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Detection of Antibiotic-resistant Bacteria in the Raw and Tap Water of the Nile River in Dakahlia Region, Egypt

M. R. Zaki[1], T. E. Farrag[2], A. H. Mohamedin[3] ,W. I. A. Saber[4], M.I. El-Bana[5]

[1]Dakahlia central lab for drinking water, Mansoura, Egypt.
[2]Chemical Engineering Department, Faculty of Engineering, Port Said University, Port Said, Egypt.
[3]Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt.
[4]Microbial Activity Unit, Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center (ID: 60019332), Giza (P.N. 12619), Egypt.
[5]Department of Botany, Faculty of Sciences, Port Said University, Port Said, Egypt.
***Corresponding author**:alfad2004@gmail.com

ABSTRACT

Antibiotic-resistant bacteria have become a major public health issue. It happens naturally, though human and animal overuse of antibiotics has accelerated the process. A drinking water treatment system is specifically intended to remove germs and diseases from water. The current study investigated the presence of antibiotic-resistant bacteria in the Nile River's surface water in Dakahlia's drinking water as well as. The results recorded a total of 39 isolates belong to 14 isolates coliforms, 13 isolates fecal coliforms, and 12 isolates fecal enterococci. Ten antibiotics; amoxicillin, ampicillin, nitrofurantoin, chloramphenicol, amoxicillin-clavulanic acid, levofloxacin, clarithromycin, ciprofloxacin, vancomycin, and sulbactam-ampicillin, were used for the determination of antibiotic resistance profiles of these isolates. The most resistant antibiotics for the Nile waters isolates were amoxicillin (95%), and ampicillin (90%), For tap water, were clarithromycin (85%), amoxicillin (83%), and ampicillin (81%). The MARI values for the Nile raw water were three times greater than the permitted limit (<0.2). The MARI values for the tap water in the majority of the investigated samples ranged from 0.37 to 0.85 and were higher than those of Nile raw. The results indicated a high risk of contamination of drinking water as affected by human activities related to urbanization, accumulation of microbial contamination during various water transfers from source to homes as well as misuse and greater exposure to antibiotics in human treatment, in poultry, and livestock farms and agriculture, which may pose a high ecological risk to the waters and public health. The study suggested adding a phase (activated carbon) inside the water treatment plant and also inside homes to get rid of antibiotic-resistant bacteria.

Keywords:
Bacteria, Resistant, Antibiotic, MARI, Water

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1. INTRODUCTION

Antibiotic resistance is estimated to kill 700,000 people every year. By 2050, if the problem is not addressed. It is expected to result in 10,000,000 deaths every year [1]. Antibiotic resistance occurs naturally over time, usually through genetic changes that is closely related to the widespread use of antibiotics in the treatment of humans and animals. In particular, more than half of the antibiotics used in the United States are given to livestock to promote growth or treat infections. It leads to its spread to humans, animals, food, plants, and the environment (in water, soil, and air). Antibiotics are not always entirely broken down by the body and are frequently excreted in their original form into the environment [2]. There is a growing problem with the excretion of antibiotic residues into the environment including rivers water, which causes pollution and arisen antibiotic resistance in bacteria due to the common use of antibiotics [2,3].

In developing countries, access to safe drinking water remains a major public health concern [4] Water, especially in rivers is hampered by pollution which renders it unusable [5]. Currently, rivers water is highly contaminated with a large number of antibiotic-resistant bacteria, which are transmitted from sewage drainage, especially from hospitals, agricultural drainage, slaughterhouses, clinics, and animal husbandry [6,7]. In Egypt, the concentrated population along the Nile River and delta is mainly dependent on its water for drinking [8]. However, the Nile River receives large amounts of polluted water that flows into the Nile. For example, Omar Bek drain is one of the largest and longest drains in the Gharbia Governorate. Discharged directly to the Damietta branch, which is the main source of water pollution along the Damietta Branch of the Nile and receives about 600,000 m3 (158.503,200 gallons) of untreated household, agricultural, hospital and industrial waste [9].

On the other hand, the design and structure of plants for treatments of drinking and waste water are considered a suitable incubator for the growth and widespread of resistant bacteria [10]. During the processes of treatments, chlorination initially reduces the total number of bacteria, and may significantly increase the proportions of those resistant to one or more antibiotics and thus facilitate the transfer of resistance to other potentially pathogenic strains [11].

This study aimed to determine antibiotic-resistant total coliform, fecal coliform, and fecal enterococci bacteria in the Nile River and tap water in Mansoura city and its villages.

2. MATERIALS AND METHODS

2.1 Samples collection and analysis: During January – December 2019, the water samples were collected a site from nearby the New Mansoura water treatment plant, and random samples of drinking tap water from Mansoura city along with its villages. All water samples were carried out within 3-4 h of collection by using 500 ml sterile screw-capped borosilicate bottles containing 1 mL of 10 % sodium thiosulfate to neutralize free residual chlorine.

One hundred milliliters of water samples were vacuum-filtered through 0.45 µm pore size cellulose nitrate-gridded membranes (Sartorius, Germany). The samples were then aseptically inoculated to the following media: m. Endo agar, MFC agar, and m-Enterococcus agar (Merck, Germany) to detect total coliform, fecal coliform, and fecal enterococcus bacteria, respectively. The plates were incubated for 24 hours at 35 ± 2°C and 44.5 ± 0.5°C for total coliform, and fecal coliform, respectively. For fecal enterococcus the plates were incubated for 48 hours at 35±2°C, according to the Standard Methods for Examination of Water and Wastewater [12]. All colonies that give positive were isolated and purified on tryptic soy agar (Merck, Germany). Colonies were then streaked onto tryptic soy agar slants and all colonies had been stored.

2.2 Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing was carried out for all isolates by the disc diffusion method [2] on Mueller Hinton agar (Merck, Germany). The bacterial suspension of each bacterial isolate was prepared using sterile saline and compared according to the 0.5 McFarland turbidity standard [13]. Then, the inoculated and streaked on the surface of Mueller Hinton agar. The plates were allowed to dry for about 10 minutes, then deposited the antibiotic discs were gently on their surfaces. Ten widely used antibiotics (Sigma, USA), in medical and veterinary practices, named amoxicillin (AMX, 25 mg/ml), ampicillin (AM, 25 mg/ml), nitrofurantoin (FT, 30 mg/ml), chloramphenicol (C, 30 mg/ml), amoxicillin + clavulanic acid (AMC, 30 mg/ml), levofloxacin (LEV, 5 mg/ml), clarithromycin (CLR, 25 mg/ml), ciprofloxacin (CIP, 5 mg/ml), vancomycin (VA, 10mg/ml) and sulbactam + ampicillin (SA, 20 mg/ml) were used for testing the susceptibility of the isolated strains. Plates were incubated at 35 ± 0.5 °C for 24 ± 2 h. The zone of inhibition for each antibiotic was measured in millimeters with a clean ruler from the center of the antibiotic disc to the edge of the area with zero growth at the end of the 24-h incubation period. The antibiotic resistance was accessed with the Kirby Bauer chart, and the antibiotic-resistant isolates were recorded.

2.3 Multiple antibiotic-resistant phenotypes (MARPs): Multiple antibiotic-resistant phenotypes (MARPs) for each sampling location were then generated for isolates that showed resistance to three or more antibiotic used (Tandra and Sudha 2014) [14]. Resistance pattern, number of antimicrobials resistant to isolates, frequencies and percentages were obtained from the antimicrobial susceptibility test result. The antibiotic resistant index (ARI) for each sampling site was also determined using the formula described by Wose et al. (2010) [15]. It was determined using the formula: **ARI = A/N(Y)** where "**A**" is the total number of resistant isoletes recorded in a population of size "**N**" (number of total isolates) for the specified location and Y is the total number of antibiotics tested in the sensitivity test. The multiple antibiotic resistance index (MARI) for each sampled location was equally derived using the mathematical expression by Al-Badaii and Abdul Halim 2021 [4], as follows **MAR index = a/n(b).** Where "**a**" represented the average number of antibiotics resistant for each site, "**b**" is the number of antibiotics tested, and "n" is the number of isolates in each the site.

3. RESULTS

Detection of antibiotic-resistant bacteria in the raw water of the Nile River in Dakahlia region.

39 isolates were obtained from 7 samples collected from Nile River water. These isolates belong to coliforms (14 isolates) fecal coliforms (13), and fecal enterococci (12). The number of antibiotic-resistant bacteria were ranged from the minimal resistant for levofloxacin (12 isolates, 31%) to the highest resistant for Amoxicillin (37 isolates, 95%) (Table 1). The others resistant bacterial isolates in descending order were ampicillin (35 isolates, 90%), chloramphenicol and nitrofurantoin (31 isolates for each, 79%), amoxicillin-clavulanic acid (25 isolates, 64%), ciprofloxacin (23 isolates, 59%), sulbactam-ampicillin (15 isolates, 38%), and vancomycin (14 isolates, 36%). The MAR percentages of total coliform, fecal coliform and fecal enterococci were 59%, 64%, and 71%, respectively (Table 1).

Table (1): Incidence of antibiotic resistance among bacteria isolated from Nile River raw water.

	LEV	CIP	AMC	C	FT	AMX	AM	VA	SA	CLR	Total	MAR%
No. isolate resist	12	23	25	31	31	37	35	14	15	28	251	
Isolate resist	31%	59%	64%	79%	79%	95%	90%	36%	38%	72%		64.4%
Total coliform	2	5	9	11	10	13	13	6	4	10	366	59%
Fecal coliform	5	8	8	10	10	12	11	4	6	9	52	64%
Fecal enterococci	5	10	8	10	11	12	11	4	5	9	60	71%

LEV, levofloxacin; **CIP**, ciprofloxacin; **AMC**, amoxicillin-clavulanic acid; **C**, chloramphenicol; **FT**, nitrofurantoin; **AMX**, Amoxicillin, **AM**, ampicillin; **V**, vancomycin; **SA**, sulbactam-ampicillin; **CLR**, clarithromycin

Compilation of the MAR phenotypes of raw Nile water showed that out of the total 39 bacterial isolates, 36 isolates (92.3%) were MAR that was resistant to more than three antibiotics. However; the low resistant isolates were 3 (7.7%) resistant to three or fewer antibiotics, and susceptible were zero (0%) isolates sensitive to all antibiotics. The ARI values of the studied months ranged from 0.083 in May, November, and December to 0.1 in January, March, July, and September. MARI on the other hand, fluctuated from 0.57 on May to 0.77 on December for the same year (Table 2; Fig 2).

Table (2): Multiple antibiotic-resistant phenotypes (MARPs) of raw water

Sampling date (2019)	Total no. of Isolates	Susceptible	Low resistant	MAR	(ARI)	No. of antibiotic-resist for all isolate	MARI
January	5	0	0	5	0.1	35	0.7
March	5	0	0	5	0.1	29	0.58
May	6	0	1	5	0.083	34	0.57
July	6	0	0	6	0.1	37	0.62
September	5	0	0	5	0.1	34	0.68
November	6	0	1	5	0.083	40	0.67
December	6	0	1	5	0.083	46	0.77
	39	0	3	36			
		0%	7.7%	92.3%			

NO. MAR: No. of Multiple Antibiotic Resistant isolate – **ARI:** Antibiotic Resistant index – **MARI:** Multiple Antibiotic Resistant index

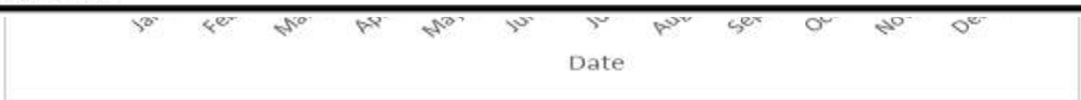

Figure 1: Multiple Antibiotic Resistant index for Raw water

Detection of antibiotic-resistant bacteria in the tap water in Dakahlia region, Egypt.

The tested 1776 tap water samples for bacterial contamination showed that 29 samples gave positive results with a total of 80 isolates, which belong to total coliforms (63), fecal coliforms (8), and fecal enterococci (9) (Tables 3 and 4). The MAR percentage for all isolates was 60% (Table 3). However, The MAR percentages were 63% for total coliform, and 70% for each fecal coliform, and fecal enterococci (Table 3). The highest number of antibiotic-resistant isolates was for clarithromycin (68 isolates 85%), followed by amoxicillin (66 isolates, 83%), ampicillin (65 isolates, 81%), amoxicillin-clavulanic acid (49 isolates, 61%). sulbactam-ampicillin (9 isolates, 49%), vancomycin (37 isolates, 46%), and levofloxacin (24 isolates, 30%) (Table 3).

Table (3): Incidence of antibiotic resistance among bacteria isolated from tap water.

	LEV	CIP	AMC	C	FT	AMX	AM	VA	SA	CLR		MAR%
No. isolate resistant	24	51	62	49	54	66	65	37	39	68	**478**	
Isolate resistant	30%	64%	78%	61%	68%	83%	81%	46%	49%	85%		60%
Total coliform	18	38	48	38	39	50	50	30	30	55	366	63%
Fecal coliform	3	5	7	5	7	8	7	4	5	5	52	70%
Fecal enterococci	3	8	7	6	8	8	8	3	4	8	60	70%

LEV, levofloxacin; **CIP**, ciprofloxacin; **AMC,** amoxicillin-clavulanic acid; **C**, chloramphenicol; **FT**, nitrofurantoin; **AMX**, Amoxicillin, **AM**, ampicillin; **V**, vancomycin; **SA**, sulbactam-ampicillin; **CLR**, clarithromycin

Table (4): Bacterial counts of total coliform, fecal coliform and fecal streptococci, isolated from different sites at Mansoura city and nearby villages.

	Site	Site code	Total Coliform	Fecal Coliform	fecal Streptococci	Total
Mansoura city	Gdela	R1	6	2	1	9
	El-Obour	R2	4	2	-	6
	El-Salam	R3	4	-	2	6
	Kafer Elbadamas	R4	4	1	-	5
	El- Teraa	R5	5	-	-	5
	Sandoub	R6	1	1	-	2
Mansoura village	Meet Khamis	R7	1	2	-	3
	Elbaqlia	R8	8	-	1	9
	Belgai	R9	5	-	-	5
	Gemizate Belgai	R10	4	-	2	6
	Meniat Sandoub	R11	3	-	-	3
	Shawa	R12	1	-	2	3
	Salant	R13	5	-	-	5
	Barq Elezz	R14	2	-	-	2
	Salamon Elqomash	R15	5	-	1	6
	Kafer Elamshoty	R16	2	-	-	2
	El- Danabiq	R17	2	-	-	2
	Meet Khairon	R18	1	-	-	1
	Total		63	8	9	80

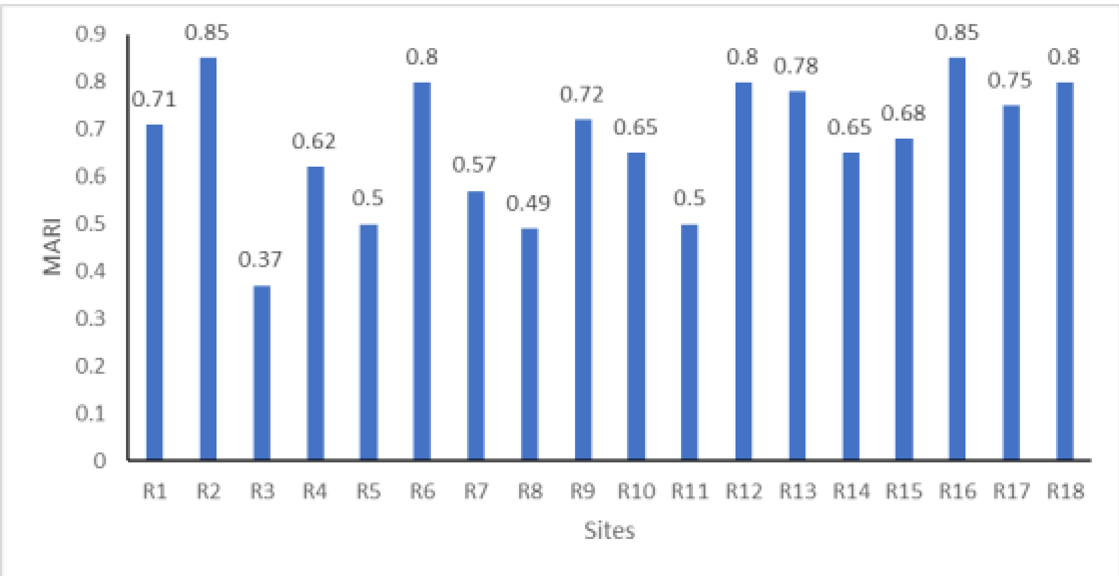

Figure 2: Multiple Antibiotic Resistant index for tap water

Table 5: Multiple antibiotic-resistant phenotypes (MARPs) of tap water

Site code	Total no. of Isolates	Susceptible	Low resistant	MAR	(ARI)	No. of antibiotic-resist for all isolate	MARI
R1	9	0	1	8	0.089	64	0.71
R2	6	0	0	6	0.100	51	0.85
R3	6	0	2	4	0.067	37	0.37
R4	5	0	0	5	0.10	35	0.62
R5	5	0	2	3	0.060	25	0.5
R6	2	0	0	2	0.100	16	0.8
R7	3	0	0	3	0.100	17	0.57
R8	9	1	4	4	0.044	39	0.49
R9	5	0	0	5	0.100	36	0.72
R10	6	0	3	3	0.050	39	0.65
R11	3	0	1	2	0.067	15	0.5
R12	3	0	0	3	0.100	24	0.8
R13	5	0	0	5	0.100	39	0.78
R14	2	0	0	2	0.100	13	0.65
R15	6	1	1	4	0.067	34	0.68
R16	2	0	0	2	0.100	17	0.85
R17	2	0	0	2	0.100	15	0.75
R18	1	0	0	1	0.100	8	0.8
Total	80	2	14	64			
		2.5%	17.5%	80%			

NO. MAR: No. of Multiple Antibiotic Resistant isolate – **ARI:** Antibiotic Resistant index – **MARI:** Multiple Antibiotic Resistant index

Data analysis of MAR phenotype isolated bacteria revealed that approximately 80% of all isolates in this study were resistant to 4-10 antibiotics., followed by 17.5% resistant to 1-3 antibiotics, and 2.5% sensitive to all antibiotics. The ARI values of all months ranged from the least 0.044 to the highest 0.1 (Table 5; Fig 2).

4. Discussion

Contaminated drinking water is a source of outbreaks of many waterborne gastro-intestinal diseases [18-19]. Consumption of drinking water contaminated with antibiotic-resistant bacteria is a major public health concern, and also contributes to its continued spread in the environment [20]. The prevalence of antimicrobial resistance has increased during the recent decades and may be due to selection pressure caused by the indiscriminate use and misuse of antimicrobials [21]

The current study detected a wide presence of antibiotic-resistant bacteria at the Damietta branch of River Nile, in front of the new Mansoura water station. The relatively high level of resistance to antimicrobial agents is a consequence of the misuse of antibiotics in the surrounding environment [17,22]. The antibiotic resistance patterns of total coliform, fecal coliform, and fecal enterococci isolates showed significant resistance in different proportions to the ten examined antibiotics. The increasing resistance to these antibiotics is believed to be due to the fact that it is an ancient antibiotic that was discovered and used extensively in agricultural, industrial, veterinary, and hospital uses [23]. Antibiotic resistance rates can be detected in bacteria isolates from Nile water samples in the Rosetta branch as a result of receiving a large load of liquid sewage [24]. [1,19] reported that surface water is contaminated mainly from livestock operations and human wastewater, and reduced livestock access to surface water reduced fecal coliform levels by an average of 94%. [25] recorded a high resistant pattern among different species of bacteria in Lake El-Manzala which is highly contaminated from different sources such as sewage, and industrial and agricultural wastes. [26] suggested that antibiotic resistance may be due to the deterioration of water quality in the Semenyih River due to agricultural and industrial activities, domestic sewage, intensive, livestock farms, untreated sewage from rural areas, and urban runoff. The results showed the existence of high antibiotic-resistant isolate bacteria in the examined tap water than that isolated from raw water, indicating the re-growth of bacteria in drinking water distribution systems, or/and presence of a leak in the distribution pipe system that was reported by [16]. They identified twenty-six bacterial isolates from the tap water of El-Baqlia village and concluded that water distribution systems can act as an incubator for the growth of antibiotic-resistant bacteria. Such distribution water system gives enough time to acquire the free broken free gene circulating in drinking water because the bacterial genes are not effectively removed by the filtration system, and the presence of biofilms in the distribution lines that are periodically released free due to the selective pressure of chlorination in drinking water [27]. It facilitates the spread of opportunistic bacteria that are resistant to antibiotics in drinking water [28]. [20], [29] and [10], reported the presence of 16s rRNA in all tap water due to chlorination kills most bacteria and promotes the release of free DNA into the water, developing resistance by spontaneous mutation or obtaining resistance genes from the environment by acquiring the resistance gene through horizontal transfer. [6] reported the presence of several antibiotic-resistant bacteria in raw water as *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas, Enterococcus, Staphylococcus*, and *Bacillus spp*. However, the bacteria were completely removed in the treated water and tap water due to the efficiency of the water treatment plant in removing bacteria, but 16S rRNA was detected in raw water, treated water and tap water due to the inefficiency of the traditional filtration method in removing the small fragment of bacterial DNA.

The MAR index in all the Nile River raw water samples through the year of the study was found to be higher than the limit (0.2) by three or four times especially for months 1/2019, and 12, 2019. This indicates high-risk contamination of surface water could be due to the arrival of huge quantities of household, agricultural, veterinary, and hospital waste into the Nile River waters near the study area by the Omar Bek drain and the low level of water in the Nile during this period of the year. [9,16,17] reported that Omar Bek drain near the new Mansoura water treatment plant is considered one of the largest sources of pollution affecting the Damietta branch of the Nile water, which carries many of the domestic, agricultural and industrial waste. [18] attributed the high average of MAR index of Rosetta branch is (0.55 to 0.65) for all of their studied isolates and all sites due to the received heavy contamination from discharge waste drains. [30] reported a gradual increase in the incidence of antibiotic-resistant bacteria from Lake Nasser in the south to Rosetta branch at the north of Nile River. [15] recorded (MAR) index greater than 0.2 for bacterial isolates from the Lake Lanao surface water, the Philippines, and attributed that for its higher contamination than that of commercial poultry farms, cattle, and pigs where antibiotics are often used for infection treatments. [31,32] reported a high MAR index observed for all samples indicating that there has been an indiscriminate use of these antibiotics in the area for management of bacterial infections. The results indicated that the MAR index of all sampled tap water was higher than that of the Nile River samples. All MARI values above 0.5 indicated the high contamination of tap water by multiple-antibiotic resistant bacteria [33].

Maybe due to the high effect of chlorine which accelerates gene exchange in or between bacterial genera, so that the chlorine-injured opportunistic pathogens can be transferred from non-antibiotic resistant bacteria to antibiotic-resistant bacteria through a natural transformation during chlorination, which poses a potential risk to disseminate antibiotic resistance in the water[34,35] also, antibiotic-resistant bacteria survived longer than antibiotic-sensitive organisms when exposed to free chlorine in a contacttime[14,36,37]. Chlorine was also a much more powerful oxidizing agent than any antibiotic so chlorine-tolerant bacteria were more resistant to any antibiotic which increasing MARI. [11] discovered more bacteria in tap water systems in regions where chlorine may be less effective. [38] Chlorine and chlorine byproducts have been shown to promote horizontal antibiotic resistance gene transfer and multidrug resistance in bacteria in the environment. [2,39,40] antibiotic-resistant E. coli had a higher frequency of chlorine tolerance than antibiotic-sensitive E. coli cultured in the presence of chlorine. [41] noticed bacterial populations in drinking water exhibit differential resistance to monochloramine. [42] observed that residual chlorine in a drinking distribution system after long-distance distribution or at the point of tap water use frequently drops to sub-inhibitory or even undetectable levels, resulting in an increase in antibiotic-resistant bacteria and antibiotic-resistance genes in sterile drinking water. [43] While chlorination may initially reduce the total number of bacteria in wastewater, it has been hypothesized that it may considerably increase the proportions of pathogenic and antibiotic-resistant species.

5. CONCLUSION

Evaluation of the Nile River, as a source of water for the new Mansoura water treatment plant, in terms of existence of bacterial pathogens and their antibiotic-resistant to selected antibiotics was done. Tap water, supplied Mansoura city and its villages, showed contamination with antibiotic-resistant pathogens which represented a huge problem to human health. The majority of the isolates retrieved from the Nile River water contained MAR and MARI, which was three time higher than the normal index, due to contamination of the Nile water by the Omar-Bek Drain which carries many households, and polluted water from hospitals, agricultural and veterinary pollutants. Also, an evaluation of tap water in Mansoura city and its villages found that all isolates are resistant to antibiotics and that MARI is much higher than Nile water as well. Therefore, instead of discharging Omer-Bek drain water into the Nile and diverting the flow, anther option must be considered. It's also suggested that an additional stage (activated carbon) be added to the new Mansoura water treatment facility to remove antibiotic- resistant bacteria and damaged DNA, as well as the usage of activated carbon filters in homes to remove bacteria and DNA from tap water.

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