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## Abstract

Free living Amoebae (FLA) are opportunistic pathogens which can occur in variety of environments. They are associated with infrequent but noxious diseases such as amoebic encephalitis and keratitis. Swimming pools and recreational waters are important sources where humans can come into contact with these amoebae. Therefore sampling and characterization of these sources for the possible presence of these amoebae is important for human safety.

In this study, six samples were collected from some important recreational freshwater resources of Lahore District. Isolated amoebae were cultured on non-nutritive agar plates and morphological and molecular methods were adopted to identify cultured amoebae.

Morphological characterization revealed the presence of oval shaped trophozoites with acanthopodia (*Acanthamoeba*) in JL, MP1, MP2, FP and C samples, rod like trophozoites (*Hartmannella*) in FP sample and binucleate trophozoites (*Sappinia*) in PU sample. Round double walled cysts with smooth outer margins (*Hartmannella*) appeared in JL, FP, MP1 and MP2 samples whereas star shaped double walled cysts (*Acanthamoeba*) appeared in FP and PU samples. PCR amplification and sequencing of 18S ribosomal RNA gene of FLA in FP sample showed its maximum homology with *Vermamoeba vermiformis* (*Hartmannella vermiformis*). Furthermore, PCR with specific primers for *Naegleria fowleri* showed the absence of this most lethal amoebic species.