Biochemical Characterization of Pathogenic free-living amoebae from Different Sources of Water

Wafaa M. Hikal

Biology Department, Faculty of Science, University of Tabuk; P.o. Box 741 Tabuk, Saudi Arabia
Parasitology Lab; Water pollution Research Department, National Research Centre, Egypt

Abstract

Objectives: Occurrence of heat-tolerant free-living amoebae in the Nile, tap and swimming-pool waters in the Nile Delta region, Egypt. Morpho-physiological, biochemical characterization of the isolated strains of free-living amoebae.

Materials & Methods: Cultivation of free-living amoebae on non-nutrient agar. Identification of the isolated strains based on the morphology of cyst and trophozoite forms as well as temperature and osmotolerance assays. Biochemical characterization of the isolated amoeba strains using quantitative and qualitative (SDS-PAGE) assays as well as qualitative determination of proteolytic activity in zymograph analysis.

Results: Potentially pathogenic free-living amoebae were isolated from all of the examined water sources. Colorimetric assays showed protease activity in heat-tolerant isolates of *Acanthamoeba*. All pathogenic isolates exhibited higher protease activity than non-pathogenic ones did. The zymographic protease assays showed various banding patterns for different strains of *Acanthamoeba*.

Objective

There are few data on the occurrence of these pathogenic free-living amoebae in the aquatic environment of Egypt. So, the main objective of the present work is to illustrate the occurrence and identification of pathogenic free-living amoebae in different types of water using morpho-physiological characteristics A secondary objective is to characterize the potential pathogenicity of the isolated strains using biochemical assays.

Methods

Samples and sampling sites

Water samples (3 liters each) were collected from different localities in Delta region, Egypt for the detection and isolation of freshwater amoebae using the membrane filtration technique (Gradus *et al. 1989)*.. Samples were collected from the Nile River, tap water and swimming pool water in clean, dry autoclavable polypropylene containers and sent to the laboratory in icebox and processed at the same day of collection (Hikal, 2010) (Table 1).

Biochemical characterization of isolated free-living amoebae :

Grown amoebae were characterized by quantitative assays for proteinase activity using chromomeric substrates. Also Qualitative determination of proteolytic activity in zymograph analysis (gelatin sodium dodecyl sulphate-polyacrylamide gel electrophoresis, SDS-PAGE gels) were demonstrated (Bahgat et al., 2006)

Table 1. Samples and sampling sites

Locality	Water type
Cairo	Nile, swimming pools and tap
Giza	Nile and tap
Qalubeya	Nile and tap
Behera	Nile and tap
Gharbeya	Nile and tap
Dakahleya	Nile and tap
Helwan	Nile, swimming pools and tap
Kafr -Elshikh	Tap
Sharkeya	Tap
Minofeya	Tap

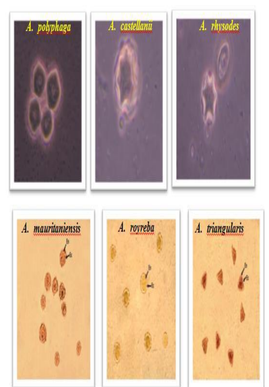

Figure 1 cyst form of *Acanthamoeba* spp

Results

Prevalence of heat-tolerant *Acanthamoeba* spp. in different types of water

Heat-tolerant *Acanthamoeba species were isolated from 56.0, 58.6 and 49.2% of the examined Nile water, tap water and swimming pools water samples, respectively.*

Morpho-physiological characteristics of isolated *Acanthamoeba* species

Identification of the different species of *Acanthamoeba was performed according to the shape and size of cysts in addition to the number, shape, size and arrangement of the cyst pores. (Figure1).*

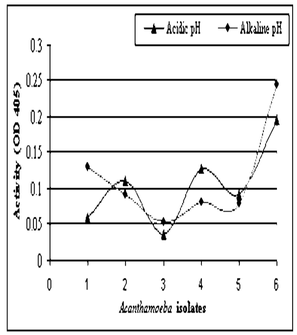

Fig.2. Tryptase activity in individual *Acanthamoeba isolates at both acidic and alkaline pH*

Quantitatively absolute enzyme activity in *Acanthamoeba* isolates

The examined *Acanthamoeba isolates were clas-sified according to absolute trypsin-like proteolytic activities into relatively pathogenic and non-pathogenic at both acidic and alkaline pH (figure 2)*

Qualitatively proteolytic activity in lysates of different Acanthamoeba isolates visua-lized by gelatin SDS-PAGE

The proteolytic profile of prepared lysates from bacterial control sample containing no *Acanthamoeba was totally different from that of different Acanthamoeba isolates. (Figure 3)..*

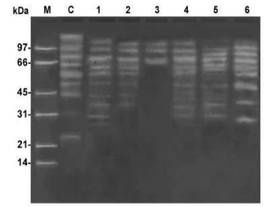

Fig.3. Serine-like protease activity in *Acanthamoeba isolates was visualized in gelatin SDS-PAGE*

Conclusions

The incidence and prevalence of the pathogenic free-living amoebae in different populations using parasitological and biochemical diagnostic tools will provide baseline data against which the risk factors associated with waterborne transmission can be identified.

.

References

Gradus M, Koenig S, Hyndiuk R, De Carlo J. Filter-culture technique using amoebae saline transport medium for the noninvasive diagno-sis of *Acanthamoeba keratitis. Am J Clin Pathol. 1989; 92: 682-685.*

Bahgat M, Sorgho H, Ouédraogo J. Enzyme-linked immunosorbent assay with worm vomit and cercarial secretions of *Schistosoma mansoni to detect infections in an endemic focus of Burkina Faso. J Helminthol. 2006, 80:19-23.*

Dr. Wafaa M. Hikal *wafaahikal@gmail.com*