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Antimicrobial Resistance Profiles of Enterobacteriaceae Isolated from Rosetta Branch of River Nile, Egypt

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Abstract: This study deals with antibiotic resistance profiles in Enterobacteriaceae family isolates from River Nile at Rosetta branch. A total of 293 enteric bacterial isolates could be recovered from fifteen sites of Rosetta branch (5 drains outfalls, 130 isolates and 10 along Rosetta branch, 163 isolates). Among the isolates, 187 (63.8%) are *Escherichia coli,* 59 (20.1%) *Proteus vulgaris,* 25 (8.5%) *Salmonella typhi* and 22 (7.5%) *Citrobacter freundii*. Twenty antibiotics; Amoxycillin/Clavulanic acid, Ampicillin, Carbenicillin, Methicillin, Piperacillin, Cephalothin, Cefotaxime, Ceftriaxone, Vancomycin, Amikacin, Tobramycin, Kanamycin, Tetracycline, Erythromycin, Clindamycin, Norfloxacin, Ofloxacin, Trimethoprim/Sulfamethoxazole, Nitrofurantoin and Chloramphenicol were used for determination of antibiotic resistance profiles of the isolates. All Enterobacteriaceae isolates exhibited 100% resistance to Ampicillin, Carbenicillin, Methicillin, Vancomycin, Erythromycin, Clindamycin, Trimethoprim/Sulfamethoxazole and Tetracycline. Also, they failed to exhibit resistance to Norfloxacin and Ofloxacin. However, multiple antibiotic resistance (MAR) in enteric bacterial isolates from all the sites could be detected which is possibly due to sewage discharge and input from other anthropogenic sources along the branch.

Key words: Antibiotic resistance profiles · Enterobacteriaceae · River Nile · Rosetta branch · Pollution · Egypt

INTRODUCTION

In Egypt, River Nile is the main source of drinking water, irrigation and industry. Egypt's annual quota of the Nile water is 55.5 billion cubic meters; River Nile covers 96% Egypt's fresh water demand [1]. Unfortunately, in spite of its vital role, River Nile receives a variety of wastes coming from sanitary drainage (sewage), industrial discharges from factories located on its shores as well as agrochemicals (fertilizers, herbicides and pesticides) coming along with soil runoff. River Nile, the primary source of fresh water in Egypt, is also the primary receptor of wastewater and drainage generated by different activities [2]. Drinking water must meet specific criteria and standards to ensure that water supplied to the public is safe and free-from pathogenic microorganisms as well as hazardous compounds [3]. Indeed the microbial quality of potable water should not exceed the limits specified in the water quality guideline [4]. Enteric bacterial pathogens have been reported to thrive for long periods in water in spite of a large number of antagonistic populations [5]. These pathogens are variously incriminated in cases of diarrhea which in turn accounts for a substantial degree of morbidity and mortality in different age groups worldwide [6,7]. Rosetta branch, the concern of the present study, receives heavy microbial load from agriculture, industrial and domestic wastewater as well as anthropogenic activities [8]. Isolation of these pathogens from water sources which are considered a serious public health risk to consumers was conducted. This risk is expected by the widely reported cases of resistance of enteric bacterial pathogens to several antibiotics [9].

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Before the abusive antimicrobial use age, only a slight resistance level had been detected among enteric bacterial pathogens. Nowadays, their susceptibility to antimicrobials has changed and resistant patterns have been used as epidemiologic markers [10]. Overuse and sometimes misuse of antibiotics in human and veterinary medicine are major promoters for the development and spread of multi-resistant bacteria worldwide [11, 12]. Liquid manure of animals and human excretions has led to dissemination of resistant enteric bacteria in the environment [13].

The present study was designed to generate baseline data on antibiotic resistance profiles in Enterobacteriaceae family isolates from River Nile at Rosetta branch. With this purpose, water samples collection took place at fifteen different points along Rosetta branch of River Nile, Egypt. Enteric bacteria were isolated from these samples and their antimicrobial resistance patterns were determined.

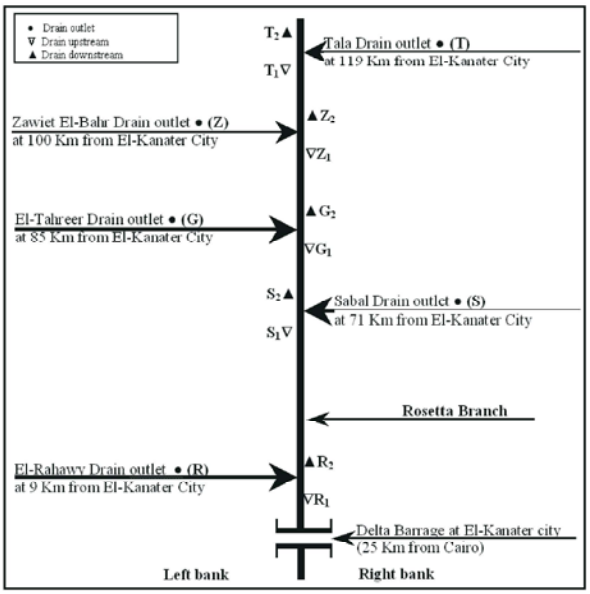


Fig. 1: Schematic diagram for water sampling sites.

MATERIALS AND METHODS

Sampling Sites: For conducting this study, Rosetta branch was subdivided into five reaches for sampling, which were carefully chosen based on locations of known waste inputs. Totally fifteen sites were chosen, three from each reach: five drain outfalls (El-Rahway, Sabal, El-Tahreer, Zawiet El-Bahr and Tala) and ten site at Rosetta Branch (five upstream and five downstream those drains) as illustrated in Fig. 1.

Samples Collection: Sampling was carried out in two seasons (summer, 2010 and winter, 2011) from Rosetta Nile branch and five drains outlets located on its sides. Water sampling was carried out according to Standard Methods for Examination of Water and Wastewater [14]. The water samples were collected from the subsurface layer (at depth 50 cm) of the midstream of the branch in sterilized 1 litter stopper polyethylene plastic bottles. These collected samples were stored in an iced cooler box and delivered immediately to the laboratory for analyses.

Isolation and Identification of *Escherichia coli*: The membrane filtration method was carried out according to American Public Health Association (APHA) [14]. In this procedure, water samples were filtered through sterile, white, grid-marked, 47 mm diameter membrane (pore size, 0.45µm), which retained bacteria. After filtration, the membrane containing bacteria was spread on M-FC agar medium (Difco, U.S.A) and incubated at 44.5°C/24hrs. After incubation, Colonies developing various shades of blue were picked up for identification. *E. coli* identification was carried out according to Pettibone [15] by using multiple tube fermentation technique in which Lauryl tryptose broth with MUG (4-methyl umbelliferyl-â-D-glucuronide) medium (Difco, U.S.A) was used in test tubes containing inverted fermentation vials. The tubes were inoculated with appropriate colonies and incubated at 44.5°C/24hrs. Tubes exhibiting gas formation with growth is considered a positive indication of *E. coli*, from which several loopfulls were streaked onto MacConkey agar (Difco, USA) plates for testing lactose fermentation ability and also onto Eosin Methylene Blue (EMB) agar medium (Difco, USA) and the plates were incubated at 35°C/24hrs. Further identification of isolates was based on Gram stain and biochemical tests (IMVIC tests) according to Collins and Lyne [16] and Cheesbrough [17]. Separately isolated pure colonies were picked up and further confirmed using the API 20E assay (bioMérieux, France) according to Juang and Morgan [18].

Isolation and Identification of *Salmonella* and other Enteric Bacteria: The membrane filtration method was carried out according to American Public Health Association (APHA) [14]. In this procedure, the concentrated sample (approximately 500-1000 ml) was enriched in a nutrient medium. This was carried out by immersing the membrane used in the concentration technique in a test tube containing 25 ml tetrathionate broth supplemented with iodine solution (6 g iodine and

5 g potassium iodide in 20 ml dis. H_2O) [14]. The tube containing the membrane filter was thoroughly mixed and incubated at 35°C for 5 days with repeated streaking from the same tube several times daily on selective growth medium. This was performed using well dried Bismuth sulfite agar (Difco, USA) plates incubated at 35°C/24hrs: Typical colonies of *Salmonella typhi* usually develop a black color with or without metallic sheen. Gram-negative enteric bacteria other than *Salmonella* produce different colors [14]. All developed colonies were picked up and further streaked on: (1) Xylose Lysine Desoxycholate (XLD) agar medium (Lab M, UK): Suspected *S. typhi* produce black centred red colonies. (2) Triple sugar iron (TSI) agar medium (Lab M, UK): colonies of *S. typhi* usually develop red slant and yellow butt with slight H_2S production [14]. Furthermore, the different developed colonies on bismuth sulfite agar were picked up and identified by using the API 20E assay (bioMérieux, France). Complete identification of Enterobacteriaceae was achieved by use of the tests in (Bergey's Manual of Systematic Bacteriology) [19].

Antimicrobial Antibiotics Susceptibility Testing: The standard Kirby-Bauer disk diffusion method [20] was used to determine antimicrobial sensitivity profiles of tested bacterial isolates for 20 antimicrobial antibiotics: Amoxycillin/Clavulanic acid (30µg), Ampicillin (10µg), Carbenicillin (100 µg), Methicillin (5µg), Piperacillin (75µg), Cephalothin (30), Cefotaxime (30µg), Ceftriaxone (30µg), Vancomycin (30µg), Amikacin (30µg), Tobramycin (10µg), Kanamycin (30µg), Tetracycline (30µg), Erythromycin (10µg), Clindamycin (30µg), Norfloxacin (10µg), Ofloxacin (10µg), Trimethoprim/Sulfamethoxazole (25µg), Nitrofurantoin (300µg) and Chloramphenicol (30µg). The discs were obtained from Oxoid, UK. Four to five similar colonies from overnight growth plate were transferred aseptically in saline solution and vigorously agitated to give a density of 0.5 McFarland turbidity standards (approximately 10^8 CFU/ml). Within 15 minutes, sterile cotton swab dipped into the culture suspension was used for inoculating the surface of solidified Mueller-Hinton agar (Oxoid, UK) plates. Then, antibiotic discs were placed 30 mm apart and 10 mm from the edge of the plate. Plates were incubated at 37°C for 18-20 hrs. The resulted diameters of inhibition zones around the antibiotic discs were measured to nearest whole mm and interpreted according to protocols standardized for the assay of antibiotic compounds as guided by National Committee for Clinical Laboratory Standards "NCCLS". The results were categorized as: R (resistant), I (intermediate sensitive) and S (sensitive) [21].

Multiple Antibiotic Resistance (MAR) Indexing: The MAR index was performed to evaluate the health risk of the environments. Multiple antibiotic resistance index (MAR) (number of antibiotics to which test isolate displayed resistance divided by total number of antibiotic to which the test organism has been evaluated for sensitivity) for each test isolate was calculated as recommended by Krumperman [22].

RESULTS

Isolation of Enterobacteriaceae: A total of 293 enteric bacterial isolates were recovered from water samples collected during summer, 2010 and winter, 2011 from five drains outfalls (130 isolates) and ten sites along Rosetta branch (163 isolates). These isolates belong to four genera and included 187 isolates of *E. coli*, 22 of *Citrobacter freundii*, 59 of *Proteus vulgaris* and 25 of *Salmonella typhi. E. coli, C. freundii* and *P. vulgaris* were recovered from drains and Rosetta branch with percentages (100%, 100% and 33.3 % of sampling sites), respectively. *S. typhi*, the causative agent of typhoid fever, was obtained only from drains and this could be attributed to contamination of these drains by feces of infected humans or animals and especially from poultry farms (Tables 1 and 2).

Table 1: Total number and percentages of Enterobacteriaceae isolates from drains outlets and Rosetta branch.

	Summer				Winter			
	Drains outlets		Rosetta branch		Drains outlets		Rosetta branch	
Enteric bacterial groups	No.	%	No.	%	No.	%	No.	%
Escherichia coli	33	23.7	56	40.4	38	24.7	60	39
Citrobacter freundii	3	2.2	6	4.3	7	4.5	6	3.9
Proteus vulgaris	12	8.6	17	12.2	12	7.8	18	11.7
Salmonella typhi	12	8.6	ND	ND	13	8.4	ND	ND
Total	60	43.1	79	56.9	70	45.4	84	54.6
		139 (47.4%)				154 (52.6%)		

ND: not detected

Table 2: Enterobacteriaceae isolates from various water sampling sites

	Enteric bacteria isolated and number of strains										
	E. coli		*C. freundii*		*P. vulgaris*		*S. typhi*[a]		MAR index[b]		
Sampling sites	S	W	S	W	S	W	S	W	S	W	AV[c]
R_1	5	6	-	2	2	2	-	-	0.65	0.6	0.62
R	9	8	-	-	3	4	4	2	0.55	0.58	0.56
R_2	8	8	-	-	2	3	-	-	0.7	0.7	0.7
S_1	4	6	-	-	2	1	-	-	0.6	0.7	0.65
S	8	8	-	-	3	1	1	8	0.7	0.47	0.58
S_2	4	8	-	-	2	1	-	-	0.59	0.6	0.59
G_1	5	6	2	-	1	1	-	-	0.6	0.75	0.67
G	6	7	-	5	1	2	2	1	0.6	0.63	0.61
G_2	9	7	2	2	1	1	-	-	0.6	0.68	0.64
Z_1	6	5	-	2	3	3	-	-	0.7	0.62	0.66
Z	5	7	3	2	2	1	-	1	0.6	0.76	0.68
Z_2	7	3	-	-	1	1	-	-	0.6	0.9	0.75
T_1	2	5	2	-	1	2	-	-	0.6	0.73	0.66
T	5	8	-	-	3	4	5	1	0.6	0.68	0.64
T_2	6	6	-	-	2	3	-	-	0.8	0.63	0.71
Total	89	98	9	13	29	30	12	13	0.63	0.66	0.65

[a] *E. coli, Escherichia coli*; *C. freundii, Citrobacter freundii*; *P. vulgaris, Proteus vulgaris* and *S. typhi*, *Salmonella typhi.* [b] MAR index: multiple antibiotic resistance index. [c] S, summer; W, winter and AV, Average.

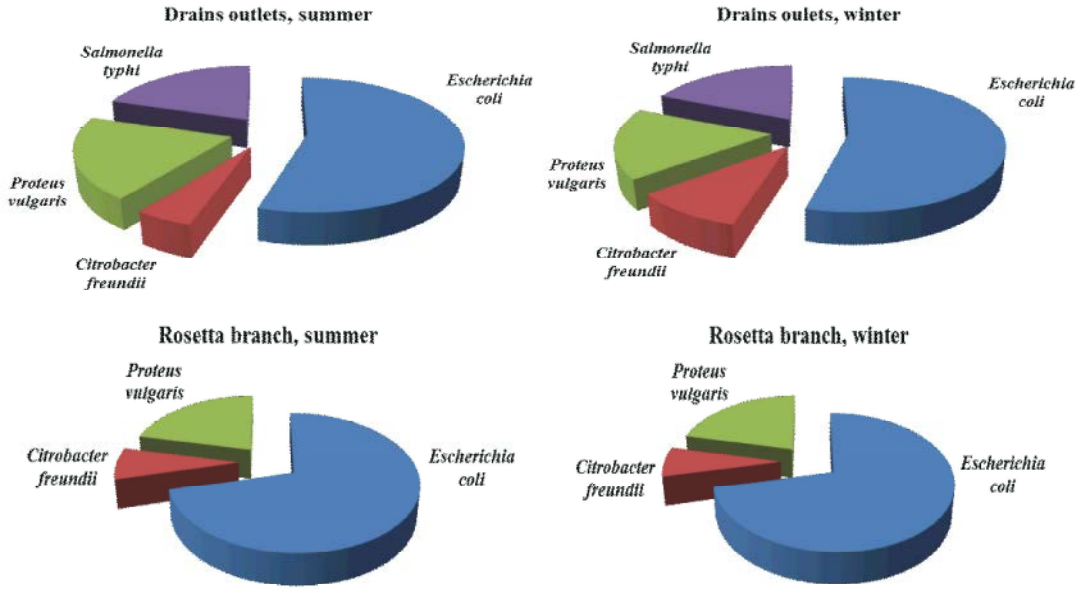


Fig. 2: % of Enterobacteriaceae isolates in drains outlets and Rosetta branch.

In summer, *E. coli* is the predominant species recovered, constituting 64.1% of all isolates. The second most common bacterium is *P. vulgaris*, 20.8% followed by *S. typhi*, 8.6% and *C. freundii* 6.5%. On the other hand, enteric bacterial isolates obtained in winter revealed closely related percentages to that of summer season, the predominant species is *E. coli* followed by *P. vulgaris, S. typhi* and finally *C. freundii* (63.7, 11.7, 8.4 and 8.4 respectively) (Fig. 2). It is worth to mention that, higher levels of enteric bacterial isolates could be obtained in winter compared to summer.

Table 3: Antibiotic resistance profile of Enterobacteriaceae isolates from five drains outlets

Resistance of bacteria	Resistance *E. coli*		*C. freundii*		*P. vulgaris*		*S. typhi*	
Antibiotics	S(n=33)	W(n=38)	S(n=3)	W(n=7)	S(n=12)	W(n=12)	S(n=12)	W(n=13)
Amoxycillin/Clavulanic acid	0	18.4	0	0	100	100	8.3	7.7
Ampicillin	100	100	100	100	100	100	100	100
Carbenicillin	100	100	100	100	100	100	100	38.5
Methicillin	100	100	100	100	100	100	100	100
Piperacillin	15.2	39.5	0	71.4	50	58.3	25	7.7
Cephalothin	24.2	18.4	100	100	100	100	41.7	100
Cefotaxime	18.2	0	0	0	50	58.3	0	15.4
Ceftriaxone	18.2	0	0	71.4	50	58.3	0	7.7
Amikacin	48.5	0	0	0	100	100	0	15.4
Tobramycin	66.7	0	0	71.4	100	100	33.3	23.1
Kanamycin	15.2	0	100	0	100	100	75	20.8
Vancomycin	100	100	100	100	100	100	100	100
Tetracycline	57.6	39.5	100	100	100	100	100	100
Erythromycin	100	100	100	100	100	100	100	100
Clindamycin	100	100	100	100	100	100	100	100
Norfloxacin	0	18.4	0	0	0	0	100	15.4
Ofloxacin	0	18.4	0	0	0	0	100	15.4
Trimethoprim/Sulfamethoxazole	39.4	18.4	100	100	100	100	100	100
Nitrofurantoin	24.2	0	100	100	100	100	100	100
Chloramphenicol	0	0	100	100	66.7	100	100	100

Table 4: Antibiotic resistance profile of Enterobacteriaceae isolates from Rosetta branch of River Nile, Egypt

Resistance of bacteria	Resistance *E. coli*		*C. freundii*		*P. vulgaris*	
Antibiotics	S(n=56)	W(n=60)	S(n=6)	W(n=6)	S(n=17)	W(n=18)
Amoxycillin/Clavulanic acid	10.7	21.6	0	33.3	100	100
Ampicillin	100	100	100	100	100	100
Carbenicillin	100	100	100	100	100	100
Methicillin	100	100	100	100	100	100
Piperacillin	21.4	48.3	0	66.7	23.5	50
Cephalothin	21.4	28.3	100	100	100	100
Cefotaxime	37.5	28.3	0	0	47.1	88.9
Ceftriaxone	37.5	40	0	0	47.1	61.1
Amikacin	0	26.7	0	33.3	100	100
Tobramycin	85.7	45	0	33.3	100	100
Kanamycin	19.6	25	0	33.3	100	100
Vancomycin	100	100	100	100	100	100
Tetracycline	44.6	48.3	0	100	100	100
Erythromycin	100	100	100	100	100	100
Clindamycin	100	100	100	100	100	100
Norfloxacin	0	5	33.3	0	17.6	22.2
Ofloxacin	0	5	66.7	0	23.5	44.4
Trimethoprim/Sulfamethoxazole	24.8	78.3	100	100	100	100
Nitrofurantoin	0	5	100	100	100	100
Chloramphenicol	0	5	100	100	52.9	100

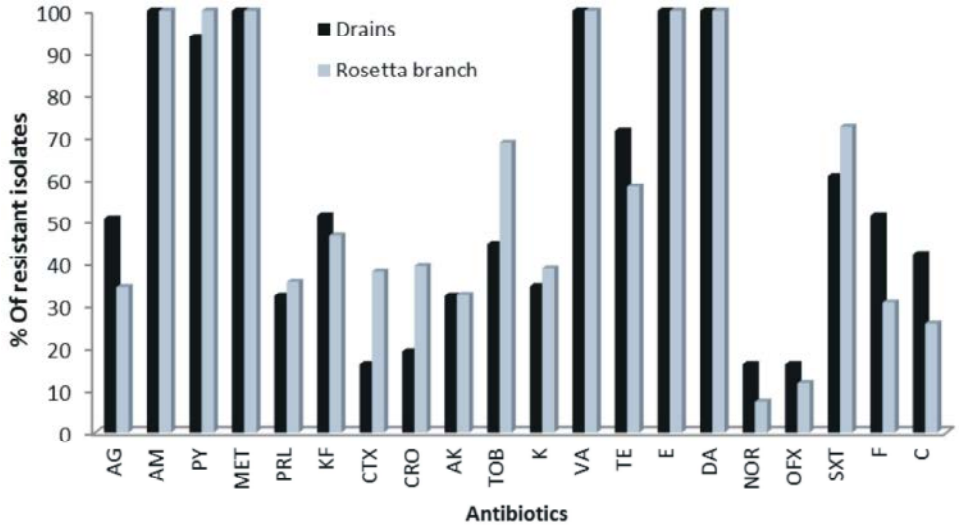


Fig. 3: Frequencies of antibiotic resistance of the Enterobacteriaceae isolates in surface water from River Nile at Rosetta branch and in wastewater from five drains outlets.
AG, Amoxycillin/Clavulanic acid; AM: Ampicillin; PY, Carbenicillin; MET, Methicillin; PRL, Piperacillin; KF, Cephalothin; CTX, Cefotaxime; CRO, Ceftriaxone; AK, Amikacin; TOB, Tobramycin; K, Kanamycin; VA, Vancomycin; TE, Tetracycline; E, Erythromycin; DA, Clindamycin; NOR, Norfloxacin; OFX, Ofloxacin; SXT, Trimethoprim/Sulfamethazole; F, Nitrofurantoin& C, Chloramphenicol.

Antibiotic Resistance: Antibiotic resistance profile results of various isolates (Tables 3 and 4) indicate multiple antibiotic resistance by all tested isolates; all enteric bacterial isolates revealed 100% to at least six antibiotics namely Ampicillin, Carbenicillin, Methicillin Vancomycin, Tetracycline and Clindamycin. Enteric bacterial isolates from drains exhibited resistance higher than that revealed by Rosetta branch isolates. The highest antibiotic resistance could be attained by *P. vulgaris* followed by *C. freundii, S. typhi* and *E. coli. Escherichia coli* isolates from drains and Rosetta branch were 100% resistant to Ampicillin, Carbenicillin, Methicillin, Vancomycin, Erythromycin and Clindamycin. The other antibiotics revealed higher activity being the maximum for Norfloxacin, Ofloxacin and Chloramphenicol. They followed by Nitrofurantoin, Amoxycillin/Clavulanic acid, Piperacillin and Ceftriaxone. Other antibiotics indicate intermediate activity and some isolates are resistant. *E. coli* isolates are resistant to about 77.5 and 82.5% of the tested antibiotics in summer and winter season respectively and the majority of them are multiple antibiotic resistant (MAR).

All *Proteus vulgaris* isolates revealed 100% resistance against fifteen used antibiotics: Amoxycillin/Clavulanic acid, Ampicillin, Carbenicillin, Methicillin, Cephalothin, Amikacin, Tobramycin, Kanamycin, Vancomycin, Tetracycline, Erythromycin, Clindamycin, Trimethoprim/Sulfamethoxazole, Nitrofurantoin and Chloramphenicol. Variable resistance could be recorded against Piperacillin, Cefotaxime and Ceftriaxone. *P. vulgaris* isolates from drains failed to exhibit resistance (0%) against Norfloxacin and Ofloxacin. *P. vulgaris* isolates are resistant to about 95% of the tested antibiotics and majority of isolates are MAR. *Citrobacter freundii* and *Salmonella typhi* revealed 100% resistance against 12 (60%) of tested antibiotics. Other antibiotics exhibited intermediate activity and some isolates are resistant. *C. freundii* isolates are resistant to about 60 and 75% of the tested antibiotics in summer and winter seasons, respectively. *S. typhi* isolates are resistant to about 85 and 100% of the tested antibiotics in summer and winter season, respectively. All tested isolates, except *S. typhi* of summer, failed exhibit resistance to Norfloxacin and Ofloxacin. All enteric bacterial isolates revealed multiple antibiotic resistances (MAR) that may have ecological and public health implications. Frequency of the resistance percentages towards the twenty tested antibiotics is nearly close to each other in drains and Rosetta branch and this refer to the same origin of resistance. Generally, Resistance is highest to Ampicillin, Carbenicillin and Methicillin, Vancomycin, Erythromycin, Clindamycin, Trimethoprim/Sulfamethoxazole and Tetracycline. Whereas, it is least to Norfloxacin and Ofloxacin (Table 5 and Fig. 3).

Table 5: Total number and percentages of enteric bacterial isolates in wastewater from drains and surface water from Rosetta branch of River Nile, Egypt

	Resistant enteric isolates			
	Drains (130 isolates)		Rosetta branch (163 isolates)	
Antibiotics	Total No.	%	Total No.	%
Amoxycillin/Clavulanic acid	66	50.8	56	34.4
Ampicillin	130	100	163	100
Carbenicillin	122	93.8	163	100
Methicillin	130	100	163	100
Piperacillin	42	32.3	58	35.6
Cephalothin	67	51.5	76	46.6
Cefotaxime	21	16.2	62	38
Ceftriaxone	25	19.2	64	39.3
Amikacin	42	32.3	53	32.5
Tobramycin	58	44.6	112	68.7
Kanamycin	45	34.6	63	38.7
Vancomycin	130	100	163	100
Tetracycline	93	71.5	95	58.3
Erythromycin	130	100	163	100
Clindamycin	130	100	163	100
Norfloxacin	21	16.2	12	7.4
Ofloxacin	21	16.2	19	11.7
Trimethoprim/Sulfamethoxazole	79	60.8	118	72.4
Nitrofurantoin	67	51.5	50	30.7
Chloramphenicol	55	42.3	42	25.8

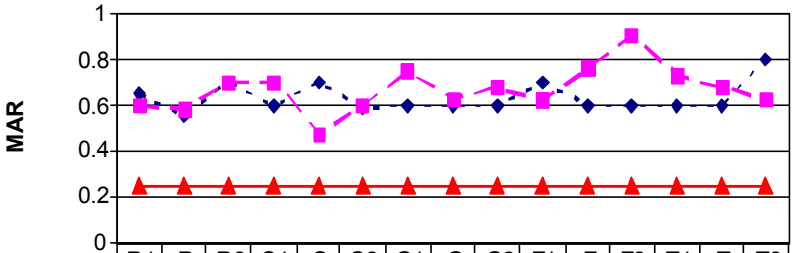
Table 6: Calculation of multiple antibiotic resistant (MAR) index for enteric bacteria isolated from water

	Summer				Winter			
Seasons	No. of Resistances		MAR index		No. of Resistances		MAR index[a]	
Enteric bacterial species	D[b]	R[c]	D	R	D	R	D	R
Escherichia coli	306	516	0.46	0.46	293	606	0.38	0.5
Citrobacter freundii	36	66	0.6	0.55	92	78	0.65	0.65
Proteus vulgaris	194	274	0.8	0.8	201	318	0.83	0.88
Salmonella typhi	166	0	0.69	0	153	0	0.58	0

[a] MAR index, Multiple antibiotic resistance index; [b] D, drains outlets; [c] R, Rosetta branch.

In drains: Resistance is 100% for Ampicillin, Methicillin, Vancomycin, Erythromycin and Clindamycin. This followed by Carbenicillin (93.2%), Tetracycline (71.5%), Trimethoprim/Sulfamethoxazole (60.8%), Cephalothin and Nitrofurantoin (51.5%), Amoxycillin/Clavulanic acid (50.8%), Tobramycin (44.6%), Chloramphenicol (42.3%), Kanamycin (34.6%), Piperacillin and Amikacin (32.3%), Ceftriaxone (19.2%) and finally Cefotaxime, Norfloxacin and Ofloxacin (16.2%). On the other hand in Rosetta branch: Resistance is 100% for Ampicillin, Carbenicillin and Methicillin, Vancomycin, Erythromycin and Clindamycin. This followed by Trimethoprim/Sulfamethoxazole (72.4%), Tobramycin (68.7%), Tetracycline (58.3%), Cephalothin (46.6%), Ceftriaxone (39.3%), Kanamycin (38.7%), Cefotaxime (38%), Piperacillin (35.6%), Amoxycillin/Clavulanic acid (34.4%), Amikacin (32.8%), Nitrofurantoin (30.7%), Chloramphenicol (25.8%), Ofloxacin (11.7%) and finally the least resistance was also recorded against Norfloxacin (7.4%).

Multiple Antibiotic Resistance (MAR) Indexing: This study was conducted to give highlight on the applicability of the multiple antibiotic resistance (MAR) index with the aim to identify the origin of resistance and offering information about the source of enteric bacteria pollution as a very useful tool for water management. MAR values are nearly related to each other regionally and seasonally (Table 6). In summer, calculations of MAR for individual bacterial species revealed that, the most pronounced MAR values in drains and Rosetta branch respectively could be achieved by *P. vulgaris* (0.80, 0.80).



	R1	R	R2	S1	S	S2	G1	G	G2	Z1	Z	Z2	T1	T	T2
summer	0.65	0.55	0.7	0.6	0.7	0.59	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.8
winter	0.6	0.58	0.7	0.7	0.47	0.6	0.75	0.63	0.68	0.62	0.76	0.9	0.73	0.68	0.63
high risk level	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

Fig. 4: Calculated MAR index for all studied sites in summer and winter seasons.
R1, upstream El-Rahawy drain; R, El-Rahawy drain outlet; R2, downstream El-Rahawy drain; S1, upstream Sabal drain; S, Sabal drain outlet; S2, downstream Sabal drain; G1, upstream El-Tahreer drain; G, El-Tahreer drain outlet; G2, downstream El-Tahreer drain; Z1, upstream Zawiet El-Bahr drain; Z, Zawiet El-Bahr drain outlet; Z2, downstream Zawiet El-Bahr drain; T1, upstream Tala drain; T, Tala drain outlet; T2, downstream Tala drain.

This was followed by *S. typhi* (0.69, 0), *C. freundii* (0.60, 0.55) and *E. coli* (0.46, 0.46). On the other hand in winter, the most pronounced MAR values for individual bacterial species in drains and Rosetta branch, respectively cold are recorded by *P. vulgaris* (0.83, 0.88). This was followed by, *C. freundii* (0.65, 0.65), *S. typhi* (0.58, 0) and *E. coli* (0.38, 0.50). To make this conclusion more effective, the MAR index for each sampling points was calculated separately (Table 2 and Fig. 4).

DISCUSSION

Contaminated drinking water is a major source of gastrointestinal microbial pathogens and causes numerous waterborne disease outbreaks. The presence of drug resistant bacteria in surface water is a major public health concern as drug resistant bacteria could be transferred to humans via consumption of contaminated drinking water which then contributes to the spread and persistence of antibiotic resistance bacteria (ARB) in environment [23]. The present study revealed a wide presence of antibiotic resistant bacteria at Rosetta Nile branch which considered as a drinking water source for Delta region. The relatively high level of resistance to antimicrobial agents is a reflection of misuse and abuse of these agents in the environment [24]. The antibiotic resistance patterns of the Enterobacteriaceae isolates exhibited high resistance to eight antibiotics, no resistance to two antibiotics and intermediate resistance to others. The drug resistance pattern suggests that most isolates had multiple drug resistance. According to Krumperman [22] and Florea [25] values of MAR higher than 0.25 pose a high risk source of contamination. Unfortunately, all the calculated values of MAR index were obviously exceeding the high risk level (0.25) with different extents, demonstrating that the area of study is considered a high risk source of contamination environment. The average MAR index is 0.55 and 0.65 for all the isolates and all sites, respectively. This may reflect that, Rosetta branch receives heavy contamination by those five drains. The present findings are in agreement with those reported by Ezzat [9] that identified Rosetta branch and associated drains as high risk contaminated area. Eight years before, Heikal [26] found a gradual increase in the incidence of antibiotic resistant bacteria from Lake Nasser to Rosetta branch. The author also identified Rosetta branch as high risk contaminated area (MAR value was 0.37). Similar study was carried out by Florea [25] who concluded that Aries River in Romania is a high risk of cotaminated environment due to high values of calculated MAR index. This indicates that the phenomenon of multiple antibiotic resistant bacteria in aquatic environment is of global concern, since it is an international rather than national problem [27].

Presence of multiple drug resistance (MDR) in enteric bacteria isolates from aquatic environment has been reported previously by Florea [25], Olaniran *et al.* [28], Abdo *et al.* [29] and Emmanuel *et al.* [30]. Olaniran *et al.* [28] investigated antibiotic resistance profiles of *E. coli* isolates from two rivers in Durban, South Africa and found that 71-97% of the isolates are resistant to the antibiotics tested. Abdo *et al.* [29] investigated the antibiotic susceptibility of pathogenic bacteria (*E. coli, Salmonella choleraesuis and Streptococcus faecium*) isolated from River Nile at Ismailia canal water, Egypt and found that these tested bacteria are resistant against most

applied antibiotics (13 antibiotics). Emmanuel *et al*. [30] evaluated the antibiogram of enteric bacterial isolates from water sources in Nigeria. They reported that all isolates are resistant to three used antibiotics. Multiple antibiotic resistances were also found in strains of Enterobacteriaceae isolated from the Venda Region Rivers in South Africa [7], Aksu River in Turkey [31], rivers in Malaysia [32], River Nile, Egypt [9], Pearl Rivers in South China [23] and Almendares River in Cuba [33].

CONCLUSIONS

The present study provides a baseline data on antibiotic resistance profiles in River Nile at Rosetta branch and main drains located on its sides. In general, elevated antibiotic resistance rates could be detected in the Enterobacteriaceae isolates from water samples of drains outfalls and Rosetta Nile branch, which receives heavy load of domestic wastewater effluent caused by that drains. Moreover, water in River Nile exhibited the lowest antibiotic resistance compared to that of drains outlets. Based on the observed similarity of antibiotic resistance profiles found in wastewater and surface water, drains discharge are most probably the main cause of widespread presence of antibiotic resistant bacteria in the Rosetta Nile branch. The results revealed that pollution can create antibiotic resistant traits. Thus, polluted River Nile may become reservoir for AR genes that can, under natural conditions, be transferred to water-borne pathogens. The presence of high number of MAR bacteria in River Nile may have ecological and public health implications. This emphasizes the need for further studies, especially in relation to the gene encoding resistance in different bacterial species as well as on the possibility of the returning of resistance genes to the human population through water usage. Because of resistance of enteric bacterial isolates against most applied antibiotics, the use of traditional antibiotics should be decreased. It is recommended to prevent unregulated use of antibiotics in different aspects (human and animal therapy, as growth promotes in animal production or in agriculture) to restrict the dissemination of multiple antibiotic resistant bacteria in environment.

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