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Aeromonas and *Pseudomonas*: antibiotic and heavy metal resistance species from Iskenderun Bay, Turkey (northeast Mediterranean Sea)

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Abstract We studied the susceptibility patterns to 15 different antibiotics and six heavy metals in *Aeromonas* spp. and *Pseudomonas* spp. isolated from Iskenderun Bay, Turkey (northeast Mediterranean Sea). A high percentage of *Aeromonas* isolates showed resistance to cefazolin (66.6%) and trimethoprim–sulphamethoxazole (66.6%). Amongst the *Pseudomonas* isolates, there was a high incidence of resistance to nitrofurantoin (86.2%), cefazolin (84.8%) and cefuroxime (71.7%). Most isolates showed tolerance to different concentrations of heavy metals, and minimal inhibition concentrations ranged from 25 to >3,200 μg/ml. The *Aeromonas* spp. and *Pseudomonas* spp. showed high resistance to copper of 98.3% and 75.4%, respectively, and low resistance to lead of 1.7% and 7.2%, respectively. Our results show that antibiotic and heavy metal resistant *Aeromonas* spp. and *Pseudomonas* spp. were widespread in Iskenderun Bay in 2007 and 2008. The increasing presence of antibiotic and heavy metal resistant *Aeromonas* spp. and *Pseudomonas* spp. may become a potential human health hazard.

Keywords *Aeromonas* · *Pseudomonas* · Antibiotic resistance · Heavy metal resistance · Human health

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Introduction

Aquatic bacteria that are resistant to multiple antibiotics are of great importance in many areas of the world. Many aquatic bacteria are responsible for various types of serious diseases; for this reason, they have been the focus of numerous studies (Calomiris et al. 1984; Messi et al. 2005; Lobova et al. 2008; Matyar et al. 2008). Hospitals discharge large quantities of untreated antibiotic wastes in to the environment, which has lead to an increase in bacteria having multiple antibiotic resistances (MARs) and to an increase in more virulent pathogens.

Aeromonas spp., autochthonous inhabitants of aquatic environments, are widespread in natural habitats such as soil, fresh and brackish water, sewage and wastewater (Araoju et al. 1991). These bacteria cause both gastrointestinal and, to a lesser extent, extra-intestinal infections in humans (Janda and Abbott 1998).

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Amongst the 14 species of *Aeromonas* known to date (Abbott et al. 2003), *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas veronii* biotype *sobria* have most commonly known in human infections and produce a variety of virulence factors such as haemolysins, cytotoxins, enterotoxins, proteases, leukocidin, phospholipases, endotoxins, outer membrane proteins and fimbriae (Chopra and Houston 1999). Pseudomonads are a ubiquitous group of environmental Gram-negative bacteria, amongst which *Pseudomonas aeruginosa* is the third most common pathogen responsible for hospital-acquired (nosocomial) infections (Schaberg et al. 1991).

Fluvial waters receive human and animal wastewater discharges, which are expected to contain anti-microbial agents likely to exert a selective pressure and commensally resistant bacteria capable of transferring their resistances to autochthonous bacteria (Goñi-Urriza et al. 2000). Although the antibiotic sensitivity of food and clinical isolates of *Aeromonas* spp. have been extensively studied (Monteil et al. 2004; Demerta et al. 2004; Palú et al. 2006; Wu et al. 2007), there is limited information about the presence of aeromonads in the marine environment.

Industrial and agricultural activities have led to substantial release of toxic metals in the environment, which can constitute a major hazard for ecosystem as well as human health (Nriagu and Pacyna 1988). In many aquatic systems, trace metals are significant contaminants, due in part to anthropogenic sources such as industry and mines inputs. Coal-fired power plants generate large quantities of ash through combustion, and this waste contains residues of multiple trace metals such as arsenic and selenium. The ash residue mixed with the water before being deposited in settling basins and then discharge into aquatic system (Rowe et al. 2002).

Ecological studies have reported that antibiotic and metal resistances are becoming a global importance (Hassen et al. 1998; Benka-Coker and Ekundayo 1998; Ansari and Malik 2007). Plasmids are known to carry resistance to antibiotics and heavy metals (Smith et al. 1993; Sobecky 1999). Marine bacteria adsorb, accumulate and transform heavy metals in the most food chains (Chan and Dean 1988).

We focused our present studies on Iskenderun Bay on the south coast of Turkey, lying in northern east of Mediterranean Sea (36°38′ E, 36°05′ N). Domestic wastes, including hospital wastes, are discharge into the bay. In addition, factories, like iron and steel, a fertiliser factory, a refinery and a coal-fired power plant discharge a high amount of processed or unprocessed wastes into the bay. Despite these environmental pressures, the bay is an important region for fishing and aquaculture. Recent studies have shown that there are high levels of accumulated heavy metals in fish from this bay (Türkmen et al. 2006; Atli and Canli 2007).

To the best of our knowledge, this is the first research determining the presence as well as resistance to antibiotics and heavy metals of *Aeromonas* spp. and *Pseudomonas* spp. isolated from Iskenderun Bay. The specific aims of this study were (a) to identify the *Aeromonas* spp. and *Pseudomonas* spp. strains recovered in Iskenderun Bay, (b) to determine the level of antibiotic resistance rates against widely used antibiotics in Turkey, (c) to determine the heavy metal resistance of the bacteria and (d) to investigate relationship between the antibiotic and heavy metal resistance (if present).

Materials and methods

Sampling

Our studies were conducted in three different sites along the southeast coast of Iskenderun Bay, Turkey which is located in the northeast of the Mediterranean Sea (Fig. 1). The geographic coordinates of the sampling sites were 1:35°56′ E, 36°28′ N; 2:36°11′ E, 36°36′ N and 3:36°10′ E, 36°42′ N.

Water samples (250 ml) were collected from March 2007 to February 2008. Samples were collected into sterile bacteriological sample bottles and brought to the laboratory in an ice chest (APHA 1992). The samples were processed within 4 h of collection. A total of 40 samples (13, 12 and 15 from sites 1, 2 and 3, respectively) were examined for the presence of *Aeromonas* spp. and *Pseudomonas* spp.

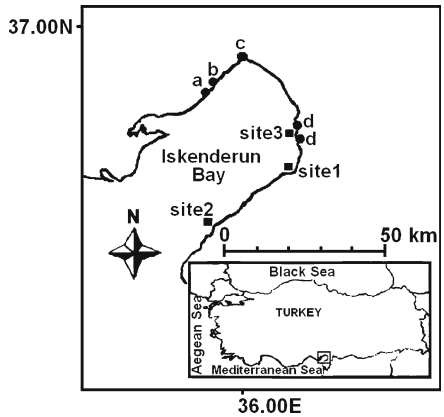

Fig. 1 Map of Turkey and Iskenderun Bay showing the three different sampling sites; *a* coal-fired power plant, *b* oil refinery, *c* fertiliser factory, *d* iron-steel factory

Bacterial isolation and identification

For isolation of *Aeromonas* and *Pseudomonas* spp., 25 ml of each water samples were inoculated in 225 ml alkaline pepton water (pH 8.6) with 1% of NaCl (*w*/*v*) and incubated at 30°C for 24 h. The samples were then plated onto selective media (ampicillin dextrin agar (ADA) medium and *Pseudomonas* F agar (Becton and Dickinson, Sparks Glencoe, MD, USA)) and then incubated at 30°C for 24–72 h. The ADA medium was freshly prepared having the following composition (grams per liter) according to Havelaar et al. (1987): tryptose (Difco), 5; dextrin (Merck), 10; yeast extract (Merck), 2; sodium chloride (Merck), 3; potassium chloride (Merck), 2; magnesium sulphate.$7H_2O$ (Merck), 0.2; ferric chloride.$6H_2O$ (Merck), 0.1; sodium desoxycholate (Merck), 0.1; bromothymol blue (Merck), 0.04; agar (Oxoid), 15 and ampicillin (ICN Biomedicals), 0.01, with pH of 8.0.

Presumptive *Aeromonas* spp. isolates were obtained from ADA medium by colony morphology (yellow, circular, convex, 1–3 mm diameter colonies). The genus *Aeromonas* was identified based on the findings of Gram-negative rods, positive oxidase test, fermentation of D-glucose, motile and the absence of growth in 6.5% sodium chloride (Ko et al. 2000).

Isolates were considered to be presumptive *Pseudomonas* spp. if they were Gram-negative rods that produced fluorescent colonies on *Pseudomonas* F agar, were oxidase- and catalase-positive and were glucose oxidative and positive for citrate. Presumptive *Aeromonas* and *Pseudomonas* colonies were subcultured on tryptone soy agar (Oxoid) at 30°C for 24 h to obtain at least two consecutive pure cultures. Identity was confirmed by using the Becton and Dickinson Crystal E/NF ID system (BBL, Cockeysville, MD, USA). These strains were identified by using the E/NF identification software (BBL, Cockeysville, MD, USA). A number of isolates (20%) were reexamined to check the test reproducibility.

Test for antibiotic resistance

Antibiotic resistance was determined by an agar diffusion test (NCCLS 1997) using Mueller–Hinton agar (Difco). Fifteen different antibiotics (representing eight classes) were used. The antibiotics tested and their sensidisk concentrations were amikacin (AN; 30 μg), streptomycin (S; 10 μg), gentamicin (GM; 10 μg), kanamycin (K; 30 μg), imipenem (IPM; 10 μg), meropenem (MEM; 10 μg), cefazolin (CZ; 30 μg), ceftizoxime (ZOX; 30 μg), cefuroxime (CXM; 30 μg), cefepime (FEP; 30 μg), chloramphenicol (C; 30 μg), nitrofurantoin (F/M; 300 μg), nalidixic acid (NA; 30 μg), tetracycline (TE; 30 μg) and trimethoprim–sulphamethoxazole (SXT; 1.25 and 23.75 μg). Overnight nutrient broth cultures of the test strains were used and the turbidity of the inoculum as adjusted in phosphate-buffered saline (pH 7.4) to a 0.5 McFarland opacity standard (Becton and Dickinson). These cultures were then streaked onto Mueller–Hinton agar plates (Difco) using a sterile cotton swab. The antibiotic discs were dispensed using a disc dispenser (Becton and Dickinson) sufficiently separated from each other so as to avoid overlapping of inhibition zones. After 30 min, the plates were inverted and incubated at 37°C for 16–18 h. The isolates were considered sensitive according to the manufacturer's instructions.

Reference strains of *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, as recommended by NCCLS (1997), were used as control organisms for verification of the anti-bacterial effect of the discs on Mueller–Hinton agar plates.

All discs were purchased from Becton and Dickinson.

For all isolates, we calculated the MAR index values (a/b, where a represents the number of antibiotics the isolate was resistant to and b represents the total number of antibiotics the isolate was tested against). A MAR index value >0.2 is observed when isolates are exposed to a high risk sources of human or animal contamination, where antibiotics use is common. In contrast, a MAR index value ≤0.2 is observed when antibiotics are seldom or never used (Krumperman 1983).

Determination of the MIC of heavy metals

The minimal inhibitory concentration (MIC) of each isolate was determined by the plate dilution method (Summers and Silver 1972). Six different heavy metals (Cd^{+2}, Cu^{+2}, Cr^{+3}, Pb^{+2}, Mn^{+2} and Zn^{+2}) were used as $CdCl_2.2H_2O$, $CuSO_4.5H_2O$, $CrCl_3$, Pb $(NO_3)_2$, $MnCl_2.2H_2O$ and $ZnCl_2$ (Merck), each in ten concentrations ranging from 12.5 to >3,200 μg/ml and added to Mueller–Hinton agar (Difco). The isolates were considered resistant if the MIC values exceeded that of the control organism. *E. coli* K-12 strain was used as the control organism as described by Malik and Jaiswal (2000) and Akinbowale et al. (2007).

Results and discussion

Aeromonas and *Pseudomonas* species and isolates

A total of 198 isolates were obtained, representing two *Aeromonas* species and their 60 isolates and six *Pseudomonas* species with their 138 isolates (Table 1). The frequency of the isolates varied markedly between sites, with most *Aeromonas* isolates recorded from site 1 and most of the *Pseudomonas* isolates recorded from sites 2 and 3 (Table 1).

Species characterisation revealed that amongst the *Aeromonas* spp., *A. hydrophila* was the dominant species (95.0%) amongst three sampling sites accounting for 57 of the 60 *Aeromonas* isolates; only three isolates of *A. caviae* were found. This agree with the results of Havelaar et al. (1990) from The Netherlands, which reported that *A. hydrophila* was the most frequently isolated species from distribution waters, whereas *A. caviae* and *A. sobria* were predominant in only a few water systems. *A. hydrophila* is a ubiquitous and opportunistic pathogen, which is part of the normal microbial flora of aquatic animals including fish, and *A. hydrophila* strains producing cytotoxins, proteases and aerolysin were commonly isolated from both healthy and moribund fish (Cahill 1990). Sewage and effluents from domestic water purification plants have been suspected as vehicles for transmission of pathogenic aeromonads in a number of clinical cases (Monfort and Baleux 1991).

Table 1 Numbers of *Aeromonas* spp. and *Pseudomonas* spp. isolated from three sites in Iskenderun Bay, Turkey (northeast Mediterranean Sea)

Species	Sources			Total
	Site 1	Site 2	Site 3	
Aeromonas				
A. hydrophila	39	7	11	57
A. caviae	–	–	3	3
Pseudomonas				
P. aeruginosa	8	4	16	28
P. cepacia	–	1	–	1
P. diminuta	–	1	–	1
P. fluorescens	6	25	3	34
P. putida	–	27	3	30
P. stutzeri	11	4	29	44
Total	64	69	65	198

In our study, amongst the *Pseudomonas* isolates, *Pseudomonas stutzeri* was the most prevalent species (31.9%), followed by *Pseudomonas fluorescens* (24.6%), *Pseudomonas putida* (21.7%) and *P. aeruginosa* (20.3%) (Table 1); only single isolates of *P. cepacia* and *P. diminuta*, were recorded.

Pseudomonas stutzeri is distributed widely in the environment, occupying diverse ecological niches, and has also been isolated as an opportunistic pathogen from humans (Lalucat et al. 2006). The second most prevalent *Pseudomonas*, *P. fluorescens*, is an emerging pathogen closely related to *P. aeruginosa*. *P. fluorescens* produces

a cholin-esterase (Rochu et al. 1998), and some strains possess a high affinity γ-aminobutyric acid binding protein (Guthrie et al. 2000) that could adversely affect neurotransmission.

The third most prevalent *Pseudomonas*, *P. putida*, is a ubiquitous aerobic Gram-negative bacillus. It can be found in aquatic environments, in soils, on vegetation and even on some animals. It is widespread in the domestic and hospital environments, particularly in sites related to water and plumbing, and has been reported from patients with cystic fibrosis.

Kueh et al. (1992) investigated the possibility of wound infection with sewage-related organisms following exposure to contaminated sea water. These authors found that *A. hydrophila*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Vibrio cholerae* were amongst the bacteria able to cause different kind of infections, and they further observed that *Vibrionaceae* were present in coastal waters, even at unpolluted sites.

Antibiotic resistance patterns

In Iskenderun Bay, a high proportion of *Aeromonas* and *Pseudomonas* isolates were resistant to different anti-bacterial agents, although resistance to aminoglycosides, carbapenems and fourth-generation cephalosporins was relatively infrequent. *Aeromonas* isolates showed a high percentage resistance to cefazolin (66.6%) and trimethoprim–sulphamethoxazole (66.6%). A low percentage of *Aeromonas* isolates were resistant to amikacin (3.3%), gentamicin (13.3%), chloramphenicol (13.3%) and nalidixic acid (13.3%; Table 2). Ko et al. (1996) found that a high proportion of *Aeromonas* were resistant to trimethoprim–sulfamethoxazole and tetracycline.

There were some marked differences in the degree of antibiotic resistance between sites (Table 2). For *Aeromonas* strains, all from site 1 were susceptible to amikacin, gentamicin and chloramphenicol; all from site 2 were susceptible to amikacin, imipenem and tetracycline, whereas those from site 3 showed variable resistance to the antibiotics tested in this study. *Aeromonas* isolates from site 3 showed higher resistance to cefazolin (85.7%) than did those from site 1 (59%) and site 2 (71.4%); this may be because site 3 is nearer the settlement and industry area than are zones 1 and 2 (Fig. 1).

Recent studies have demonstrated that the presence of *Aeromonas* spp. in drinking water is a potential risk, since some species can produce a wide range of virulence factors (Warburton et al. 1994; Kühn et al. 1997; Brandi et al. 1999). A further concern is the increasing incidence of multidrug resistance amongst *Aeromonas* spp. isolates that has been observed worldwide (Petersen and Dalsgaard 2003; Ottoviani et al. 2006; Matyar et al. 2007). Besides their potential pathogenicity to humans, many *Aeromonas* species have also been described as important fish pathogens (Hänninen et al. 1997; Doukas et al. 1998).

Amongst the *Pseudomonas* isolates in our study, a high percentage were resistant to nitrofurantoin (86.2%), cefazolin (84.8%) and cefuroxime (71.7%), and a low percentage were resistant to kanamycin (5.8%), meropenem (7.2%) cefepime (8.7%), amikacin (12.3%) and imipenem (16.7%). Resistance to imipenem was similar to the results of Blandino et al. (2004). Susceptibility to aminoglycosides and carbapenems was reported previously by Sader and Jones (2005) and is similar to our results. From the site 1, none of the *Pseudomonas* isolates was found resistant to amikacin.

Multiple antibiotic resistance index

The MAR index values ranged from 0.2 to 0.60 for *Aeromonas* strains and from 0.2 to 0.73 for the *Pseudomonas* strains (Fig. 2). A significant proportion of *Aeromonas* strains isolated from site 1 were resistant to seven antibiotics (30.8%), a significant proportion of *Pseudomonas* strains from site 2 were resistant to five antibiotics (42.5%) and a significant proportion of the *Pseudomonas* strains isolated from site 3 were resistant to six antibiotics (31.4%). A variety of profiles, involving resistance to tetracycline and chloramphenicol and other antibiotics, have been described by Radu et al. (2003), who detected multiple resistance of *Aeromonas* spp.

There was a similar level of resistance to nine antibiotics in *Aeromonas* strains (3.3%)

Table 2 Percentage of resistant isolates of *Aeromonas* spp. and *Pseudomonas* spp. from three sites in Iskenderun Bay, Turkey (northeast Mediterranean Sea) to 15 antibiotics belonging to the eight classes

Classes of antibiotics	Antibiotics	Site 1		Site 2		Site 3		Total *Aeromonas* spp. ($N = 60$)	Total *Pseudomonas* spp. ($N = 138$)
		Aeromonas spp. ($N = 39$)	*Pseudomonas* spp. ($N = 25$)	*Aeromonas* spp. ($N = 7$)	*Pseudomonas* spp. ($N = 62$)	*Aeromonas* ($N = 14$)	*Pseudomonas* spp. ($N = 51$)		
		Percentage of resistant isolates							
Aminoglycosides	AN (30 μg)	–	–	–	6.4	14.3	23.5	3.3	12.3
	S (10 μg)	2.5	12	42.8	40.3	35.7	43.1	15.0	36.2
	GM (10 μg)	–	8	42.8	12.9	35.7	33.3	13.3	19.6
	K (30 μg)	23	16	14.2	1.6	14.2	5.8	20.0	5.8
Carbapenems	IPM (10 μg)	56.4	16	–	8	14.2	27.4	40.0	16.7
	MEM (10 μg)	51.2	12	14.2	4.8	21.4	7.8	40.0	7.2
Cephalosporins	CZ (30 μg)	59	68	71.4	90.3	85.7	86.2	66.6	84.8
	ZOX (30 μg)	61.5	28	14.2	1.6	35.7	17.6	50.0	12.3
	CXM (30 μg)	56.4	64	71.4	80.6	42.8	64.7	55.0	71.7
	FEP (30 μg)	53.8	28	42.8	3.2	14.2	5.8	43.3	8.7
Chloramphenicol	C (30 μg)	–	52	42.8	67.7	35.7	54.8	13.3	60.8
Nitrofurantoin	F/M (300 μg)	5.1	80	71.4	95.1	50	78.4	23.3	86.2
Quinolones	NA (30 μg)	5.1	8	14.2	25.8	35.7	37.2	13.3	26.8
Tetracyclines	TE (30 μg)	5.1	60	–	40.3	21.4	39.2	15.0	43.5
Trimethoprim–sulphamethoxazole	SXT (1.25 and 23.75 μg)	76.9	72	42.8	77.4	50	49	66.6	65.9

Number of isolates is shown at the top each column

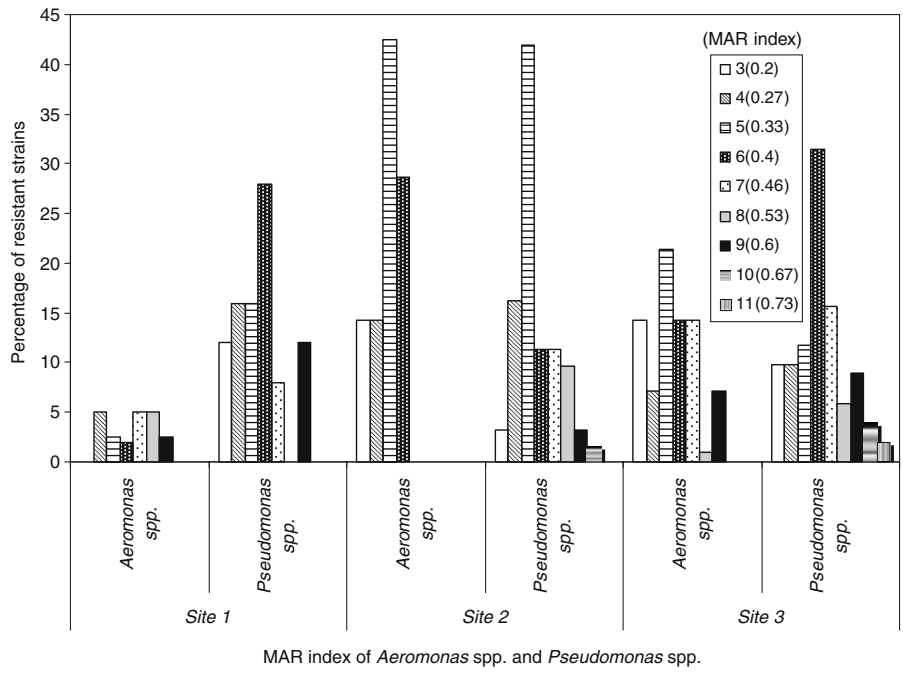

Fig. 2 Anti-bacterial multi-resistance of *Aeromonas* spp. and *Pseudomonas* spp. isolated from three different sites in Iskenderun Bay, Turkey

and *Pseudomonas* strains (5.0%). A high multi-resistance incidence, similar to or higher than those found in the present study, has been reported in aquatic environments and fish isolates (Vivekanadhan et al. 2002; Matyar et al. 2007).

According to Anderson and Levin (1999), the frequency and rates of ascent and dissemination of antibiotic resistance in bacterial populations can be directly related to the applied compounds. De Vicente et al. (1990) reported that the number of strains multi-resistant to several antibiotics was high in seawater samples containing low levels of fecal indicators. A high incidence of antibiotic resistance in *Pseudomonas* spp. was reported by Jones et al. (1986) for lake waters.

Resistance to heavy metals

Trends in heavy metal resistance varied depending on the isolates for *Aeromonas*, Cu > Mn > Cr > Cd = Zn > Pb (Table 3) and for *Pseudomonas*, Cu > Cd > Mn > Zn > Cr > Pb (Table 4). Other studies have shown the following order of resistance for *Aeromonas* isolates, Cd > Cu > Hg > Cr (Miranda and Castillo 1998) and for *Pseudomonas* and *Micrococcus* isolates Mn > Cu > Pb > Zn > (Benka-Coker and Ekundayo 1998). In the present study in Iskenderun Bay, resistance to six heavy metals was as follows: for the *Aeromonas* and *Pseudomonas* isolates to cadmium, 35.0% and 56.5%; copper, 98.3% and 75.4%; chromium, 38.3% and 31.9%; lead, 1.7% and 7.2%; manganese, 43.3% and 44.9% and zinc 35.0% and 41.3%, respectively (Tables 3 and 4).

The MICs of the isolates ranged from 25 to >3,200 μg/ml. The *Aeromonas* isolates showed a higher resistance to copper and chromium than did the *Pseudomonas* isolates, and the *Pseudomonas* isolates showed a higher resistance to cadmium, lead, manganese and zinc than did the *Aeromonas* isolates. Tolerance to the maximum MIC (>3,200 μg/ml) for copper was 2.1% for *Aeromonas* and 1.6% for *Pseudomonas* isolates. *Aeromonas* strains from site 3 showed higher resistance to chromium, lead and manganese than did the *Aeromonas* strains from sites 1 and 2. Similarly, the *Pseudomonas* strains from site 3 showed higher resistance to chromium, lead, manganese and zinc than did *Pseudomonas* strains from sites 1 and 2. A possible explanation for these differences is that site 3 is nearer to the iron-steel factories than are sites 1 and 2 (Fig. 1). Higher proportion of metal resistance detected in the *Aeromonas* and *Pseudomonas* strains from site 3

Table 3 Heavy metal tolerance in *Aeromonas* spp. from three sites in Iskenderun Bay, Turkey (northeast Mediterranean Sea)

Heavy metal	Site of isolates	Number of total isolates	Metal concentrations (μg/ml) with number of tolerant isolates										Resistant isolates	
			12.5	25	50	100	200	400	800	1,600	3,200	>3,200	*n*	%
Cadmium						a								
Aeromonas	Site 1	39			11	15	2	11					13	33.3
Aeromonas	Site 2	7				1		4	1	1			6	85.7
Aeromonas	Site 3	14		2	9	1	2						2	14.3
Total		60		2	20	17	4	15	1	1			21	35.0
Copper							a							
Aeromonas	Site 1	39						39					39	100.0
Aeromonas	Site 2	7					1		1		5		6	85.7
Aeromonas	Site 3	14								1	12	1	14	100.0
Total		60					1	39	1	1	17	1	59	98.3
Chromium									a					
Aeromonas	Site 1	39							29	10			10	25.6
Aeromonas	Site 2	7					1	4	2				–	–
Aeromonas	Site 3	14							1	2	11		13	92.8
Total		60					1	4	32	12	11		23	38.3
Lead										a				
Aeromonas	Site 1	39							12	27			–	–
Aeromonas	Site 2	7							4	3			–	–
Aeromonas	Site 3	14						1	8	4	1		1	7.1
Total		60						1	24	34	1		1	1.7
Manganese										a				
Aeromonas	Site 1	39						7	14	4	14		14	35.9
Aeromonas	Site 2	7							4	1	2		2	14.3
Aeromonas	Site 3	14					1	2		1	10		10	71.4
Total		60					1	9	18	6	26		26	43.3
Zinc							a							
Aeromonas	Site 1	39				12	9	3	15				18	46.1
Aeromonas	Site 2	7				4		2			1		3	42.8
Aeromonas	Site 3	14			1	6	7						–	–
Total		60			1	22	16	5	15		1		21	35.0

a minimal inhibition concentration of standard strain *E. coli* K12, *n* total number of tolerant isolates

could be the result of heavy metal contamination from iron-steel factories. Both Gram-positive and Gram-negative bacteria can resist heavy metals (Silver and Walderhaug 1992). Resistance of toxic metals in bacteria probably reflects the degree of environmental contamination with these substances and may be directly related to exposure of bacteria to them (Aiking et al. 1984).

Natural ecosystems containing high concentrations of heavy metals are also frequent. Not surprisingly, heavy metal resistance genes are commonly found in environmental bacteria (Silver and Phung 1996).

The present study is one of only a few that addresses the incidence of antibiotic and heavy metal resistance in *Aeromonas* spp. and *Pseudomonas* spp. With all six of the metals tested, there was a high frequency of cefazolin resistance amongst all the isolates.

The *Aeromonas* strains which were metal resistant also showed a high resistance to antibiotics as follows: from site 1, high resistance to cefazolin, cefuroxime and trimethoprim–sulphamethoxazole; from site 2, high resistance to cefazolin and cefuroxime and from site 3, high resistance to cefazolin. The *Pseudomonas*

Table 4 Heavy metal tolerance in *Pseudomonas* spp. from three sites in Iskenderun Bay, Turkey (northeast Mediterranean Sea)

Heavy metal	Site of isolates	Number of total isolates	Metal concentrations (μg/ml) with number of tolerant isolates										Resistant isolates	
			12.5	25	50	100	200	400	800	1,600	3,200	>3,200	*n*	%
Cadmium						a								
Pseudomonas	Site 1	25		1	11	8	3	2					5	20.0
Pseudomonas	Site 2	62		3	8	8	11	22	5	5			43	69.3
Pseudomonas	Site 3	51		2	12	7	4	4	19	2	1		30	58.8
Total		138		6	31	23	18	28	24	7	1		78	56.5
Copper							a							
Pseudomonas	Site 1	25					2	17			6		23	92.0
Pseudomonas	Site 2	62					8	11	7	9	27		54	87.1
Pseudomonas	Site 3	51				3	21	1	9		14	3	27	52.9
Total		138				3	31	29	16	9	47	3	104	75.4
Chromium									a					
Pseudomonas	Site 1	25						13	12				–	–
Pseudomonas	Site 2	62				1	1	22	32	3	3		6	24.0
Pseudomonas	Site 3	51						2	11	20	18		38	74.5
Total		138				1	1	37	55	23	21	3	44	31.9
Lead										a				
Pseudomonas	Site 1	25			1		3	13		8			–	–
Pseudomonas	Site 2	62			1			23	15	20	3		3	4.8
Pseudomonas	Site 3	51					1	7	18	18	7		7	13.7
Total		138			2		4	43	33	46	10		10	7.2
Manganese										a				
Pseudomonas	Site 1	25					7	11	4	1	2		2	8.0
Pseudomonas	Site 2	62					1	8	14	10	29		29	46.8
Pseudomonas	Site 3	51						9	7	4	29	2	31	60.8
Total		138					8	28	25	15	60	2	62	44.9
Zinc							a							
Pseudomonas	Site 1	25			2		19	3	1				4	16.0
Pseudomonas	Site 2	62				7	34	10		4	7		21	33.9
Pseudomonas	Site 3	51				10	9	5	3	21	2	1	32	62.7
Total		138			2	17	62	18	4	25	9	1	57	41.3

a minimal inhibition concentration of standard strain *E. coli* K12, *n* total number of tolerant isolates

strains which were metal resistant also showed a high resistance to antibiotics as follows: from site 1, high resistance to cefazolin, cefuroxime, nitrofurantoin, tetracycline and trimethoprim–sulphamethoxazole; from site 2, showed a high resistance to five antibiotics: cefazolin, cefuroxime, chloramphenicol, nitrofurantoin and trimethoprim–sulphamethoxazole and from site 3, high resistance to four antibiotics: cefazolin, cefuroxime, chloramphenicol and nitrofurantoin.

Industrial activities, mining and intensive farming are causing dramatic changes in natural ecosystems. Amongst the novel selective pressures that face environmental bacterial populations are discharges of heavy metals, xenobiotic compounds, antibiotics and organic solvents, all of which can have a significant impact on the environmental selection of antibiotic resistance genes (Alanso et al. 2001). Some of these microorganisms that possess resistance genes could cause important diseases.

Conclusion and recommendations

The presence, antibiotic resistance and heavy metal resistance patterns of *Aeromonas* strains

and *Pseudomonas* strains in a marine environment at northeast of Mediterranean Sea were investigated. These results suggests that Iskenderun Bay, Turkey, is a reservoir for antibiotic and metal resistant *Aeromonas* and *Pseudomonas* strains and might be increasing the numbers of resistant bacteria in the Bay with possibilities of transferring of their resistance determinants to other pathogenic bacteria. Although Chang and Bolton (1987) found that plasmid-mediated antibiotic resistance in *Aeromonas* spp. was not frequent, McIntosh et al. (2008) have found an important incidence of R-plasmids in multiple antibiotic and mercury resistant *Aeromonas* isolates from Atlantic salmon. The study of Rhodes et al. (2000) provided evidence that related tetracycline-resistance encoding plasmids have been transferred between different *Aeromonas* species and *E. coli* and between the hospital and aquaculture environments in distinct geographical locations.

Thus, plasmid-mediated antibiotic and heavy metal resistance in *Aeromonas* spp. in the Mediterranean Sea should be further elucidated. Further works are needed to understand better metal and antibiotic resistant *Aeromonas* spp. and *Pseudomonas* spp. along with other parts of the Turkish coasts on the Mediterranean and Aegean Seas.

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References

Abbott, S. L., Cheung, W. K. W., & Janda, J. M. (2003). The genus *Aeromonas*: Biochemical characteristics, atypical reactions, and phenotypic identification schemes. *Journal Clinical Microbiology, 41*, 2348–2357.

Aiking, H., Stinamn, A., Van-Ganderen, C., Van-Heerikhuizen, H., & Vant-Riet, J. (1984). Inorganic phosphate accumulation and cadmium detoxification in *Klebsiella aerogenes* NCTC 418 growing in continuous culture. *Applied and Environmental Microbiology, 47*, 374–377.

Akinbowale, O. L., Peng, H., Grant, P., & Barton, M. D. (2007). Antibiotic and heavy metal resistance in motile aeromonads and pseudomonads from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. *International Journal of Antimicrobial Agents, 30*, 177–182.

Alanso, A., Sanchez, P., & Martinez, J. L. (2001). Environmental selection of antibiotic resistance genes. *Environental Microbiology, 3*, 1–9.

Anderson, D. I., & Levin, B. R. (1999). The biological cost of antibiotic resistance. *Current Opinion in Microbiology, 2*, 489–493.

Ansari, M. I., & Malik, A. (2007). Biosorption of nickel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial wastewater. *Bioresource Technology, 98*, 3149–3153.

APHA Microbial Examination (1992). In A. E. Greenberg, L. S. Clesceri, & A. D. Eaton (Eds.), *Standard methods for the examination of on water and wastewater* (18th ed.). Washington DC: American Public Health Association.

Araoju, R. M., Arribas, R. M., & Pares, R. (1991). Distribution of *Aeromonas* species in waters with different level of pollution. *Journal of Applied Bacteriology, 71*, 182–186.

Atli, G., & Canli, M. (2007). Natural occurrence of metallothionein-like proteins in liver tissues of fish species from the northeast Mediterranean Sea. *Water Environment Research, 9*, 958–963.

Benka-Coker, M. O., & Ekundayo, J. A. (1998). Effects of heavy metals on growth of species of *Micrococcus* and *Pseudomonas* in a crude oil/mineral salts medium. *Bioresource Technology, 66*, 241–245.

Blandino, G., Marchese, A., Ardito, F., Fadda, G., Fontana, R., Cascio, G. L., et al. (2004). Antimicrobial susceptibility profiles of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated in Italy from patients with hospital-acquired infections. *International Journal of Antimicrobial Agents, 24*, 515–518.

Brandi, G., Sisti, M., Giardini, F., Schiavano, G. F., & Albano, A. (1999). Survival ability of cytotoxic strains of motile *Aeromonas* spp. in different types of water. *Letters in Applied Microbiology, 29*, 211–215.

Cahill, M. M. (1990). A review, virulence factors in motile *Aeromonas* species. *Journal of Applied Bacteriology, 69*, 1–6.

Calomiris, J. J., Armstrong, J. L., & Ramon, J. S. (1984). Association of metal tolerance with multiple antibiotic resistances of bacteria isolated from drinking water. *Applied and Environmental Microbiology, 47*, 1238–1242.

Chan, K. Y., & Dean, A. C. R. (1988). Effects of cadmium and lead on growth, respiration and enzyme activity of the marine bacterium *Pseudomonas marina*. *Chemosphere, 17*, 597–607.

Chang, B. J., & Bolton, S. M. (1987). Plasmids and resistance to antimicrobial agents in *Aeromonas sobria* and *Aeromonas hydrophila* clinical isolates. *Antimicrobial Agents and Chemotherapy, 31*, 1281–1282.

Chopra, A. K., & Houston, C. W. (1999). Enterotoxins in *Aeromonas* associated gastroenteritidis. *Microbes and Infection, 1*, 1129–1137.

Demerta, A., Huys, G., Tonolla, M., Swings, J., & Peduzzi, R. (2004). Polyphasic taxonomic study of "*Aeromonas eucrenophila*-like" isolates from clinical and environmental sources. *Systematic and Applied Microbiology, 27*, 343–349.

De Vicente, A., Avilés, M., Codina, J. C., Borrego, J. J., & Romero, P. (1990). Resistance to antibiotics and heavy metals of *Pseudomonas aeruginosa* isolated from natural waters. *Journal of Applied Bacteriology, 68*, 625–632.

Doukas, V., Athanassopoulou, F., Karagouni, E., & Dotsika, E. (1998). *Aeromonas hydrophila* infection in cultured sea bass, *Dicentrarchus labrax L.*, and *Puntazzo puntazzo* Cuvier from the Aegean Sea. *Journal of Fish Diseases, 21*, 317–320.

Goñi-Urriza, M., Pineau, L., Capdepuy, M., Roques, C., Caumette, P., & Quentin, C. (2000). Antimicrobial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers. *Journal of Antimicrobial Chemotherapy, 46*, 297–301.

Guthrie, G. D., Nicholson-Guthrie, C. S., & Leray, H. L. (2000). A bacterial high-affinity GABA binding protein: Isolation and characterization. *Biochemical and Biophysical Research Communications, 268*, 65–68.

Hänninen, M. L., Oivanen, P., & Koski, V. P. (1997). *Aeromonas* species in fish, fish eggs, shrimp and freshwater. *International Journal of Food Microbiology, 34*, 17–26.

Hassen, A., Saidi, N., Cherif, M., & Boundabous, A. (1998). Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. *Bioresource Technology, 65*, 73–82.

Havelaar, A. H., During, M., & Versteegh, J. F. M. (1987). Ampicillin-dextrin agar medium for enumeration of Aeromonas species in water by membrane filtration. *Journal of Applied Bacteriology, 62*, 279–287.

Havelaar, A. H., Versteegh, J. F. M., & During, M. (1990). The presence of *Aeromonas* in drinking water supplies in The Netherlands. *Zentralblatt für Hygiene und Umweltmedizin, 190*, 236–256.

Janda, J. M., & Abbott, S. L. (1998). Evolving concepts regarding the genus *Aeromonas*: An expanding panaroma of species, disease presentations, and unanswered questions. *Clinical Infectious Diseases, 27*, 332–344.

Jones, J. G., Gardener, S., Simon, B. M., & Pickup, R. W. (1986). Antibiotic resistant bacteria in Windemere and two remote upland tarns in the English Lake District. *Journal of Applied Bacteriology, 60*, 443–453.

Ko, W. C., Yu, K. W., Liu, C. Y., Huang, C. T., Leu, H. S., & Chuang, Y. C. (1996). Increasing antibiotic resistance in clinical isolates of *Aeromonas* strains in Taiwan. *Antimicrobial Agents Chemotherapy, 40*, 1260–1262.

Ko, W. C., Lee, H. C., Chuang, Y. C., Liu, C. C., & Wu, J. J. (2000). Clinical features and therapeutic implications of 104 episodes of monomicrobial *Aeromonas* bacteremia. *Journal of Infection, 40*, 267–273.

Krumperman, P. H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied and Environmental Microbiology, 46*, 165–170.

Kueh, C. S. W., Kutarski, P., & Brunton, M. (1992). Contaminated marine wounds-the risk of acquiring acute bacterial infection from marine recreational beaches. *Journal of Applied Bacteriology, 73*, 412–420.

Kühn, I., Allestam, G., Huys, G., Jahnssen, P., Kersters, K., Krovacek, K., et al. (1997). Diversity, persistence, and virulence of *Aeromonas* strains isolated from drinking water distribution system in Sweden. *Applied and Environmental Microbiology, 63*, 2708–2715.

Lalucat, J., Bennasar, A., Bosch, R., Garcia-Valdes, E., Palleroni, N. J. (2006). Biology of *Pseudomonas stutzeri*. *Microbiology and Molecular Biology Reviews, 70*, 510–547.

Lobova, T. I., Barkhatov, Y. V., Salamantina, O. V., & Popova, L. Y. (2008). Multiple antibiotic resistance of heterotrophic bacteria in the littoral zone of Lake Shira as an indicator of human impact on the ecosystem. *Microbiological Research, 163*, 152–160.

Malik, A., & Jaiswal, R. (2000). Metal resistance in *Pseudomonas* strains isolated from soil treated with industrial wastewater. *World Journal of Microbiology and Biotechnology, 16*, 177–182.

Matyar, F., Kaya, A., & Dincer, S. (2007). Distribution and antibacterial drug resistance of *Aeromonas* spp. from fresh and brackish waters in Southern Turkey. *Annals of Microbiology, 57*, 443–447.

Matyar, F., Kaya, A., & Dincer, S. (2008). Antibacterial agents and heavy metal resistance in Gram-negative bacteria isolated from seawater, shrimp and sediment in Iskenderun Bay, Turkey. *Science of the Total Environment, 407*, 279–285.

McIntosh, D., Cunningham, M., Ji, B., Fekete, F. A., Parry, E. M., Clark, S. E., et al. (2008). Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of *Aeromonas salmonicida* subsp. *salmonicida* is associated with carriage of an IncA/C plasmid similar to the *Salmonella enterica* plasmid pSN254. *Journal of Antimicrobial Chemotherapy, 61*, 1221–1228.

Messi, P., Guerieri, E., & Bondi, M. (2005). Antibiotic resistance and antibacterial activity in heterotrophic bacteria of mineral water origin. *Science of the Total Environment, 346*, 213–219.

Miranda, C. D., & Castillo, G. (1998). Resistance to antibiotic and heavy metals of motile aeromonads from Chilean freshwater. *Science of the Total Environment, 224*, 167–176.

Monfort, P., & Baleux, B. (1991). Distribution and survival of motile *Aeromonas* spp. in brackish water receiving sewage treatment effluent. *Applied and Environmental Microbiology, 57*, 2459–2467.

Monteil, C. H., Prévost, G., & Monteil, H. (2004). Virulence factors of clinical *Aeromonas caviae* isolates. *Pathologie Biologie, 52*, 21–25.

NCCLS—National Committee for Clinical Laboratory Standards (1997) *Approved standards M2-A6. Performance standards for antimicrobial disk susceptibility tests* (6th ed.). Wayne: NCCLS.

Nriagu, J. O., & Pacyna, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature, 333*, 134–139.

Ottoviani, S., Santarelli, S., Bacchiocchi, L., Masini, C., Ghittino, C., & Bacchiocchi, I. (2006). Occurrence and characterization of Aeromonas spp. in mussels from the Adriatic Sea. *Food Microbiology, 23*, 418–422.

Palú, A. P., Gomes, L. M., Miguel, M. A. L., Almeida, A. C. F., & Solares de Oliveria, S. (2006). Antimicrobial resistance in food and clinical *Aeromonas* isolates. *Food Microbiology, 23*, 504–509.

Petersen, A., & Dalsgaard, A. (2003). Antimicrobial resistance of intestinal *Aeromonas* spp. and *Enterococcus* spp. in fish cultured in integrated broiler-fish farms in Thailand. *Aquaculture, 219*, 71–82.

Radu, S., Ahmad, N., Ling, F. H., & Reezal, A. (2003). Prevalence and resistance to antibiotics for *Aeromonas* species from retail fish in Malaysia. *International Journal of Food Microbiology, 81*, 261–266.

Rhodes, G., Huys, G., Swings, J., McGann, P., Hiney, M., Smith, P., et al. (2000). Distribution of oxytetracyline resistance plasmids between aeromonads in hospital and aquaculture environments: Implication of Tn1721 in dissemination of the tetracycline resistance determinant Tet A. *Applied and Environmental Microbiology, 66*, 3883–3890.

Rochu, D., Rothlisberger, C., Taupin, C., Renault, F., Gagnon, J., & Masson, P. (1998). Purification, molecular characterization and catalytic properties of the *Pseudomonas* fluorescens enzyme having cholinesterase-like activity. *Biochimica et Biophysica Acta, 1385*, 126–138.

Rowe, C., Hopkins, W. A., & Congdon, J. D. (2002). Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: A review. *Environmental and Monitoring Assessment, 80*, 207–276.

Sader, H., & Jones, R. N. (2005). Antimicrobial susceptibility of uncommonly isolated non-enteric Gram-negative bacilli. *International Journal of Antimicrobial Agents, 25*, 95–109.

Schaberg, D. R., Culver, D. H., & Gaynes, R. P. (1991). Major trends in the microbial etiology of nosocomial infection. *American Journal of Medicine, 91*, 72–75.

Silver, S., & Phung, L. T. (1996). Bacterial heavy metal resistance: New surprises. *Annual Reviews of Microbiology, 50*, 753–789.

Silver, S., & Walderhaug, M. (1992). Regulation of chromosomal and plasmid cation and anion transport systems. *Microbiology Reviews, 56*, 195–213.

Smith, J. J., Howington, J. P., & McFeters, G. A. (1993). Plasmid maintenance and expression in *Escherichia coli* exposed to the Antarctic marine environment. *Antarctic Journal of the United States, 28*, 123–124.

Sobecky, P. A. (1999). Plasmid ecology of marine sediment microbial communities. *Hydrobiologia, 401*, 9–18.

Summers, A. O., & Silver, S. (1972). Mercury resistance in a plasmid bearing-strain of *Escherichia coli*. *Journal of Bacteriology, 112*, 1228–1236.

Türkmen, A., Türkmen, M., Tepe, Y., Mazlum, Y., & Oymael, S. (2006). Metal concentrations in blue crab (*Callinectes sapidus*) and mullet (*Mugil cephalus*) in Iskenderun Bay, northern east Mediterranean, Turkey. *Bulletin of Environmental Contamination and Toxicology, 77*, 186–193.

Vivekanadhan, G., Savithamani, K., Hatha, A. A. M., & Lakshmanaperumalsamy, P. (2002). Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. *International Journal of Food Microbiology, 76,* 165–168.

Warburton, D. W., McCormick, J. K., & Bowen, B. (1994). Survival and recovery of *Aeromonas hydrophila* in water: Development of methodology for testing bottled water in Canada. *Canadian Journal of Microbiology,* 40, 145–148.

Wu, C. J., Wu, J. J., Yan, J. J., Lee, H. C., Lee, N. Y., Chang, C. M., et al. (2007). Clinical significance and distribution of putative virulence markers of 116 consecutive clinical *Aeromonas* isolates in southern Taiwan. *Journal of Infection, 54*, 151–158.