

Occurrence of small free-living amoebae from natural water resources

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Abstract

Small free-living amoebae are also called as amphizoic amoebae, because of their capability to exist both as free-living as well as opportunistically pathogenic. Some of the species of *Naegleria* and *Acanthamoeba* are known to cause fatal disease (Primary Amoebic Meningoencephalitis= PAM and Granulomatous Amoebic Encephalitis= GAE) of central nervous system affecting brain and amoebic keratitis affecting eyes. The purpose of our study was to find out whether small free-living amoebae were also found in natural resource of water such as river and pond from Lucknow region. A total 68 samples of water were collected from six different sites of Gomti River and four different ponds from Lucknow city. A total 38 samples were found to be positive for small free-living amoebae. The most common amoebae present were species of *Naegleria*, *Schizopyrenus*, *Acanthamoeba*, *Hartmannella* and *Vannella*. Out of total 47 water samples from Gomti River, 27 samples were positive for amoebae and out of 21 samples of ponds examined 11 were found to be positive. The occurrence of these amoebae in natural water sources such as river and pond in Lucknow region poses a threat to human being for meddling with water without taking adequate care. Since the pathogenic strains of amoebae are known to infect human being via nasal route during swimming and/or via cut in the skin/ body via haematogenous route. Exposure of eyes with contaminated water may cause amoebic keratitis in human being. A great awareness among masses is essential to educate about these new amoebic disease, their possible preventive measures and occurrence of these pathogenic amoebae from natural water resources.

Keywords:- Free-living, Amphizoic, *Naegleria*, *Acanthamoeba*, PAM, GAE

Introduction

Small free-living amoebae are widely distributed in the human environment (soil, water and air). These amoebae have been isolated from various natural water sources such as rivers, pond, lake etc. (Kasprzak and Mazur, 1974 and Jonckheere, 1981), from thermal acidic stream (Sheehan *et al.*, 2003), from artificial well (Shenoy *et al.*, 2002) and from domestic tap water (Kilvington *et al.*, 2004, Pandey and Sharma, 2006).

These amoebae are called "amphizoic" because of their capability to exist both as free-living as well as opportunistically pathogenic (Page, 1974). People probably have more frequent contact with these organisms particularly with their resistant cysts and

temporary amoeboid-flagellate stage (Martinez and Visvesvara, 1997). The species of *Naegleria*, *Acanthamoeba* and *Balamuthia* have been identified as opportunistic pathogens of human beings and other domestic animals and are known to cause a spectrum of infections in immunocompromised individuals including persons with AIDS (Denny *et al.*, 1997 and Sison *et al.*, 1995).

Occurrence of Primary Amoebic Meningoencephalitis (PAM) has also been reported from India. Pan and Ghosh (1971) from Calcutta, first reported the occurrence of PAM in two children. Third case was reported by Bedi *et al.* (1972) from Udaipur, Rajasthan. One more case has been reported, in an infant of five months old from Mangalore, south India (Shenoy *et al.*, 2002).

Recently, Jain *et al.* (2002) from Chandigarh also reported survival of a patient after the infection of

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Naegleria. Recently, Kaushal *et al.* (2008) reported one more case of primary amoebic meningoencephalitis from Ludhiana, who had the history of taking bath in the village pond. Till date a total of nine cases have been reported between 1971- 2008 from India. More than 200 cases of PAM have been reported so far abroad and most of them are from America. This may be because of the greater awareness about these diseases rather than the incidence. The aim of our investigation was to detect these amphizoid amoebae from aquatic environment used by human being and to know the distribution of the pathogenic and non-pathogenic free-living amoebae in natural water resources.

Materials and Method

The sampling sites included Gomti River at different point and pond of different localities in ten parts of Lucknow city. Using sterile 500 ml screw-cap bottle, 200 ml surface water samples were collected at proposed collecting sites. Water temperature and pH were recorded at the sampling sites. The water samples were filtered through a sterile filter paper using sterile conical funnel. The residue was collected in the inner side of the cone of filter paper and the 1.00 cm cone of this filter paper was cut by sterilized scissors and was placed in the center of non-nutrient agar petri-dish plates already pre-seeded with *Escherichia coli* (as food for amoebae) keeping the inner side of paper downward. These plates were incubated at 37.00 °C for 8-10 days or longer for the growth of amoebae for their detailed study and biological characterization.

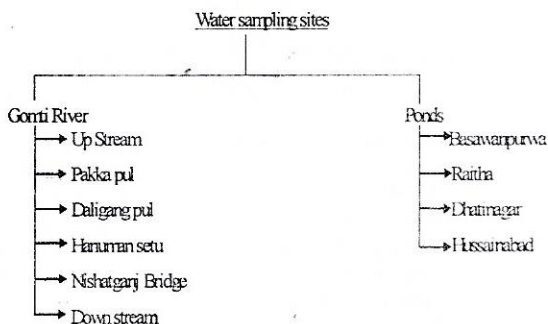
Amoeba Identification

Amoebae were identified on the morphological basis of trophozoites, cysts and flagellates following Singh (1985) and the latest classification of protozoa (Levine *et al.*, 1980).

Results and Discussion

All the samples were collected throughout the year. Total 68 samples were collected from different points of Gomti River and various ponds of

The Experimental design of water sampling sites is giving below



All samples were collected throughout the year

Lucknow city and surrounding areas. The pond water samples were collected from the villages of Bakshi-Ka-Talab area of Lucknow. Out of total 68 water samples, 38 samples were found to be positive for small free-living amoebae (57%). Out of total 47 samples from river, 27 were positive (57%) and out of 21 samples of pond examined, 11 samples were found to be positive for amoebae (52%) (Fig. 1 and 2). The water temperature recorded was in the range of 29.00 °C-37.00 °C and pH from 5.00 to 7.00. *Acanthamoeba* strains and other free-living amoebae could be recovered from pH range of 5.00 to 7.00 and temperature range (27.00 °C to 35.00 °C) of water, where as *Naegleria* strains were found in the water temperature from 28.00 °C to 35.00 °C and at the pH range of 6.50-7.00.

In our investigation, the strains of free-living amoebae isolated from different water samples were identified as different strains and species of *Naegleria*, *Acanthamoeba*, *Schizopyrenus* and *Hartmannella* (Plate-1 and 2).

The identification of the amoebic isolates was done following their detailed biological characterization (studying the morphology of cysts and trophozoite, locomotion, nuclear division and pathogenicity) using the method of Singh, 1985 (Table-1 and Plate-



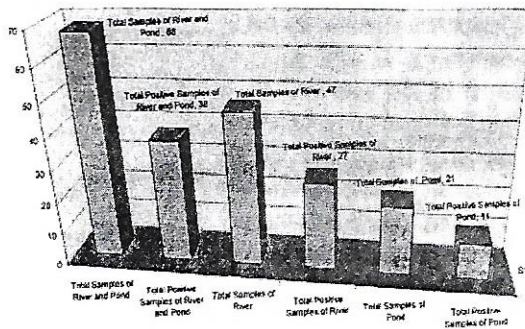


Fig. 1: Number of positive sample from river and ponds in ten different site of Lucknow

1 and 2). In pathogenicity test using *Albino* mice (weight approximately 12 gm) the strain (R-4) of *Naegleria fowleri* was found to be highly pathogenic. The occurrence of small free-living amoebae from water sources has also been reported from different parts of India and abroad (Singh and

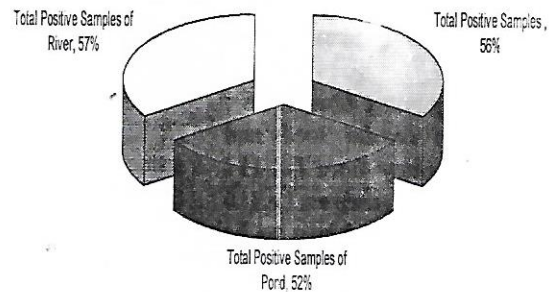


Fig. 2: Percentage of positive water samples for free-living amoebae from river and pond of Lucknow

Das, 1972a; Gogate *et al.*, 1984; Pandey and Sharma, 2006). Human infection by *Naegleria* and *Acanthamoeba* has been reported world wide (Nacapunchai *et al.*, 1999). The environmental sources of amoebic isolates range from air to water and to soil (John and Howard, 1995).

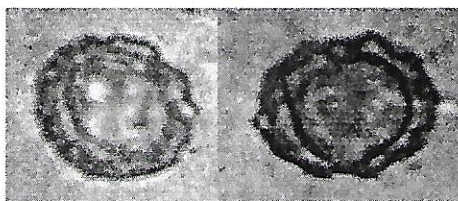
Table-1: Isolation of amphizoic amoebae from water resources (River and pond) in Lucknow

Site name	Site code	Water sample		A m o e b a
		Total sample	Positive sample	
River and Pond				
Up-stream	R - 1	9	6	A , N , H
Pakka pul	R - 2	5	3	A , N , V n
Daligang pul	R - 3	6	3	A , V , H
Hanuman setu	R - 4	12	7	A , N , S c z
Nishat ganj Bridge	R - 5	9	5	A , N , H , V n
Down -stream	R - 6	6	3	N , A
Basawan Purwa pond	P - 1	5	3	A , N , H
Raith pond	P - 2	7	4	A , N , V n
Dhatingara pond	P - 3	5	2	A , N , S c z
Hussainabad pond	P - 4	4	2	A , N , S c z
Total		68	38	A , N , S c z , H , V n

Note: N = *Naegleria*, Scz = *Schizopyrenus*, A = *Acanthamoeba*, Vn = *Vannella*, H = *Hartmannella*

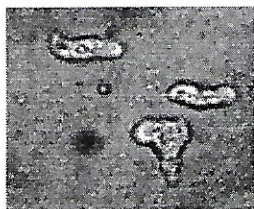


Cyst

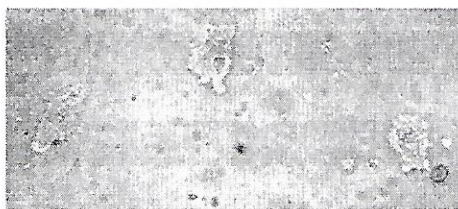


Trophozoites

Naegleria spp.



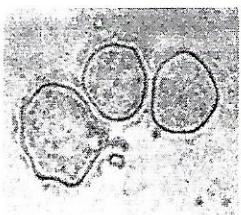
Cyst



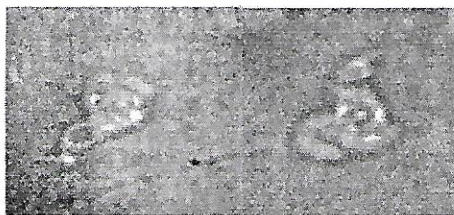
Trophozoites

Acanthamoeba spp.

Plate no. 1

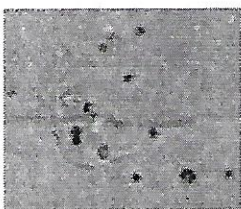


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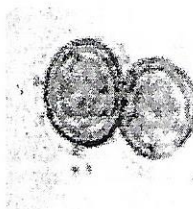


Trophozoites

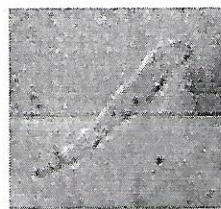
Schizopyrenus spp.



Trophozoites
Vanella



Cyst



Trophozoites
Hartmannella spp.

Plate no. 2

Our results are inconformity with that of specific characteristic of amoebae isolates by other workers (Pandey and Sharma, 2006; Singh and Das, 1972a, Martinez *et al.*, 1997 and Rohr *et al.*, 1998).

Conclusion

These finding serve as additional evidence for the presence of either pathogenic or non-pathogenic free-living amoebae responsible for human diseases under natural conditions, therefore, these results may have profound implication with regard surveillance of water system for amoebae and especially for the prevention of diseases, either directly or indirectly. A greater awareness among masses is essential to educate about the new amoebic diseases and occurrence of these pathogenic amoebae in natural water sources like rivers, ponds, lake etc.

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