

Molecular and morphological identification of pathogenic free-living amoebae in superficial water in Shiraz, Iran

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Introduction: Among the many genera of free-living amoeba that exist in the nature, members of only four genera (*Negleria*, *Acanthamoeba*, *Balamuthia*, *Sappinia*) have an association with human infection. These genera are known as causative agents of many clinical manifestations such as encephalitis and keratitis. Infective forms of these pathogenic organisms distribute in wide geographical regions of the world and consider in water, soil and air. There are only a few studies have done about these protozoa in Iran. **Materials and methods:** 30 samples were collected from superficial water of Shiraz in summer 1388. The samples were filtered and their sediments were cultured on NNE medium and were incubated at three different temperatures; 22 °C, 37 °C, 44 °C. The media were inspected with Invert microscopy and amoeba were identified by phase-contrast microscopy and were evaluated by light microscopy after trichrom staining. Polymerase chain reaction (PCR) was performed for molecular detection of amoebae. **Results:** Among 30 samples, 29 out of them were identified as *Acanthamoeba* by microscopy and confirmed by PCR. The growth rate of amoeba in 22 °C were more than 37 °C. Eight of 30 samples grew at 44 °C, but flagellate forming test of them were negative and showed any band with *N. fowleri* specific primers. Two of samples were identified morphologically as *Balamuthia* and *Sappinia*. **Conclusion:** Due to the wide distribution of free living amoeba in superficial waters in Shiraz, further investigations about various aspects of this important opportunistic protozoa especially for establishment of appropriate prevention tools is recommended.

Key words: Free –living amoeba, *Acanthamoeba*, PCR, Shiraz