



Isolation, identification and biological characterization of *Acanthamoeba polyphaga* from suspected cases of eye patients and its electron microscopy

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Abstract

Corneal scrapings and tear drop of 90 patients with suspected cases of keratitis and eye infection attending the Govt. Hospital and rural areas patients of Lucknow city were screened for amoebic pathogens including *Acanthamoeba*. Of these, 6 (6.66percent) were positive for *Acanthamoeba*, which fulfilled the criteria for suspecting *Acanthamoeba* keratitis. These were distributed among all ages with the maximum numbers in the 20-45 year age group. None of the patients were using contact lenses and were mostly agricultural labourers and had recent history of swimming in ponds and river and also associated with water sports. The predisposing factor found in this study was trauma of varying degrees. The result of present study confirmed that Amphizoic amoebae colonized almost every conceivable aquatic habitat. This is alarming signal that shows presence of Amphizoic amoebae. Incidences of infection during warm season have been traced in patients suffering from eye infection. Preventive measures include public awareness and maintenance of water body and adequate chlorination.

Keywords: *Acanthamoeba* , Keratitis, Amphizoic Amoebae, Chlorination

Introduction

Acanthamoeba is a free-living, opportunistic protozoan parasite of human beings. *Acanthamoeba* can cause a fatal Meningoencephalitis disease, but its few species have been found to be commonly associated with eye infections, typically, *Acanthamoeba* keratitis associated with contact lens use (Anisah *et al*, 2005). The increasing prevalence of this eye disease is thought to be linked to the increased use of contact lenses (Kamel *et al*, 2003). *Acanthamoeba* keratitis is usually diagnosed after viral and bacterial causes have been eliminated (and, as a result, there is often a significant delay before appropriate treatment is administered). Because of the severity of *Acanthamoeba* keratitis, a significant loss of visual acuity is common and in many cases total loss of sight in the infected eye occurs (Khan, 2003). Current methods of detection involve the culture and microscopic identification. These methods are time consuming, laborious, and open to error. The development of a rapid, simple detection method for *Acanthamoeba* is thus important. *Acanthamoeba* species have been

isolated from diverse sources, such as freshwater, seawater, chlorinated water from swimming pools, dental treatment units, and contact lens cases (Anisah *et al*, 2004 and Visvesvara, 2010). Most of the strains found are not pathogenic. Some pathogenic forms are known to survive for extended periods in fresh water. The presence of pathogenic *Acanthamoeba* organisms in the atmosphere is an important factor in the prevalence of *Acanthamoeba* keratitis, although this is not its main cause (Siddiqui and Khan, 2012). Patients with *Acanthamoeba* keratitis usually are users of daily-wear disposable, soft contact lenses. The use of tap water to rinse contact lenses allows deposits of lime scale to accumulate, and this lime scale often contains pathogenic *Acanthamoeba* species. Lenses create a corneal abrasion, facilitating entry of *Acanthamoeba*. The organism can survive in contact lens cases and solutions.

Material and Methods

For the diagnosis of *Acanthamoeba* keratitis, sample (tear or corneal scraping) isolated from the patients of different age group ranging from 3year to 17 years. All samples, tear and corneal scrap were collected in sterile screw capped small tubes

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and brought to the lab and tested for culture on non nutrients agar plate pre seeded with *E.coli* in accordance with a standard method (Visvesvara,2010). Microscopy and culture for amebic, bacterial, mycobacterial, and fungal organisms were performed. Swabs from the cornea were inoculated onto two *Escherichia coli*-seeded, 1% non nutrient agar (ECNNA) plates. In addition; the patient's disposable contact lenses were replaced onto two ECNNA plates. Ten milliliters of the patient's contact lens-disinfecting solution was centrifuged at 3,000 rpm for 10 min, and the sediment was inoculated onto two ECNNA plates. All of the ECNNA plates were incubated at 25 and 37°C for 20 days. The disinfecting solution was cultured for bacteria. The *Acanthamoeba* isolate was cloned by diluting a suspension of cysts in sterile ameba saline, spreading them on agar under a microscope, and selecting individual cysts by using low magnification. A piece of agar bearing the selected cyst was cut out and transferred face down to a fresh ECNNA plate. Several plates were prepared in this manner from sequential cultures, and each time the block of agar was carefully examined under the microscope to make sure that only one cyst was present.

Results and Discussion

Acanthamoeba Keratitis is a seldom recorded infection; however, it is a serious condition hence it should not be overlooked or taken lightly. This infection is influential on the eyes and if not treated timely, then it may lead to ocular impairment or blindness on permanent basis (Hammersmith, 2006). It is studied that the infection is caused due to free-living microscopical amoeba which are also known as *Acanthamoeba*. When these microorganisms, *Acanthamoeba*, infects the cornea of the eye (external transparent layer of the eye) it leads to *Acanthamoeba* keratitis. Humans are at greater risk of developing this infection, as these kinds of amoebae are frequently found in various water bodies such as lakes, soil air etc. This condition was first diagnosed in the 1973, wherein approximately 90% of affected individuals were contact lenses users. There are several factors that may lead to *Acanthamoeba* keratitis. For example, using contaminated water from sources such as tap or well may increase the risk of such infection.

Also infected contact lenses may cause such issues (Shoff *et al.*, 2007). Avoid wearing contact lenses while in hot tub or swimming in pool or even while taking shower as these factors may also result in *Acanthamoeba* infection (Shoff *et al.*, 2008). Adopting inappropriate measures for storing contact lenses may cause the virus to settle on the lenses and then infect your eyes on wearing them. Six positive cases out of 90 examined (6.666%) for suspected cases of amoebic keratitis showed positive growth from their eye scrapings /tear drops. Identification and biological characterization of amoebic isolates was done following the patterns of Singh and Hanumaiah (1979) and Levine *et al* (1980). All the trophic characters observed were similar to the typical of *Acanthamoeba sp.* strain. However the cyst was double-walled and polyhedral. The endocyst (inner) was stellate or polygonal and ectocyst was wrinkled with ripples. In Scanning Electron Microscopy study, ridges and grooves were present on the surface of cyst and pores were present on the cyst wall (Plate-1 fig1, 2&3, 4). Cysts were uninucleate with a prominent nucleolus. During excystment trophozoite emerged from the pore that was present on cyst wall. Trophozoites also failed to produce temporary amoeboid flagellate stage on repeated efforts (Plate2, Fig 1, 2&3). Thus on the basis of above observations of trophozoites and cysts, the strains isolated from eye of suspected patients were also identified as *Acanthamoeba polyphaga* because of their close similarity in the observation of trophic, cystic characters and locomotion & behaviour. Free-living amoebae (FLA) are the main predators of bacterial populations in the environment, playing a major role in maintaining the ecological balance of many environmental systems by feeding on bacterial population, which is present nearly in all water bodies, due to which humans are directly exposed to the risk of amoebic infection through water (Carter, 1968; Culbertson *et al.*, 1972 and Singh, 1985). As already mentioned, these amoebae are ubiquitous in nature and present in all water bodies. During study, samples were taken from the suspected patients of amoebic infection (i.e. cerebrospinal fluid and nasal swab of suspected cases of meningitis and respiratory tract infection and tear drops/eye scrapings from suspected cases of keratitis) Pathogenic free-living amoebae are known as Amphizoic amoebae because they are



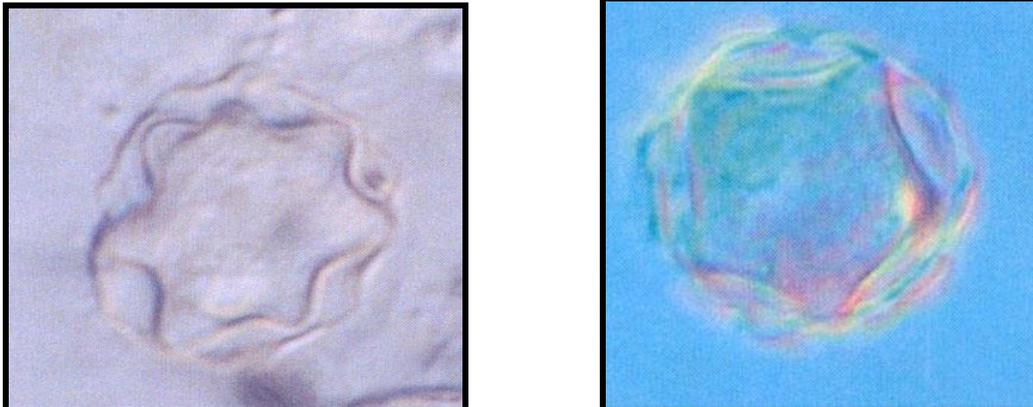


Figure 1 Shows Photographs of Cyst of *Acanthamoebae polyphaga*

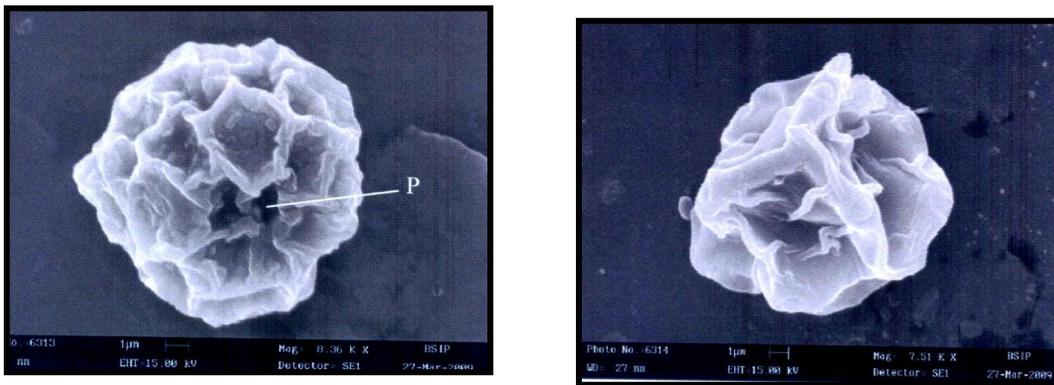


Figure 2 Shows SEM of *Acanthamoebapolyphaga*

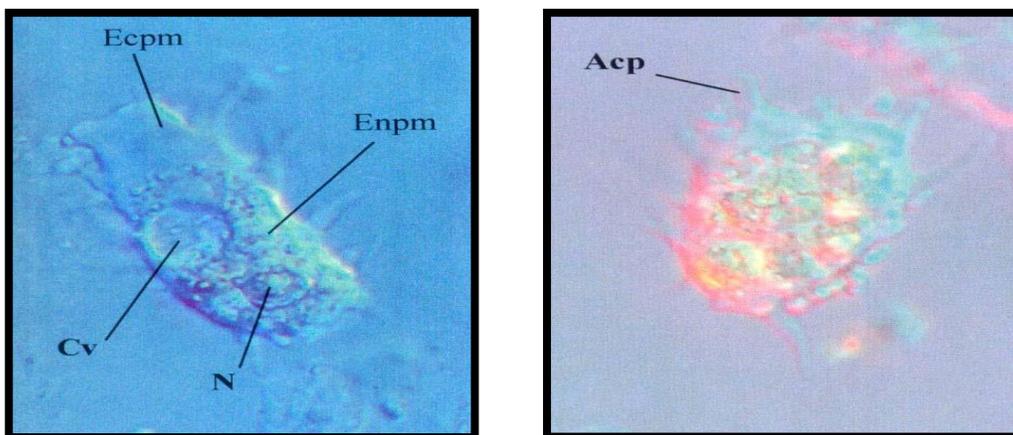


Figure 3 Shows Trophozoite of *Acanthamoeba polyphaga*



Figure 4 Shows SEM of *Acanthamoeba polyphaga*

able to survive freely in nature or endozoically inside mammalian hosts. It is because of this reason, samples were also taken from suspected patients of amoebic infection, who were using contaminated water for bathing, drinking, swimming and cleaning of their contact lenses. These amoebae also infect the eyes by using contaminated lenses or splashing unclean water. Infection has been known to be associated with wearing contact lenses during swimming (Stehr-Green *et al.*, 1989).

Acanthamoeba polyphaga (strain E₁, E₂ and E₃) isolated from tears of eye. Marciano-Cabral *et al.*, (2000) and Kilvington *et al.*, (1990) demonstrated the isolation of amoebae from a eye patient's cornea, contact lenses container, saline rinsing solution and kitchen cold tap water were identical, thus implicating domestic tap water as the source of *Acanthamoeba* sp. in Keratitis. *Acanthamoeba* have also isolated from CSF, human brain and nasal passage of healthy humans from different countries of world (Sadaka *et al.*, 1994; Shenoy *et al.*, 2002; Pisani *et al.*, 2003; Ahmad *et al.*, 2007; Kaushal *et al.*, 2008, Rai *et al.*, 2008 and Ahmad Al-Herrawy *et al.*, 2014). Amphizotic amoebae have also been reported from tears, corneal scrapings of

eye patients (Sharma *et al.* 2000; Schroeder *et al.* 2001; Parija *et al.* 2001; De Jonckheere, 2003, Kirwood, 2007, Safar, 2010 and Mafi M. *et al.* 2014). Kilvington *et al.*, (2004) demonstrated that isolates from a patient's cornea contact lens container, saline rinsing solution, kitchen cold tap water were identified, thus the study implicated, for the first time, domestic tap water as the sources of *Acanthamoeba* species in keratitis. Chynn *et al.* (1997) also published an incidence of contaminated tap water exposure in *Acanthamoeba keratitis* patients. Khan and Paget (2002) have also reported the isolation of *A. polyphaga* from tap water in Leicester, U.K. Kamelet *et al.*, (2004) first time reported a non contact lens related *Acanthamoeba* keratitis from Malaysia, in a 28 years old Indonesian male construction worker who had a trauma of the right eye during work. His eye was struck by sand and dust particles after which he quickly washed with water from an open tank at the construction site. Tap water may be the origin of the infection via contact lenses and poor contact lens hygiene (Kilvington *et al.*, 2004).

The result of present study showed that clinical recognition of the signs and risk of factors for

Acanthamoeba keratitis among non-contact lens wearer, as early diagnosis and prompt treatment are associated with improved clinical outcome.

Effective drug therapy was attributable to these free-living amoebae to cause corneal infection was improved and new drugs are needed, particularly when a resistance to classical therapeutics is encountered.

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References

- Ahmad, K., Dil, A.S., Ahmad, I., Hussain, S. and Lakhnana, N.A. 2007. Primary Amoebic Meningoencephalitis: Report of a case. *International Journal of Pathology*. 5 (1): 34-36
- Ahmad Al-Herrawy, Mahmoud Bahgat, Abd-Elhafez Mohammed, Ameen Ashour and Wafaa Hikal 2014. *Acanthamoeba* species in swimming pools of Cairo, Egypt. *Iranian J. Parasitol* Vol. 9, No. 2, pp. 194-201.
- Anisah, N., Yusof, S., Wan Norliana, A., Noraina, A.R. and Norhayati, M. 2004. *Acanthamoeba* sp. Isolated from salt water in the west coast of peninsular Malaysia. *Tropical Biomedicine*. 21(1): 109-111.
- Carter, R.F. 1968. Primary amoebic meningoencephalitis: clinical, pathological and epidemiological features of six fatal cases. *Journal of Pathology and Bacteriology*. 96:1-5.
- Chynn, E.W., Talamo, J.H. and Seligman, M.S. 1997. *Acanthamoeba* keratitis: is water exposure a true risk factor? *CLAO Journal* 23(1): 55-56.
- Culbertson, C.G., Ensminger, P.W. and Overton, W.M. 1972. Amoebic cellulocutaneous invasion by *Naegleria aerobia* with generalized visceral lesions after subcutaneous inoculation: an experimental study in guinea pig. *Am.J.Clin.Patj*. 57:375-386.
- De Jonckheere, J.F. 2003. Epidemiological typing of *Acanthamoeba* keratitis isolated from keratitis cases in Belgium. *Bull. Soc. Belg. Ophthalmol*. 287: 27-33.
- Elmeya H. Safar 2010. Involvement of the eye with *Acanthamoeba*. *JASMR*, 5(2): 191-198.
- Hammersmith K.M. 2006. Diagnosis and management of *Acanthamoeba* keratitis. *Curr Opin Ophthalmol*. 17:327-331.
- Kamel, A.G.M., Saleba, Anisah Nordin, Yusof Suboh, Norhayati Mokhtar and Norazah 2004. Isolation of *Acanthamoeba* spp. Daripadapersekitaran akuatik. Prosiding Simposium Sains Kesihatan Kebangsaan Ke 5, Marriot Putrajaya, 25-26 Mei, 497-501.
- Kaushal, V., Chhina, D.K., Ram, S., Singh, G., Kaushal, R.K. and Kumar, R. 2008. Primary Amoebic Meningoencephalitis due to *Naegleria fowleri*. *JAPI*, vol. 56.
- Khan, N.A. and Paget, T.A. 2002. Molecular tools for speciation and Epidemiological studies of *Acanthamoeba*. *Current Micro*. 44: 444-449.
- Khan, N.A (2003). Pathogenesis of *Acanthamoeba* infections. *Micob. Pathog*. 34, 277-285.
- Kilvington, S., Gray, T., Dart, J., Morlwt, N., Beeching, J.R., Frazer, D.G. and Matheson, M. 2004. *Acanthamoeba* keratitis The role of domestic tap water contamination in the United Kingdom. *Invest. Ophthalmol. Vis. Sci*. 45:165-169.
- Kilvington, S., Larkin, D.F.P., White, D.G. and Beeching, J.R. 1990. Laboratory investigation of *Acanthamoeba* keratitis. *J. Clin. Microbiol*. 28: 2722-2725.
- Kirwood, B.J. 2007. *Acanthamoeba* keratitis; an overview. *The Journal of the American Society of Ophthalmic Registered Nurses, Ind*. Vol. XXXII, No.3, 16-20.
- Levine, N.D., Corless, J.O., Cox, F.E.G., Deroux, G., Grain, J., Honigberg, B.M., Leedale, G.F., Loeblick, A.R., Lom, J., Lynn, D., Merinfeld, E.G., Page, F.C., Polijansky, G., Sprague, V., Vavra, J. And wallance, F.C. 1980. A newly revised classification of the protozoa. *J. Protozool*. 27:37-58.
- Mafi M., Niyayati M., and Haghighi A. 2014. Evaluation of *Acanthamoeba* species from Tehran surface water sources using Real Time PCR Method. International Conference on Food Biological and Medical Sciences (FBMS-2014), Bangkok (Thailand): 69-71.
- Maraciano-cabral, F.R., Puffenbarger, R. And Carbal, G.A. 2000. The increasing importance of *Acanthamoeba* infection. *J. Eukaryot. Microbiol*. 47: 29-36.
- Parija, S.C., Shiv Prakash, M.R., Rao, V.A. and Vellaniparambil, R.J. 2001. *Acanthamoeba* keratitis in Pondichery. *J. Comun. Dis*. 33(2): 126-129.
- Pisani, F., Costa, C., Oteri, G. And Loli, A. 2003. Identification of amoebae in the CSF in a patient with meningoencephalitis. *Journal of Neurosurgery and psychiatry*, 74: 1445-1446.
- Rai R., Singh, D.K., Srivastava, A.K. and Bhargava, A. 2008. A case report of primary amoebic meningoencephalitis. *Indian Pediatrics*. 1004, vol. 45



- Sadaka, H.A., El-nassery, S.F., Abou, Samra, L.M. and Awadalla, H.N. 1994. Isolation and identification of free-living amoebae from some water sources in Alexandria. *Journal of the Egyptian Society of Parasitology*, 24: 247-257.
- Schroeder, J.M., Booton, C.G., Ingrid, J.H.I., David, S.V., Markus, B.M., Fuerst, A.P. and Byers, J.T. 2001. Use of subgenetic 18S ribosomal DNA PCR and sequencing for genus and genotype identification of *Acanthamoeba* from humans with keratitis and from sewage sludge. *J. Clin. Microbiol.* 39(5): 1903-1911.
- Siddiqui, R. and Khan, N.A 2012. Biology and pathogenesis of *Acanthamoeba*. *Parasites and Vectors*. 5:6. doi:10.1186/1756-3305-5-6.
- Sharma, A.K., Sharma, U.D. and Singh, S. 2000. First case of *Acanthamoeba* keratitis in a contact lens wearer from India. *Biol. Memoirs*. 26(2): 44-47.
- Shenoy, S., Godwin, W., Prashanth, H.V., Vidyakshmi, K., Dhanashree, B. And Bharath, R. 2002. Primary Meningoencephalitis by *Naegleria fowleri*; first reported case from Mangalore, South India. *J. Clin. Microbiol.* 40 (1):309-310.
- Shoff M, Rogerson A, Schatz S, Seal D. 2007 Variable responses of *Acanthamoeba* strains to three multipurpose lens cleaning solutions. *Optom Vis Sci*. 84:202–207.
- Shoff ME, Rogerson A, Kessler K, Schatz S, Seal DV. 2008 Prevalence of *Acanthamoeba* and other naked amoebae in South Florida domestic water. *J Water Health*. 6:99–104.
- Singh, B.N. 1985. Free living soil amoebae as human pathogen. *Proc. Indian Natn. Sci. Acad.* B 51(3):297-312.
- Singh, B.N. and Hanumaiah, V. 1979. Studies on pathogenic and nonpathogenic amoebae and the bearing of nuclear division and locomotive form and behaviour on the classification of order Amoebida. Monograph No.1 of the Association of Microbiologist of India. *Indian J. Microbiol.* 1-80.
- Stehr-Green, J.K., Bailey, T.M. and Visveavara, G.S. 1989. The epidemiology of *Acanthamoeba* keratitis in the United States. *Am. J. Ophthalmol.* 107:331-336.
- Visvesvara, G.S 2010. Free-living Amoebae as opportunistic Agents of Human Disease. *Journal of Neuroparasitology*. Vol. (1), Article IDN 100802, 1-13.

