table 4: present cases of PAM survivors along with the treatment they received during different time period.

YEAR	TREATMENT/ MEDICATION	RECOVERED PEOPLE
2002	Antibiotic Rifampicin, amphotericin B, imidazole application for three weeks.	27 years old woman
2006	Fluconazole and orally intake of rifampicin	9-year-old boy
2007	Treatment duration of three weeks amphotericin B, chloramphenicol and rifampicin	9-month-old baby
2012	Treatment with Dexamethasone and miltefosine	13-year-old young girl
2016	Application amphotericin B, metronidazole and rifampicin.	10 years old young boy
2017	Fluconazole, dexamethasone	17-year-old young girl
2018	Fluconazole, dexamethasone and miltefosine	11-year-old boy

Discussion

The comprehensive study provide thoroughly explores various aspects of *Naegleria Fowleri* infections, covering the pathogens biology epidemiology clinical symptoms immune response treatment options and associated risk factors (Jahangeer et al., 2020). The detailed analysis emphasizes the seriousness of the threat posed by this uncommon yet lethal amoeba and underscore the necessity for ongoing research and public health initiative to combat its spread and minimize its consequences. A significant insight highlight ion this study is the intercalate interaction between the immune system and *N. Fowleri* infections. Research has uncovered complex mechanism through which both innate and humoral immunity reach to the presences of the amoeba, revealing the body efforts to defend against pathogens (Maciver *et al.,* 2020). However, the effectiveness of these immune response particularly regarding humoral mechanism, is viral for developing effectiveness therapeutic approaches and potential vaccines. The study emphasis the global distribution of *N. Folwer* and its correlation with various environmental and cultural factors, the identification of risk elements such as water source, recreational activities and religious underscores the necessity for targeted intervention and public health education efforts especially in regions where the pathogen is prevalent. Implementing measures such as water treatment improved sanitations practice and enhanced surveillance can help reduce the incidence of *N. Fowleri* infections and safeguard vulnerable populations (Ekici *et al.,* 2022). The advancement in treatment options including the investigations of nano-formulation and combination therapies. The effectiveness of drug like amphotericin B, Fluconazole and miltefosine either individually or combination underscore the importance of multidisciplinary approaches to managing *N. Fowleri* infections. The identification of survivors of primary amebic meningoencephalitis and their treatment protocols offers valuable insight into potential therapeutic strategies for future case. Despite this advancement several challenges and limitations persist (Carlson *et al.,* 2021). The dynamic nature of *N. Folweri* and the evolving understanding of its biological and epidemiology require ongoing research and surveillance efforts. Additionally addressing social and economic factors contributing to the spread of infection such as access clean water and healthcare infrastructure remains essential for effective preventions and control measure (Wang *et al.,* 2021). This study emphasizes the urgent need for sustained vigilance collaboration and innovation in the battle against *N. Fowleri* infections. By advancing our knowledge of the pathogen enhancing diagnostic and treatment options, and implementing target public health interventions, we can strive to mitigate the impact of this rare yet devastating disease and protect vulnerable populations worldwide.

Conclusion:

The examination of *Naegleria Fowleri* infections highlights the pressing need for continued vigilance, collaboration and innovation to combat this rare yet lethal disease. By delving into various aspects such as the pathogens biology epidemiology clinical manifestations immune response, treatment options, and associated risk factors the severity of the threat posed by *N. Fawleri* becomes clear. Key finding from the review underscores the intricate interaction between

the immune system and *N. fowleri* infections, emphasizing the ongoing discussion and further exploration required regarding the efficacy of immune response, particularly humoral immunity. Furth more the global prevalence of *N. fowleri* and its correlation with immunity. Furth more the global prevalence of *N. fowleri* and its correlation with environmental and cultural elements stress the significant of targeted interactions and public health awareness campaigns to mitigation infections and safeguard vulnerable communities. Progression in treatment modalities including advancements like nano-formulations and combinations therapies, holds promise for managing *N. Fowleri* infections. Insights gleaned from survivors and their treatments protocols offer valuable guidance for future cases.

Limitations

It is vital to note that the research on *Naegleria fowleri* in progress which may possibly develop novel evolution in future that may challenge the knowledge in this article.

Moreover, this information is on the biology, avoidance and therapeutics for *N. fowleri* which doesn't explore the social or economic factors which involved in spread of infection.

Future Prospect

- To advance the comprehension of *Naegleria fowleri* in biology entails continued monitoring and vigilance to detect and respond to outbreaks of primary GIs, especially in endangered populations.
- To effectively deal with this fatal infection, it is essential to implement combined efforts among researchers, public health authorities, and communities.
- Moreover, it is imperative to explore the potential use of lectins, sugar-binding proteins, for vaccine and diagnostic purposes.
- Additionally, it is necessary to delve deeper into the role of neutrophils in immunity against *N. fowleri*, including the potential use of TNF-α to increase neutrophil response to the ameba.

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