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ORIGINAL ARTICLE

Antibiotic and heavy metal resistance in Gram-negative bacteria isolated from the Seyhan Dam Lake and Seyhan River in Turkey

Fatih Matyar · Osman Gülnaz · Gulcihan Guzeldag · H. Aysun Mercimek · Sayim Akturk · Afet Arkut · Melis Sumengen

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Abstract This study aimed to determine the pattern of antibiotic and heavy metal resistance in Gram-negative bacteria isolated from five different sites in the Seyhan Dam Lake and Seyhan River in Adana, Turkey. The susceptibility of 268 isolates to 16 different antibiotics and five heavy metals was investigated by agar diffusion and dilution methods, respectively. The most common species isolated from the samples were *Aeromonas hydrophila* (17.5 %), *Aeromonas caviae* (8.9 %) and *Citrobacter freundii* (8.9 %). There was a high incidence of resistance to ampicillin (80.2 %), streptomycin (71.6 %) and cefazolin (60.4 %). Multiple antibiotic resistance indices ranged from 0.2 to 0.81, suggesting exposure to antibiotic contamination. The isolates showed tolerance to different concentrations of heavy metals. These results indicate that antibiotic and heavy metal resistance among the Seyhan Dam Lake and Seyhan River bacteria may pose a risk to the fish population and public health. At the same time, the finding in the aquatic environments of different combinations of resistance genes suggests their involvement in the spread of multidrug-resistant strains.

Keywords Seyhan Dam Lake · MAR index · Gram-negative bacteria · Heavy metal · Antibiotic resistance

F. Matyar (✉) · O. Gülnaz
Faculty of Education, Department of Science and Technology Education, Cukurova University, 01330 Saricam, Adana, Turkey
e-mail: fmatyar@cu.edu.tr

G. Guzeldag · H. A. Mercimek
Faculty of Arts and Science, Department of Molecular Biology and Genetics, Kilis 7 Aralık University, 79000 Kilis, Turkey

S. Akturk · A. Arkut · M. Sumengen
Faculty of Arts and Science, Department of Biology, Cukurova University, 01330 Saricam, Adana, Turkey

Introduction

Humans often adversely affect ecosystems including soil and, brackish and fresh waters. The use of antibiotics to control infectious diseases in humans and livestock is increasing steadily worldwide, and the uncontrolled release of antibiotics coupled with industrial pollution may cause an increase in antibiotic- and heavy-metal-resistant bacteria. In particular, hospitals discharge large quantities of untreated antibiotic waste into the environment, which has contributed to the emergence of bacteria with multiple antibiotic resistances and increased virulence (Matyar et al. 2010). These bacteria are able to spread their resistance genes to water-indigenous bacteria. Pathogenic bacteria associated with waterborne diseases include *Vibrio cholerae*, *Vibrio parahaemolyticus*, pathogenic *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Clostridium botulinum*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Legionella pneumophila*, *Helicobacter pylori*, and *Leptospira interrogans*. These bacteria have several ways of infecting humans, including by ingestion, inhalation or contact with a wound, and the World Health Organisation (WHO) estimates that 3.4 million people, mostly children, die from water-related diseases every year (Wilkes et al. 2009). The potential for developing of antibiotic-resistant bacteria has raised social concerns leading to extensive investigation of the influence of antibiotics on human and ecosystem health (Kim et al. 2011).

Aquatic environments may also be contaminated with toxic metals from agrochemicals, industrial wastewaters (e.g., paper and chlor-alkali industries) and gas and coal mining (Matlock et al. 2002). Toxic metals are hazardous because they accumulate through the food chain and cause environmental hazards (Rani and Mahedevan 1993). Heavy metals (Fe, Zn, Mn, Co, Cu, Ni, V, Mo) are essential micronutrients for bacteria because they are incorporated into enzymes and cofactors. However, they are toxic in high concentrations because they

bind to enzymes and DNA and produce oxygen radicals through the Fenton reaction (Lopez-Maury et al. 2002). Microbial metal resistance mechanisms include the precipitation of metals as phosphates, carbonates and sulphides; metal volatilization via methyl or ethyl group addition; physical exclusion by electronegative components in membranes and exopolymers; energy-dependent metal efflux systems; and intracellular sequestration with low molecular weight, cysteine-rich proteins (Silver 1998).

Various reports have suggested that antibiotic-and heavy-metal-resistant bacteria in water environments are increasing annually worldwide (Miranda and Castillo 1998; Matyar 2007; Gul-Seker and Mater 2009; Matyar 2012).

This study focused on the Seyhan Dam Lake and Seyhan River. The Seyhan River is 560 km long and has three hydroelectric power plants. One of them—the Seyhan Dam Lake—is an important fresh water source for Adana City. The lake has a depth of 53.20 m, a surface area of 67.82 km^2 and a volume of 1,200 hm^3. There are two slaughterhouses, a hospital and a sheep intestine-processing factory that discharge their wastes into the river. In addition, both sides of the river are impacted by agricultural activities that use different agrochemicals. Domestic and hospital wastes are also discharged into the river.

The present study aimed to investigate the level of microbial pollution in the Seyhan Dam Lake and along the Seyhan River and to identify Gram-negative isolates, determine their antibiotic and heavy-metal resistances, and assess their MAR (multiple antibiotic resistance) indices and the frequent association between heavy metals and antibiotic resistance.

Materials and methods

Sampling sites

Samples were collected from June to October 2011 from the Seyhan Dam Lake (Site 1) and along the Seyhan River (Sites 2–5). The geographic coordinates of the sampling sites were the following: Site 1: 35°16′21.60″E, 37°4′24.20″N; Site 2: 36°20′8.80″E, 36°58′48.60″N; Site 3: 35°19′46.45″ E, 36° 57′ 18.85″ N; Site 4: 35°1′39.90″E, 36° 45′26.65″N; and Site 5: 34°55′10.50″E, 36°44′02.13″N (Fig. 1). The water samples were collected 5–6 m away from the shore and 0–20 cm below the surface, using 250-mL sterile bottles (APHA 2005). A total of 81 samples was examined: (16, 17, 17, 15 and 16 samples from Sites 1, 2, 3, 4 and 5, respectively). All samples were brought to the laboratory in an ice chest, and processed within 3 h.

Bacteriological examination and culture conditions

The spread plate method was used for isolating the bacteria. Water sample (1 mL) was serially diluted in sterile water and

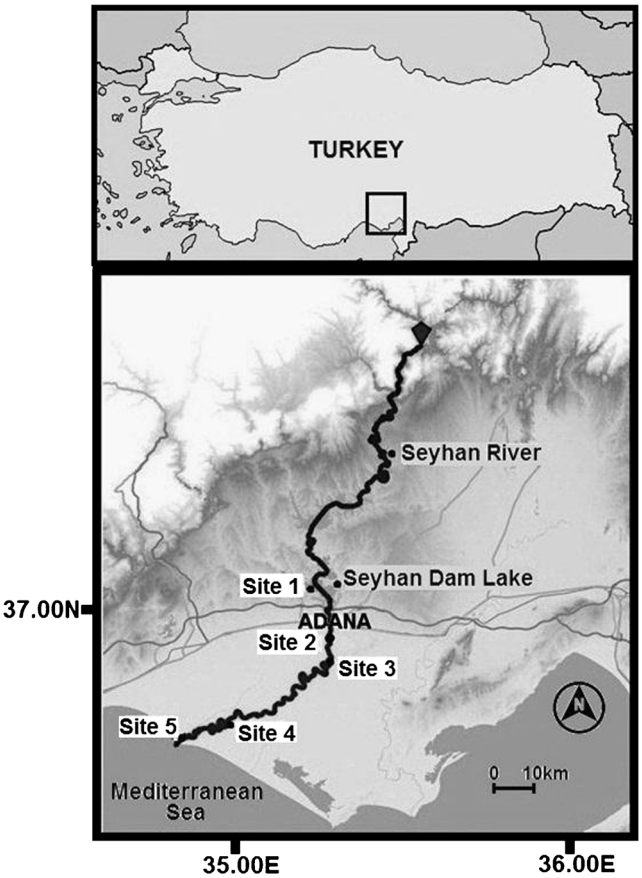

Fig. 1 Sampling sites

plated. The counts for the Gram-negative bacteria were made using MacConkey agar (McC) (Merck, Darmstadt, Germany). The bacteria were then inoculated with the appropriate dilution, and incubated for 24–72 h at 35°C. To evaluate the incidence of resistant bacteria, media supplemented with ampicillin (50 μg mL^{-1}), streptomycin (25 μg mL^{-1}), tetracycline (25 μg mL^{-1}) and chloramphenicol (25 μg mL^{-1}) were used (Table 1). A total of 268 Gram-negative bacterial isolates were selected randomly from the plates containing antibiotics: 36 isolates from Site 1, 63 from Site 2, 60 from Site 3, 54 from Site 4 and 55 from Site 5. The isolates were purified on McC agar and then maintained in nutrient agar (Oxoid, Merck). All isolates were characterized phenotypically, namely by Gram staining, oxidase and catalase reactions, motility, and glucose and gelatin liquefaction tests (Lemos et al. 1985). The isolates were then identified using Becton Dickinson Crystal E/NF identification software (BBL, Sparks, MD).

Antibiotic sensitivity test

Antibiotic sensitivity was tested using the disc diffusion method (NCCLS 2002). A total of 16 different antimicrobial agents were selected as representatives of the nine classes used in this study as shown below. Amikacin (AN, 30 μg), ampicillin

Table 1 Bacterial count on McConkey agar (McC). *CFU* Colony forming units

Site	Total viable count (CFU ml^{-1})	Resistant viable count (%)			
		Ampicillin (50 μg mL^{-1})	Chloramphenicol (25 μg mL^{-1})	Streptomycin (25 μg mL^{-1})	Tetracycline (25 μg mL^{-1})
1	1.0×10^3 ±149.9	6.3	11.4	5.6	1.6
2	2.4×10^3 ±86.2	13.9	18.5	16.6	7.1
3	1.3×10^5 ±387.6	9.5	50.1	7.2	10.7
4	3.5×10^4 ±278.9	8.3	18.3	5.3	3.0
5	7.0×10^4 ±355.9	7.5	31.4	11.4	5.1

(AM, 10 μg), nalidixic acid (NA, 30 μg), chloramphenicol (C, 30 μg), tetracycline (TE, 30 μg), nitrofurantoin (F/M, 300 μg), streptomycin (S, 10 μg), gentamicin (GM, 10 μg), imipenem (IPM, 10 μg), cefazolin (CZ, 30 μg), ceftizoxime (ZOX, 30 μg), meropenem (MEM, 10 μg), cefuroxime (CXM, 30 μg), cefepime (FEP, 30 μg), kanamycin (K, 30 μg) and trimethoprim-sulphamethoxazole (SXT, 1.25 and 23.75 μg) were used. For each strain tested, a standard inoculum was prepared by adjusting the bacterial suspension in LB broth (Merck) to a final optical density of 0.5 McFarland units.

The reference strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control organisms to verify the antibacterial effect of the discs, as recommended by the NCCLS (1997). All discs were purchased from Becton Dickinson; the susceptibility/resistance of the isolates was determined according to the information supplied by the manufacturer (BBL).

The MAR index was calculated as the ratio (a/b) between the number of antibiotics to which the isolate was resistant (a) and the total number of antibiotics tested (b). A MAR index value >0.2 is observed when the isolates are exposed to high risk sources of human or animal contamination, where antibiotics use is common; in contrast, a MAR index value ≤0.2 observed when antibiotics are seldom or never used (Krumperman 1983).

Determination of the minimal inhibitory concentration of heavy metals

The heavy metal resistance of the strains was determined using Mueller-Hinton agar (Merck) supplemented with various concentrations of five different heavy metals (Cd^{+2}, Cu^{+2}, Cr^{+3}, Pb^{+2} and Ni^{+2}). The metals used were $CdCl_2\cdot2H_2O$, $CuSO_4\cdot5H_2O$, $CrCl_3$, $Pb(NO_3)_2$, and $NiCl_2\cdot6H_2O$ (Merck), each in ten concentrations ranging from 12.5 μg mL^{-1} to >3, 200 μg mL^{-1}. The isolates were considered to be resistant if the minimal inhibitory concentration (MIC) value exceeded that of the control organism. *E. coli* K-12 strain was used as the control organism, as described by Akinbowale et al. (2007).

Results and discussion

Isolated bacteria

A total of 268 isolates was obtained (36, 63, 60, 54, and 55 from Sites 1, 2, 3, 4 and 5, respectively) representing 23 Gram-negative bacterial genera and 45 species (Table 2). Four species were found at a high frequency: *Aeromonas hydrophila* (17.5 %), *Aeromonas caviae* (8.9 %), *Citrobacter freundii* (8.9 %) and *Escherichia coli* (7.5 %). The most prevalent species was *A. hydrophila*, which represented 34.0 % of the isolates from Site 2 and 31.9 % of those from Site 3. Site 2 is near the hospital, and Site 3 is near the sheep intestine-processing factory. *Aeromonas hydrophila* is a pathogenic organism that has been isolated frequently from many sources, including freshwater habitats, throughout the world (Matyar et al. 2010). The high environmental prevalence of these bacteria should be regarded as an important threat to public health, as *Aeromonas* infections are generally acquired through the consumption of water and food (Borrell et al. 1998). The pathogenicity of *A. hydrophila* infection has been related to a number of possible virulence factors and different studies have reported a significant correlation between diarrhoeal diseases and the production of a variety of extracellular products, including enterotoxins, haemolysins and cytolytic proteins (Cumberbatch et al. 1979). The next most prevalent species were *Aeromonas caviae* and *Citrobacter freundii*. *Aeromonas caviae* was isolated frequently from Site 5 (79.2 %) near the agricultural area. *Citrobacter freundii* was isolated commonly from Site 1 (41.7 %). In the environment, *Citrobacter* is found commonly in the soil, water, food and intestinal tracts of animals and humans. In particular, *Citrobacter freundii* can cause a range of infections, such as urinary tract infection, neonatal sepsis, brain abscess, meningitis, bloodstream infection, intra-abdominal sepsis and pneumonia (Pepperell et al. 2002). The fourth most prevalent species, *E. coli*, a faecal bacteria, was isolated extensively (60.0 %) from Site 3. As indicated previously, Site 3 is located near the sheep intestine-processing factory, and significant agricultural activities also occur near this site. Although many strains of *E. coli* occur as commensal members of the

Table 2 Distribution of bacteria isolated from five different sites

Species	Source					No
	Site 1	Site 2	Site 3	Site 4	Site 5	
Acinetobacter baumannii				1	1	2
Acinetobacter lwoffi		14				14
Aeromonas caviae		5			19	24
Aeromonas hydrophila	1	16	15	6	9	47
Aeromonas sobria	4			14		18
Aeromonas veronii		2		1	1	4
Burkholderia cepacia		2		5		7
Burkholderia gladioli	1					1
Chromobacterium violaceum			1			1
Chryseobacterium meningosepticum			1			1
Citrobacter freundii	10	3	8		3	24
Citrobacter koseri					2	2
Edwardsiella hoshinae		2		1		3
Enterobacter aerogenes				2		2
Enterobacter asburiae					2	2
Enterobacter cancerogenus				3		3
Enterobacter cloacae	6	5		5		16
Escherichia coli		3	12		5	20
Flavimonas oryzihabitans	2			1		3
Klebsiella oxytoca	2	1		6	7	16
Klebsiella pneumoniae spp *ozaenae*				1		1
Klebsiella pneumoniae spp *pneumoniae*				5	2	7
Morganella morganii			2			2
Pantoea agglomerans	1	1		1		3
Pasteurella multocida						
Plesimonas shigelloides				1		1
Proteus vulgaris			1			1
Providencia alcalifaciens			4			4
Providencia rustigianii			1			1
Providencia stuartii			1			1
Pseudomonas aeruginosa	5					5
Pseudomonas fluorescens	1					1
Pseudomonas putida	2	1		1		4
Serratia ficaria			2			2
Serratia fonticola			2		1	3
Serratia liquefaciens			1			1
Serratia plymuthica					1	1
Shewanella putrefaciens			3			3
Shigella species			1			1
Stenotrophomonas maltophilia	1	2	1			4
Vibrio alginolyticus		1				1
Vibrio cholerae			1			1
Vibrio metschnikovii		1	1		2	4
Yersinia enterocolitica Group		1	2			3
Yersinia pestis		1				1
Yersinia pseudotuberculosis		2				2
Total	36	63	60	54	55	268

microbiota in the intestinal tracts of animals and humans, some strains are important pathogens that cause a wide spectrum of diseases, ranging from self-limiting to life-threatening intestinal and extra-intestinal illnesses (Kaper et al. 2004).

Antibiotic resistance patterns

The viable counts for the total Gram-negative bacteria and the resistant bacteria from Site 3 were higher than the other sites (Table 1). There are two slaughterhouses, and a sheep intestine processing factory near Site 3. These processing units discharged their untreated wastes directly into the river.

Among our isolates (Fig. 2), a high proportion was resistant to ampicillin (80.2 %), streptomycin (71.6 %), cefazolin (60.4 %) and trimethoprim-sulphamethoxazole (44.0 %). The high degree of resistance to ampicillin in the present study was similar to the findings of Vaseeharan et al. (2005). Cardonha et al. (2004) also found a high incidence of ampicillin, cephalothin and trimethoprim-sulfamethoxazole resistance in aquatic environments.

In the present work, the resistance trend against the different antibiotics was as follows: AM>S>CZ>SXT>TE>NA>GM>C>K>CXM>IPM>FM>MEM>ZOX>AN>FEP. This trend was in overall agreement with data reported by Matyar (2012) regarding the trend of resistance to 16 antibiotics of bacterial isolates from a coastal area of the Mediterranean Sea. Isolates from Site 2, which is located near the hospital, showed variable resistance to all of the tested antibiotics. Hospitals discharge large quantities of untreated antibiotic waste into the environment, which has contributed to the increase in bacteria, including pathogens, with multidrug resistance (Matyar et al. 2010). Among Site 2 isolates, a high percentage of bacteria were resistant to streptomycin (88.9 %), ampicillin (76.2 %), cefazolin (76.2 %) and trimethoprim-sulphamethoxazole (69.8 %), and a low percentage were resistant to cefepime (3.2 %), meropenem (6.3 %) and imipenem (9.5 %), while Cardonha et al. (2004) found uniform sensitivity to gentamicin and imipenem among the bacteria recovered from similar samples.

Multiple antibiotic resistance index

If the bacterial isolates were resistant to four or more antibiotics, they were designated as multi antibiotic resistant (MAR). Overall 34.5 % of the bacteria from Site 5 has a MAR index ranging from 0.25 to 0.31. Among the bacteria isolated from Site 3, 16.7 % were resistant to four and 10.0 % were resistant to five antibiotics. Similar resistance was found for Site 1 and Site 2 isolates to four antibiotics (22.2 % and 19.0 %, respectively). Isolates from Site 2 showed resistance to 10, 12 and 13 antibiotics with a frequency of 1.6 % for all three conditions; none of the isolates from sites 1, 3, 4 and 5 were resistant to ≥9 antibiotics (Fig. 3). The species with the highest MAR index were *Aeromonas hydrophila*, *Aeromonas caviae*, *Citrobacter freundii* and *Escherichia coli*. Fernàndez-Delgado and Suàrez (2009) found enteric bacteria isolates from the Caribbean Sea that were resistant to 5 or more of the 20 antibiotics tested. In addition, Pathak and Gopal (2007) found *E. coli* isolates from glacial water resistant to seven of ten antibiotics. Our findings are in agreement with these results.

The MAR index ranged from 0.25 to 0.44 for Site 1 and 4 isolates, from 0.25 to 0.5 for Site 3 and 5 isolates and from 0.25 to 0.81 for Site 2 isolates (Fig. 3). Similar results can be

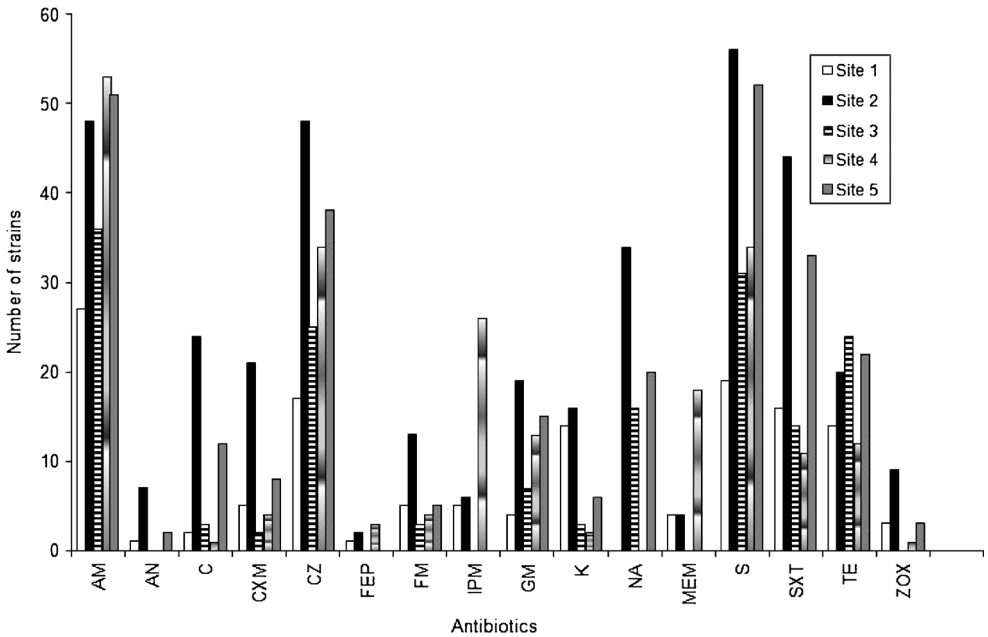

Fig. 2 Antibacterial resistance of bacteria isolated from five different sites

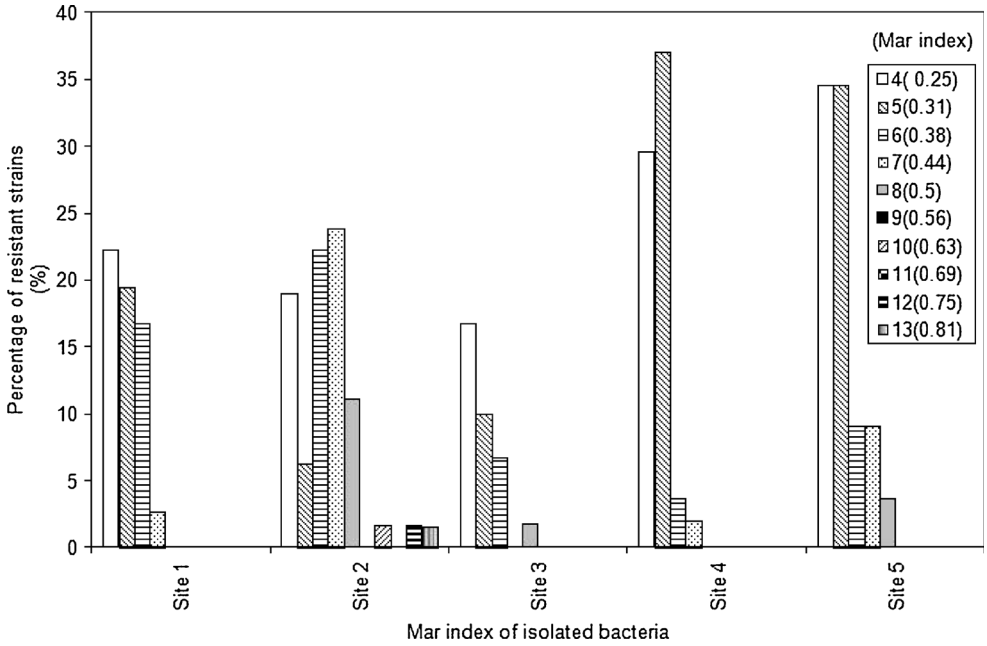

Fig 3 Multiple antibiotic resistance (MAR) index of isolated bacteria

found in the literature. For example, Lee et al. (2009) reported that the MAR index ranged from 0.44 to 0.67 for *Vibrio* spp. in their study performed in a giant freshwater prawn hatchery in Malaysia.

The high frequency of antibiotic-resistant bacteria raises questions regarding the origin of the resistant genes. In this respect, antibiotic-resistant fecal bacteria in domestic sewage discharged in to the Seyhan River might transfer their antibiotic resistance to the indigenous fish flora, provoking its spread and prevalence in the aquatic environment.

Multiple antimicrobial resistances may result partly from the spread of genetic elements, including plasmids, transposons, and integrons that may confer resistance to numerous antimicrobials (Obi et al. 2004). Aquatic environments may be incubators for new combinations of antibiotic resistances that can result in an increase in environmental multi-resistant bacteria. These types of gene combinations can lead to an increase in multidrug resistant bacteria in the above mentioned environments.

Heavy metal resistance

The susceptibility to five heavy metals (Cd^{+2}, Cr^{+3}, Cu^{+2}, Pb^{+2} and Ni^{+2}) at a range of concentrations was recorded for all the isolates (Table 3), revealing the resistance patterns associated with the different sampling sites: Site 1, Ni>Cd>Cu>Pb>Cr; Site 2, Cu>Cd=Ni>Pb>Cr; Site 3, Cu>Cr>Ni>Cd>Pb; Site 4, Cd>Ni>Cu>Cr>Pb; and Site 5, Cd>Cu>Ni>Pb>Cr. Gul-Seker and Mater (2009) investigated the effects of five heavy metals on marine bacteria and observed the following order of resistance for Marmara Sea and Black Sea isolates: Cd=Cu>Cr>Pb>Mn and Cd>Cu=Cr>Pb>Mn respectively. Altug and Balkis (2009) found resistance to seven heavy metals in the order Cu>Mn>Ni>Zn>Pb>Cd>Fe for sea water isolates; our findings from Site 3 were similar to these results.

Resistance to five heavy metals was as follows for Site 1, 2, 3, 4 and 5 isolates, respectively: cadmium, 44.4 %, 15.9 %, 53.3 %, 92.6 % and 100.0 %; chromium, 19.4 %, 1.6 %, 58.3 %, 14.8 % and 12.7 %; copper, 41.7 %, 25.4 %, 71.7 %, 33.3 % and 56.4 %; lead, 22.2 %, 11.1 %, 41.7 %, 11.1 % and 38.2 %; and nickel, 61.1 %, 15.9 %, 55.0 %, 57.4 % and 41.8 %. The minimal inhibitory concentrations (MIC) of the isolates ranged from 12.5 μg mL^{-1} to 3,200 μg mL^{-1}. Site 3 isolates showed a higher resistance to chromium, copper and lead than the others. The highest resistance to cadmium was found among Site 5 isolates (100 %), and the highest resistance to nickel was found among Site 1 isolates (61.1 %). The resistance to lead was similar between Site 2 and 4 isolates. Tolerance to the highest MIC (>3,200 μg mL^{-1}) of cadmium, copper and lead was found among Site 5 isolates and to chromium and nickel for Site 3 isolates. The isolates from Sites 3, 4 and 5 that the highest resistance to heavy metals were *Aeromonas hydrophila*, *Escherichia coli* and *Klebsiella oxytoca*, respectively. There are extensive agricultural activities near Sites 3, 4 and 5. The uncontrolled use of different agrochemicals, such as pesticides and herbicides, occurs around these sites.

The uncontrolled use of pesticides in agriculture alters various relationships among the different components of the natural environments. Aquatic microorganisms, bacteria in particular, play an important role in the environmental fate of pesticides. One property of pesticides and their metabolites, that may influence the induction or inhibition of enhanced

Table 3 Heavy metal tolerance in bacteria from five different sites

Metal/environment	Metal concentrations (μg/mL) with number of tolerant isolates											Resistant	
	No	12.5	25	50	100	200	400	800	1,600	3,200	>3,200	No	%
Cadmium					_[a]								
Site 1	36		4	10	6	15	1					16	44.4
Site 2	63		7	30	16	3	4			1	2	10	15.9
Site 3	60			1	27	20	10		1	1		32	53.3
Site 4	54			4		11	22	14	1	2		50	92.6
Site 5	55					3	30	13	2	1	6	55	100.0
Chromium								_[a]					
Site 1	36			5	12		9	3	1	4	2	7	19.4
Site 2	63			31	10	17	2	2		1		1	1.6
Site 3	60	2				1	3	19	11	10	14	35	58.3
Site 4	54		1	5	10	7	23		4	4		8	14.8
Site 5	55		1	15	2	9	21			4	3	7	12.7
Copper						_[a]							
Site 1	36				17	4	4	8	3			15	41.7
Site 2	63			3	26	18	5	10	1			16	25.4
Site 3	60	3			8	6	17	14	12			43	71.7
Site 4	54				19	17	9	6	3			18	33.3
Site 5	55				8	16	15		13	1	2	31	56.4
Lead									_[a]				
Site 1	36						3	12	13	4	4	8	22.2
Site 2	63						10	29	15	5	2	7	11.1
Site 3	60						1	11	23	18	7	25	41.7
Site 4	54					1	19	23	5	5	1	6	11.1
Site 5	55						2	18	14	7	14	21	38.2
Nickel	60						_[a]						
Site 1	36			2	2	4	6	16	2	1	3	22	61.1
Site 2	63				3	5	13	21	11	4	6	10	15.9
Site 3	60		3			2	22	14	10	2	7	33	55.0
Site 4	54				9	8	6	17	11	2	1	31	57.4
Site 5	55				2	27	3	7	11	3	2	23	41.8

[a] Minimal inhibition concentration of standard strain *Escherichia coli* K12

microbial degradation is their toxicity to soil and water microbes responsible for degradation (Somasundaram et al. 1990).

Interaction of metal resistance and antibiotic resistance

Metal-resistant isolates from Site 2 also showed a high resistance to four antibiotics: streptomycin, ampicillin, cefazolin and trimethoprim-sulphamethoxazole. Similarly, Site 4 isolates that were metal resistant also showed a high resistance to four antibiotics: streptomycin, ampicillin, cefazolin and imipenem. Site 5 isolates that were metal resistant also showed a high resistance to four antibiotics: streptomycin, ampicillin, cefazolin and trimethoprim-sulphamethoxazole. In contrast, heavy-metal resistant Site 1 isolates showed resistance to only two antibiotics: ampicillin and streptomycin. Site 3 isolates that were metal resistant were also resistant to ampicillin. Bacterial isolates resistant to the heavy metals tested showed a high frequency of resistance to streptomycin and ampicillin. Metal and antibiotic resistances are the most common features that bacteria gain due to the bio-essentiality or abuse of metals and/or antibiotics (De Sousa et al. 2006). Recent studies (Alonso et al. 2001; Summers 2002; Matyar et al. 2008) have suggested that the presence of metal contamination in natural environments could play a role in the maintenance and proliferation of antibiotic resistance.

This study investigated the antibiotic and heavy metal resistance patterns of Gram-negative bacteria in the Seyhan

Dam Lake and Seyhan River. The results showed that the Seyhan River water sources were of poor microbiological quality and the bacterial isolates had gained resistance to several antibiotic agents. In addition, the increasing numbers of heavy-metal-resistant bacteria could be the result of agrochemical and industrial pollution.

In conclusion, we show that, in the aquatic environments analyzed, which were impacted heavily by human activities, antibiotic- and heavy-metal-resistant bacteria can be found, constituting a risk to public health. Moreover, they might represent a reservoir of multiresistant genetic elements carrying new associations of resistance genes.

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