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Antibiotic Resistant Bacteria: A Factor to be Considered in Safe Drinking Water

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Abstract

Wastewater management has been subjected to different treatment technologies using chemical and biological techniques for several decades in order to protect the environment and public health. Biological treatment is based on microbial capability to reduce the pathogens load to achieve the lowest environmental as well as public health risks. However, such treatment is often neglecting the fact that bacteria resistant ability to antibiotics may increase during sewage treatment processes. This study aims to demonstrate the evidence of antibiotic resistant or multiple-antibiotic resistant (MAR) bacteria in water even after treatment, which can contribute to the increase their existence among the bacterial population. The study objective is extend to prove that the public health risk from the high existence of MAR population in drinking water begin to be much more complicated if the antibiotic resistance character is transferable from non-pathogenic bacteria to pathogens or opportunistic pathogens. The study showed that most bacterial species isolated from chlorinated water at two districts in Cairo, Egypt were resistance to ampicillin, sulfaguanidine and streptomycin. MAR bacteria represented 62.4 to 98% of the isolates. Antibiotic resistant bacteria could represent 40% to 70% of the isolates from the distributed drinking water. Bacterial isolates from wells at three water works in Cairo showed resistance to penicillin, 2-sulfanilamide pyrimidine, tetracyclin, chloramphenicol and neomycin. Study on the impact of activated carbon application in a pilot water treatment plant showed that the incidence of coliforms resistant strains among isolates varied significantly according to the source of water samples. MAR was not always high in the same samples in which the overall resistance was high. The antibiotic resistance character was mostly transferable. Accordingly and as a conclusion, the incidence of antibiotic resistant bacteria should be considered as valuable parameter in both wastewater treatment evalauation and in the new drinking water standards.

Keywords

Wastewater Treatment, Antibiotic Resistant Bacteria in Water, Water Treatment Efficiency, Resistance Transfer

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1. Introduction

Antibiotics consumption worldwide lie between 100,000 to 200,000 ton per year (Wise, 2002). Usage patterns may be different from country to another (Kummerer, 2008). Selection of resistant organisms in nature may result from natural production of antibiotics by soil organisms, presence of antibiotics in pharmaceutical industrial wastes, runoff from animal feed or crops, or treated waste products from animal or human (Witte, 1998; Hirsch *et al*., 1999; Larsson *et al*., 2007; Li *et al*., 2008 a,b; Thomas, 2008). On average, 30% of the antibiotics used is metabolized and 70% is excreted into wastewater (Kummerer and Henninger, 2003). Previous studies showed that inadequately treated sewage and wastes are the main sources of antibiotic resistant bacteria in the environment (Hanwood *et al.,* 2001; Inversen *et al.,* 2002). Bacterial antibiotic resistance may arise by spontaneous mutations or extra-chromosomal inheritance in man and animals brought by selective pressure of antibiotics used in therapy and prophylaxis or for growth promotion

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(Walsh, 2000; Gaskins *et al*., 2002; Kummerer and Henninger, 2003). The presence of antibiotic resistant bacteria in aquatic environment throughout the world has been documented (Ash *et al*., 2002; Schwartz *et al*., 2006; Pontes *et al*., 2009). Relatively harmless microbes which pass through water treatment systems could be allowing dangerous bacteria such as those have resistance as well as multiple resistances to antibiotics to reproduce in drinking water supplies. The discovery that resistance characteristics can be transferred to sensitive recipients' cells via R-factor plasmid vectors has emphasized the magnitude of the problem (Mitsuhashi, 1977). Francy *et al*. (2006) concluded that because humans exposed to antibiotics, the *Escherichia coli* (*E. coli)* they harbor will likely be more resistant to antibiotics than those found in the gastrointestinal tracts of wildlife. Natural reservoirs of resistance genes may provide a source of transferable traits for emerging pathogens (Elsas *et al*., 2000). Feary *et al*. (1972) reported that the incidence of antibiotic-resistance among total and fecal coliforms reached more than 40% in potable water samples from the tested spring and wells, while it was increased to 60% in streams and seawater samples.

The introduction of granular activated carbon (GAC) filtration step in the water treatment train has been advocated to remove objectionable organic contaminants, including oil, gasoline, phenol, or Trihalomethanes (THMs) and their precursors (National Research Council: Drinking Water and Health, 1977; 1980). It has been shown that GAC can adsorb bacterial nutrients and be colonized by bacteria (Camper *et al.,* 1986). Health problems may arise when colonized carbon particles penetrate treatment barriers (Stewart *et al.,* 1990) or if bacteria biofilm formed sloughed or sheared off filter bed by hydraulic forced and enter effluent water (Schwartz *et al.,* 1998). Bacteria released in the effluent of GAC contactor may include pathogens, opportunistic pathogens and antibiotic resistant bacteria (Reasoner, 1991). Antibiotic resistant strains which carry transferable resistance and present in drinking water may represent a source of public health hazards for patients using immunosuppressive drugs or undergoing chemotherapy (Geldreich, 1991). So, if bacteria from GAC filters can reach drinking water, the public health significance of these organisms must be considered.

The microbiological criteria for drinking water quality have been directed towards protection of the consumers from the possibility of health hazards that resulted from microbial pollution. Therefore, the main bacteriological examinations for drinking water are based on tests for heterotrophic plate count (HPC) population, total coliforms, fecal coliforms and fecal streptococci (USEPA, 2009; 2010; WHO, 2008).

This study aims to demonstrate the evidence of antibiotic resistant or multiple-antibiotic resistant (MAR) bacteria in water even after treatment, which can contribute to the increase existence among the bacterial population. The study objective is extend to prove that the public health risk from the high existence of MAR population in drinking water begin to be much more complicated if the antibiotic resistance character is transferable from non-pathogenic bacteria to pathogens or opportunistic pathogens.

2. Materials and Methods

2.1. Drinking Water Sampling

Drinking water samples were collected, in duplicate, weekly for a month from El-Dokki and Nasr City, Cairo. The water source of El-Dokki water treatment plant is the Nile River. The main water sources of Nasr City are ground water and fresh water from Ismailia Canal. In both water treatment plants, surface water first flocculated, coagulated with alum, pre-chlorinated, filtered through rapid sand filters and post chlorinated before pumping through the distribution system.

2.1.1. Test for Standard Plate Count (SPC)

The collected water samples were checked for total viable bacterial counts content. According to the method described in American Public Health Association (APHA, 2005), membrane filter (MF) technique was followed for SPC determination (duplicate plates from each sample) using modefied-Standard Plate Count (m-SPC) agar and incubation at 35°C for 48 hrs.

2.1.2. Antibiotic Resistance Determination

Antibiotic-containing media were prepared by adding sterilized antibiotic solution to melt and tempered (at 48 to 50°C) Muller Hinton agar (Difco). The antibiotics used through this study are the widely used antibiotics in Egypt and the concentrations are the levels accepted as constituting clinical resistance. The antibiotics used and their final concentrations per mL in the media were as follows: ampicillin (15µg), tetracycline hydrochloride (15µg), chloramphenicol (25 µg), streptomycin sulfate (15 µg) and the chemotherapeutic agent sulfaguanidine (350 µg). Bacterial isolate colonies were randomly selected from the m-SPC filters for antibiotic resistance tests and inoculated onto m-SPC agar plates. After the incubation period, the master plates were replicated onto the antibiotic containing medium. Five antibiotic plates and one as a control plate (without antibiotic) were incubated at 35°C for 24 h and antibiotic resistance was determined. An organism was considered resistant to an antibiotic if it grew as well on the antibiotic plate as on the control plate. Any sign of growth inhibition was scored as sensitive to that antibiotic.

2.1.3. Identification of Multiple Antibiotic Resistant (MAR) Strains

MAR strains were picked from the control plates and inoculated into nutrient broth (Difco), incubated for 24 h at 35°C and streaked onto nutrient agar (Difco) plates. A single isolated colony was used for identification. Cell morphology, Gram-staining, catalase and oxidase reactions, motility, urease, indole and glucose oxidation/fermention (O/F) tests were carried out. According to the schemes of Buchanan and Gibbons (1974), Lennette *et al*. (1974) and LeChevallier *et al*. (1980), the strains were placed into genera or groups.

2.2. Underground Water Sampling

Sixty-five ground water samples were collected from thirteen wells under use in three water works at Cairo. Total and fecal coliforms were determined for the water samples according to methods described in the American Public Health Association (APHA, 2005). Nonfecal bacterial isolates were streaked on Eosin Methylene Blue (EMB) agar for purification and the isolates were classified into genera or groups according to the scheme given by LeChevallier *et al*. (1980). Resistance to chloramphenicol, tetracycline hydrochloride, neomycin sulfate and penicillin G-sodium salt and 2-sulfanilamide pyrimidine was tested. Melted and tempered nutrient agar was provided with the antibacterial agent to give final concentration of 50 µg/mL. Duplicate plates of media containing the antibiotic or the chemotherapeutic were streaked in parallel rows with test organisms and incubated at 35°C for 48 h. An organism was considered resistant to the agent when its grow normally on both the the antibiotic and the control plates.

2.3. Granular Activated Carbon (GAC) Test

2.3.1. Water Treatment Plant

Water sample was taken from the Ohio River at Cincinnati, OH, USA; pumped to settling basin, flows by gravity to Chemical House where chlorine, fluoride, lime, iron sulphate, activated carbon and soda ash were used. From the flocculating compartment, water flows to the building which houses the filters. Between the coagulating basins and the filter house, powdered activated carbon and chlorine was added when needed. In the filter house, there are 47 rapid sand filters; each has a normal operating capacity of 1.32 million m^3 day^{-1}. After filtration, the water is stored in the clear wells of 5.23 and 1.72 million m^3 capacities, ready for distribution.

2.3.2. GAC Pilot Plant Bacteriological Analysis

GAC pilot plant is connected to the filter house and feed with a part of the filter effluent, flow rate, 185 thousand m^3 day^{-1}; depth, 4.57 m; diameter. 3.35 m, activated carbon capacity, 19050 kg; empty bed contact time (EBC) 15 min. and 21 min. Water samples were collected from sand filter effluent, GAC effluent 15 min and 21 min empty bed contact time (EBC). The primary areas of bacterial concern were:

*Heterotrophic Plate Counts (HPC) determination at 20°C (7 days incubation) and 28°C (3 days incubation) by the spread plate technique on R2A medium (Reasoner and Geldreich, 1985).

*Total coliforms by Membrane Filter Technique (MF) using m-Endo LES medium.

*Fecal coliform by MF technique using M-FC medium.

*Biochemical identification of bacteria isolated from GAC effluents using API-20E, Rapid NFT strips (API System Products, Plainview, N.Y.) and OXI/FERM TUBE (ROCHE Diagnostic Systems, N.J., USA).

2.3.3. Antibiotic Resistant Pattern

Antibiotic resistance pattern for bacteria isolated from the water treatment processes using Muller Hinton or R2A medium (Difco) and the following antibiotic susceptibility test disks (Difco): naladixic acid (NA) 30 µg, chlormphenicol (C) 30 µg, tetracycline (TE) 30 µg, oxytetracycline (T) 30 µg, ampicillin (AM) 10 µg, colistin (CL) 10 µg, kanamycin (K) 30 µg, streptomycin (S) 10 µg, nitrofurantoin (FD) 300 µg, neomycin (N) 5 µg polymyxin B (PB) 300 units, chlortetracycline (A) 30 µg.

2.3.4. Transferability of Antibiotic Resistant Characters

Antibiotic resistant characters were assayed for transferability by mating the resistant strains with two recipient strains Naladixic acid resistant (N^r) 25 µg:

**E. coli* ATCC 27662-1 NED, SUB 2 Pa-ttee K-12F nal^r, nitrosoguanidine-induced mutant derived from ATCC 14948, and

**E. coli* CGSC strain No. 4204 nal^r A designation KL 166-K.B. Low strain- Sex: Hfr-Origin: PO 45 Hfr KL 16-Chromosomal markers: gyrA1,rel A1, thy A 24, spo T 1, thi-1, deo B13-suppressor free organism (Supplied by Dr. Barbara J.Bachmann, EW- Coli Genetic Center, Dept. of Biology, Yale Univ., New Haven, CT).

3. Results

The antibiotics resistant frequencies of 563 isolates from El-Dokki drinking water were presented in the following order: ampicillin > sulphaguanidine > tetracycline > streptomycin> chloramphenicol. A quite similar pattern was obtained for the 397 isolates from Nasr City drinking water which were:

ampicillin> sulfaguanidine> streptomycin> tetracycline> chloramphenicol (Table 1). These observations may reflec the usage patterns of these antibiotics. Of the total 960 screened antibiotic resistant strains, the percentages of single, double, triple, quadruple and quintuple resistant strains were 11.0, 42.6, 31.8, 10.1 and 0.006, respectively (Table 2).

Table 1. Percentage of antibiotic resistant bacteria among SPC isolates from drinking water.

Source	SPC range (cfu/100 mL^{-1})	No of strains examined	Percentage of isolates resistant to:				
			Amp.	Tet.	Cm.	Str.	Sug.
El-Dokki	40 to 3800	563	84.0	33.3	11.3	15.5	67.6
Nasr City	20 to 340	397	89.7	35.6	6.4	56.9	78.1

Amp: Ampicillin; Cm: Chloramphenicol; Tet: Teracycline; Str: Streptomycin; Sug: Sulfaguanidine

Table 2. Percentage of bacterial isolates carrying different numbe of resistance determinates (R).

Source	Percentages of strains carrying different R determinants					Percentages of MAR isolate
	1R	2R	3R	4R	5R	
El-Dokki	14.9	45.4	26.2	7.6	0.8	80.0
Nasr City	5.5	38.5	39.7	13.6	0.2	92.0
% of total isolates	11	42.6	31.8	10.1	0.006	85.2

Table 3. Identities of some MAR strains isolated from drinking wate samples.

Identity	El-Dokki		Nasr City	
	No of isolates	% of total	No of isolates	% of total
1. Gram-positive non- fermentative rods:		2.5		1.2
Acinetobacter spp.	1		0	
Alcaligenes spp.	3		0	
Moraxella ssp.	1		0	
Flavobacterium spp.	0		2	
2. Gram-negative fermentative rods:		3.5		13
Aeromonas spp.	1		12	
Citrobacter freundii	1		3	
Enterobacter aerogenes/Cloacae	4		6	
Hafnia	1		0	
3. Gram-positive cocci:		1		8.6
Micrococcus spp.	2		14	
4. Gram-positive rods:		93		77.2
Bacillus spp.	172		125	
Corynebacterium	15		0	
Total number of identified isolates	201		162	
Total MAR	450		364	

Thus, approximately 85% of the tested strains were considered as MAR (Table 2).The percentages of strains carrying 1R, 2R and 5R in the water samples from El-Dokki district were higher than those of Nasr City. The percentages of MAR strains isolated from El-Dokki and Nasr City water samples were 80 and 92%, respectively (Table 2).

The identities of some isolated MAR phenotypes (363 strains) are presented in table 3. Four major groups were identified and Gram-positive rods constituted the largest portion of MAR and represented 93 and 77.2% of the total identified strains isolated from El-Dokki and Nasr City water samples, respectively. Gram-negative fermentative rods represented the second group, while the Gram-positive cocci and Gram- negative nonfermentative rods constituted the third and fourth groups of the identified MAR strains (Table 3).

The majority of isolates (77) representing flora from 13 wells were found to be penicillin resistant. These were followed by sulfanilamide pyrimidine (64), tetracycline (18), chlor-amphenicol (16) and finally neomycin (10) (Table 4). Results revealed that, 19 isolates belongs to different genera were sensitive to all of the tested compounds, while only 18 isolates were resistant to only one compound. The percentages of doubly, triply, quadraply and quintuply resistant strains are 31.7%, 14.9%, 12.9% and about 4%, respectively. It becomes evident that the majority of the

isolated strains (63%) are of MAR ability (Table 4).

Within a specific genus or species different strains showed differences in resistance and/or sensitivity to the compounds used (Table 5). The identities of the MAR phenotypes (64/101 strains) are presented in table 6. Four major groups were identified. Generally, Gram-negative nonfermentative rods constituted the largest portion of MAR isolated from El-Maadi wells and represented 63.8%. Gram-negative fermentative rods represented 33.3, 19.1 and 62.5% while, Gram-positive cocci represented 55.5%, 10.6% and 31.5% of the MAR identified strains isolated from Mostrod, El-Maadi and El-Marg wells, respectively. Finally, Gram-positive rods represented 11.1% and 6.3% of the MAR isolated from Mostrod and El-Maadi, respectively (Table 6).

Table 4. Antibiotic resistance patterns for strains isoilated from well water.

Location	Antibiotic resitance					No of isolates	% of Location isolates
Mostrod	-	-	-	-	-	7	31.8
	-	-	-	+	-	3	13.6
	-	-	-	-	+	3	13.6
	+	-	-	+	-	1	4.5
	-	+	-	+	+	3	13.6
	+	+	-	+	+	3	13.6
	+	+	+	+	+	2	9.1
El-Maadi	-	-	-	-	-	4	6.3
	-	-	-	+	-	11	17.4
	-	+	-	-	-	1	1.6
	-	-	-	+	+	29	46.0
	+	-	-	+	+	3	4.7
	-	+	-	+	+	4	6.3
	+	+	-	-	+	1	1.6
	-	+	+	+	+	4	6.3
	+	+	-	+	+	4	6.3
	+	+	+	+	+	2	3.2
El-Marg	-	-	-	-	-	8	50.0
	-	+	-	+	-	2	12.5
	-	+	-	+	+	4	25.0
	+	+	-	+	+	2	12.5
No of total isolates						101	
No of + isolates	16	18	10	77	64		

Cm: Chloramphencol Tet: Terracycline Neo: Neomycin, Pen: Penicillen Sulf:Sulfanilamide pyrimidene, (-) Sensitive (+) Resistant

Table 5. Antibiotic resistance pattern for some strains isolated from underground water.

Genera or Group	No of isolates	Antibiotic resistance				
		Cm	Tet	Neo	Pen	Sulf
Bacillus	2	-	-	-	+	-
	2	-	-	-	-	-
	1	-	-	-	-	+
Moraxella	2	-	-	-	+	-
	2	-	-	-	-	-
Pseudomonas	7	-	-	-	+	-
	4	-	-	-	-	-
Micrococcus	1	-	-	-	-	+
	1	+	-	-	-	-
	8	-	-	-	-	-

+ Resistant, - Sensitive

Table 6. Identities of MAR strains isolated from well water.

Identity	Mostrod		El-Maadi		El-Marg	
	No of isolates	% of total MAR	No of isolates	% of total MAR	No of isolates	% of total MAR
Gram-negative non-fermentative rods:		0		63.8		0
Morexcella spp.	0		10.0		0	
Flavobacterium spp.	0		1.0		0	
Pseudomonas / *Alcaligenes*	0		19.0		0	
Gram-negative fermentative rods:		33.3		19.0		62.5
Aeromonas	2.0		4.0		0	
Enterobacteriaceae	1.0		5.0		5.0	
Gram positive cocci:		55.5		10.6		37.5
Micrococcus spp.	1.0		1.0	10.6	0	37.5
Staphylococcus spp.	4.0		4.0		3.0	
Gram positive rods:		11.11		6.3		0. 0
Bacillus spp.	1.0		3.0		0.0	
Total MAR	9.0		47.0		8.0	

Regarding to the Cincinatti water treatment plant that includes the pilot of GAC, a total of 11 samples from each step of treatment processes were collected. For water that feed the sand filters, the maximum HPC counts was 10^4 cfu mL^{-1}, while both total and fecal coliforms densities showed wide range of fluctuation reaching 28 to 29000 and 4 to 1072 cfu 100 mL^{-1}, respectively. On the base of the geometric means, the removal that was achieved in HPC, total coliforms and fecal coliform reached (40% at 20°C and 47% at 28°C), 89% and 78%, respectively as a result of chemical treatment before sand filtration. The results of the effluent of sand filtration showed a progressive reduction in bacterial densities (Table 7).

Table 7. Changes in bacteriological parameters through water treatment process.

Site	HPC (cfu ml^{-1}) at		Coliforms (cfu 100 mL^{-1})	
	20° C	28° C	Total	Fecal
Raw water	($2.4x10^3$ -$8.7x10^4$)	($4.7x10^2$- $8.4x10^4$)	($2.8x10$ -$2.9x10^4$)	($0.4x10$-$1.07x10^3$)
	$2.2\ x10^{4*}$	$1.4x10^{4*}$	$1.9\ x10^{3*}$	$1.46\ x\ 10^{2*}$
Sand filter influent	($1.7x10^2$ -$5.3x10^4$)	($7.0x10$ -$2.8\ x10^4$)	($0.4\ x10$-$1.2x10^3$)	($0.1x10$-$7.4x10^1$)
	$1.3\ x10^{4*}$	$7.3\ x\ 10^{3*}$	$2.06x10^{2*}$	3.2x10*
Sand filter effluent	($2.9x10^2$-$4.7x10^4$)	($1.0x10^{\ 2}$-$1.0x10^4$)	($0.1x\ 10$ -$3.8x10$)	($0.1x10$-$1.4x10$)
	$8.5x10^{3*}$	$4.9\ x10^{3*}$	2.1x10*	0.4x10*
GAC 15 min EBC	($4.0x10^2$ -$2.3x10^4$)	($1.0x10^2$ -$2.8x10^3$)	($0.1x10$ -$2.0x10^1$)	< 1
	$5.7x10^{3*}$	$2.3x10^{3*}$	0.5x10*	-
GAC 21 min EBC	($5.0x10^2$ -$1.5x10^4$)	($3.0x10$ -$5.9x10^3$)	($0.1x10$ -$2.2x10^1$)	< 1
	$4.5x10^{3*}$	$1.4x10^{3*}$	0.4x10*	-

( ) Range for 11 samples., *Geonetric mean.

It was observed that high percentage of the HPC bacteria that initially grew on R2A, has failed to grow in subculture to Tryptic Soy Broth (TSB) with 0.3 yeast extract that suggested for API identification system (Table 8).

Table 8. Culturable isolates in TSB

Source	No of isolates	% test positives
HPC 28° C on R2A medium	1346	38.70
HPC 20 C on R2A medium	1310	38.85
Total coliforms (m-Endo LES medium)	723	87.40
Fecal coliform (m-FC)	450	91.50

chemical treatment for water before sand filtration. In addition, the percentage of antibiotic resistant bacteria was found to be lower among isolates from the GAC effluent than that of bacteria in corresponding sand filtered water. Selection for bacteria exhibiting resistance to neomycin (N) and polymyxin B (PB) was achieved by filtration through GAC. No differences were observed in the antibiotic resitance pattern (resistance for N and PB) for the HPC population isolated from the effluent of GAC reactor by increasing the EBC from 15 to 21 min. Sand filter and GAC reactors may represent sites for selection and reproduction for bacteria exhibiting MAR characters (Table 9).

The effluents of sand filters and GAC 15 min EBC reactor have the highest percentages of total coliform population that showed antibiotic and multiple antibiotic resistant characters. Isolates of total coliforms showed high reistance to ampicillin. neomycin, polymyxin B and colistin. However, fecal coliform isolates showed somewhat low frequency of resistance. Most isolates were resistant to ampicillin (Table 10).

Table 9. Changes in antibiotic resistant HPC in water treatment process.

Water sample	No of isolates	% Antibiotic resistant	% MAR	Highly resistant to:
Raw water	96 (20º C)	84.3	82.7	AM-S-FD
	96 (28º C)	68.6	50.8	N-PB-AM
Sand filter influent	80 (20º C)	90.0	79.1	PB-N-AM
	91 (28º C)	92.3	77.3	N-PB-S
Sand filter effluent	81 (20º C)	93.8	90.7	N-PB-AM
	72 (28º C)	90.2	83.0	PB-N-S
GAC effluent 15 min	84 (20º C)	80.9	91.1	PB-N-S
	50 (28º C)	84.0	54.7	PB-N
GAC effluebt 21 min	59 (20º C)	81.3	81.2	PB-N
	78 (28º C)	79.4	91.9	PB-N

AM: Ampicillin S: Streptomycin FD: Nitrofurantoin N: Neomycin PB: Polymyxin

Table 10. Behaviour of antibiotic resistant coliforms in water treatment processes.

Water sample	No of isolates	% Antibiotic resistant	% MAR	Highly resistant to:
A-Total coliforms:				
Raw water	53.0	60.3	46.8	AM-N-PB
Sand filter influent	57.0	52.6	33.3	AM
Sand filter effluent	44.0	70.4	54.8	AM-Cl
GAC efflu. 15 min.	27.0	74.0	80.0	N
GAC efflu. 21 min.	25.0	28.0	28.0	AM
B- Fecal coliform:				
Raw water	73.0	38.9	44.4	AM-T
Sand filter influent	45.0	28.8	23.0	AM
Sand filter effluent	9.0	11.1	00.0	AM

AM: Ampicillin N: Neomycin PB: Polymyxin B Cl: Colisyin T: Oxytetracycline

Table 11. Predominate antibiotic resistant species in water treatment processes.

Isolates from:	Site 1	Site 2	Site 3	Site 4	Site 5
	E.coli	***E.coli***	***E.coli***		
Fecal coliforms		*K.pneumoniae*			
	Serratia				
	E.coli	*Aeromonas*	*Serratia*	*Chromobacter*	*Pseidomonas*
Total coliforms	Klebsiells	*Klebsiella*	*Enterobacter*	*Serratia*	*Aeromonas*
		Enterobacter	*Citrobacter*		*Enterobacter*
	Moraxella	*Moraxella*	*Moraxella*	*Moraxella*	*Moraxella*
HPC at 20ºC	Pseudomonas	*Pseudomonas*	*Pseudomonas*	*Pseudomonas*	*Pseudomonas*
		Serratia	*Flavobacteria*		*Serratia*
	Serratia	*Enterobacter*		*Enterobacter*	*Moraxella*
HPC at 28ºC	*Klebsiella*	Pseudomonas	*Moraxella*	*Pseudomonas*	*Agobacter*
	GRP 17				
	CDC Ent.		*Pasturella*	*Aeromonas*	*Enterobacter*

Site 1: Raw water; Site 2: Sand Filter Infl.; Site 3: Send filter effl.; Site 4: GAC Effl. 15 min.; Site 5: GAC Effl. 21 min.

Table11 showed the identification of representative isolates that exhibited antibiotic resistance.Mating experiments showed that antibiotic susceptibility patterns were transferred partially or completely to the recipients. The results revealed that the transfer of antibiotic resistance was expressed much more by using *E. coli* ATCC 27662-1 than *E. coli* KL 166-CGSC.

4. Discussion

According to different legistlation and different degrees of importance ascribed to the use of antibiotics, reliable data providing information on the total use and the patterns of antibiotic use and per capita consumption exist for only a few countries. Antibiotic prescription rates and intake without prescription vary markedly between countries (Molstad *et al*., 2002). It is emphasized that antibiotic concentrations used for screening the resistant bacteria are the levels accepted as constituting clinical resistance (Matsen and Barry, 1974). Comparing our results with other published work is quite complicated due to differences in types and concentrations of the tested antibiotics. The percentages of MAR bacteria observed in drinking water of the two Cairo districts are quite higher than that reported in USA by Armstrong *et al*., (1981). They reported percentages ranged between 27.4% and 86.1% for MAR isolates among SPC bacteria from drinking water of 6 communities in Oregon, USA. The majority of MAR organisms are nonpathogenic. However, the presence of opportunistic pathogens such as *Acinetobacter* spp., *Moraxella* spp. and *Flavobacterium* spp. could represent a potential health hazard for patients in hospitals, clinics, nurseries and rest homes (Geldreich, 1991). *Bacillus* spp. causes spoilage problems in food products, beverages, cosmetics and drug industries (Dunnigan, 1969). These antibiotic resistant bacteria may transfer their resistance factors to other pathogenic microorganisms that present in the intestinal tract of the consumer.

Generally, through the study that carried out on underground water that used as source of drinking water, the depth of wells ranged between 45 m and 65 m depth. The soil of El-Maadi area is clay and an open drain of high loads of organic wastes exists 40 m away from the wells, and represent a source of water pollution. The depth of the wells is in the range mentioned.

El-Maadi water works was constructed on a clay loamy soil, 100 m away from the eastern bank of the Nile River. Each of the tested wells is between 55 and 65 m.

Differences in depth, hydrogeologic formation and sources of possible pollution are sufficient to separate the data according to the location from which the strains are identified. For the previous reasons one can expect that difference in MAR frequency in bacteria from wells sampled at the three sites must exist. Our data confirmed this expectation. The isolation of Gram-negative non-ferementative rods from El-Maadi wells and not from the other two locations may be due to the low numbers of isolates examined from both Mostrod and El-Marg wells. The high incidence of certain patterns of resistance involving penicillin, sulfanilamide pyrimidine and tetracycline was observed and believed to be a reflection of drugs frequently used in clinical therapy.

The results of GAC contactors contrast with those reported by Trulear and Characklis (1982) and based on the observed deterioration in the treated water quality when bacteria were sloughed from the biofilm which build up and adhered or accumulated on GAC and appeared in effluent water. Note that all these results were based on samples received from drinking water treatment facilities that are running well and in compliance with established operation regulations.

Subculturing bacteria from low-nutrient medium (R2A) to high-nutrient medium (TSB) may cause suppression, stress, injury or even inhibition for the subcultured and called viable but nonculturable strains (Reasoner and Geldreich, 1985). This resulted in loss of high percentage of bacterial isolates that were picked for antibiotic resistance test and to be identified as a part of this study. The results of coliforms antibiotic resistant are in agreement with the results of Niemi *et al*. (1983).

Antibiotic resistant bacteria showed some resistance towards chlorination process and chlorine may be play as a selective factor for antibiotic resistant bacteria (Wolf *et al.,* 1985**)**

5. Conclusions

* The antibiotic resistant bacteria represented high percentage of heterotrophic plate count (HPC) bacteria of drinking water whether the source is surface water or undergroundwater.

* Large portion of antibiotic resistant bacteria that isolated was multiple antibioti resistant (MAR).

* Filtration process of drinking water was not effective against antibiotic resistant bacteria.

* Chlorine treatment was a selective factor for anti-biotic resistant bacteria in treated water.

* High percentage of isolated bacteria from HPC population using low nutrient media (R2A) was not culturable on the media recommended for identification by API system.

* Identification of some MAR bacterial strains that isolated from water showed that they were belong to opportunis-tic pathogens.

* The antibiotic resistance character was mostly transferable.

* The application of granular activated carbon (GAC) in water treatment train has no additional impact on the presence of antibiotic resistant bacter in the treated water.

* The high presence of antibiotic resistant bacteria in drinking water with SPC complying with the standards is

supporting for the request of setting limits for the presence of antibiotic resustat bacteria among the SPC.

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