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Occurrence of Antibiotic-Resistant and Plasmid DNA Harboursing Bacterial Pathogens in Stressed Polluted Water Environment of Lake Manzala, Egypt

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Abstract: This study aims at characterization of microbial pollution of Lake Manzala, bacteriological investigation of water and fish and isolation of antibiotic resistant and plasmid harbouring bacterial strains. The study revealed high levels of pollution in the water and fish samples taken from the most important sites (Kapoty, Bashtier and Mataryia areas), representative of human activity and the different ecosystems in the Lake water environment. The testing for total suspended solids (TSS), ammonia and nitrates, demonstrated that figures exceeded national and international standards. The fish-tissue samples gathered from two different sites yielded high concentration of bacterial count by plate count method. Total viable bacteria (TVB) reached more than 10^4 cfu mL⁻¹ in water samples and 10^5 cfu g⁻¹ in fish samples, particularly in Kapoty and Mataryia areas. Faecal coliform counts reached 10^2 cfu mL⁻¹ in water samples and 10^3 cfu g⁻¹ in fish samples. The API-20E test kit was used for the identification of eighty isolates of different bacteria strains. The bacterial strains *Stentrophomonas maltophilia*, *Proteus mirabilis*, *Escherichia coli* and *Erwinia* sp. were common species found in the samples of the study and demonstrated multi-drug resistance. These strains harbored β -Lactamases and plasmid DNA; characteristics that can be attributed to the stressed water environment of the polluted Lake Manzala.

Key words: Water pollution, Lake Manzala, *Stentrophomonas maltophilia*, *Proteus mirabilis*, antibiotics resistance, plasmid DNA

INTRODUCTION

Lake Manzala is a shallow costal lake that consisting of thirty basins with depths ranging from 0.7 to 3 m in depth, the deepest areas resting in the navigation canal (Zaky, 2006). It is situated east of the Nile River's Delta, between the Damitta branch of the Nile River and the Suez Canal. The Mediterranean Sea is immediately north of the narrow peninsula which separates the two bodies of water. Lake Manzala did not exist before the 6th century AD, before then it was an area of cultivated land with fertile soil, tectonic plate movement caused an earthquake that created the lake (Zaky *et al.*, 2005). Creation of canals and drains such as the Bahr El-Bakar drain, the Sirw drain, the Ramsis drain and the Hadous drain has created a eutrophic condition and low salinity levels in the lake. The areas around the drain outlets in the south and west are characterized by brackish water and the areas in the northeast, near the sea outlets, are saline (Zaky, 2006).

The lake suffers from water pollution induced by agricultural drainage, industrial wastes and sewage and has been shown to be contaminated by persistent organochlorine pollutants (Abbassy *et al.*, 2003). This pollution condition of the lake has increased bacterial content particularly that of pathogenic bacterial indicators, such as the fecal coliforms, *E. coli*, *Enterococci* and *Clostridium perfringes* and is manifested in the water as well as in the fish populations (El-Sarangawy, 1990;

Zaky, 2006). Pathogenic species such as, *Aeromonas hydrophila* and *Aeromonas sobria*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Vibrio anguillarum*, were present in the gills, intestines and flesh of the fish samples. The specimens exhibited toxigenic characteristics as well as multi-drug resistance which could explain the marked reduction of fish and the increase of diarrhoeal diseases among human populations residing in the north eastern coast of Egypt (El-Gaber *et al.*, 1997; Sulayman, 1999; Zaky *et al.*, 2005). This study aims at characterization of microbial pollution of Lake Manzala, focusing on the antibiotic resistant bacterial strains.

MATERIALS AND METHODS

From April to July of 2003, 10 water samples were taken from Kapoty, Bashtier and Mataryia areas, fish samples were collected from El-Bashtier Area and El-Mataryia Area. The selected sites are host to significant populations around the lake and receive various types of pollutants which negatively affect the condition of the lake and human health.

Sampling Sites

El-Kapoty area samples were taken from the end of the junction canal, which connects the Suez Canal with Lake Manzala, the main source of pollution in this area comes from the city of Port-Said. Effluents such as sewage water and industrial wastes from multiple factories are disposed of in this area of the Lake. This site is close to El-Kapoty village, a fishing village in Port-Said, where they dispose of raw sewage directly into the Lake water. El-Mataryia area is considered the fresh-water part of the Manzala Lake; however, it receives high amounts of different types of pollutants. Untreated sewage from the El Dakahlyia governorate empties here as well as 6 million m³/day of industrial and agricultural waste from the El-Siwr, Hadous, Ramsis and Bahr El-Bakar drains. The drains empty into the El Genka reservoir, a part of the lake that is characterized by vast vegetation composed of reeds and other aquatic plants like the water hyacinth, this area is particularly important for fishing, specially the fishing of *Tilapia* sp. El-Bashtier area is considered a mid point between the El-Kapoty and the Mataryia areas; it receives water currents from different directions resulting in high water levels. The depth reaches three meters and is part of the navigation canal. The area has many islets which are inhabited by people who work in fishing and raise animals (Fig. 1).

Sampling Methods

Ten water samples from each site were taken in clean sterile one liter glass bottles and transported from the lake to the laboratory within 6 h. The bottles were kept in ice bags and ice jackets for direct examination. Ten fish samples were taken from the El Bashtier area and the El Mataryia area. Once the fish samples were collected they were immediately packed in sterile polyethylene cases and preserved in ice. All samples collected were transported, homogenized and prepared for immediate bacteriological analysis, using 0.8% saline for the pour plate method.

Water samples were directly diluted with 0.8% saline distilled water for bacterial counting using the dilution method and pour plates method. The fish were dissected and 1 g of the intestinal contents was aseptically stripped out with sterile forceps. Samples of gill lamellae and intestinal contents (1 g) were aseptically re-suspended in 100 mL of 0.1% w/v chilled peptone water (pH 7.0), homogenized and poured with different dilutions into Petri dishes (Zaky *et al.*, 2005).

Physicochemical Characteristics of Water Samples

Physicochemical characteristic of each water sample were evaluated in the field. The temperature of the water samples was determined by using a mercury thermometer. The pH values were determined using a recombination of a pH electrode (Eil Series 1180) and a pH meter (Eill, Model 7030), which



Fig. 1: Map of Lake Manzala, showing the three different sampling sites and their respective sources of pollution

was calibrated against pH 4, 7 and 9 buffers. The salinity of the water was determined using a refractometer model Leica No. 10419. The levels of dissolved oxygen in the water samples were determined using the Oxygen Meter model YSI 58. The chemical analysis to determine the total suspended solids (TSS), ammonia, nitrates, calcium, magnesium and chloride levels were done using the recommended standard methods of water analysis (APHA, 1995).

Microbiological Analysis

Counts of total viable bacteria were determined utilizing nutrient agar with 5 g of peptone, 5 g of sodium chloride, 3 g of beef extract and 15 g of agar L⁻¹ of distilled water. The incubation period was 24 h and the temperature was kept at 37°C. Counts of faecal coliforms were estimated utilizing endo agar medium was composed of 10 g of lactose, 10 g of peptone and 3.5 g of dipotassium hydrogen phosphate, 2.5 g of sodium sulfite, 0.5 g of basic fuchsin and 15 g of agar L⁻¹ of distilled water. The incubation period was 24 h and the temperature was kept at 44°C. Bacterial isolates were further identified by taking typical colonies from the agar medium and identifying them according to the recommended API 20 E system (bioMerieux) for identification of the family Enterobacteriaceae.

Resistance to Antimicrobial Agents

The disc diffusion method was used to find antimicrobial resistance. The bacterial strains were grown overnight in nutrient broth, then 1 mL of the broth was poured into a Petri dish and finally the

nutrient agar media (50°C) was poured. Different antibiotic discs (oxoid) were inserted into the plates, before the complete solidification of the media and the plates were incubated at 37°C for 24 h. The absence of a colony was an indication of the resistance of the bacteria to the following antibiotics; chloramphenicol (30 mcg), ampicillin (10 mcg), penicillin G (10 mcg), streptomycin (10 mcg) and Gentamycin (10 mcg) (Zaky *et al.*, 2005).

Isolation of Plasmid DNA

The alkaline lysis method was used in mini preparation (Maniatis *et al.*, 1982). For antibiotic resistant bacterial strains isolated from water and fish samples.

RESULTS

Physicochemical Properties of Water

Physical and chemical properties were estimated for all water samples collected from the El-Kapoty, El Bashtier and El Mataryia sites seasonally from autumn to summer. Most samples taken from these sites exceed the limits of pollution standards, such as total suspended solids TSS, ammonia and nitrate, respective to their different levels of salinity (Table 1).

Bacteriological Characteristics of Water

Bacterial counts were high in most of the water samples, particularly in those belonging to the El Mataryia area. The mean value of total viable bacterial counts reached more than 15,000 cfu mL⁻¹ and the mean value of fecal coliform counts reached more than 200 cfu mL⁻¹ (Table 1).

Seasonal variation in bacterial counts was obvious where. Summer is the highest season. Total viable bacterial counts for all sites reached a mean value of 33 733.33 cfu mL⁻¹ (SD±20,096.10). Summer faecal coliform counts (Table 2) for all sites reached a mean value of 776.33 cfu mL⁻¹ (SD±1,163.83).

Table 1: Mean values of the physicochemical parameters of the water in the different sampling sites of Lake Manzala

Parameters	Kapoty	Bashtier	Mataryia
Temperature (°C)	21.000±0.00	22.000±0.00	20.000±0.00
pH	8.400±0.12	8.100±0.12	8.500±0.90
Salinity	18.250±1.22	9.000±1.08	4.000±0.88
Dissolved oxygen (mg L ⁻¹)	1.975±0.00	1.825±0.00	1.675±0.00
Total suspended solids (mg L ⁻¹)	139.500±8.19	80.500±8.39	61.000±8.25
Ammonia (mg L ⁻¹)	0.450±0.02	0.500±0.20	0.800±0.11
Nitrate (mg L ⁻¹)	0.150±0.02	0.125±0.00	0.300±0.18
Chlorides (mg L ⁻¹)	4,332.500±8.17	2,057.220±2.10	1,061.150±1.45
Total viable bacteria (cfu mL ⁻¹)	3,350.000±17.57	4,350.000±9.26	15,150.000±313.23
Faecal coliforms (cfu mL ⁻¹)	54.400±5.31	41.250±6.69	212.500±1.68

Data are expressed as Mean±SD

Table 2: Mean values and standard deviation of different bacterial counts (cfu g⁻¹) in different anatomical parts of fishes in tow sampling sites of Lake Manzala

Site	Total viable bacteria	Faecal coliforms
Bashtier		
Intestine	1,422.5±10.26	64.25±15.50
Gill	607.5±7.06	33.75±5.23
Flesh	460.0±12.91	28.75±6.67
Mataryia		
Intestine	12,500.0±851.47	65.00±10.00
Gill	1,487.5±6.75	25.50±5.29
Flesh	2,830.0±1178.42	12.00±3.16

Data expressed as Mean±SD

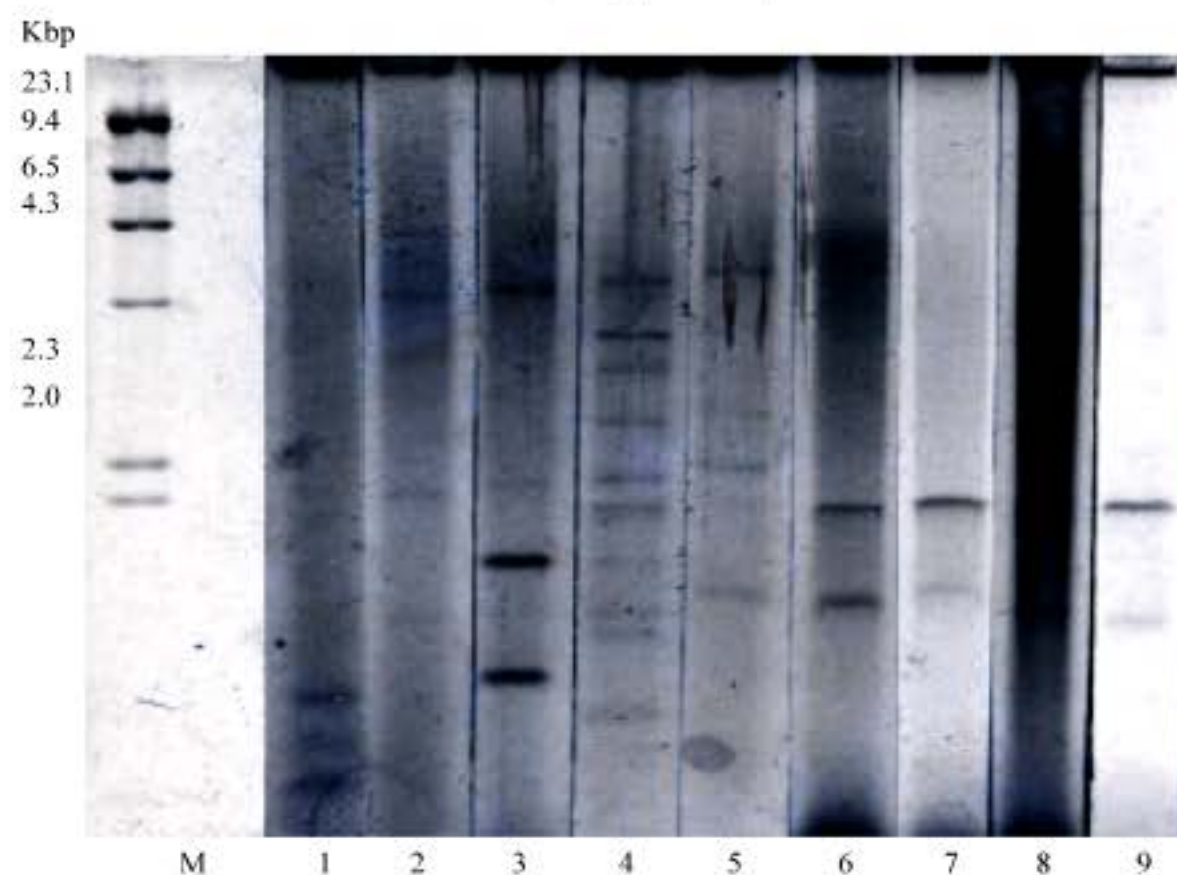


Fig. 2: Plasmid DNA profiling, of different bacterial strains, isolated from water and fish of Lake Manzala. (M) λ DNA digested with Hind III, (1) *Pseudomonas* sp., (2) *Pasturella* sp., (3) *Erwinia* sp., (4) *Citrobacter freundii*, (5) *Sphomonas paucimobilis*, (6) *Stentrophomonas maltophilia*, (7) *Stentrophomonas maltophilia*, (8) *Proteus mirabilis* and (9) *Escherichia coli*

Table 3: Type of antibiotic resistance by bacterial strain from samples of Lake Manzala water and fish

Isolates	Source	Ampicillin	Penicillin G	Gentamycin	Streptomycin	Chloramphenicol
<i>Pseudomonas</i> sp.	W	R	R	S	S	S
<i>Pasturella</i> sp.	W	R	R	S	S	S
<i>Erwinia</i> sp.	W	S	S	S	S	R
<i>Citrobacter freundii</i>	F	R	R	R	S	R
<i>Sphomonas paucimobilis</i>	F	S	S	S	S	S
<i>Stentrophomonas maltophilia</i>	F	R	R	S	R	S
<i>Proteus mirabilis</i>	W	R	R	S	S	R
<i>Escherichia coli</i>	F	R	R	S	R	R

R: Resistant, S: Susceptible, W: Water, F: Fish

Bacteriological Characteristics of Fishes

There were obvious variations found in the total viable bacteria and fecal coliform counts in the different anatomical parts of the fish and by site sampled. The highest count mean value recorded of total viable bacteria was 14000 cfu g⁻¹ and was recovered from the fish of the Mataryia area (Table 3).

Identification of Bacterial Strains

A total of 80 isolates were identified using the API 20E system. The following bacterial strains were found: *Stentrophomonas maltophilia* 18%, *Proteus mirabilis* 16%, *Escherichia coli* 14%, *Erwinia* sp. 13%, *Citrobacter freundii* 12%, *Pseudomonas* sp. 10%, *Sphomonas pucimabilis* 6% (Fig. 2).

DISCUSSION

There are many studies that have looked at the polluted condition of Lake Manzala and the deterioration of the physicochemical and bacteriological conditions which have adversely affected the Lake's ecosystem. Including the evolution of the lake water from marine brackish to brackish fresh. The eutrophication of Lake Manzala has been in response to increased freshwater inputs and

nutrients from agricultural run-off and urban waste disposal (Shaheen and Yousseff, 1978). This has resulted in a dynamic ecology of Lake ecosystem, which was noted in the changing of the fish population from a mixed brackish water species to a population that is primarily constituted of a single species of *Tilapia* (Khalil, 1990).

The effluents of fresh water from drains like the Bahr El-Bakar drain, oppose the current from the Mediterranean Sea resulting in slow water movement to the point where the lake is almost stagnant or at least without currents (Zaky *et al.*, 2005). The heavy loads of suspended solids and organic matter have raised the bed of the lake and have created shallow water depths. The high densities of microbial populations are noteworthy, particularly those of bacteria.

This study revealed high records of pollutants, like ammonia and nitrates, the presence of these can be explained by the anoxic conditions created by the depletion of oxygen. In addition high suspended particles whether of organic or mineral origin, adsorb bacteria to their surfaces. The adsorption of debris particles not only provide microbes a more favorable nutritional environment than those found in free water but also neutralize inhibitory and toxic substances. Thus the suspended particles have a favorable effect on the bacterial growth.

Fecal coliform counts, well known water pollution indicators, were performed on the different anatomical parts of the fish samples from the Kapoty and Mataryia areas. These counts were used in the study to assess and understand the level of pollution of Lake Manzala.

The classical method for the enumeration of heterotrophic bacteria was used in the study; this method accomplishes the separation of individual microbes from the multitude of micro-organisms in a natural microbial community allowing for the individual phenotypes to be determined, which is the basis of the pure culture method and are the mainstay of microbiology (Levin *et al.*, 1992).

The deterioration of the environmental conditions in Lake Manzala can be attributed to the people inhabiting the region and their role as an element of the ecosystem. The complaints and ever increasing recorded cases of diarrhea, gastroenteritis, kidney failure and other diseases may be partially explained by the aforementioned lake condition. The Lake Manzala water samples as well as the fish samples were found to have very high pathogenic bacteria contents; some of these pathogens produce dangerous extra cellular products that are virulent. It is worthy to note that many of these pathogens belong to different taxonomic groups like the families Enterobacteriaceae and Vibrionaceae, with species such as *E. coli*, *Salmonella* sp., *Shigella* sp., *Vibrio* sp. and *Aeromonas* sp. These pathogens have been reported to exist in Lake Manzala (Uerbia *et al.*, 2003; Zaky *et al.*, 2005).

The statistical analysis of earlier studies revealed a strong correlation between counts of *Aeromonas* sp. and fecal coliforms in Lake Manzala, a brackish water environment. Thus *Aeromonas* sp. can be a powerful tool for the assessment of the microbial pollution in the brackish water environments (Zaky, 2006; Zaky *et al.*, 2005).

A study (Dumontet *et al.*, 2003; Pianietti *et al.*, 2004) revealed that *Aeromonas hydrophila* exhibited a resistance to a number of antibiotics, particularly to ampicillin and penicillin. The resistance is an indication of the presence of β -Lactamases which are common in bacterial pathogens found in polluted water environments and the Egyptian environments. These environments are rich in nutrients like calcium, magnesium and chlorides; there is a strong correlation between the existence of these nutrients and *Aeromonas* sp. counts in brackish water environments (Zaky, 2006).

This study, revealed the occurrence of different multi-drug resistant strains of *Pseudomonas* sp., *E. coli*, *Proteus mirabilis* and *Stentrophomonas maltophilia*. The latter was isolated for the first time from polluted water in Egypt through this study; the organism is an important opportunistic pathogen that is isolated world wide from the rhizosphere of some plants, waste water and drinking tap water (Critchley *et al.*, 2003; Hoefel *et al.*, 2005).

Stentrophomonas maltophilia is used in biotechnology for biological control of plant pathogens and bioremediation (Berg *et al.*, 1999). Recently it has been recorded however, that its unregulated

presence in natural environments is dramatically increasing, causing bacteraemia, pneumonia, infections of the urinary tract and soft tissues, as well as many nosocomial infections in children and adults (Boktour *et al.*, 2006; Deletro *et al.*, 2006). The presence of this organism poses a risk to the health of the populations residing along the lake in this important coastal area of Egypt. The organism harbours a DNA plasmid and is β -Lactamase resistant characteristics which are of primary importance in the epidemiology of infectious diseases and clinical (nosocomial) infections. More studies which focus on the transmission of opportunistic bacterial pathogens like *Stenotrophomonas maltophilia* are needed.

REFERENCES

- Abbassy, M.S., H.Z. Ibrahim and H.M. Abdel-Kader, 2003. Persistent organochlorine pollutants in the aquatic ecosystem of Lake Manzala, Egypt. *Bull. Environ. Contam. Toxicol.*, 70: 1158-1164.
- APHA, 1995. Standard Methods for Examination of Water and Wastewater. 19th Edn., American Public Health Association, Washington, DC. USA.
- Berg, G., N. Roskot and K. Smalla, 1999. Genotypic and phenotypic relationships between clinical and environmental isolates of *Stenotrophomonas maltophilia*. *J. Clin. Microbiol.*, 37: 3594-3600.
- Boktour, M., H. Hanna, S. Ansari, B. Balma and R. Hachen *et al.*, 2006. Central venous catheter and *Stenotrophomonas maltophilia* bacteremia in cancer patients. *Cancer*, 106: 1967-1973.
- Critchley, M.M., N.J. Cromar, N.C. McClure and H.J. Fallowfield, 2003. The influence of the chemical composition of drinking water on cuprosolvency by biofilm bacteria. *J. Applied Microbiol.*, 94: 501-507.
- Deletro, M.D., J. Rodriguez-Bano, L. Martinez-Martinez, A. Pascual, R. Perez-Carola, E.J. Perea and M.A. Muniain, 2006. Epidemiology, clinical features and prognosis of infections due to *Stenotrophomonas maltophilia*. *Enferm Infect. Microbiol. Clin.*, 24: 4-9.
- Dumontet, S., V. Pasquale, M. Mancino, G. Normanno and K. Krovacek, 2003. Incidence and characterization of *Aeromonas* sp. in environmental and human samples in southern Italy. *New Microbiol.*, 26: 215-225.
- El-Gaber, G.A., M. Naguib and E. El-Aziz, 1997. *Vibrio* species infections in *Oreochromis niloticus* and *Mugil cephalus*, sodium chloride tolerance pathogenicity, serological relatedness and antibiograms sensitivity of recovered vibrios. *Vet. Med. J.*, 45: 87-99.
- El-Sarangawy, D.A., 1990. Pathogenic indicators of water in six wastewater resources in Lake Manzala, North Egypt, Part I, Bahr El-Bakar. *Zagazig Vet. J.*, 18: 26-34.
- Hoefel, D., P.T. Monis, W.L. Grooby, S. Andrews and C.P. Saint, 2005. Profiling bacterial survival through a water treatment process and subsequent distribution system. *J. Applied Microbiol.*, 99: 175-186.
- Khalil, M.T., 1990. The physical and chemical environment of Lake Manzala, Egypt. *Hydrobiologia*, 196: 193-199.
- Levin, M.A., R.J. Seidler and M. Rogul, 1992. *Microbial Ecology*. 1st Edn., McGraw-Hill, New York, ISBN: 0-07-037506-2, pp: 11-25.
- Maniatis, T., E.F. Fritsch and J. Sambrook, 1982. *Molecular Cloning a Laboratory Manual*. Cold Spring Harbor Laboratory, N.Y., USA., ISBN: 087969-136-0, pp: 149-171.
- Pianietti, A., L. Sabatini, F. Bruscolini, F. Chiaverini and G. Cecchetti, 2004. Faecal contamination indicators, *Salmonella*, *Vibrio* and *Aeromonas* in water used for the irrigation of agricultural products. *Epidemiol. Infect.*, 132: 231-238.
- Shaheen, A.H. and S.F. Youssef, 1978. The effect of the cessation of Nile flood on the hydrographic features of lake Manzala, Egypt. *Arch. Hydrobiol.*, 48: 339-367.

- Sulayman, Z.I., 1999. Antibigram of some bacteria contaminating *Tilapia* fish at El-Manzala Lake in Port-Said Governorate. *Vet. Med. J.*, 27: 19-27.
- Uerbia, D., O. Arzuza, G. Young, E. Para, R. Castro and M. Paullo, 2003. Rotavirus type A and other enteric pathogens in stool samples from children with acute diarrhoea on the Colombian Northern coast. *Int. Microbiol.*, 6: 27-32.
- Zaky, M.M., A. Salem and F.A. Mansour, 2005. Environmental and health significance of toxigenic *Aeromonas sobria* and *Aeromonas hydrophila* in polluted water of Lake Manzala, Egypt. Proceedings of the 26th African Health Congress, Nov. 29-Dec. I, Egypt.
- Zaky, M.M., 2006. Environmental factors influencing multi-drug resistant and harboring plasmid DNA *Aeromonas hydrophila* isolated from polluted water of Lake Manzala, Egypt. Proceedings of the 19th Iaps International Conference on Environment, Health and Sustainable Development, Sept. 11-16, Alexandria, Egypt, pp: 159-160.