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Determination of microbial quality and plasmid-mediated multidrug resistant bacteria in fountain drinking water sources in Turkey

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Abstract

The bacterial contamination as the total aerobic bacteria, coliform and fecal coliform numbers were determined and analyzed for temperature, pH, conductivity and dissolved oxygen in seasonally collected water samples from fifteen different stations placed in Adana- Tufanbeyli road line during March 2008- January 2009. In addition, antibiotic resistance profiles of isolates were examined against frequently used antibiotics, and analyzed plasmid DNA of multiple antibiotic resistant (MAR) isolates. Total aerobic bacteria in fountain water samples was determined as $3x10^3$ CFU ml^{-1} and total and fecal coliforms were determined 460 MPN/100 ml. Results obtained from biochemical analysis showed that 121 of the isolates were *Proteus vulgaris*, 69 *Escherichia coli*, 51 *Pseudomonas aeruginosa* and 28 *Citrobacter* spp. According to these results, the existence of *Vibrio parahaemolyticus* in stations 2 and 10, and *Streptococcus faecalis* in stations 11 and 15 respectively were confirmed. *Clostridium perfringens* was not detected in water samples. A total of 273 isolates were tested for antimicrobial susceptibility by agar disc diffusion methods. A total of sixteen antibiotics were used for determination of antibiotic resistance of isolates. Resistance to bacitracin, vancomycine, cephalothin and ampicillin was detected in 77, 77, 63 and 50%, respectively. Multiple antibiotic resistance (MAR) value $\geq$ 0.25 was determined in 68.4% of identified 273 isolates and meaning of this percentage were resistant to four and more antibiotics. Plasmid DNA was isolated from 22 isolates with multiple antibiotic resistance index ranged from 0.3 to 0.6 taken randomly by agarose-gel electrophoresis, some of them contain a high-molecular weight plasmid DNA. Highlight of our study that the appearance of potential antibiotic resistances in fountain drinking water requires increased surveillance for risk assessment and prevention strategies to protect public health.

Key words

Fountain drinking water sources, Antibiotic resistance, Fecal coliform, Plasmid profile

Introduction

Water is essential to all known life forms and is required for hydration to sustain health, sanitation needs, as a solvent to wash everday items, for industrial applications and as a thermal transfer agent, among others. Inadequate water treatment has a significant and devastating impact on public on health. About 1.2 billion people, worldwide, lack access to safe drinking water, and twice that many lack adequate sanitation. As a result, the World Health Organization (WHO, 1996a) estimates that 3.4 million people, mostly children, die every year from water- related diseases (Wilkes *et al*., 2009). Water sources are frequently exposed to microbial contamination from humans, animals and the environtment (Lehloesa and Muyima, 2000). Fecal coliforms are used as indicators of enteric pathogenic organisms in aquatic environments, although recent studies indicate that they are not reliable predictors of the presence of patogenic viruses (Noble and Fuhrman, 2001). Wildlife, sewage effluents, failing septic systems and runoff from farm animal feedlots, agricultural

lands are important sources of fecal coliform bacteria in waters (Hunter *et al*., 1999; Crowther and Wyer, 2002). The increase of faecal pollution in source water is a problem in developing as well as in developed countries (ASMCR, 1999). Water – borne bacterial pathogens such as *E. coli* 0157, *Salmonella* spp., *Shigella* spp. and *Vibrio cholerae* can lead to diarrhoeal outbreaks that may have serious medical and economic implications (WHO, 1996b; WHO, 2000). This problem is further copounded by the increasing incidence of pathogens with and/or drug resistance (Depaola *et al*., 1995). A lot of various antibiotic resistance genes to a broad range of antibiotics have been found in microorganisms distributed not only in hospital wastewaters and animal production wastewaters, but also in sewage, wastewater treatment plants, surface water, groundwater, and even in drinking water (Zhang *et al*., 2009). The fast dissemination of drug-resistant bacteria is an alarming and increasing problem, complicating the treatment of infections. Much of the problem is the result of antibiotic resistance genes transferring among bacterial species, carried by plasmids and transposons (Stokes and Hall, 1989).

The objectives of the study were to: determine the bacterial contamination as the total aerobic bacteria, coliform and fecal coliform numbers in fountain drinking water sources at Adana- Tufanbeyli road line, along with determination of temperature, pH, conductivity and disolution oxygen in drinking water. In addition, antibiotic resistance profiles and MAR and plasmid DNA profiles were also determined.

Materials and Methods

Collection of water samples : 15 different fountain drinking water stations determined at Adana - Tufanbeyli road line and water samples collected on March, June, September 2008, January 2009 period. Water samples were collected from all the stations in 100 ml sterile bottle. Water samples analysed immediately after thorough

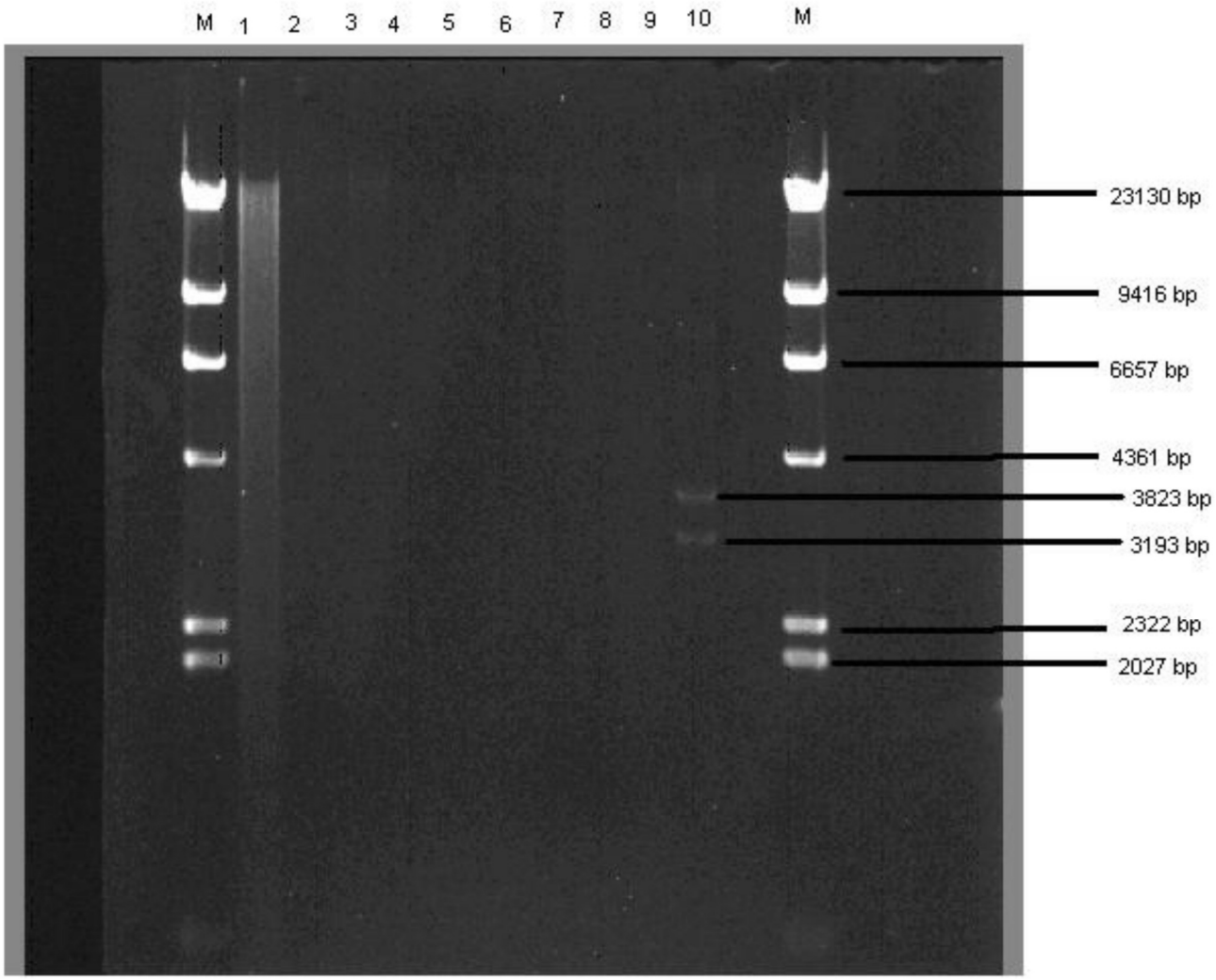

Fig.1: Plasmid profiles of multidrug resistant isolates in agarose gel electrophoresis-1

mixing and stored at 4 °C for further use. Temperature, pH, conductivity, and dissolved oxygen parameters of the water samples were measured immediately and the water samples were stored at 4 °C for further studies (Toroglu *et al*., 2005).

Enumeration of total aerobic bacterial populations : The total colony count of aerobic bacterial was done by the pour plate method using plate count agar (PCA) (Nwachukwu, 2000). To determine total aerobic bacterial populations, 1 ml water samples were spread on two plate count agar (PCA) plates using sterile glass bar and incubated for 24 hr at 37 °C. After incubation of the two plates, the colonies were screened and identified on the taxonomic schemes and descriptions by Holt *et al*.(1994).

Enumeration of total and feacal coliform populations : Multiple tube method was carried out to enumerate the total coliform and faecal coliform populations. Numbers of coliform bacteria were determined by most probable number (MPN) methods (Madden and Gýlmour, 1995). Total and faecal coliform population was expressed as most probable number (MPN 100 ml^{-1}). Faecal (thermotolerant) coliforms constitute a subset of total coliforms.

***Escherichia coli* :** Individual colonies from the MPN tubes were subcultured on to eosin methylene blue (EMB) agar and incubated for 24 hrs at 37 °C. Colonies showing a green metallic sheen on EMB agar were confirmed for *Escherichia coli* using biochemical tests as described by Forbes *et al*. (1998) and Leclerc *et al*. (2001).

***Pseudomonas aeruginosa* :** *Pseudomonas* spp., was isolated by incubating at 42, 37 and 20°C on *Pseudomonas* selective medium (Oxoid CM 457). Then they were tested at these temperatures for casein hydrolysis and pigment production on milk-agar with cetrimide

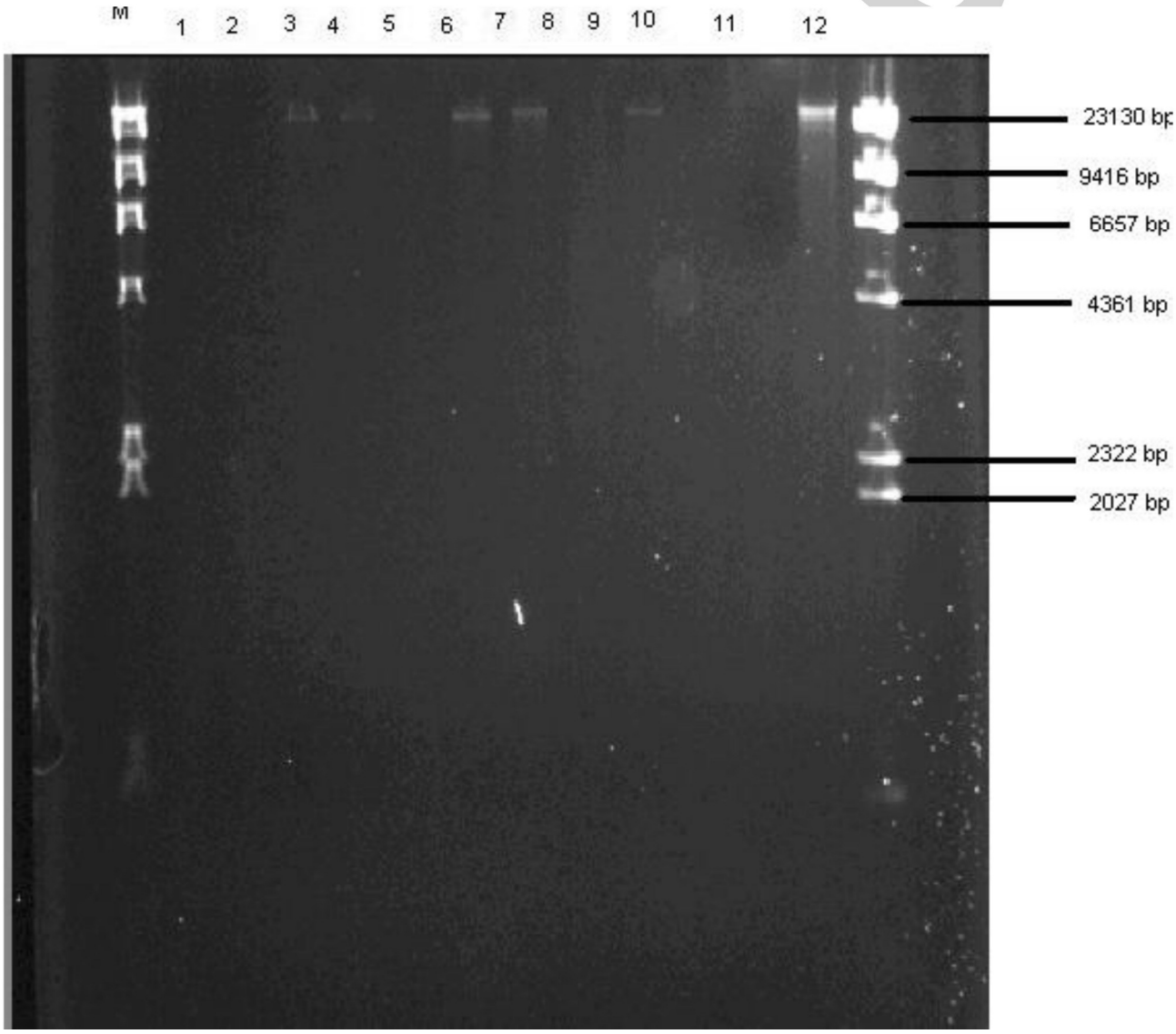

Fig.2 : Plasmid profiles of multidrug resistant isolates in agarose gel electrophoresis-2

Table 1 : Isolates in fountain drinking water samples of 15 stations in Turkey

Bacteria species	Total aerobic bacteria	Total coliforms	Faecal coliforms	Number of strains	Ratio (%)
E. coli	-	49	20	69	25.2
Citrobacter spp.	-	20	8	28	10.2
Proteus vulgaris	121	-	-	121	44.3
Pseudomonas aeruginosa	51	-	-	51	18.6
Vibrio parahaemolyticus	2	-	-	2	0.7
Streptococcus faecalis	2	-	-	2	0.7
Total	**174**	**71**	**28**	**273**	**100**

(Brown and Foster, 1970) and for a positive oxidase reaction and were catalase-positive and were glucose oxidative and positive for citrate and positive for lipase for only *Pseudomonas aeruginosa* (MacFaddin, 2000). In addition, confirmation of isolates was performed by using classic biochemical tests (Gilligan, 1995; Toroglu *et al*., 2005).

***Streptococcus feacalis* :** The samples were inoculated in azide dextrose broth. One ml volume of water samples mixed with 5 ml volume of azid dextrose broth was incubated at 37 ºC for 24-48 hrs. The samples in the test tubes were scored positive, if the turbidity form occured between 24-48 h (Anonymous, 1996).

***Vibrio* spp. :** TCBS agar is used in the diagnosis of *Vibrio cholerae* (Anonymous, 1996). Previously, for enrichment, a few drops of water samples was incubated for 6-8 hrs in taurocholate-peptone broth (10 ml) (pH 8.0-9.0); organisms from this culture can be stained or subcultured (Jawetz *et al*., 1995). A loop full of selective enriched broth from previously incubated water sample were spread on the solid surface of TCBS agar medium. For succesful isolation of typical colonies, triplicate plates of TCBS agar medium for all samples were carried out. Typical colonies can be picked in 18 hours. All of the plates were incubated for 24 hours at 37 ºC. After incubation, for *Vibrio cholerae,* 2-4 mm diameter yellow luminous colonies on TCBS are positive. For *Vibrio parahaemolyticus,* 2 mm diameter green circular colonies are positive.

***Clostridium perfringens* :** Water samples were inoculated into thioglycolate medium and onto blood agar plates incubated anaerobically. The growth from one of the media was transferred into milk. A clot torn by gas in 24 hrs indicated presence of *C. perfringens.* Once pure cultures were obtained by selecting colonies from anaerobically incubated blood plates, and identified by biochemical tests (various sugars in thioglycolate, action on milk), hemolysis and colony form. Lecithinase activity was evaluated by the precipitate formed around colonies on egg yolk media (Jawetz *et al*., 1995).

Antibiotic sensitivity tests : The antibiotic sensitivity of the isolates were determined using the disc diffusion method (Bauer *et al*., 1966). Standardized inoculum of the overnight grown LB broth cultures were spread on Mueller- Hinton agar plates using sterile swabs. The plates were dried at room temperature for 2 hrs before placing the antibiotic discs at equidistance. The plates were incubated for 24 hrs at 37 °C and the diameter of zone of inhibition was measured. All discs were obtained from Oxoid and interpretation of the followed criteria recommended in the NCCLS (1997). A total of 16 antibiotics (belonging to 9 classes): Ampicillin (AMP:25µg) (Penicillins); Streptomycin (STR:10µg), Gentamicin (GEN:30µg), Tobramycin (NN:10µg), Amikacin (AN:30µg) (Aminoglycosides); Tetracycline (TE:30µg) (Tetracycline); Cefuroxime (CEF:30µg; first-generation cephalosporin), Cephalothin (CF:30µg; second-generation cephalosporin), Ceftizoxime (ZOX:30µg; third-generation cephalosporin), Cefepime (FEP:30µg; fourth-generation cephalosporin) (β-lactam antibiotic- Cephalosporins); Vancomisin (VA:30µg), Bacitrasin (B:10µg) (Glicopeptides); Ertyromycin (E:15µg) (Macrolids); Chloramphenical (C:30µg) (Chloramphenicol); Trimethoprim- sulfamethokzol (SXT:25µg) (Trimethoprim-sulfamethoksol); Meropenem (MEM:10µg) (Carbapenem) were used in this study.

For all isolates, the MAR index values (a/b, where a represents the number of antibiotics the isolate was resistant to, b represents the total number of antibiotics the isolate was tested against) were calculated. A MAR index value $\geq$ 0.2 is observed when isolates are exposed to high risk sources of human or animal contamination, where antibiotics use is common; in contrast a MAR index value < or = 0.2 observed when antibiotics are seldom or never used (Krumperman, 1985).

Isolation and analysis of plasmid DNA : The plasmid DNA was prepared and isolated according to standard protocols using a Roche plasmid isolation kit (Roche applied science high pure plazmid isolation kit. Cat no: 11 754 777 001). Separated by 0.7 % agarose gel electrophoresis (agarose gels in a Tris-HCl-Boric acid-EDTA: TBE buffer) at room temperature at 5- 10 V cm^{-2}. 20 µl of plasmid DNA was mixed with 5 µl gel Loading Buffer (Brom phenol blue and glycerole) and was loaded into the individual well of the gel. The gel was stained with ethidium bromide (EtBr 1 µg ml^{-1}) for 30-45 min at room temperature and then destained with 1 mM $MgSO_4$ for 15 min (Meyers *et al*., 1976, Maniatis *et al*., 1982). DNA bands were

Table 2. Features of fountain drinking water samples of 15 stations in Turkey

Sampling months 2009	Sampling station(No.)	pH	Temperature (°C)	Conductivity (µs cm^{-1})	Dissolved O_2 (mg l^{-1})	Total aerobic bacteria (CFU ml^{-1})	Total coliform (CFU 100 ml^{-1})	Fecal coliform (CFU 100 ml^{-1})
	1	7.96	10	346	6.81	0	240	240
	2	7.50	11	384	6.92	5	43	0
	3	7.60	12	460	7.03	0	0	0
	4	7.70	13	502	7.13	800	43	0
	5	7.45	12	631	7.10	1	9	0
	6	7.87	14	402	7.33	2000	15	0
1st period	7	7.74	15	481	7.40	1	9	0
March 2008	8	7.42	13	791	7.62	0	0	0
	9	7.46	12	676	7.62	3000	4	0
	10	7.64	14	371	7.89	2	9	0
	11	7.43	13	975	7.92	3	0	0
	12	7.38	15	628	6.57	3	0	0
	13	7.45	18	618	8.16	2	9	0
	14	7.55	15	516	7.03	1	4	0
	15	7.04	17	860	7.15	1000	0	0
	1	8.05	11	335	6.66	2	240	240
	2	7.64	12	511	7.06	2	240	0
	3	7.66	12	613	6.61	0	0	0
	4	7.98	13	450	7.18	8	23	0
	5	7.58	12	649	7.00	3	75	75
	6	8.08	15	392	6.70	7	15	0
2nd period	7	7.85	16	496	7.11	3	43	43
June 2008	8	7.58	19	817	7.03	25	0	0
	9	7.62	17	660	6.93	8	240	240
	10	7.80	15	367	7.03	2	43	0
	11	7.66	20	964	6.54	210	7	0
	12	7.62	16	597	6.34	40	240	0
	13	7.93	18	371	6.59	50	0	0
	14	7.60	16	550	7.00	1	4	0
	15	7.49	22	880	6.31	1000	0	0
	1	8.17	10	378	6.34	20	0	0
	2	7.62	11	469	6.67	250	240	240
	3	7.55	12	583	6.44	0	0	0
	4	7.96	10	479	6.70	8	23	0
	5	7.58	12	623	6.38	2	43	43
	6	8.09	15	363	6.59	4	43	43
3rd period	7	7.86	15	474	6.69	100	15	0
September 2008	8	7.52	18	797	6.53	20	0	0
	9	7.64	17	614	6.49	5	150	150
	10	7.72	12	347	6.22	8	15	0
	11	7.92	20	654	6.40	40	460	460
	12	8.00	17	621	6.40	10	240	240
	13	7.90	17	340	6.51	2	15	0
	14	7.58	16	860	6.62	0	0	0
	15	7.66	20	865	6.48	18	0	0
	1	8.23	8	316	6.15	2	0	0
	2	7.90	8	476	6.78	3	460	0
	3	8.05	10	613	6.18	0	0	0
	4	8.19	11	513	6.77	3	43	0
	5	7.79	10	617	7.18	15	4	0
	6	8.39	12	655	7.00	2	9	0
4th period	7	8.26	14	572	6.71	0	0	0
January 2009	8	7.80	10	844	6.39	3	0	0
	9	7.90	15	387	6.88	530	9	0
	10	8.00	10	500	6.75	2	21	0
	11	8.02	12	855	7.21	2	23	0
	12	8.28	12	652	6.52	2	43	43
	13	8.06	16	588	6.48	0	0	0
	14	8.00	14	628	6.67	0	0	0
	15	8.27	15	883	6.77	3	0	0

CFU: Colony Forming Unit

Table 3 : Antibiotics resistances levels of isolates in at different stations in Turkey

Bacterial groups	Period (No.)	Isolates (No.)	Resistance to antibiotics (%)															
			VA	AMP	B	STR	AN	TE	E	SXT	ZOX	MEM	C	GEN	FEP	CEF	NN	CF
E. coli	1	38	90	50	70	50	0	0	70	30	20	0	15	0	0	0	0	75
Citrobacter spp.	1	2	50	80	80	40	0	30	30	10	0	0	0	0	0	40	0	80
Proteus vulgaris	1	18	76	53	82	20	0	0	32	0	16	0	18	8	0	40	0	85
Pseudomonas aeruginosa	1	5	100	60	100	30	0	0	20	27	20	0	20	0	0	0	8	90
Vibrio parahaemolyticus	1	2	50	50	50	0	0	0	50	0	0	0	50	0	0	0	0	50
Streptococcus faecalis	1	2	50	0	50	50	50	0	50	0	0	0	0	0	0	0	0	0
Total	**1**	**65**	**69**	**48**	**72**	**31**	**8**	**5**	**42**	**11**	**9**	**0**	**17**	**1**	**0**	**13**	**1**	**63**
E. coli	2	15	85	40	93	70	0	0	70	25	10	0	20	0	0	25	4	83
Citrobacter spp.	2	8	100	70	80	60	0	40	80	50	30	0	50	0	0	30	0	90
Proteus vulgaris	2	38	94	65	92	30	0	39	70	34	32	0	31	10	0	50	10	76
Pseudomonas aeruginosa	2	8	100	100	100	30	0	0	84	87	20	0	45	0	0	30	8	100
Vibrio parahaemolyticus	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus faecalis	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	**2**	**71**	**63**	**45**	**60**	**31**	**0**	**13**	**67**	**32**	**15**	**0**	**24**	**1**	**0**	**22**	**3**	**58**
E. coli	3	6	100	50	100	50	0	10	40	10	10	0	10	0	0	45	0	100
Citrobacter spp.	3	13	82	60	76	0	0	0	30	40	10	0	15	0	0	20	0	75
Proteus vulgaris	3	39	94	60	86	40	0	15	48	10	0	0	20	14	0	60	0	77
Pseudomonas aeruginosa	3	29	100	80	100	40	0	0	58	27	0	0	11	0	0	20	0	74
Vibrio parahaemolyticus	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus faecalis	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	**3**	**87**	**62**	**41**	**60**	**20**	**0**	**4**	**29**	**14**	**3**	**0**	**9**	**2**	**0**	**24**	**0**	**54**
E. coli	4	10	85	40	100	30	0	30	60	15	0	0	11	0	0	30	20	57
Citrobacter spp.	4	5	100	70	100	20	0	10	60	20	0	0	35	0	0	30	0	80
Proteus vulgaris	4	26	96	50	100	10	0	26	70	10	0	0	0	0	0	50	10	88
Pseudomonas aeruginosa	4	9	100	80	100	20	0	20	30	0	0	0	0	0	0	30	0	76
Vibrio parahaemolyticus	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus faecalis	4	0	0	0	0	00	0	0	0	0	0	0	0	0	0	0	0	0
Total	**4**	**50**	**63**	**40**	**66**	**13**	**0**	**14**	**36**	**7**	**0**	**0**	**7**	**0**	**0**	**23**	**5**	**50**

AMP: Ampicillin; STR:Streptomycin; GEN: Gentamicin; NN: Tobramycin; AN: Amikacin; TE:Tetracycline; CEF:Cefuroxime; CF:Cephalothin; ZOX:Ceftizoxime; FEP:Cefepime; VA:Vancomisin; B:Bacitrasin; E: Ertyromycin; C:Chloramphenical; SXT:Trimethoprim- sulfamethokzol; MEM:Meropenem

visualized and calculated using GelQuant programme with MiniBis Bio-Imaging System. The approximate sizes of the plasmids were estimated by comparison to plasmid marker (Vivantes, NM046).

Results and Discussion

In this study, 273 strains belonging to 3 bacterial genera (*Escherichia coli, Citrobacter* spp., *Proteus vulgaris*) of the *Enterobacteriaceae,* 1 bacterial genera (*Pseudomonas aeruginosa*) of the *Pseudomonas*, 1 bacterial genera (*Vibrio parahaemolyticus*) of the *Vibrios*, 1 bacterial genera (*Streptococcus faecalis*) of the *Streptococci*, in the water samples taken from the fountains were isolated. *Clostridium perfringens* was not determinated in water samples. Among the isolates, 121 strains were *Proteus vulgaris* (44.3%), followed by 69 strains of *Escherichia coli* (25.2%), 51 strains of *Pseudomonas aeruginosa* (18.6%), 28 strains of *Citrobacter* spp. (10.2%), 2 strains of *Vibrio parahaemolyticus* (0.7%), and 2 strains of *Streptococcus faecalis* (0.7%) (Table 1). These bacteria are defined as a indication of fecal contamination in areas polluted by urban, industrial and domestic wastes by other studies and markedly pathogens of human capable of causing a diversification of diseases. (Dinçer *et al*., 2001; Matyar *et al*., 2008; Toroglu and Toroglu, 2009).

In the present study, *Proteus vulgaris* was the most frequently detected microorganism and important nosocomial

pathogen. The second most prevalent microorganism was *E. coli*, an indicator of faecal contamination in water environments. The third and fourth most prevalent microorganism were *Pseudomonas aeruginosa* and *Citrobacter* spp. While the minimal microorganisms observed were *Vibrio parahaemolyticus* and *Streptococcus faecalis.*

The bacterial contamination level was determined higher in water of 2nd, 4th, 6th, 7th, 9th,11th,13th,15th sampling stations. The maximum aerobic bacterial contamination was 3 x 10^3 CFU ml^{-1} in the 9th station. These situations are based on several reasons: first one, sewage system is to be insufficient or to be gone without; second one, because of inapplicability of acreage structure, house wastewater is not sufficiently convened in cesspools; thirdly, wastewaters is not filtered, and interfered environmental waters;

Table 4 : Antibiotic resistances (%) of bacterial genera

Antibiotics	*E.coli*	*Citrobacter* spp	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Vibrio parahaemolyticus*	*Streptococcus faecalis*	Total (%)
VA	90	83	90	100	50	50	77
AMP	45	70	57	80	50	0	50
B	90	84	90	100	50	50	77
STR	50	30	50	30	0	50	35
AN	0	0	0	0	0	50	8
TE	10	20	20	5	0	0	9
E	60	50	55	48	50	50	52
SXT	20	30	13	35	0	0	16
ZOX	10	10	12	10	0	0	7
MEM	0	0	0	0	0	0	0
C	14	25	16	19	50	0	20
GEN	0	0	8	0	0	0	1
FEP	0	0	0	0	0	0	0
CEF	25	30	50	20	0	0	20
NN	6	0	5	4	0	0	2
CF	80	80	85	85	50	0	63

AMP:Ampicillin; STR:Streptomycin; GEN:Gentamicin; NN:Tobramycin; AN:Amikacin; TE:Tetracycline; CEF:Cefuroxime; CF:Cephalothin; ZOX:Ceftizoxime; FEP:Cefepime; VA:Vancomisin; B:Bacitrasin; E:Ertyromycin; C:Chloramphenical; SXT:Trimethoprim- sulfamethokzol; MEM:Meropenem

Table 5 : Multiple antibiotic resistance (MAR) distribution of bacterial genera

MAR index		*E.coli*	*Citrobacter* spp.	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Vibrio parahaemolyticus*	*Streptococcus faecalis*	Number of tgotal resistant isolates
1/16	0.062	2	0	6	1	0	0	9
2/16	0.125	10	1	19	5	0	0	35
3/16	0.187	17	1	19	4	0	1	42
4/16	0.25	14	6	28	10	1	1	60
5/16	0.312	10	8	18	6	1	0	43
6/16	0.375	5	4	7	8	0	0	24
7/16	0.437	1	1	6	5	0	0	13
8/16	0.5	5	3	5	8	0	0	21
9/16	0.562	3	1	5	2	0	0	11
10/16	0.625	2	2	7	2	0	0	13
11/16	0.687	0	1	1	0	0	0	2
Total		**69**	**28**	**121**	**51**	**2**	**2**	**273**

fifth, domestic animals and other animals is used fountains; sixth, basic sources of fountains and theirs mobility system is not sufficient; seventh, cesspools is presenced on nearby or upper of fountains'acreage.

The coliform and faecal coliform contamination levels showed determined high values in June 2008 and September 2008 periods. The results show that there was a significant increase in the coliform and faecal coliform rates in June 2008 and September 2008 periods compared to the other periods. Furthermore, there was a significant increase in the water temperature (°C) values in these periods compared to the other periods. It was also contained coliform bacilli with high MPN (460). The water temperature value changed from 10 to 22 °C between June 2008 and September 2008 period. However it changed from 8 to 18 °C between March 2008 and January 2009 period. Jarlier *et al.* (1988) found that the water temperature is an essential factor for resistance development. On the other hand, higher water temperature could be an augmenter factor for the coliform and faecal coliform rates and for the transfer of resistance gene during hot seasons (Then *et al*., 1983; Jarlier *et al*., 1988).

The pH value in the water samples changed from 7.04 and 8.39. Water conductivity value in the water samples ranged from 316 and 975 µs cm^{-1}. Dissolved O_2 value in the water samples changed from 6.15 to 8.16 mg l^{-1}. It was found out that these values of Dissolved O_2 (mg L^{-1}) changed from 6.15 to 8.16. The change in dissolved oxygen did not affect increase or decrease in number of bacteria as these values were below normal values as per guidelines for drinking water quality (WHO, 1996b). But, Coyne and Howell (1994) reported that pH values between 4 and 9 cause an increase in fecal contamination (Coyne and Howell, 1994) (Table 2).

The resistant bacteria from the second- June 2008 and third- September 2008 periods were higher than the other periods. Because stations in those periods have both drainage water and garbage and house wastewater and higher the average water temperatures. The frequencies of Bacitrasin and Vancomisin (Glicopeptides) resistant bacteria from all periods were higher than all other resistant bacteria. Among the isolates, a high percentage of bacteria were resistant Vancomisin (100%), Bacitrasin (100%) in *Pseudomonas aeruginosa* at first- March 2008 period, and resistant Vancomisin (100%) in *Citrobacter* spp.; Vancomisin (100%), Ampicillin (100%), Bacitrasin (100%), Cephalothin (100%) in *Pseudomonas aeruginosa* at the second- June 2008 period, and resistant Vancomisin (100%), Bacitrasin (100%), Cephalothin (100%) in *E.coli*; Vancomisin (100%), Bacitrasin (100%) in *Pseudomonas aeruginosa* at the third- September 2008 period, and resistant Bacitrasin (100%) in *E.coli*; Vancomisin (100%), Bacitrasin (100%) in *Citrobacter* spp; Bacitrasin (100%) in *Proteus vulgaris;* Vancomisin (100%), Bacitrasin (100%) in *Pseudomonas aeruginosa* at the fourth- January 2009 period, whereas a low percentage of bacteria were resistant to the other antibiotics. There were no resistance Meropenem and Cefepime in all isolates. When resistance levels of isolates were compared, *Pseudomonas aeruginosa* isolates were highly resistant in proportion to the other isolates. When resistance levels of periods were compared, there were no resistance 2 different antibiotics at 1st period March 2008, 3 different antibiotics at the second- June 2008 period, 4 different antibiotics at the third- September 2008 period, 5 different antibiotics at the fourth- January 2009 period. Another remarkable point is the resistance to Amikacin in only *Streptococcus faecalis* isolates, to Tetracycline in only *Citrobacter* spp isolates, to Gentamicin in only *Proteus vulgaris* isolates, to Tobramycin in only *Pseudomonas aeruginosa* isolates at1st period March 2008, to Gentamicin in only *Proteus vulgaris* isolates at the second- June 2008 period, to Gentamicin in only *Proteus vulgaris* isolates at the third- September 2008 period, 65 bacterial strains were sampled at first period, mostly showed resistance against B (72%), VA (69%), CF (63%), AMP (48%), E (42%), STR (31%), C (17%), CEF (13%), SXT (11%), ZOX (9%), AN (8%), TE (5%), GEN (1%), NN (1%), respectively and sensitivity for MEM, FEP. 71 bacterial strains were sampled at second period, mostly showed resistance against E (67%), VA (63%), B (60%), CF (58%), AMP (45%), SXT (32%), STR (%31), C (24%), CEF (22%), ZOX (15%), TE (13%), NN (3%), GEN (1%), respectively and sensitivity for AN, MEM, FEP. 87 bacterial strains were sampled at third period, mostly showed resistance against VA (62%), B (60%), CF (54%), AMP (41%), E (29%), CEF (24%), STR (20%), SXT (14%), C (9%), TE (4%), ZOX (3%), GEN(2%), respectively and sensitivity for AN, MEM, FEP, NN. 50 bacterial strains were sampled at fourth period, mostly showed resistance against B (66%), VA (63%), CF (50%), AMP (40%), E (36%), CEF (23%), TE (14%), STR (13%), SXT (7%), C (7%), NN (5%), respectively and sensitivity for AN, ZOX, MEM, GEN, FEP. (Table3).

The presence of antibiotic resistant bacteria in water (Ozgumus *et al*., 2009) and surface water (Toroglu *et al*., 2005; Matyar *et al*., 2008; Toroglu and Toroglu, 2009) is a subject of public health concern. The asset of these resistance bacteria in water, no matter where they come from, is a real risk factor for acquiring such bacteria in the environments. Precautions should be increased at fastest for dissemination of these resistant bacteria in the environment.

Of all the isolates assessed, 77% were resistant to B and VA, 63% to CF, 52% to E, 50% to AMP, 35% to STR, 20% to CEF and C,16% to SXT, 9% to TE, 7% to ZOX, 8% to AN, 2% to NN and 1% to GEN, respectively. Whereas none of them were resistant to MEM and FEP. (Table 4) The high degree of resistance to ampicillin (50%) in the present study was similar to the findings of other researchers (Matyar *et al*., 2008; Ozgumus *et al*., 2009; Vaseeharan *et al*., 2005). The findings of the present study is not consistent with previous reports of Koksal *et al.* (2007) and Matyar *et al.* (2008) where low level resistance to ampicillin, cefemine and meropenem were observed in bacterial strains. Recently, *E.coli* isolates from drinking water sources in jordan have been detected high resistance levels for ampicillin, trimethoprim/sulfamethoxazole, gentamicin, and tetracycline (Shehabi *et al*., 2006).

MAR index of isolates were determined from 0.062 to 0.687. When MAR index of 121 *Proteus vulgaris* and 69 *E.coli* isolates were analyzed, MAR index of 15% of both groups showed 0.5 and above value. Besides, 51 *Pseudomonas aeruginosa* and 28 *Citrobacter* spp. isolates showed between 0.5 and 0.687 value in 23% and 25% MAR index. The value of 0.5 - 0.687 in MAR index showed that fountain drinking water contained antibiotics coming from animal and human origins. In total 273 isolates identified from fountain drinking waters, 17% contained 0.5 - 0.687 MAR index. 187 isolates MAR index were determined 0.25 and above (Table 5). The value of 0.25 and above value in MAR index of isolates is very useful information about origin of contamination. If MAR index of isolate is above 0.2, the isolate are from human body or of veterinery origins.

Similarly, MAR for kanamycin, nalidixic acid, tetracycline, and trimethoprim have been shown in fecal coliforms in India (Pathak and Gopak, 2008). Our results are in general agreement with that showed by these researchers. Cernat *et al.* (2002) have showed that all aquatic R^+ strains they isolated from the river water transferred two or more antibiotic resistance markers (Cernat *et al*., 2002). The occurence of coliforms with high-level resistance to ampicillin and to other antibiotics has been proposed to reflect human affects in the environments (Andersen and Sanda, 1994).

Ozgumus *et al*. (2009) reported that antibiotic-resistant coliforms were widespread in water samples from ten rivers in northern Turkey (Ozgumus *et al*., 2009). Some researchers exhibited that drinking waters in public use in same region were contaminated with multiple antibiotic-resistant *E.coli*, and these water sources are supplied mainly from the local rivers after as chlorination (Alpay-Karaoglu *et al*., 2007; Ozgumus *et al*., 2007). Toroglu *et al*. (2005) reported that 40% of the coliforms isolated from Aksu river in Southern Turkey indicated multiple antibiotic resistance (MAR). But, Pathak and Gopak,2008) have been showed that coliforms were present in treated drinking water samples (Pathak and Gopak, 2008). Similarly, we detected the coliforms in fountain water samples.

In the present study, 22 isolates with MAR index ranging from 0.3 to 0.6 were identified for the presence of plasmid DNA (Fig. 1, 2). Plasmid profiles of isolates were detected by comparing the fragment size of marker (Vivantes, NM046). The molecular weights of plasmids from *Proteus* spp., were determined 3823 bp and 3193 bp (figure 1 line 10).

In the study, most of the isolates contained plasmid size 12215 bp to 20654. We determined only one plasmid in *Proteus* spp., that molecular weight was 20640 bp in line 3, only one plasmid in *Proteus* spp., that molecular weight was 19466 bp in line 4, only one plasmid in *Pseudomonas* spp., that molecular weight was 17254 bp in line 6, only one plasmid in *Proteus* spp., that molecular weight was 14020 bp in line 7, only one plasmid in *Proteus* spp., that molecular weight was 12215 bp in line 10, only one plasmid in *E. coli* that molecular weight was 20654 bp in line 12.

The findings of our study are consistent with the report of Tauxe's (1997) where in *E.coli* has been generally accepted as the predominant vehicle for the dissemination of resistance genes and vectors due to its abundance in environments. In addition to, Davies (1994) reported that plasmid and transposon-mediated resistance is widely transmitted between different bacterial species, and genera including human pathogens.

Our study conclude that multiple resistant *Proteus* spp. isolates, *Pseudomonas* spp., and *E.coli* isolate and plasmid containing multidrug resistant genes are present in the fountain water samples and the water may act as a possible source of transfer of these highly resistant pathogens and their genes to human and represent a significant microbial disease health risk. In conclusion, water-born microbial diseases can cause significant public health problems.

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