Journal of Bacteriology and Parasitology

Zaky and Salem, J Bacteriol Parasitol 2014, 6:6
DOI: 10.4172/2155-9597.1000249

Research Article Open Access

Environmental Factors Influencing Antibiotic Resistant Bacterial Pathogens in Polluted Lake Manzala, Egypt

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Abstract

Lake Manzala is considered one of the most important Costal lakes, in the northern part of Egypt. It suffers from high load of pollutants from different sources such as sewage, industrial and agricultural wastes. In this study, physicochemical characterization of lake water revealed high levels of pollution in different sampling sites such as, pH, TSS, TDS, ammonia ,nitrates, sulfates, alkalinity, chlorides, calcium and magnesium.

Bacterial count such as TVB and fecal coliform of water and fishes of the lake revealed high contamination in Lake Manzala, a total of 90 isolates were identified and resulted in different bacterial pathogens such as, *E. coli*, *Proteus mirabilis*, *Sphomonas paucimobilis*, *Citrobacter freunii*, *Erwinia* sp., *Pasturella* sp. and *Pseudomonas* sp.

Antibiogram was done for all isolates using eight antibiotics such as penicillin, ampicillin, cefotaxime, chloramphenicol, rifampin, tetracycline, streptomycin and gentamicin.

The result showed a high resistant pattern among different species which are harboring plasmid DNA. This is an indication that these bacterial pathogens have risk factors in the communities around Lake Manzala.

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. 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.

Although the lake is still considered as the largest of the Egyptian Delta Lakes, its area has been gradually decreased. Extensive land reclamation during the last century has reduced the lake surface area to less than half of its original size. In 1900's, its area was estimated at about 1,709.4 km^2. By the end of the previous century, the area of the lake was estimated to be 1000 km^2. The area of the open water is only 742.8 km^2 due to the presence of a large number of islets in the lake. During the last decade, the reclamation is progressing at an accelerating pace where land had subsequently been created and islands enlarged. Creation of canals and drains such as the Bahr El-Bakar drain, the Sirw drain, the Ramsis drain and the Hadous drain has created an 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 [1].

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 [2].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 population [3]. pathogenic species such as, *Aeromonas hydrophila* and *Aeromonas sobria*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescence* 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 [4-6]. This study aims at characterization of microbial pollution of Lake Manzala, focusing on the antibiotic resistant bacterial strains.

Materials and Methods

Seasonally during the year 2014, water samples were taken from Kapoty, Bashtier, Mataryia and Gamil outlet 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-Bashtier Area is considered a midpoint 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 the fishing and raise animals, El-Mataryia area is considered the fresh-

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Received August 31, 2015; **Accepted** November 09, 2015; **Published** November 12, 2015

Citation: Zaky MMM, Salem MAM (2015) Environmental Factors Influencing Antibiotic Resistant Bacterial Pathogens in Polluted Lake Manzala, Egypt. J Bacteriol Parasitol 6: 249. doi: 10.4172/2155-9597.1000249

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 m^3/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, especially the fishing of *Tilapia* spp.

Gamil outlet is considered the adjoining part of the lake with sea water coming from Mediterranean Sea (Figure 1).

Sampling methods

Water samples from each site were taken in clean sterile one litre glass bottles and transported from the lake to the laboratory within six hours. The bottles were kept in ice bags and ice jackets for direct examination. 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 plate 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.

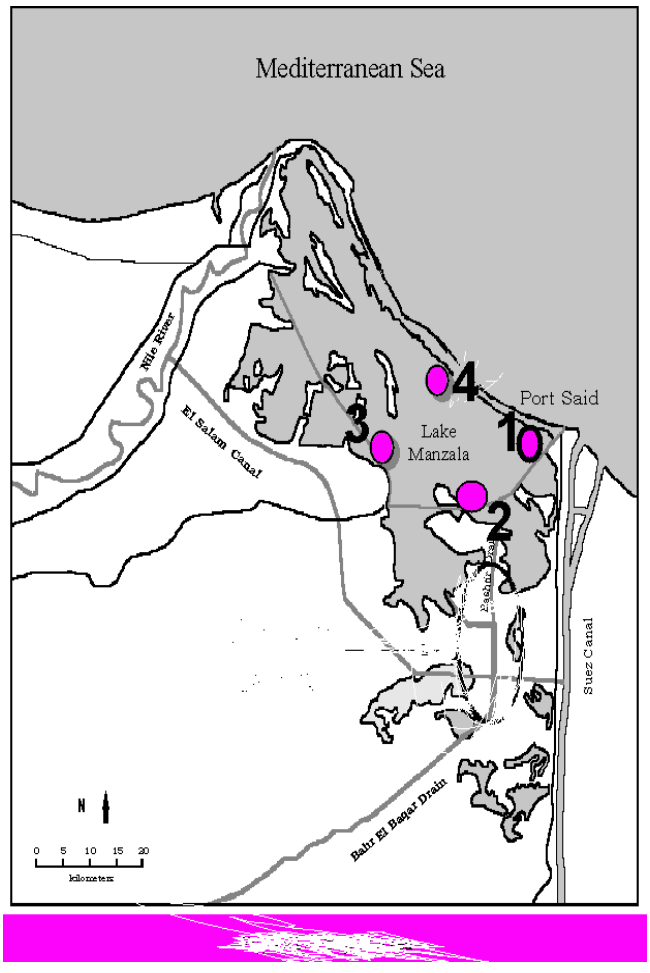

Figure 1: Map of Lake Manzala, showing the three different sampling sites and their respective sources of pollution.

Physicochemical characteristics of water samples

Physicochemical characteristic of each water sample was 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 was calibrated against pH 4, 7 and 9 buffers. 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, Sulphates, Alkalinity, calcium, magnesium and chloride levels were done using the recommended standard methods of water analysis (mg/L).

Microbiological analysis

Counts of total viable bacteria (TVB), Total anaerobic and total anaerobic spore formers were determined utilizing nutrient agar. Total coliforms and of faecal coliforms *Shigella* spp on S. S. agar. *Vibrio* spp. on T.C.B.S agar. *Aeromonas* spp. on Aeromonas differential agar. Counts of water cfu/ml and of fish cfu/g.

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.*

Statistical analysis

The correlation matrix is the relationship between a number of data sets that are scaled to be independent of the unit of measurement. It is used to determine whether data sets move together; that is whether large values of one set are associated with large values of the other (positive correlation); whether small values of one set are associated with large values of the other (negative correlation); or whether the values of the sets are unrelated [7].

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) were 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 hrs. The presence of a colony was an indication of the resistance of the bacteria to the following antibiotics; Chloramphenicol (30 µg), Ampicillin (10 µg), Penicillin G (10 µg), Streptomycin (10 µg), Gentamycin (10 µg), Cefotaxime (5 µg), Rifampcin (5 µg) and Tetracyclin (30 µg).

Isolation of plasmid DNA

The alkaline lysis method was used in mini preparation [8]. For antibiotic resistant bacterial strains isolated from water and fish samples.

Results

Physicochemichal parameters and bacteriological characteristics, showed seasonal variations in water samples in different sites

El Kapoty site: The statistical analysis of the data in El-Kapoty site

showed that there was a very strong positive (+ve) correlation between temperature and TVB, T. An, S.S, *Aermonas* and *Vibrio* (r=0.99, 0.92, 0.97, 0.92 and 0.97; p<0.05) respectively. Also, there was a strong positive (+ve) correlation between temperature and T.C, F.C and F.S (r=0.70, 0.86 and 0.84; p<0.05) respectively (Table 1).

The statistical analysis showed that there was a very strong negative (-ve) correlation between pH and T.C (r=-0.94, p<0.05). Also, there was a strong negative (-ve) correlation between pH and *Vibrio* (r=-0.70, p<0.05). Also, there was a modest negative (-ve) correlation between pH and TVB, T. An and S.S (r=-0.66, 0.65 and 0.52; p<0.05) respectively (Table 2).

Bashtier site: The statistical analysis of the data in Bashtier site showed that there was a very strong positive (+ve) correlation between temperature and *Vibrio* (r=0.96; p<0.05). Also, there was a strong positive (+ve) correlation between temperature and T.V.B, T. An, T.C, S.S and *Aermonas* (r=0.86, 0.87, 0.77 and 0.86; p<0.05) respectively (Table 3).

The statistical analysis showed that there was a strong negative (-ve) correlation between pH and T.V.B, T. An, T.C, S.S, *Aermonas* and *Vibrio* (r=-0.77, -0.79, -0.87, -0.73, -0.72 and -0.76; p<0.05) respectively (Table 4).

Mataryia site: The statistical analysis 0f the data on Mataryia site showed that there was a very strong positive (+ve) correlation between temperature and TVB and *Aermonas* (r= 0.94 and 0.92; p<0.05) respectively. Also, there was a strong positive (+ve) correlation between temperature and T.C and *Vibrio* (r= 0.78 and 0.80; p<0.05) respectively (Table 5).

Parameters	Autumn	Spring	Summer	Winter	Average ± SD
Temp.	22.7	20	29.3	13.8	21.5 ± 6.4
pH	7	8.4	8.3	9.5	8.3 ± 1.0
E.C	3983.3	2383	1716.7	1225	2327.0 ± 1201.9
D.O	2.7	1.8	1.6	1.7	2.0 ± 0.5
T.S.S	110.3	173	179.6	95.5	139. 6 ± 42.9
T.D.S	16026.7	9846	17882	8304	13014.7 ± 4654.5
NH_4^+-N	0.7	0.2	0	0.8	0.4 ± 0.4
NO_3^--N	0.2	0.2	0	0.1	0.1 ± 0.1
SO_4^{--}	2003.1	1600.6	1718	1415.9	1684.4 ± 246.2
Cl^-	5636.8	4959	7873	3980	5612.2 ± 1653.5
Alkal	253.3	207.5	205	252.5	229.6 ± 27.0
Total Hardness	3046.7	2200	3314.7	1850	2602.9 ± 691.1
Ca^{++}	344.2	282.6	326	344.7	324.4 ± 29.2
Mg^{++}	531.7	363.4	608.3	240.8	436.1 ± 165.5

Table 1: The seasonal variations of different physicochemical parameters from El-Kapoty Site.

Parameters	Autumn	Spring	Summer	Winter	Average ± SD
T.V.B	6500	3500	9200	350	4887.5 ± 3817.1
T. An	4200	700	5500	150	2637.5 ± 2619.0
T. An. sp	5	220	22	1.5	62.1 ± 105.6
T.C	1200	300	780	15	573.8 ± 523.4
F.C	48	25	440	0	128.3 ± 208.8
F.S	1.7	0	40	0	13.9 ± 22.6
S.S	88.3	40.8	140	22	72.8 ± 52.8
Aermonas	78.7	25.5	190	21.5	78.9 ± 78.5
Vibrio	63.7	39.3	76	11	47.5 ± 28.7

Table 2: The seasonal variations of the count of different Bacterial species in El-Kapoty Site.

Parameters	Winter	Summer	Spring	Autumn	Average ± SD
Temp.	14.5	29.7	20.8	23.2	22.1 ± 6.3
pH	8.9	8.2	8.4	8.6	8.5 ± 0.3
E.C	975	1516.7	2100	2812.3	1851.0 ± 788.5
D.O	1.4	1.4	1.75	3.1	1.9 ± 0.8
T.S.S	76.5	83	114.7	45.7	80.0 ± 28.3
T.D.S	3572	11542.7	5225.3	6488	6707.0 ± 3437.8
NH_4^+-N	1.4	0.2	0.2	0.14	0.5 ± 0.6
NO_3^--N	0	0.06	0.2	0.1	0.1 ± 0.1
SO_4^{--}	630.2	3180.2	949.4	2138.7	1724.6 ± 1167.5
Cl^-	1541.2	4779.5	2159.3	1966.5	2611.6 ± 1468.1
Alkal	302.5	191.7	212.5	276.7	245.9 ± 52.3
Total Hardness	500	2146.7	1215	1860	1430.4 ± 732.5
Ca^{++}	200.4	331.3	284.5	307	280.8 ± 56.9
Mg^{++}	218.7	321	122.8	265.9	232.1 ± 84.0

Table 3: The seasonal variations of different physicochemical parameters in Bashtier Site.

Parameters	Winter	Summer	Spring	Autumn	Average ± SD
T.V.B	3000	43000	6000	6400	14600.0 ± 18994.0
T. An	600	24000	3200	3300	7775.0 ± 10888.6
T. An. sp	1.5	25	120	18.3	41.2 ± 53.5
T.C	8	150	70	1	57.3 ± 69.2
F.C	80.5	68	45	0	48.4 ± 35.4
F.S	0	0	0	0.3	0.1 ± 0.2
S.S	37	320	39.5	70	116.6 ± 136.4
Aermonas	42	310	39.5	84.3	119.0 ± 129.0
Vibrio	8.5	110	34.7	80.3	58.4 ± 45.4

Table 4: The seasonal variations of the count of different Bacterial species in Bashtier Site.

Parameters	Autumn	Spring	Summer	Winter	Average ± SD
Temp.	23.3	20.5	28.7	15	21.9 ± 5.7
pH	8.06	8.5	7.9	7.5	8.0 ± 0.4
E.C	1708.3	1616.7	991.7	880	1299.2 ± 423.7
D.O	1.75	1.6	1.4	1.9	1.7 ± 0.2
T.S.S	41.3	98.7	49.3	53.5	60.7 ± 25.8
T.D.S	3039.3	3257.3	2951.3	2863	3027.7 ± 169.1
NH_4^+-N	1.05	0.6	0	4	1.4 ± 1.8
NO_3^--N	0.3	0.2	0.2	0.5	0.3 ± 0.1
SO_4^{--}	907.4	1048.6	947.5	635.5	884.8 ± 176.5
Cl^-	953	1181.9	1905	1113.7	1288.4 ± 422.1
Alkal	321.7	262.5	276.7	312.5	293.4 ± 28.3
Total Hardness	856.7	870	756.7	870	838.4 ± 54.8
Ca^{++}	176.1	262.5	165.6	256.3	215.1 ± 51.4
Mg^{++}	101.2	52.3	83.5	55.9	73.2 ± 23.3

Table 5: The seasonal variations of different physicochemical parameters in Mataryia Site.

The statistical analysis showed that there was a very strong negative (-ve) correlation between pH and F.S (r=-0.95, p<0.05). Also, there was a strong positive (+ve) correlation between pH and *F.C* (r= 0.70, p<0.05) (Table 6).

El Gamil site: The statistical analysis showed that there was a very strong positive (+ve) correlation between temperature and *Vibrio* (r=0.99; p<0.05). Also, there was a strong positive (+ve) correlation between temperature and T.V.B, T. AN sp, and *Aermonas* (r=0.85, 0.87, and 0.77; p<0.05) respectively (Table 7).

Parameters	Autumn	Spring	Summer	Winter	Average ± SD
T.V.B	29000	20000	92000	1700	35675.0±39230.4
T.An	16000	120000	11000	1000	37000.0 ± 55683.6
T.An.sp	3.3	34	8.3	10	13.9 ± 13.7
T.C	78.3	210	430	80	199.6 ± 165.5
F.C	1.7	560	150	8.5	180.1 ± 262.4
F.S	1.7		0.7	21	7.8 ± 11.4
S.S	430	130	450	91.5	275.4 ± 190.9
Aeromonas	180	130	180	100	147.5 ± 39.5
Vibrio	110	73.3	89	17	72.3 ± 39.8

Table 6: The seasonal variations of the count of different Bacterial species in Mataryia Site.

The statistical analysis showed that there was a strong negative (-ve) correlation between pH and F.S ($r=-0.88$, $p<0.05$) (Table 8).

Bacteriological characteristics of fishes

Identification of bacterial strains: A total of 90 isolates were identified using the API 20E system, 21 different strains with different ratios.

Plasmid DNA profiling: Plasmid profiling for the different strain, which are resistant to antibiotics, contains a variety of plasmids (Figure 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 [9]. 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 with a population that is primarily constituted of a single species of Tilapia [10].

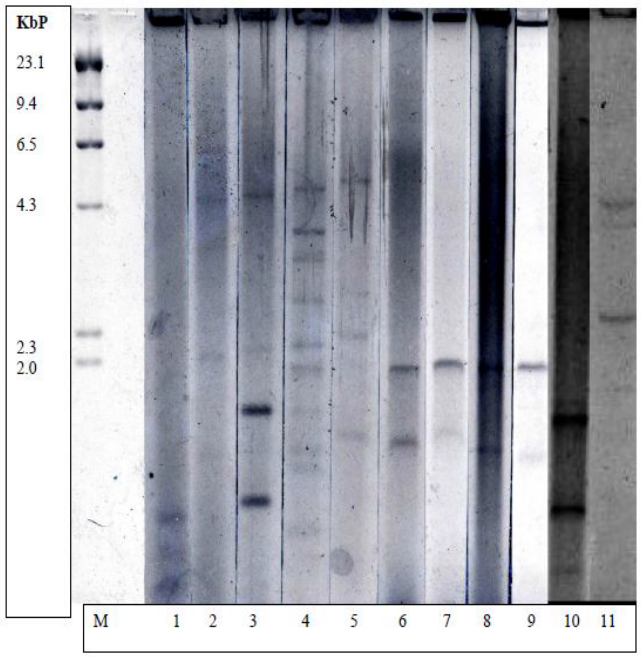

Figure 2: Plasmid DNA profiling, of different bacterial strains, isolated from water and fish of Lake Manzala. M) λ DNA digested with Hind III, **1)** *Pseudomonas* sp., **2)** *Pasturella* sp;, **3)** *Erwinia* sp.; **4)** *Citrobacter freundi,* **5)** *Sphomonas paucimobilis;* **6)** *Stentrophomonas maltophilia;* **7)** *Stentrophomonas maltophilia;* **8)** *Proteus mirabilis;* **9)** *Eschericia coli;* **10)** *Aeromonas hydrophila;* **11)** *Aeromonas sobria*

Parameters	Autumn	Spring	Summer	Winter	Average ± SD
Temp.	24.7	22.7	31	15	23.4 ± 6.6
pH	8.5	8.1	7.9	8	8.1 ± 0.3
E.C	3950	2566.7	1533.3	1350	2350.0 ± 1193.6
D.O	2.7	1	1.4	2.8	2.0 ± 0.9
T.S.S	176.7	332	125.7	302.5	234.2 ± 98.8
T.D.S	21676.7	11881.3	14060	19267	16721.3 ± 4529.3
NH_4^+-N	2.8	1	0.9	1.2	1.5 ± 0.9
NO_3^--N	0.3	0.2	0.1	0	0.2 ± 0.1
SO_4^{--}	4203	1959.7	1145.2	1748.5	2264.1 ± 1337.9
Cl^-	4779.3	4948.9	4500.9	5176.9	4851.5 ± 284.9
Alkal	333.3	392.5	300	400	356.5 ± 48.0
Total Hardness	2746.7	2445	2333.3	3020	2636.3 ± 309.7
Ca^{++}	1349.3	288.5	316.6	348.7	575.8 ± 516.3
Mg^{++}	462	419.3	234.2	522.9	409.6 ± 124.4

Table 7: The seasonal variations of different physicochemical parameters El Gamil Site.

Parameters	Autumn	Spring	Summer	Winter	Average ± SD
T.An	38000	130000	100000	3000	67750.0 ± 57714.1
T.An.sp	60.7	49	330	2	110.4 ± 148.6
T.C	110	190	60	24.5	96.1 ± 71.7
F.C	23	200	100	0.5	80.9 ± 90.1
F.S	18.3	5	6.7	1	8.7 ± 8.8
S.S	230	50.2	150	40.5	117.7 ± 89.8
Aeromonas	220	43.2	290	88	160.3 ± 114.5
Vibrio	69.3	63	120	15	66.8 ± 43.0

Table 8: The seasonal variations of the count of different Bacterial species in El Gamil Site.

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. The heavy loads of suspended solids and organic matter have raised the bed of the lake and have created shallow water depths. The highest 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. This in line with other studies on pollution of Lake Manzala [11,12].

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 (Table 9).

The classical method for the enumeration of heterotrophic bacteria was used in the study; this method accomplishes the separation of

Site	Part of fish	No. of tests	T.V.B	T.An	T.An. Sp	T.C	F.C	F.S	S.S	*Aeromonas* sp.	*Vibrio* sp.
	Intestine	4	142250 ± 11638	75332.5 ± 74978	1122 ± 1233	41732 ± 44685	8662 ± 9293	26500 ± 41868	31750 ± 29420	30757 ± 46863	11270 ± 14256
Bashtier	**gills**	4	60750 ± 33519	47750 ± 44214	427 ± 320	2762 ± 1829	3272.5 ± 2385	24083 ± 21494	4320 ± 4535	21817 ± 17707	2280 ± 2569
	flesh	4	44212 ± 50719	36200 ± 30193	6025 ± 10079	745 ± 727	2000 ± 2041	12443 ± 18742	17987 ± 16038	15142 ± 13189	1757 ± 1622
	Intestine	4	94750 ± 74186	808250 ± 1461293	106170 ± 189827	17800 ± 19163	5000 ± 9345	10143 ± 11460	89000 ± 47511	26250 ± 22276	18875 ± 30123
Mataryia	**gills**	4	138750 ± 198761	46850 ± 37444	10007 ± 15486	3557 ± 5678	1987.5 ± 2666	7673 ± 5136	58000 ± 46209	22892 ± 15807	2375 ± 2098
	flesh	4	30737 ± 42541	23212.5 ± 23114	5362 ± 9098	1150 ± 1618	717.5 ± 1435	3576 ± 3706	16925 ± 17407	14782 ± 19433	1010 ± 1166

Table 9: The average and standard deviation values of different bacterial counts in three parts of fish samples in two sampling sites (Bashtier and Mataryia).

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 [13].

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 extracellular 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* spp., *Shigella* spp., *Vibrio* spp. and *Aeromonas* spp. These pathogens have been reported to exist in Lake Manzala [14,15].

The statistical analysis of previous studies revealed a strong correlation between counts of *Aeromonas* spp. and fecal coliforms in Lake Manzala, a brackish water environment. Thus *Aeromonas* spp. can be a powerful tool for the assessment of the microbial pollution in the brackish water environment [6].

A study [16,17] 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 environment and the Egyptian environment. These environments are rich in nutrients like calcium, magnesium and chlorides; there is a strong correlation between the existence of these nutrients and *Aeromonas* spp. counts in brackish water environment [1].

Statistical analysis indicates that Temperature and pH are two main factors influencing bacterial pathogens in Lake Manzala. 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 worldwide from the rhizosphere of some plants, waste water and drinking tap water [18,19] (Table 10).

Stentrophomonas maltophilia is used in biotechnology for biological control of plant pathogens and bioremediation [20]. Recently it has been recorded however, that its unregulated presence in natural environments

No	Strain	Source	Number of isolates	Percentage
1	*Acinetobacter* sp.	Water	1	1.1%
2	*Erwinia* sp.	Water	4	4.4%
3	*Stentrophomonas maltophilia*	Fish	2	2.2%
4	*Aeromonas hydrophila*	Fish	16	17.7%
5	*Aeromonas sobria*	Fish	15	16.6%
6	*Chrysomonas lutcola*	Water	1	1.1%
7	*Flavobacterium oryzihabitans*	Water	2	2.2%
8	*Favobacterium odoratum*	Water	1	1.1%
9	*Flavobacterium maningosepticum*	Water	1	1.1%
10	*Bacillus* sp.	Water	8	8.8%
11	*Serratia* sp.	Water	1	1.1%
12	*Vibrio* sp.	Water	1	1.1%
13	*Pseudomonas* sp.	Water	1	1.1%
14	*Pasteurella* sp.	Water	1	1.1%
15	*Klebsiella pneumonia*	Fish	3	3.3%
16	*Citrobacter freundii*	Fish	2	2.2%
17	*Sphomonas paucimebilis*	Fish	1	1.1%
18	*Proteus mirabilis*	Fish	9	10%
19	*E. coli*	Fish	10	11.1%
20	*Enterobacter amnignus*	Fish	1	1.1%
21	*Streptococcus* sp.	Water	3	3.3%

Table 10: Identification of bacterial strain from samples of Lake Manzala water and fish.

No.	Strain	Cefotaxim	Chloramphenicol	Streptomycin	Refampcin	Ampecillin	Tetracyclin	PenicillinG	Gentamicin
1	*Acinetobacter* sp.	S	S	R	S	R	S	R	S
2	*Erwinia* sp.	S	R	S	S	S	R	S	S
3	*Stentrophomonas maltophilia*	S	S	R	S	R	S	R	S
4	*Aeromonas hydrophila*	S	S	R	R	R	R	R	S
5	*Aeromonas sobria*	S	R	R	S	R	R	R	R
6	*Chrysomonas lutcola*	S	S	S	R	R	S	R	S
7	*Flavobacterium oryzihabitans*	S	S	R	S	R	S	R	S
8	*Favobacterium odoratum*	S	S	S	S	S	R	S	S
9	*Flavobacterium maningosepticum*	S	R	S	S	R	S	R	S
10	*Bacillus* sp.	R	R	R	R	R	R	R	R
11	*Serratia* sp.	S	S	S	S	S	S	S	S
12	*Vibrio* sp.	S	R	R	S	R	S	R	R
13	*Pseudomonas* sp.	S	S	S	S	R	S	R	S
14	*Pasteurella* sp.	S	S	S	S	R	S	R	S
15	*Klebsiella pneumonia*	S	R	R	R	R	S	R	S
16	*Citrobacter freundii*	S	R	S	R	R	S	R	R
17	*Sphomonas paucimebilis*	S	S	S	S	S	S	S	S
18	*Proteus mirabilis*	R	R	R	R	R	S	R	R
19	*E. coli*	R	R	R	R	R	R	R	R
20	*Enterobacter amnignus*	R	S	S	R	R	R	R	S
21	*Streptococcus sp*	R	R	R	R	S	R	R	R

Table 11: The antibiotic resistance pattern of bacterial strains isolated of fish of Lake Manzala (S=Susceptible; R=Resistant).

is dramatically increasing, causing bacteraemia, pneumonia, infections of the urinary tract and soft tissues, as well as many nosocomial infections in children and adults [21,22]. The presence of bacterial pathogens in water and fish of Lake Manzala, 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 ß-Lactamase resistant characteristics which are of primary importance in the epidemiology of infectious diseases and clinical (nosocomial) infections. More studies which focuses on the transmission of opportunistic bacterial pathogens are needed (Table 11).

Conclusion

In this study, physicochemical characteristics revealed high pollution condition of the lake water. A bacterial count of fish parts indicates that fish of the lake not suitable for human consumption. Bacterial Identification of water and fish samples revealed the occurrence of different bacterial pathogenic species in the lake.

Aeromonas hydrophila and *Aeromonas sobria* are two dominant species in Lake Manzala. Statistical analysis revealed that temperature and pH are two important factors influencing bacterial counts Lake Manzala.

Antibiogram showed variation of resistance of bacterial pathogens to different antibiotics. Plasmid DNA profiling indicates that antibiotic resistant pathogens are genetically active in the environment.

Lake Manzala is considered a source of many bacterial pathogens which are risk for human health in this area of Egypt.

More efforts should be done for prevention and rehabilitation lake Manzala, to avoid dangerous human diseases in this important part of Egypt.

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