Antibiotic-Resistant Bacteria in Water and Fish: A Risk to Human Health

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Abstract Water pollution is one of the most severe environmental problems that affect public health and can cause diseases as gastrointestinal diseases. Microbial pollution of Lake Manzala was studied in four main localities, Kapoty, Bashtier, Mataryia and Gamil areas which receive a high load of sewage, agricultural and industrial wastes from different sources. A total of 50 bacterial isolates from water and fish were identified using API 20E system, revealed that 20% of these bacterial strains, were related to *Escherichia coli* (*E. coli*), which has been subjected to different antibiotics, such as Ampicillin, Penicillin G, Streptomycin, tetracycline, Gentamycin, Chloramphenicol, rifampicin and Cefotaxime. All strains variation of resistance pattern, harboring Plasmid DNA are an indication of risk to human health in the communities around Manzala Lake, from such bacterial pathogens, which live in polluted and stressed environment.

Keywords: *Microbial pollution, Manzala lake, antibiotics, Plasmid DNA*

Cite This Article: Mahmoud M. M. Zaky, Saly M. E. Toubar, and Ahmed Samy El-Shafey, "Antibiotic-Resistant Bacteria in Water and Fish: A Risk to Human Health." *International Journal of Celiac Disease*, vol. 9, no. 2 (2021): 77-81. doi: 10.12691/ijcd-9-2-2.

1. Introduction

Lake of Manzala is a shallow costal lake of thirty basins with depths ranging from 0.7 to 3m in depth, the deepest areas resting in the navigation canal. It is situated east of the Nile River's Delta, between the Damietta 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 [1,2]. The Lake suffers from water pollution induced by agricultural drainage, industrial wastes and sewage and is contaminated by persistent organochlorine pollutants [3].

Water is an important constituent of the life support system. No one can live and even dream to live without water. Most of our water bodies have become polluted due to industrial growth; urbanization and man-made problems mainly the result of population growth. Poor sanitation and contaminated drinking water arising from human activity and natural phenomena create serious problems in human health. The chief sources of water pollution are sewage and other waste, industrial effluents, agricultural discharges and industrial wastes from chemical industries, fossils fuel plants and nuclear power plants. They create a larger problem of water pollution rendering water no longer fit for drinking, agriculture and, as well as for aquatic life. More than 2.6 billion people--40% of the world's population--lack basic sanitation facilities and over one billion people still use unsafe drinking water sources. As a result, thousands of children die every day from diarrhea and other water, sanitation and hygiene-related diseases and many suffer and are weakened by illness [4].

Microbiological contamination is an important water-quality problem worldwide. Human impact on this category of contamination is significant and several human-related activities, and also the population explosion, have affected and are still affecting dramatically the aquatic environment. Extensive industrialization and agriculture have led to increased pollution of water [5].

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 perfringens* and is manifested in the water as well as in the fish populations [2,6].

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 diarrheal diseases among human populations residing in the northeastern coast of Egypt [7,8,9,10].

To improve water quality, it is important to set up a management strategy and plans that provide implementing strategies to address water quality. Similarly, for maintaining overall ecosystem health, it is very important to raise global awareness of the problems associated with fecal pollution of water resources [5].

2. 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.

2.1. 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 fishing and raise animals, 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, especially the fishing of Tilapia spp. Gamil outlet is considered the adjoining part of the lake with sea-water coming from the Mediterranean sea (Figure 1).
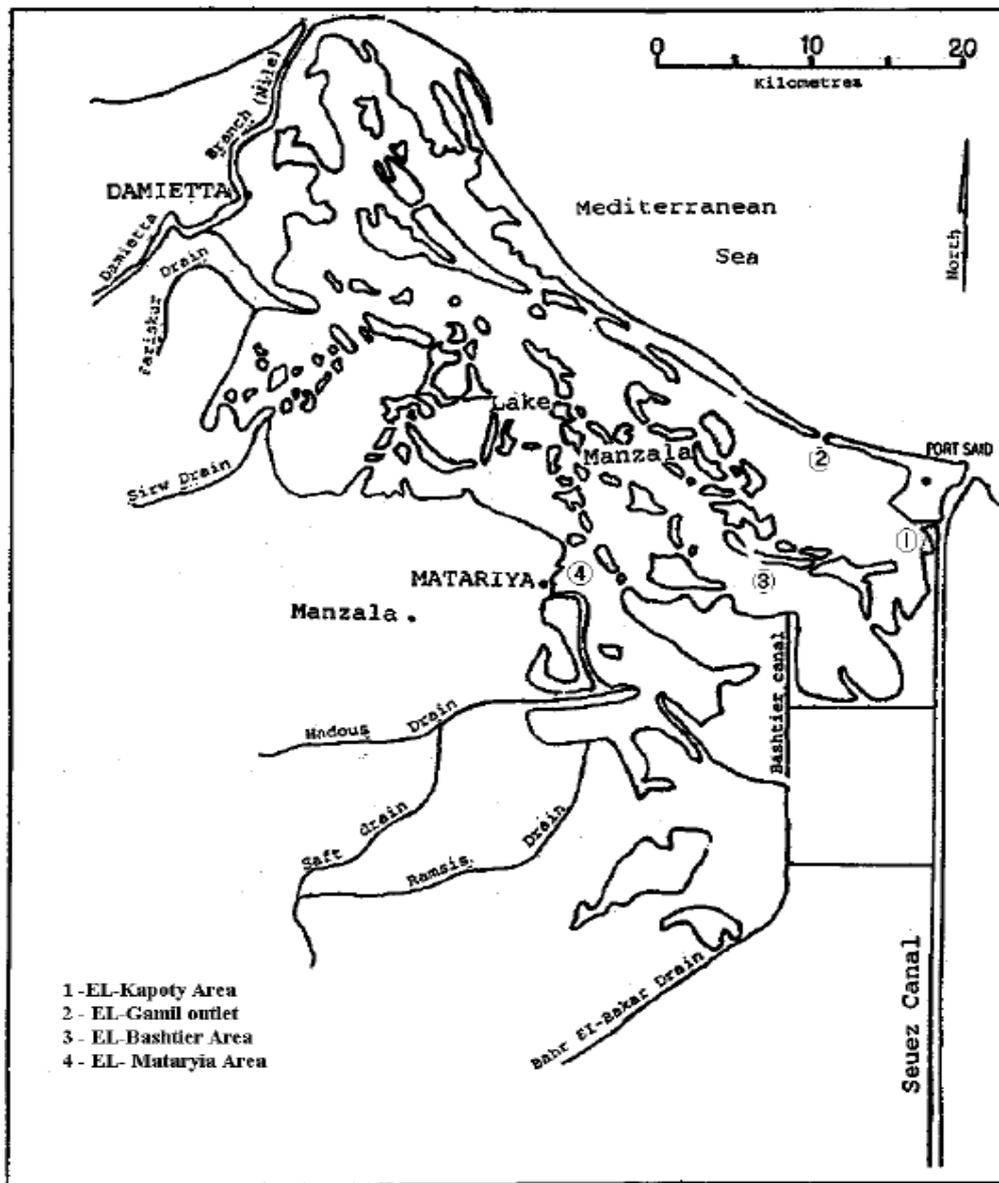

Figure 1. Map of Lake Manzala, showing the three different sampling sites and their respective sources of pollution

2.2. Sampling Methods

Water samples from each site were taken in clean sterile one liter 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 plates method. The fish were dissected and 1g. of the intestinal contents was aseptically stripped out with sterile forceps. Samples of gill lamellae and intestinal contents (1g) 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 [7].

2.3. Microbiological Analysis

Counts of total viable bacteria (T.V.B), were determined utilizing nutrient agar and fecal coliforms (F.C) were estimated utilizing endo agar 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.

2.4. Resistance to Antimicrobial Agents

The disc diffusion method was used to find antimicrobial resistance. The bacterial strains were grown overnight in nutrient broth, then 1ml 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 37o C for 24 hrs. The presence 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), Gentamycin (10 mcg), Cefotaxime (5Mcg), Rifampicin (5Mcg) and Tetracycline (30Mcg) [7].

2.5. Isolation of Plasmid DNA

The alkaline lysis method was used in mini preparation for antibiotic-resistant bacterial strains isolated from water and fish samples [11].

3. Results

Table 1 shows the isolated bacteria while E.coli is the highest number of bacterial isolates (10 isolates) representing 20% from total isolates but *Chrysomonas lutcola, Favobacterium odoratum, Flavobacterium maningosepticum, Serratia sp, Vibrio sp* and *Sphomonas*

paucimebilis are the lowest number of isolates (1 isolate for each bacteria) representing 2 % for each bacteria.

Table 1. Name, number and percentage of each bacterial isolate

NO	Stain	Number of isolates	Percentage
1	*Erwinia sp*	4	8%
2	*Stentrophomonas maltophilia*	2	4%
3	*Aeromonas spp*	5	10%
4	*Chrysomonas lutcola*	1	2%
5	*Flavobacterium oryzihabitans*	2	4%
6	*Favobacterium odoratum*	1	2%
7	*Flavobacterium maningosepticum*	1	2%
8	*Serratia sp*	1	2%
9	*Vibrio sp*	1	2%
10	*Pseudomonas sp*	3	6%
11	*Pasteurella sp*	2	4%
12	*Klebsiella pneumonia*	3	6%
13	*Citrobacter freundii*	2	4%
14	*Sphomonas paucimebilis*	1	2%
15	*Proteus mirabilis*	9	18%
16	E.coli	10	20%
17	Enterobacter amnignus	2	4%

Table 2 shows TVB and FV by cfu/ml; in Kapoty area, TVB was ranged between 1200 to 10000 and FC was ranged between 100 to 3800. In El-Gamil outlet, TVB was ranged between 100 to 3300 and FC was ranged between 50 to 100. In Bashtier area, TVB was ranged between 1000 to 5000 and FC was ranged between 3 to 100. Finally in Mataryia area, TVB was ranged between 1400 to 7200 and FC was ranged between 50 to 240.

Table 2. Bacteriological parameters in Manzala Lake

		Kapoty	El-Gamil	Bashtier	Mataryia
TVB (cfu/ml)	Autumn	1200	1500	1000	1400
	Winter	1300	2750	5000	4000
	Spring	5600	3300	4000	5600
	Summer	10000	100	3000	7200
	Mean	4525	1912.5	3250	4550
	SD	4186.8	1423.8	1707.83	2473.19
FC (cfu/ml)	Autumn	200	30	3	240
	Winter	100	100	100	100
	Spring	1500	50	50	50
	Summer	3800	100	100	100
	Mean	1400	70	63.25	122.5
	SD	17722.2	35.6	46.57	81.80

SD standard deviation of the sample.

Table 3 shows the sensitivity of each bacterial isolate to the following antibiotics; Cefotaxime, Chloramphenicol, Streptomycin, Rifampicin, Ampicillin, Tetracycline, Penicillin G and Gentamicin while E coli show multi drug-resistant

Table 3. Effect of different antibiotics on isolated strains

| NO	Strain	Cefotaxime	Chloramphenicol	Streptomycin	Rifampicin	Ampicillin	Tetracycline	Penicillin G	Gentamicin
1	*Erwinia sp*	S	R	S	S	S	R	S	S
2	*Stentrophomonas maltophilia*	S	S	R	S	R	S	R	S
3	*Aeromonas spp*	S	R	R	S	R	R	R	R
4	*Chrysomonas lutcola*	S	S	S	R	R	S	R	S
5	*Flavobacterium oryzihabitans*	S	S	R	S	R	S	R	S
6	*Favobacterium odoratum*	S	S	S	S	S	R	S	S
7	*Flavobacterium maningosepticum*	S	R	S	S	R	S	R	S
8	*Serratia sp*	S	S	S	S	S	S	S	S
9	*Vibrio sp*	S	R	R	S	R	S	R	R
10	*Pseudomonas sp*	S	S	S	S	R	S	R	S
11	*Pasteurella sp*	S	S	S	S	R	S	R	S
12	*Klebsiella pneumonia*	S	R	R	R	R	S	R	S
13	*Citrobacter freundii*	S	R	S	R	R	S	R	R
14	*Sphomonas paucimebilis*	S	S	S	S	S	S	S	S
15	*Proteus mirabilis*	R	R	R	R	R	S	R	R
16	*E coli*	R	R	R	R	R	R	R	R
17	*Enterobacter amnignus*	R	S	S	R	R	R	R	S
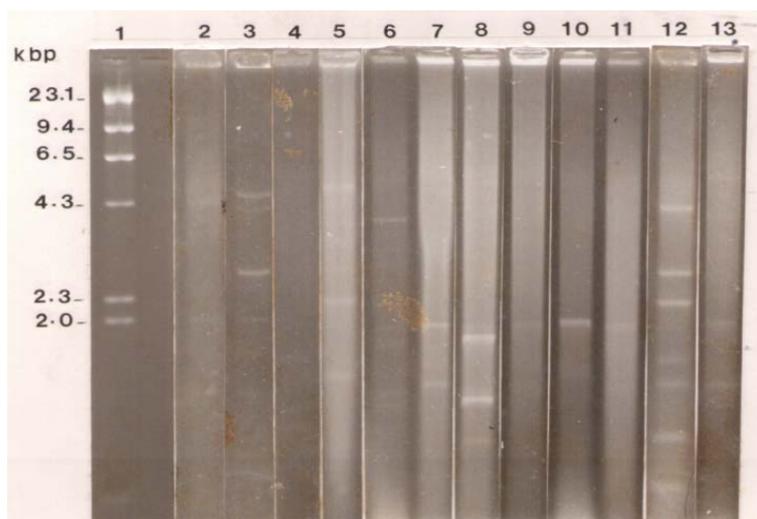
Figure 2. Plasmid DNA profiling of different bacterial isolates

Table 4. Names of isolated strains according to their number in plasmid DNA profiling

NO of strain in plasmid DNA profiling	Name of strain in plasmid DNA profiling
1	*Erwinia sp*
2	*Stentrophomonas maltophilia*
3	*Aeromonas spp*
4	*Serratia sp*
5	*Vibrio sp*
6	*Pseudomonas sp*
7	*Pasteurella sp*
8	*Klebsiella pneumonia*
9	*Citrobacter freundii*
10	*Sphomonas paucimebilis*
11	*Proteus mirabilis*
12	*E.coli*
13	*Enterobacter amnignus*

Figure 2 shows plasmid DNA profiling to the isolated bacteria while names of isolated strains according to their number in plasmid DNA profiling represented in Table 4.

4. Discussion

Lake of Manzala is considered one of the most polluted lakes on the northern coast of Egypt, due to a high load of pollutants from different sources. High bacterial counts of T.V.B and F.C, indicates high pollution of different sampling sites, particularly El Mataryia area and El Kapoty area, because of the disposal of different wastes in these areas

Isolation of different bacterial pathogens such as *E.coli, proteus spp* and other Enteric bacterial pathogens in line with other studies [12,13] which indicates that this Lake is a suitable environment to bacterial pathogens which affect negatively the aquatic organisms, particularly fishes which are the main source of protein in this area of Egypt. Antibiogram of the different strains in this study and high resistance of antibiotics agree with other studies [12,13].

Plasmid profiling of the different strains in this study could be an indication that the antibiotics resistance could be plasmid coded particularly β lactamase pathogens which gives these organisms the ability of gene transfer, particularly among enteric microorganisms, such as *E.coli* and *Proteus spp* while this action is hazardous to human health in the communities around the Lake that agree with the study [13] that approved presence of strains with harbored β-Lactamases and plasmid DNA that can be attributed to the stressed water environment of the polluted Lake Manzala.

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