

# Epidemiology of primary amoebic meningoencephalitis-related deaths due to *Naegleria fowleri* infections from freshwater in Pakistan: An analysis of 8-year dataset

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## ABSTRACT

**Objective:** This study was aimed at gathering all the data related to *Naegleria fowleri* resulted primary amoebic meningoencephalitis (PAM) deaths in Pakistan and present it in a dataset, conduct statistical analysis to report any significant finding and present it as a base for future investigations on the subject.

**Materials and Methods:** A retrospective 6-month study was designed which gathered data related to *N. fowleri* resulted PAM spread in Pakistan from 2008 to 2015. The study adhered to STROBE recommendations for observational cross-sectional studies.

**Results:** The mean (X) age of the patients was 29.47 years (standard deviation [SD]: 13.95), and adults between 26 and 45 years were most affected ( $n = 24$ , 24.5%). The mean (X) survival days after onset of symptoms till death was 4.26 (SD: 2.94). Mortality observed from 2008 till 2015 was 98 ( $n = 98$ , 100%). The majority of deaths occur in southern city of Karachi ( $n = 94$ , 95.9%) in temperature range of between 35°C and 39°C ( $n = 39$ , 60.2%). An incidence rate of 4.084e + 92 was calculated for Karachi. The chlorination of the municipality water supply of Karachi city was below the WHO recommended level of 0.5 ppm. All deaths belonged to individuals from Muslim community ( $n = 98$ , 100%) and without swimming history. Only one individual was reported to have a swimming history.

**Conclusion:** Apart from contaminated municipal water supply, the use of water for ablution directly from ground source is believed to be a major contributing factor to the increasing number of infections; however, this claim could not be statistically verified. This study suggests that concerted efforts by all stakeholders are required if Pakistan needs to bring down the number of *Naegleria* infection cases. Further research is also immediately required to uncover the role of environmental factors in the rise of *Naegleria* infection cases.

**Key words:** Amoeba, encephalitis, *Naegleria fowleri*, Pakistan, primary amoebic meningoencephalitis

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## INTRODUCTION

*Naegleria fowleri* is a single-celled organism which causes a rare but life-threatening brain infection referred to as primary amoebic meningoencephalitis (PAM), more commonly addressed as a brain-eating infection. Once it reaches the brain, the pathogen rapidly causes swelling and inflammation in the brain. Eventually, the extreme pressure in the cranium due to inflammation leads to necrosis of brain tissues. Studies have reported a fatality in 97% of the cases.<sup>[1,2]</sup> This organism is a thermophile amoeba which flourishes in warm, freshwater reservoirs such as puddles, ponds, and lakes and also thrives in nearby soils of such vicinities. In addition, it lives in nonchlorinated water reservoirs which can include swimming pools and sometimes municipality water supplies. Being a thermophile, it is more viable in hot temperatures and has also been reported to kill a number of individuals in the USA during summer season.<sup>[3,4]</sup>

Evidence indicates that it cannot infect an individual by oral route as it is reported to infect an individual only through olfactory route. When it enters the nasal cavity, the pathogen travels to brain and feeds on nerve tissues. Swimmers and individuals involved with water sports are at increased risk of contacting the pathogen. Another possible way of contacting the infection is by performing activities that render water in the nasal cavity which if contaminated can lead to an infection. Such practices might include washing the sinuses, i.e., nasal irrigation and rituals involving rinsing of nasal cavity such as ablution.<sup>[5-7]</sup> The infection not only prevails in developing countries like Pakistan but it has surfaced in developed countries such as USA, the UK and New Zealand as well.<sup>[8-11]</sup> In addition, the pathogen was also reported in the municipality water supply of St. Bernard Parish in the USA; the fatalities of which were later reported.<sup>[8,9]</sup>

The symptoms of the infection range from a simple change in smell or taste to huge indicators such as seizures and hallucinations. It begins within the first 2 days of exposure which progress rapidly resulting in death within a week or less. Other symptoms include fever, frequent headaches, stiffness in neck, nausea, vomiting, photosensitivity, imbalance, and drowsiness.<sup>[10]</sup> The signs of infection become visible as soon as the body comes in contact with the pathogen. The symptoms worsen and become more evident with each passing day.

In Pakistan, contaminated tap water and freshwater pollution is quite common, and many studies have reported the phenomenon; the resort spas swimming pools which are sometimes inadequately chlorinated are few examples of the sources of infection in the country.<sup>[12-14]</sup> However, *N. fowleri*-related infection was never reported before. Since it is occurring in Pakistan, PAM is characteristically in focus from May to September while the temperature in the country is at its extreme. This pathogen is an increasing threat, particularly when one considers the water pollution rate in Pakistan. Most rural areas and some of the major cities rely on ground water, wells, rivers, canals, and streams to fulfill their basic water needs. The majority of the people predominantly from rural background lack easy access to water and have no choice but to make use of these unfiltered, nonchlorinated ground waters and wells which may be a thriving pool for *N. fowleri*. The majority of the water resources are owned by the government while managed as publically owned resource. According to a report, 95% of shallow ground water supplies in the province of Sindh were contaminated although the presence of *N. fowleri* was negative.<sup>[14]</sup> It is true that free-living amoebae infections are most common in individuals involved in water sports; however, the statement was not so convincing in Pakistani context because so far majority of *N. fowleri*-related deaths reported in Pakistan were of individuals with no active history of swimming or water activities.

There is no study which can comprehensively report the cases of *N. fowleri* infections occurring in Pakistan since the last 8 years. Most of the information in the country is either not reported or missing. This study was aimed at gathering the data and presenting it in a primary data set with some statistical analyses to examine and present it as a base for further investigation.

## MATERIALS AND METHODS

A retrospective study was designed which was aimed at gathering the data related to *N. fowleri* infection deaths in the country since the last 8 years. The study adhered to STROBE recommendations for observational cross-sectional studies.

### Rationale of the study

The study drove its rationality from the fact that till date, there are very few literature sources available which report the phenomenon, and due to the scattered and missing information, there is not a single research

literature which presents the data of PAM-related deaths of Pakistan.

### Study objectives

The objective of the study was to collect all the data related to *N. fowleri* resulted PAM deaths in Pakistan and present it in a dataset, conduct statistical analysis to report any significant finding and present it as a base for future investigations on the subject.

### Study duration

The duration of the study was 6 months.

### Eligibility criteria and data gathering

The timeline of retrieved data was from the oldest available information on the subject to most recent, i.e., 2008–2015. All data relating to the *N. fowleri*-related deaths in Pakistan were sought from Google Scholar, PubMed, Web of Science, ScienceDirect, and local database and news agencies such as Dawn.com, the Express Tribune, and the News. The keywords used to search the data included *Naegleria fowleri*, *N. fowleri*, primary amoebic meningoencephalitis, and PAM. Some of the missing data were also sought through manual inquiry which included physical visits, telephonic conversations, and emails.

### Identified variables

The variables identified were categorized as timeline-specific variables which included years and months, mean temperature of months in degree Celsius (°C), and venue of reported cases; the other set of variables were classified as patient-specific variables such as number of reported cases, age, age group, gender, swimming history, and religious views.

### Statistical analysis and tabulation

The data gathered were expressed in frequencies, i.e., percentages (%) and sample number (*n*). Some of the variables were expressed as mean (*X*) and standard deviation (*SD*). In addition, some of the timeline and patient variables were cross tabulated, and Chi-square test was employed to detect any significant association. It is also tabulated in the form of a comprehensive data set.

### Ethical approval statement

The study does not contain any of the individual person's data and report findings with the purpose of only presenting the scattered or missing information in a single data set. It was granted exemption from the Ethical Review by the Committee on Infectious Diseases, Department of Medicine, Clifton Hospital, Karachi, Pakistan (Letter #CH-04-02). However, the

committee was updated regarding developments throughout the study.

### Conceptual framework and hypothesis

Prior to the study commencement, hypothesis was set. A one-tailed hypothesis was formulated, i.e. number of *N. fowleri* infections-related deaths rose in Pakistan during the last few years.

## RESULTS

A total of 98 patients lost their lives in Pakistan due to the infection since the year 2008. The highest number of cases reported in the last 8 years was in 2012 (*n* = 22, 22.4%). Almost a third (*n* = 29, 29.6%) of the deaths occurred in July. The mean temperature of the month was between 35°C and 39°C when most deaths occurred (*n* = 59, 60.2%). Majority of the cases (*n* = 94, 95.9%) occurred in the city of Karachi. The summary of data timeline is tabulated in Table 1.

**Table 1: Summary of data timeline**

Time	Sample ( <i>n</i> )	Percentage	<i>P</i>
Reported cases in years			
2008	2	2	<0.05
2009	11	11.2	
2010	20	20.4	
2011	13	13.3	
2012	22	22.4	
2013	3	3.1	
2014	14	14.3	
2015	13	13.3	
Total	98	100	
Reported cases in months			
April	2	2	<0.05
May	19	19.4	
June	12	12.2	
July	29	29.6	
August	14	14.3	
September	12	12.2	
October	8	8.2	
November	2	2	
Total	98	100	
Mean temperature (°C) in which deaths reported			
Between 35°C and 39°C	59	60.2	<0.05
Between 40°C and 44°C	39	39.8	
Total	98	100	
Venue of reported cases (cities)			
Karachi	94	95.9	<0.05
Thatta	1	1	
Umerkot	1	1	
Lahore	1	1	
Hyderabad	1	1	
Total	98	100	

The mean (X) age of the patients was 29.47 years (SD: 13.95) and mean (X) survival days after onset of symptoms till death was 4.26 (SD: 2.94). No individual who contacted the disease was able to survive (100% mortality). The age group of adults, i.e., between 26 and 45 years, was most affected (n = 24, 24.5%). The majority were males (n = 90, 91.8%) and had no swimming history (n = 95, 96.9%). All of the deaths occurred in the Muslim community. Table 2 gives a detailed outlook on the patient data.

Cross tabulating the variable of time, i.e. years, with patient variables such as gender and age group, revealed significance associations. Chi-square test revealed statistically significant P values, i.e., <0.05.

**Table 2: Data of patients affected by Naegleria fowleri infection**

Time	Sample (n)	Percentage	P
<b>Patient age</b>			
Children and toddlers (up to 5 years)	2	2	<0.05
Teenagers (11-18 years)	13	13.3	
Adolescents (19-25 years)	19	19.4	
Adults (26-45 years)	24	24.5	
Geriatrics (45 and above)	10	10.2	
Not reported or missing data	30	30.6	
Total	98	100	
<b>Gender</b>			
Male	90	91.8	<0.05
Female	8	8.2	
Total	98	100	
<b>Swimming history</b>			
No history	95	96.9	<0.05
Yes	2	2	
Not reported or missing data	1	1	
Total	98	100	
<b>Religious views</b>			
Islam	98	100	<0.05
Total	98	100	

From the cross tabulation, it was observed that in the beginning, PAM-related deaths occurred mainly in males. However, female deaths have also reported recently. Cross tabulating the variable of year with patient age group revealed that individuals from all ages, i.e., from infants and toddlers to elderly, were reported dead due to the pathogen. The data are expressed in Table 3.

Moreover, it was also reported that chlorination of the municipality water supply in the city of Karachi was below the WHO recommended level of 0.5 ppm. Data of 913 water samples taken from different areas of the city in 2012 reported 201 water samples to have chlorination below 0.5 ppm. In the year 2014, another 2094 water samples were drawn across the city and reported low chlorination. The subsequent year also reported similar results. Table 4 summarizes the findings.

In addition, a detailed summary of the number of *N. fowleri* cases reported in the country is presented in Table 5.

## DISCUSSION

*N. fowleri* is one of the most fatal waterborne pathogens known in the world. With an extremely high fatality rate and dearth of treatment options, it has become a matter of grave concern for the health officials. One hundred and thirty-eight cases have been recorded in the United States during 1962–2015.<sup>[57]</sup> However, in case of Pakistan, the numbers are startling. *N. fowleri* infection has resulted in the death of 98 individuals during a short period of 8 years from 2008 to 2015. In the year 2012, Pakistan had the highest number of cases reported when as many as 22 cases were recorded. This study highlights the risk factors of this

**Table 3: Cross tabulation of years with gender and age group**

	Cross tabulation of variables								P
	Years sample count (expected count)								
	2008	2009	2010	2011	2012	2013	2014	2015	
<b>Gender</b>									
Male	2 (1.8)	11 (10.9)	20 (18.4)	12 (11.9)	22 (20.2)	3 (2.8)	10 (12.9)	10 (11.9)	<0.05
Female	0 (0.2)	0 (0.9)	0 (1.6)	1 (1.1)	0 (1.8)	0 (0.2)	4 (1.1)	3 (1.1)	
<b>Age group</b>									
Children and toddlers (up to 5 years)	0	0 (0.2)	0 (0.4)	0 (0.3)	1 (0.4)	0 (0.1)	1 (0.3)	0 (0.3)	<0.01
Teenagers (11-18 years)	0 (0.3)	4 (1.5)	0 (2.7)	4 (1.7)	0 (2.9)	1 (0.4)	2 (1.9)	2 (1.7)	
Adolescents (19-25 years)	1 (0.4)	1 (2.1)	0 (3.9)	3 (2.5)	4 (4.3)	1 (0.6)	3 (2.7)	6 (2.5)	
Adults (26-45 years)	1 (0.5)	4 (2.7)	0 (2)	3 (1.3)	3 (2.2)	0 (0.3)	1 (1.4)	1 (1.3)	
Geriatrics (45 and above)	0 (0.2)	2 (1.1)	0 (2)	3 (1.3)	3 (2.2)	0 (0.3)	1 (1.4)	1 (1.3)	
Not reported or missing data	0 (0.6)	0 (3.4)	20 (6.1)	0 (4)	10 (6.7)	0 (0.9)	0 (4.3)	0 (4)	



**Table 4: Summary of chlorination status of city of municipality water supply of Karachi**

Year	Chlorine (ppm)	Samples having chlorine below WHO recommended level (total samples taken)	Percentage	Source
2008				
2009				
2010				
2011				
2012	<0.5 ppm	201 (913)	22	The Express Tribune, 2013 <sup>[15]</sup>
2013				
2014	<0.5 ppm	2094 (2094)	100	The News, 2015 <sup>[16]</sup>
2015	<0.5 ppm	113 (214)	52.8	Douglas Main, 2015 <sup>[17]</sup>

NSC=No sampling conducted

infection, especially in Karachi, which has witnessed the emergence of this pathogen at an alarming rate. Karachi is situated on the southern coast of Pakistan and experiences a hot and humid climate.<sup>[15,17,58]</sup>

Most of the cases reported worldwide were recorded in individuals with a greater involvement in recreational activities such as water sports, freshwater swimming, and diving. However, in case of Pakistan, apart from one case, none of the patients infected with *Naegleria* had history of participation in recreational activities. According to a study conducted on 13 PAM patients in Karachi, none showed any history of water activities. However, ablation through tap water was suggested as a likely risk factor, especially among the Muslim community.<sup>[13]</sup>

Data showed that all of the individuals who died due to *N. fowleri* infection belonged to the Muslim community. Scientists at the Virginia Commonwealth University mentioned ablation as one of the core reasons of *N. fowleri*-related deaths in Pakistan.<sup>[16]</sup> This is because ritual nasal cleansing is performed as part of the ablation. Hence, taking water into the nostrils as part of ritual of "ablation" is suggested as the likely route of acquiring infection.

Contaminated water supply is suggested as another possible cause of *Naegleria* infections. In Australia, the cases of *N. fowleri* were detected in private water supplies that were poorly treated and had lack of chlorination.<sup>[59]</sup> In case of Karachi, the presence of *N. fowleri* amoeba in the water sources can be attributed to various factors. First, the water from freshwater lakes is neither treated nor chlorinated properly. Second, the presence of broken pipe lines and leakages of the sewage and water lines results in outflow of sewage in the water lines in many instances. Hence, it becomes a favorable condition for the *N. fowleri* amoebae to breed in.

Apart from the contaminated municipal water supply, another possible explanation for the occurrence

of infections can be attributed to obtaining water from underground aquifers through the process of boring. It is a process in which ground is dug deep to obtain freshwater from an underground confined or unconfined aquifer.<sup>[17]</sup> This increases the likelihood of *N. fowleri* infection since it dwells in such habitat.

A close observation of the cases reported reveals that all the deaths caused by that *N. fowleri* occurred in April–September when most part of the country experiences pronounced spells of hot weather. Pakistan is experiencing changing climatic conditions. Karachi in particular has been experienced soaring temperatures, and summer temperatures are projected to continue rising. It is feared that this increase of temperature may be one of the factors potentially contributing to the recent upsurge in the number of infection cases in the city.

It is imperative to note that *N. fowleri* is found in warm water environments and has the ability to survive high temperatures (thermo-tolerant). Therefore, with elevated atmospheric temperatures, the water temperatures rise that allows the *Naegleria* amoebae to multiply in number. Hence, the risk of infection is likely to increase due to increased temperatures due to global warming effects in this part of the world. Therefore, environmental factors should be investigated in greater depth with regard to increase in *Naegleria* cases so that effective preventive strategies can be employed to curb the spread of the infection.

It is also noteworthy to observe that predominantly the cases were reported among males. Therefore, gender, a factor of susceptibility to the infection, should be investigated in greater depth. Health officials in the country have raised red flag over upsurge in the number of *Naegleria* cases. Along with the need to investigate the reasons for the rise in the cases, it is equally essential to take all the necessary efforts to prevent the occurrence of infection. Keeping in view the rapid progression of the infection, awaiting results for tests such as immunohistochemistry and polymerase chain reaction

**Table 5: Summary of yearly reported cases of *Naegleria fowleri* infection in Pakistan**

Total reported cases (n)	Year	Patient	Months	Maximum temperature in month (°C)	Patient ag (years)	Gender (male/female)	Religion	Swimming history	Duration of survival after symptoms (days)	City	Source
2	2008	1	July	37	30	Male	Islam	None	6	Karachi	Shakoor et al., 2011 <sup>[13]</sup>
211	2009	2	September	41	25	Male	Islam	None	15	Karachi	
		1	April	42	30	Male	Islam	None	3	Karachi	
		2	May	41	36	Male	Islam	None	6	Karachi	
		3	July	39	60	Male	Islam	None	7	Karachi	
		4			30	Male	Islam	None	9	Karachi	
		5			64	Male	Islam	None	7	Karachi	
		6			18	Male	Islam	None	6	Karachi	
		7	August	37	22	Male	Islam	None	5	Karachi	
		8			16	Male	Islam	None	3	Karachi	
		9	October	41	18	Male	Islam	None	3	Karachi	
		10			18	Male	Islam	None	7	Karachi	
20	2010	11	November	37	35	Male	Islam	None	6	Karachi	
		1	Between May and September	Range from 37 to 41	NR	Male	Islam	None	NR	Karachi	Siddiqui and Khan, 2012 <sup>[8]</sup>
		2			NR	Male	Islam	None	NR	Karachi	
		3			NR	Male	Islam	None	NR	Karachi	
		4			NR	Male	Islam	None	NR	Karachi	
		5			NR	Male	Islam	None	NR	Karachi	
		6			NR	Male	Islam	None	NR	Karachi	
		7			NR	Male	Islam	None	NR	Karachi	
		8			NR	Male	Islam	None	NR	Karachi	
		9			NR	Male	Islam	None	NR	Karachi	
10			NR	Male	Islam	None	NR	Karachi			
11			NR	Male	Islam	None	NR	Karachi			
12			NR	Male	Islam	None	NR	Karachi			
13			NR	Male	Islam	None	NR	Karachi			
14			NR	Male	Islam	None	NR	Karachi			
15			NR	Male	Islam	None	NR	Karachi			
16			NR	Male	Islam	None	NR	Karachi			
17			NR	Male	Islam	None	NR	Karachi			
18			NR	Male	Islam	None	NR	Karachi			
19			NR	Male	Islam	None	NR	Karachi			
20			NR	Male	Islam	None	NR	Karachi			

Contd...

**Table 5: Contd...**

Total reported cases (n)	Year	Patient	Months	Maximum temperature in month (°C)	Patient ag (years)	Gender (male/female)	Religion	Swimming history	Duration of survival after symptoms (days)	City	Source
13	2011	1	Between May and September	Range from 38 to 44	All patients aged between 16 and 64 years*	Male	Islam	None	NR	Karachi	Mahmood, 2015 <sup>[19]</sup> Hasan, 2014 <sup>[20]</sup>
		2									
		3									
		4									
		5									
		6									
		7									
		8									
		9									
		10									
		11									
		12									
		13									
22	2012	1	July	36	27	Male	Islam	None	1	Karachi	Alam, 2012 <sup>[21]</sup> Ilyas, 2012 <sup>[22]</sup> Sharif <i>et al.</i> , 2014 <sup>[23]</sup> Malik, 2012 <sup>[24]</sup> Abbasi, 2012 <sup>[25]</sup> The Express Tribune, 2012 <sup>[26,27]</sup>
		2									
		3									
		4									
		5									
		6									
		7									
		8									
		9									
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		18									
		19									
		20									
		21									
		22									
		3									
2											
3											

*Contd...*

**Table 5: Contd...**

Total reported cases (n)	Year	Patient	Months	Maximum temperature in month (°C)	Patient ag (years)	Gender (male/female)	Religion	Swimming history	Duration of survival after symptoms (days)	City	Source	
14	2014	1	May	40	25	Female	Islam	None	1	Karachi	Hasan, 2014 <sup>[20,31,32]</sup>	
		2			39	Male	Islam	None	1	Karachi	Dawn.com, 2014 <sup>[33-35]</sup>	
		3				14	Male	Islam	None		Karachi	
		4	June	42	22	Male	Islam	None	1	Karachi	Ilyas, 2013 <sup>[36]</sup>	
		5				39	Male	Islam	None		Karachi	The Express Tribune, 2014 <sup>[37-42]</sup>
		6	July	37	34	Male	Islam	None		0.5	Karachi	
		7				28	Male	Islam	None		Karachi	
		8				9 months	Female	Islam	None	2	Hyderabad	
		9	August	37	13	Male	Islam	None		7	Karachi	
		10				29	Male	Islam	None	5	Karachi	
		11				34	Male	Islam	None	1	Karachi	
		12	September	40	57	Male	Islam	None		4	Karachi	
		13				32	Female	Islam	None	NR	Karachi	
		14	October	41	24-25	Female	Islam	None		NR	Karachi	
13*	2015	1	April	41	18	Female	Islam	None	7	Karachi	Tanveer Ahmed, 2015 <sup>[43]</sup>	
		2	May	43	25	Male	Islam	None		Karachi	Dawn.com, 2015 <sup>[44-47]</sup>	
		3				37	Female	Islam	None		Karachi	
		4				16	Male	Islam	None		Karachi	
		5				40	Male	Islam	None	3	Thatta	Hasan, 2015 <sup>[48,49]</sup>
		6				22	Male	Islam	None	2	Karachi	Mandhro, 2015 <sup>[50]</sup>
		7	June	44	39	Male	Islam	None			Karachi	The Express Tribune, 2015 <sup>[51-55]</sup>
		8				22	Male	Islam	None	1	Karachi	
		9				56	Male	Islam	None	NR	Karachi	
		10	July	36	25	Male	Islam	None		5	Karachi	The News Tribe, 2015 <sup>[56]</sup>
		11				22	Female	Islam	None		Karachi	
		12				23	Male	Islam	None	03	Umerkot	
		13	November	36	30	Male	Islam	None		NR	Lahore	

\*Source of weather history ([https://www.wunderground.com/history/wmo/41780/2015/11/6/MonthlyHistory.html?req\\_city=&req\\_state=&req\\_statename=&reqdb.zip=&reqdb.magic=&reqdb.wmo=](https://www.wunderground.com/history/wmo/41780/2015/11/6/MonthlyHistory.html?req_city=&req_state=&req_statename=&reqdb.zip=&reqdb.magic=&reqdb.wmo=)).



become too risky. The health-care authorities and water supply management authorities need to take immediate steps to control the spread of infection.

Water supply management must ensure that water supplies are chlorinated and up to standard recommended level. Besides, awareness must be created among the masses about the causes as well as means of prevention of the infection. Along with the advertisements on the electronic and print media, social media can prove to be an effective tool in creating public awareness to curb this parasitic infection.<sup>[60]</sup>

In Karachi, the Water and Sewerage Board (KWSB) is the chief body to look over concerns about water supplies and distribution and to keep the city's water supply properly chlorinated. It is imperative that the water resources are given proper authorized chlorination and biological treatment to ensure the water needs are met.<sup>[15-17,58]</sup>

Recently, the health authorities have directed the KWSB to ensure proper chlorination of the municipal water supply as per the WHO recommendations and also formulated preventive strategies to curb the spread of this infection. A focal group including 6 members has been constituted and the *Naegleria* Prevention Centre is planned at a water filtration plant in Karachi. The scope of the focal group is to devise preventive strategies, conduct sampling across Karachi's water supply, and review sample test for the presence of *N. fowleri*. Furthermore, the authorities have also started an awareness campaign.<sup>[58,60]</sup>

The risk of *Naegleria* infection can be avoided through several preventive measures at home. One must make it a practice to use water only after boiling for the purpose of washing and cleansing. Sterilized and filtered water must only be used for the purpose of gargling, brushing, and ablution. Moreover, individuals should keep their faces away from shower to avoid the entrance passage via nose.

In addition, chlorine tablets, i.e., 1 tablet for 5 L of water, can be dissolved in overhead and/or underground water tanks and reservoirs that are routinely cleaned preferably at least twice every year. It is urged that one must always inquire about the sanitation facilities and only use swimming pools that are properly chlorinated and disinfected. The Karachi Metropolitan Corporation must also make sure that all the swimming pools in the city are cleaned and adequately chlorinated.

## CONCLUSION

The numbers of reported PAM cases due to *N. fowleri* are on the rise which verifies the hypothesis. Contaminated municipal water supply, improper chlorination, deteriorating water distribution systems, increase in heat waves, and a general rise in global warming are all possible explanations that attribute to an alarming increase and contribute in creating ideal niches for *N. fowleri* pathogen. Several researches also highlight the use of water for ablution directly from the ground source as another cause of *N. fowleri* outbreak. However, due to nonavailability of the data, this could not be statistically verified. Further study is recommended to establish whether ablution is the contributing factor to *Naegleria*-related deaths.

This study suggests that concerted efforts by all stakeholders are required if Pakistan wants to bring down the number of *Naegleria* infection cases. Further research is also immediately required to uncover the role of environmental factors in the rise of *Naegleria* infection cases.

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