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Occurrence of antibiotic resistance genes in culturable bacteria isolated from Turkish trout farms and their local aquatic environment

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ABSTRACT: Antibiotic resistance and presence of the resistance genes were investigated in the bacteria isolated from water, sediment, and fish in trout farms. A total of 9 bacterial species, particularly *Escherichia coli*, were isolated from the water and sediment samples, and 12 species were isolated from fish. The antimicrobial test indicated the highest resistance against sulfamethoxazole and ampicillin in coliform bacteria, and against sulfamethoxazole, imipenem, and aztreonam in known pathogenic bacteria isolated from fish. The most effective antibiotics were rifampicin, chloramphenicol, and tetracycline. The multiple antibiotic resistance index was above the critical limit for almost all of the bacteria isolated. The most common antibiotic resistance gene was *ampC*, followed by *tetA*, *sul2*, $bla_{\text{CTX-M1}}$, and bla_{TEM} in the coliform bacteria. At least one resistance gene was found in 70.8% of the bacteria, and 66.6% of the bacteria had 2 or more resistance genes. Approximately 36.54% of the bacteria that contain plasmids were able to transfer them to other bacteria. The plasmid-mediated transferable resistance genes were *ampC*, $bla_{\text{CTX-M1}}$, *tetA*, s*ul2*, and bla_{TEM}. These results indicate that the aquatic environment could play an important role in the development of antibiotic resistance and the dissemination of resistance genes among bacteria.

KEY WORDS: Antibiotic resistance gene · Coliform bacteria · Fish pathogen · Antimicrobials · Multiple antibiotic resistance index

INTRODUCTION

Aquaculture introduces land-derived microbes, nutrients, metals, and other chemicals, such as antibiotics, to the aquatic environment. Antibiotics have been used for decades in humans for therapeutic treatment of bacterial infections, and in other animals for treating and protecting health and as growth promoters (Sapkota et al. 2008). The overuse of antibiotics may cause a selection pressure on environmental microorganisms contributing to proliferating antibiotic resistance in microorganisms (Aoki 1992).

The occurrence of antibiotic resistant bacteria and antibiotic resistance genes (ARGs) has raised greater concern in recent years. Antibiotic resistant bacteria (Bhattacherjee et al. 1988) and antibiotics (Halling-Sørensen et al. 1998) are being discharged in various amounts into the environment. Antibiotic resistant bacteria have been reported in many environments such as hospital effluents and sewage treatment plants, rivers, lakes, sediments, fish farms, and other aquatic environments (Kayis et al. 2009, Boran et al. 2013, Xu et al. 2015). In aquaculture environments, release of antibiotics into the surrounding water occurs following treatment of bacterial fish diseases (Romero et al. 2012, Ozturk & Altinok 2014).

The emergence of antimicrobial resistance among fish pathogenic bacteria has become a major concern in aquaculture production for several countries during the past decades. Resistance of fish pathogens to

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drugs develops not only year by year but also seasonally within a year (Kusuda & Kawai 1998). The widespread distribution of resistance genes among environmental bacteria, and commensal bacteria in humans or animals, is an outcome of horizontal gene transfer. Horizontal gene transfer is often facilitated by mobile genetic elements such as conjugative plasmids, phages, and transposons (Levy & Marshall 2004, Nguyen et al. 2014). Unfortunately, very limited information is available regarding the development and extent of antibiotic resistance on fish farms, particularly in relation to fish farms in Turkey. In this work, we determined the presence of antibiotic resistant bacteria and their resistance genes in fish farms and their local environment (water and sediment) in Trabzon and Rize, Turkey. Thus, the aims of this study were to investigate (1) the antibiotic resistance levels among bacteria isolated from rainbow trout, water, and sediment samples, (2) the multiple antibiotic resistance index, and (3) the transferability of the resistance genes by horizontal gene transfer.

MATERIALS AND METHODS

Bacterial examination from water, sediment and fish

The study area was located in Rize and Trabzon, Turkey. Seven trout farms (including 2 in research centers) were used to examine bacterial pathogens of fish and coliform bacteria of sediment and water samples in the influent and effluent of the farms. For this purpose, fish, sediment, and water samples from each farm were aseptically collected and transported to the laboratory every 2 mo for 1 yr. The samples usually consisted of 5 fish, 6 water and 6 sediment samples (3 from influent and 3 from effluent) per farm. Each water and sediment sample was 250 ml and 10 g, respectively. Fish were randomly sampled considering absence of any noticeable disease in the ponds. All sampled fish were examined externally and internally. The gills and the body surface were examined microscopically for the presence of bacteria. If lesions were present, an incision was made with a sterile scalpel and a flamed loop was inserted in the incision to collect bacterial samples aseptically. Then the body surface was swabbed with 70% ethyl alcohol before opening to access external organs to prevent contamination by normal external bacterial flora. Subsequently, fish liver, trunk kidney, and spleen samples were aseptically streaked on tryptic soy agar (TSA; Sigma-Aldrich Chemie) and incubated at 20–25°C for 2–3 d. Ten grams of the sediments were diluted with 95 ml sterile distilled water. The suspension was mixed with an automated wrist shaker for 5 min and centrifuged at 500 × *g* for 10 min, then the supernatant was filtered (0.45 µm, Sartorius, Biotech) and the filter paper was placed on eosin methylene blue agar (EMB; Sigma-Aldrich Chemie) (Atwill et al. 2007). One hundred milliliter water samples were filtered (0.45 µm) and then filter paper was placed on EMB (Sigma-Aldrich). After incubation at 22°C (TSA) and 35°C (EMB) for 2 d, bacteria isolated from fish, water, and sediment were subcultured on the same medium to check the purity of the isolates when bacterial colonies were overloaded on the first culture medium. If different colonies were present, based on phenotypic morphology, all colonies were sampled. All the strains were biochemically characterized with API 20NE and 20E (Biomerieux) and the following biochemical tests were performed: Gram staining, cytochrome *c* oxidase, catalase, β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophan deaminase, indole production, Voges–Proskauer, gelatinase, fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, saccharose, amygdalin, methyl red, arabinose, lactose, esculin, xylose, motility, and oxidative/fermentative tests. Isolates were identified to the genus or species level by standard bacterial taxonomy procedures (Frerichs 1984, Holt et al. 1994, Austin & Austin 2007). Isolates were stored in a broth culture supplemented with 20% glycerol at −70°C. All strains of bacteria were also identified by sequencing of the 16S rRNA genes as described below.

Antibiotic susceptibility testing

Antibiotic susceptibility tests were performed by the disk diffusion method using 9 mm diameter commercial disks (Bioanalyse) on Mueller–Hinton agar plates (Oxoid) according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2011). This was done by streaking the surface of Mueller–Hinton agar plates uniformly with 0.5 McFarland standardized inoculum and thereafter exposing the plates to disks impregnated with known concentrations of antimicrobial substances. Commercial antibiotic disks used in the study included ampicillin (AMP; 25 µg), aztreonam (ATM; 30 µg), imipenem (IMP; 10 µg), ceftriaxon (CRO, 30 µg), kanamycin (K; 30 µg), gentamycin (CN; 10 µg), tetracycline (TE; 30 µg), oxytetracycline (OTC; 30 µg), sulfamethoxazole (SMZ; 100 µg), chloramphenicol (C; 30 µg), sulfamethoxazole/

trimethoprim (SXT; 25 µg), and rifampicin (RA; 30 µg). The antibiotic disks were placed on the agar using a disk dispenser (Bioanalyse). Triplicate plates were inverted and incubated at 25°C and 35°C, respectively, for 18–36 h. The inhibition zone diameters were measured to the nearest millimeter, and the isolates were characterized as susceptible or resistant to the antibiotics based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2011).

For all isolates, multiple antibiotic resistance (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) were calculated for each bacterial isolate. In general, a MAR index value >0.2 is observed when the isolate is exposed to high-risk sources of contamination where antibiotic use is common; in contrast, a MAR index value ≤0.2 is observed when antibiotics are seldom or never used (Krumperman 1983).

PCR amplification and sequencing of ARGs

All isolates were tested for the presence of ARGs. The genomic DNA was extracted from the isolates using a boiling technique. Briefly, a colony of each isolate was suspended in 100 µl distilled water and heated at 100°C for 10 min, followed by immediate cooling on ice for 2 min. The suspension was centrifuged at 17 000 × *g* for 3 min and the supernatant was stored at −20°C for PCR assays. ARGs were amplified by PCR using the primers shown in Table 1. PCR assays were performed to detect the resistance genes for tetracycline (*tetA*, *-B*, *-C*, *-D*), sulfonamide (*sul1*, *-2*, *-3*), beta-lactam (*ampC*, bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{PSE}), chloramphenicol (*cmlA*), and aminoglycoside (*aadA*) in all isolates and transconjugants.

Each 25 µl PCR reaction mixture (prepared on ice) contained 100 ng (1–2 µl) of the sample DNA, 12.5 µl of 2× Master Mix PCR mixture (QIAGEN Master PCR kit, Qiagen Molecular Biochemicals), 100 pmol of each primer, and distilled water. Thermal cycling

Table 1. Primers used in the PCR reactions

Primer name	Sequence (5′–3′)	Target gene	PCR product (bp)	Anneal temp. (°C)	Reference
Tet A FW	GCTACATCCTGCTTGCCTTC	*tetA*	210	55	Ng et al. (2001)
Tet A RV	CATAGATCGCCGTGAAGAGG				
Tet B FW	TTGGTTAGGGGCAAGTTTTG	*tetB*	659	54	Ng et al. (2001)
Tet B RV	GTAATGGGCCAATAACACCG				
Tet C FW	CTTGAGAGCCTTCAACCCAG	*tetC*	418	55	Ng et al. (2001)
Tet C RV	ATGGTCGTCATCTACCTGCC				
Tet D FW	AAACCATTACGGCATTCTGC	*tetD*	787	54	Ng et al. (2001)
Tet D RV	GACCGGATACACCATCCATC				
Sul1 FW	CGGCGTGGGCTACCTGAACG	*sul1*	433	59	Kerrn et al. (2002)
Sul1 RV	GCCGATCGCGTGAAGTTCCG				
Sul2 FW	GCGCTCAAGGCAGATGGCATT	*sul2*	293	59	Kerrn et al. (2002)
Sul2 RV	GCGTTTGATACCGGCACCCGT				
Sul3 FW	TCAAAGCAAAATGATATGAGC	*sul3*	787	48	Heuer & Smalla (2007)
Sul3 RV	TTTCAAGGCATCTGATAAAGAC				
AmpC FW	TTCTATCAAMACTGGCARCC	*ampC*	550	48	Schwartz et al. (2003)
AmpC RV	CCYTTTTATGTACCCAYGA				
TEM OT-1 FW	TTGGGTGCACGAGTGGGTTA	bla_{TEM}	465	54	Arlet & Philippon (1991)
TEM OT-2 RV	TAATTGTTGCCGGGAAGCTA				
TEM OT-3 FW	ATGAGTATTCAACATTTCCG	bla_{TEM}	859	45	Olesen et al. (2004)
TEM OT-4 RV	CAATGCTTAATCAGTGAGG				
PSE1 FW	CGCTTCCCGTTAACAAGTAC	bla_{PSE}	465	50	Zühlsdorf & Wiedemann (1992)
PSE1 RV	CTGGTTCATTTCAGATAGCG				
CmlA FW	TGTCATTTACGGCATACTCG	*cmlA*	455	52	Sáenz et al. (2004)
CmlA RV	ATCAGGCATCCCATTCCCAT				
AadA FW	TGATTTGCTGGTTACGGTGAC	*aadA*	284	52	Van et al. (2008)
AadA RV	CGCTATGTTCTCTTGCTTTTG				
CTX-M-1 FW	GGTTAAAAAATCACTGCGTC	bla_{CTX}	863	49	Saladin et al. (2002)
CTX-M-1 RV	TTGGTGACGATTTTAGCCGC				
5′-CS	GGCATCCAAGCAGCAAG	Integrase genes	Variable	50	Lévesque et al. (1995)
3′-CS	AAGCAGACTTGACCTGA				
Hep51	GATGCCATCGCAAGTACGAG	Integrase genes	Variable	55	White et al. (2001)
Hep74	CGGGATCCCGGACGGATGCA CGATTTGTA				

was done with a Thermo Hybaid thermal cycler (Thermo Electron). The PCR amplification conditions consisted of initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 95°C for 30 s, annealing at 45–59°C (see Table 1) for 30 s, and extension at 72°C for 45 s; and a final cycle of amplification at 72°C for 10 min. Controls consisted of the PCR mixture containing (1) no DNA template (reagent control) and (2) DNA from bacteria known to contain the different resistance genes tested for (positive control). Five microliters of PCR reaction mixture was subjected to electrophoresis in 1% agarose gel prepared in 0.5× Tris–Acetate–EDTA (TAE) buffer and run at 100 V for 1 h. The gels were then stained with ethidium bromide, and viewed by UV transillumination. Each of the 5 antibiotic resistant gene PCR products from different bacterial species was also confirmed by DNA sequence.

In addition to biochemical phenotyping, all strains of bacteria were also identified by DNA sequencing of their 16S rRNA gene. PCR was performed according to Drancourt et al. (2000) using primers of the highly conserved 5′ and 3′ regions of the eubacterial 16S rRNA gene. The reactions were performed with PCR master mix (Neb) in a thermal cycler (ThermoHybaid). PCR products were purified with a NucleoSpin PCR purification kit (Macherey–Nagel) and directly sequenced on an ABI PRISM 310 genetic analyzer (Applied Biosystems). Both strands of the templates were sequenced. The derived nucleotide sequences were analyzed and aligned with the BioEdit Sequence Alignment Editor (North Carolina State University, Raleigh, NC, USA). The results of the sequencing were used for homology searches using the methods of Altschul et al. (1990).

Plasmid isolation and conjugation

All isolated bacteria strains (n = 166) that contained one or more ARGs were tested for the presence of plasmid (Table 2). The plasmid isolation was performed using a QIAGEN plasmid isolation kit according to the manufacturer's instructions. Plasmids were detected and their size was estimated by elec-

Table 2. Number and percentage of isolated coliform and pathogenic bacteria at different sampling times and/or sampling points. WI: water influent; WE: water effluent; SI: sediment influent; SE: sediment effluent; N: number of bacterial isolates

Bacterial species	N	%	Sampling time						Sampling point			
			Jun	Aug	Oct	Dec	Feb	Apr	WI	WE	SI	SE
Coliforms												
Escherichia coli	109	68.55	8	12	20	29	20	20	29	28	30	22
Citrobacter diversus	14	8.81	2	1	2	3	4	2	1	2	5	6
Enterobacter cloacae	13	8.18	1	9	–	–	1	2	2	5	3	3
Klebsiella oxytoca	12	7.55	1	3	1	2	1	4	4	3	3	2
Serratia fonticola	5	3.14	1	2	2	–	–	–	1	2	2	–
Klebsiella pneumoniae subsp. *ozaenae*	3	1.89	1	–	–	1	1	–	–	2	–	1
Klebsiella pneumoniae subsp. *pneumoniae*	1	0.63	–	–	–	1	–	–	–	–	1	–
Erwinia carotovora	1	0.63	–	–	1	–	–	–	–	–	1	–
Enterobacter aerogenes	1	0.63	–	1	–	–	–	–	1	–	–	–
Total	159	100	14	28	26	36	27	28	38	42	45	34
									Isolated from			
Fish pathogens									Liver	Kidney	Spleen	Skin
Aeromonas hydrophila	6	25.00	2	2	–	–	–	2	4	6	5	1
Enterobacter sakazakii	4	16.67	–	2	–	–	2	–	4	4	4	–
Pseudomonas luteola	3	12.50	–	2	–	1	–	–	2	3	3	–
Enterobacter spp.	2	8.33	–	1	–	–	–	1	2	1	2	–
Enterobacter cloacae	2	8.33	–	1	–	–	1	–	2	2	2	–
Yersinia ruckeri	1	4.17	–	–	1	–	–	–	1	1	1	–
Citrobacter freundii	1	4.17	–	1	–	–	–	–	1	1	–	–
Pseudomonas oryzihabitans	1	4.17	–	–	–	1	–	–	1	1	1	–
Photobacterium damselae damselae	1	4.17	–	–	–	1	–	–	1	1	1	–
Escherichia vulneris	1	4.17	–	–	–	–	1	–	1	1	1	–
Burkholderia cepacia	1	4.17	1	–	–	–	–	–	–	1	1	–
Aeromonas caviae	1	4.17	–	1	–	–	–	–	1	–	1	–
Total	24	100	3	10	1	3	4	3	20	22	22	1

trophoresis of 10 µl of each purified sample in 0.8% agarose gels in 0.8% TAE with known plasmid weight standards (Neb) and run at 90 V for 2 h. Gels were stained with 0.5 mg ml^{-1} ethidium bromide and viewed by UV transillumination.

To determine the role of plasmids in antimicrobial resistance, plasmids from *Escherichia coli* (n = 38), *Klebsiella oxytoca* (n = 5), *Citrobacter diversus* (n = 3), *Klebsiella pneumoniae* subsp. *ozaenae* (n = 1), *Enterobacter cloacae* (n = 1), *Serratia fonticola* (n = 1), *Aeromonas hydrophila* (n = 2), and *Enterobacter sakazakii* (n = 1) were transformed into the wild-type *E. coli* K-12 J53-2 strain (resistant to rifampicin and sensitive to ampicillin) with the broth mating method, described by Rice et al. (1990). Transformants were selected on Luria Bertani (LB) agar plates supplemented with ampicillin (10 µg ml^{-1}) and rifampicin (150 µg ml^{-1}). Although some of the pathogenic fish bacteria were resistant to 30 µg rifampicin, all of them were sensitive to 150 µg rifampicin. Plates were then incubated at 37°C for 24 h, and transformants were detected by colony formation. The presence of plasmid was checked again from each colony present on LB agar. PCR assays as detailed above were conducted for transformants to determine plasmid-mediated ARGs.

RESULTS

Isolated bacterial species from fish, water, and sediment samples were identified to genus or species level. A total of 159 bacterial isolates (9 species) were isolated from water and sediment samples. Coliform bacteria were isolated from both water and sediment influent and effluent samples. There was no significant difference observed between influent and effluent samples in terms of bacterial species (Table 2). Although 210 fish were sampled, only 24 bacterial isolates (12 species) were isolated from fish (Table 2). The most abundant species was *Escherichia coli*, followed by *Citrobacter diversus*, *Enterobacter cloacae*, and *Klebsiella oxytoca* among coliform bacteria isolated from sediment and water. In contrast, *Aeromonas hydrophila* followed by *Enterobacter sakazaki* and *Pseudomonas luteola* were the most common fish pathogens isolated from fish. *Aeromonas hydrophila* was the only bacterium isolated from skin lesion of a fish (Table 2).

Antimicrobial susceptibility tests indicated that 92.45% of the coliform bacteria were resistant to sulfamethoxazole, followed by ampicillin (47.79%) and imipenem (28.93%). The most effective antibiotic was rifampicin (0.63%; Table 3). However, in fish, all bacteria were resistant to sulfamethoxazole, followed by imipenem (95%) and aztreonam (95%). The most

Table 3. Antibiotic resistance profiles of the bacteria isolated from fish farms. AMP: ampicillin; ATM: aztreonam; IMP: imipenem; CRO: ceftriaxon; K: kanamycin; CN: gentamicin; TE: tetracycline; OTC: oxytetracycline; SMZ: sulfamethoxazole; C: chloramphenicol; SXT: sulfamethoxazole/trimethoprim; RA: rifampicin; n: number of bacterial isolates

Bacterial species	n	Antibiotic tested, dosage per disk											
		AMP 25 µg	ATM 30 µg	IMP 10 µg	CRO 30 µg	K 30 µg	CN 10 µg	TE 30 µg	OTC 30 µg	SMZ 100 µg	C 30 µg	SXT 25 µg	RA 30 µg
Coliforms													
Escherichia coli	109	32	23	29	17	11	14	16	17	100	5	12	–
Citrobacter diversus	14	11	5	3	4	3	2	2	4	12	3	3	–
Enterobacter cloacae	13	13	5	5	1	–	2	–	–	13	1	–	–
Klebsiella oxytoca	12	10	2	3	1	1	3	3	3	12	–	–	–
Serratia fonticola	5	5	2	3	1	–	–	–	–	5	–	–	–
K. pneumoniae ozaenae	3	2	1	1	1	–	–	–	–	2	–	–	–
K. pneumoniae pneumoniae	1	1	1	1	–	–	–	–	–	1	–	–	–
Erwinia carotovora	1	1	1	1	1	1	1	1	1	1	–	–	1
Enterobacter aerogenes	1	1	–	–	1	–	–	–	–	1	–	–	–
Fish pathogens													
Aeromonas hydrophila	6	5	6	5	2	3	3	1	4	6	1	5	4
Enterobacter sakazakii	4	2	4	4	4	4	2	–	–	4	–	4	4
Pseudomonas luteola	3	3	3	3	3	2	1	1	1	3	2	3	3
Enterobacter spp.	2	–	2	2	2	1	1	–	–	2	–	2	2
Enterobacter cloacae	2	2	2	2	2	2	1	–	–	2	–	1	2
Yersinia ruckeri	1	–	1	1	1	–	–	–	–	1	–	1	–
Citrobacter freundii	1	1	1	1	–	1	–	–	–	1	1	1	1
P. oryzihabitans	1	1	–	1	1	–	–	–	–	1	–	1	1
Ph. damselae damselae	1	1	1	1	1	–	–	1	1	1	1	1	1
Escherichia vulneris	1	1	1	1	1	–	–	–	–	1	–	–	1
Burkholderia cepacia	1	1	1	1	1	1	–	–	–	1	1	1	1
Aeromonas caviae	1	1	1	1	1	–	–	–	–	1	–	–	1

effective antibiotic was tetracycline (12.5%), followed by oxytetracycline (25%) and chloramphenicol (25%; Table 3).

The highest MAR index value in the coliform bacteria was 0.83 in *E. carotovora*. This species was resistant to 10 out of 12 antibiotics. MAR values for almost all bacteria were above the critical level of 0.20, except for *Klebsiella pneumoniae* subsp. *ozaenae* (0.19; Fig. 1). Also, the MAR value in the bacteria isolated from fish was higher than that of the coliforms. All fish pathogenic bacteria had MAR index values above the 0.20 level (Fig. 2).

In addition to antimicrobial resistance of the bacteria, resistance genes of the bacteria isolated from fish, sediment, and water of trout farms were also examined. ARGs of coliform and known fish pathogenic bacterial species are summarized in Tables 4 & 5, respectively. Of these, the most common ARG was determined to be *ampC* in pathogenic fish bacteria

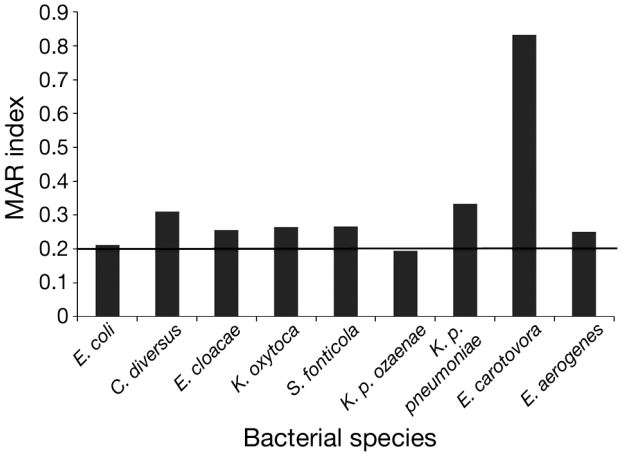

Fig. 1. Multiple antibiotic resistance (MAR) index values of coliform bacteria. Horizontal line indicates the MAR critical limit of 0.2. For species names in full, see Table 2

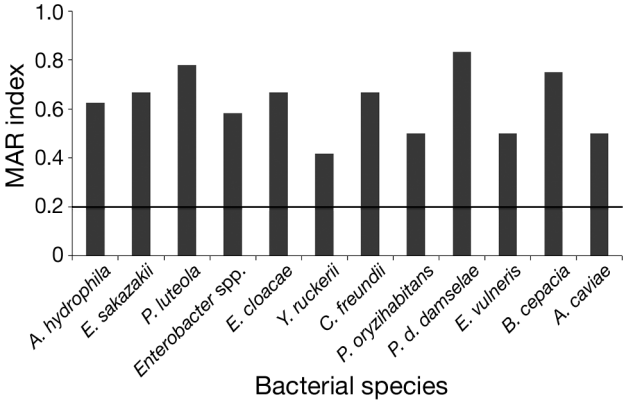

Fig. 2. Multiple antibiotic resistance (MAR) index values of fish pathogenic bacteria. Horizontal line indicates the MAR critical limit of 0.2. For species names in full, see Table 2

Table 4. Antibiotic resistance genes of coliform bacterial species. Samples were taken from different sampling points: water influent (WI), water effluent (WE), sediment influent (SI) and sediment effluent (SE). N: no. of isolates (total = 159)

Bacterial species	Sampling point	N	Antibiotic resistance gene												Integron	
			tetA	*tetB*	*tetC*	*tetD*	*sul1*	*sul2*	*sul3*	*cmlA*	*ampC*	$bla_{TEM-012}$	$bla_{TEM-034}$	bla_{PSE}	bla_{CTX-M1}	*aadA*	Class 1	Class 2
Escherichia coli	WI/WE	29/28	5/4	3/5	–/–	4/3	1/1	4/8	2/1	–/–	15/11	2/4	2/3	–/–	8/6	4/2	–/–	–/–
	SI/SE	30/22	4/3	5/2	1/3	4/4	1/–	2/6	1/1	–/–	13/10	3/2	–/1	–/–	5/3	3/1	–/–	–/–
Citrobacter diversus	WI/WE	1/6	–/–	1/–	–/–	–/–	–/–	–/1	–/–	–/–	–/–	–/1	–/1	–/–	–/1	–/–	–/–	–/–
	SI/SE	2/5	–/3	1/–	1/–	1/–	/––	2/1	–/–	1/–	3/2	1/–	–/–	–/–	1/1	1/–	1/–	–/–
Enterobacter cloacae	WI/WE	2/5	1/–	–/–	–/–	2/3	1/–	–/–	–/–	1/3	–/1	1/2	1/–	–/–	–/–	1	–/–	–/–
	SI/SE	3/3	1/–	–/–	–/–	1/–	–/1	–/–	–/–	1/–	–/–	1/2	–/–	1/1	–/–	1/–	1/1	–/–
Klebsiella oxytoca	WI/WE	4/3	2/3	–/–	–/–	–/1	–/–	1/1	–/–	–/–	1/–	–/2	–/–	–/–	1/–	1/1	–/–	–/–
	SI/SE	3/2	1/1	–/–	–/–	–/–	–/–	–/2	–/–	–/1	–/–	–/1	–/2	–/–	–/–	–/1	–/1	–/–
Serratia fonticola	WI/WE	1/2	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/1	–/–	–/–	–/–	–/–	–/–	–/–	–/–
	SI/SE	2	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	1	–/–	–/–	–/–	–/–	–/–	–/–	–/–
K. pneumoniae subsp. *ozaenae*	WI/WE	–/2	–/1	–/–	–/–	–/1	–/–	–/1	–/–	–/–	–/2	–/–	–/–	–/–	–/1	–/–	–/–	–/–
	SI/SE	–/1	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
K. pneumoniae subsp. *pneumoniae*	WI/WE	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
	SI/SE	–/1	–/–	–/1	–/–	–/–	–/–	–/–	–/–	–/1	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
Erwinia carotovora	WI/WE	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
	SI/SE	1/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
Enterobacter aerogenes	WI/WE	1/–	–/–	–/–	–/–	–/–	1/–	–/–	–/–	–/–	1/–	1/–	–/–	–/–	–/–	1/–	–/–	–/–
	SI/SE	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–

Table 5. Distribution of antibiotic resistance genes among pathogenic bacterial species isolated from fish

Bacterial species	Antibiotic resistance gene												Integron	
	tetA	*tetB*	*tetC*	*tetD*	*sul1*	*sul2*	*sul3*	*ampC*	*cmlA*	$bla_{TEM-OT12}$	$bla_{TEM-OT34}$	bla_{PSE}	*aadA*	bla_{CTX-M1}	Class 1	Class 2
Aeromonas hydrophila	1	3	1	–	2	2	–	1	1	2	1	2	3	1	2	1
Enterobacter sakazakii	–	–	–	2	2	–	–	2	2	–	–	–	–	2	–	1
Pseudomonas luteola	–	1	–	–	–	1	–	1	1	1	1	–	–	–	–	–
Enterobacter spp.	–	–	–	1	–	–	–	1	1	1	–	1	–	–	1	–
Enterobacter cloacae	–	–	–	1	1	–	–	1	1	–	–	–	–	1	–	1
Yersinia ruckeri	–	–	–	1	1	–	–	1	1	1	–	1	–	–	1	–
Citrobacter freundii	–	1	–	–	–	1	–	1	–	1	1	1	–	1	1	–
Pseudomonas oryzihabitans	–	–	–	–	–	1	–	–	–	1	–	–	–	–	–	–
Photobacterium damselae damselae	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–
Escherichia vulneris	–	–	–	1	–	–	–	–	–	–	1	–	–	–	–	–
Burkholderia cepacia	–	–	–	–	–	–	–	1	–	1	1	–	–	1	1	–
Aeromonas caviae	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–
Total	1	5	1	6	6	5	0	11	8	8	5	5	3	6	6	3

(45.83%) as well as in coliform bacteria (37.74%). The resistance gene *ampC* was followed by the *tetA* gene (18.87%) in coliform bacteria. *Sul2* (18.24%) was the most prevalent gene among *sul* genes, and it was followed by bla_{CTX-M1} (16.98%) and $bla_{TEM-OT12}$ (14.47%). Only 5.03% of the coliform bacteria had the *cmlA* gene. Among coliform bacteria (n = 159), 68.55% (n = 109) had at least one resistance gene and 42.10% (n = 67) had 2 or more. Only the *Erwinia carotovora* isolate had no ARG. ARGs of the bacteria isolated from both water and sediment influent and effluent were similar (Table 4). Furthermore, in bacterial fish pathogens, the *ampC* gene was followed by *cmlA* and bla_{TEM} (33.30%) in terms of isolation frequency. Of 24 fish pathogenic bacterial isolates, 2 isolates of *P. luteola* and *E. sakazakii* and 1 isolate of each *E. cloacae* and *A. hydrophila* did not have any ARG. Seventeen isolates (70.33%) had at least 1 ARG and 16 isolates (66.67%) had 2 or more resistance genes. The presence of plasmids in bacteria was also determined; 30.81% (49/159) of coliform and 12.5% (3/24) of pathogenic fish bacteria had plasmids ranging in size from 2 to 10 kb (Table 6).

All bacteria having plasmids were assessed by conjugation experiments with *E. coli* K12-J53-2. While 34.86% (14/38) of the *E. coli* isolates having plasmids were able to transfer their plasmids to the recipient, a transfer efficiency of 60% (3/5) was seen with *K. oxytoca*. Only one *E. sakazakii* isolate contained plasmid (1/4) and this isolate was able to transfer its plasmid. Additionally, PCR assays were conducted for transconjugants to determine plasmid-mediated ARGs. The most transferred resistance gene was *ampC*, followed by bla_{CTX-M1} and *tetA* (Table 7). The *sul2* and bla_{TEM} resistance genes were present only in T2 coded transconjugant.

Table 6. Plasmid presence (number of isolates containing plasmid(s) and %) in the bacteria isolated from fish farms. N: no. of isolates (total = 183)

Bacterial species	N	Presence	Plasmid (%)
Coliforms			
Escherichia coli	109	38	34.86
Klebsiella oxytoca	12	5	41.67
Citrobacter diversus	14	3	21.43
Klebsiella pneumoniae subsp. *ozaenae*	3	1	33.33
Enterobacter cloacae	13	1	7.69
Serratia fonticola	5	1	20.00
Fish pathogens			
Aeromonas hydrophila	6	2	33.33
Enterobacter sakazakii	4	1	25.00

Table 7. Antibiotic resistance genes detected by PCR in transconjugants

Code	Donor	Antibiotic resistance gene					
		tetA	*sul2*	*ampC*	$bla_{TEM\text{-}OT12}$	$bla_{TEM\text{-}OT34}$	$bla_{CTX\text{-}M1}$
T2	*Escherichia coli*	–	+	+	+	+	–
T7	*E. coli*	–	–	+	–	–	+
T10	*E. coli*	–	–	+	–	–	+
T16	*E. coli*	–	–	+	–	–	+
T77	*E. coli*	+	–	+	–	–	–
T84	*E. coli*	–	–	+	–	–	–
T86	*E. coli*	–	–	+	–	–	–
T87	*E. coli*	–	–	+	–	–	–
T95	*E. coli*	–	–	+	–	–	–
T96	*E. coli*	–	–	+	–	–	+
T103	*Klebsiella oxytoca*	+	–	–	–	–	–
TE14	*Enterobacter sakazakii*	–	–	+	–	–	+

DISCUSSION

Antibiotics are generally used worldwide to treat bacterial infections in humans and other animals, including fish. Inappropriately or unintentionally used antibiotics may accelerate the acquirement of ARGs in pathogenic fish bacteria and transfer of antibiotic resistance to the bacteria present in the environment around fish farms or in food chains. Genetic mechanisms involved in horizontal transfer of ARGs among environmental bacteria include conjugative transfer by mobile elements including plasmids, transposons, and integrons on plasmids or transposons (Tennstedt et al. 2003, Zhang et al. 2009). In the present study, ARGs were observed in environmental bacteria isolated in influent and effluent water and sediment, and in fish pathogenic bacteria from trout farms in Turkey. Bacteria on fish farms or in the surrounding environment may gain antibiotic resistance because they can be exposed to antibiotics eliminated into the environment from homes and/or hospitals, other fish farms, and animal farms located in their vicinity. This exposure to a wide range of antibiotics in addition to antibiotics specifically administrated for disease control on fish farms may be a reason why pathogenic fish bacteria have a higher MAR index.

Ampicillin, erythromycin, kanamycin, neomycin, oxolinic acid, and streptomycin are commonly used antibiotics in Turkey and several European countries to treat fish bacterial diseases (Costello et al. 2001). Kayis et al. (2009) indicated that resistance to these antibiotics was 67% in freshwater aquaculture in Turkey. Sulfamethoxazole is the most commonly used antibiotic in fish farms in Turkey, and 100% of the bacteria were found to be resistant to sulfamethoxazole (Kayis et al. 2009). In contrast, amphenicol antibiotics (e.g. chloramphenicol and florfenicol) were introduced to aquaculture only a few years ago; therefore, most bacteria are not yet resistant to them (authors' unpubl. data). In the present study, determination of a high number of antibiotic resistant strains may indicate the widespread use of florfenicol in intensive fish farming in Turkey. Resistance among fish pathogenic bacteria to sulfamethoxazole, imipenem, and aztreonam was determined as 100, 95, and 95%, respectively. Additionally, a recent study conducted in fish farms in Turkey has shown differences in antibiotic resistance (Kayis et al. 2009). In the present study, bacteria isolated from water, sediment and fish on farms located downstream of human settlements were more resistant to tested antibiotics than those isolated from farms not located close to settlements (authors' unpubl. data). It can be inferred that the different resistance profiles of isolated bacteria could be due to different geographical locations and sampling time. In pathogenic fish bacteria, 50% or more resistance toward some tested antibiotics was observed. This is an alarming situation considering the control of fish diseases in Turkey.

Multiple-antibiotic-resistant *Escherichia coli* strains isolated from drinking water were found to carry ARGs encoding resistance to aminoglycoside, tetracycline, and trimethoprim-sulfamethoxazole (Cernat et al. 2007). Multiple-antibiotic-resistant bacteria were also isolated in the present study. Seven of the 9 environmental coliform bacteria and all pathogenic fish bacteria except *Aeromonas caviae* had multiple ARGs. MAR index values >0.2 are regarded as an indicator of antimicrobial contamination, and index values ≤0.2 indicate that the environment of isolation has no history of or very low use of antibiotics (Krumperman 1983). MAR index values above 0.2 have been determined in many studies (Matyar 2012, Su et al. 2011). We found MAR index values ranging between 0.19–0.83 and 0.42–0.83 for coliform bacteria and pathogenic fish bacteria, respectively. These results are in agreement with previously published studies. Matyar et al. (2014) found that the MAR index in pathogenic fish bacteria ranged between 0.2 and 0.81, which suggests exposure to antibiotic contamination. Find-

ing different combinations of ARGs in both coliform and pathogenic fish bacteria suggests their involvement in the spread of multidrug-resistant strains in aquatic environments. In the present study, pathogenic fish bacteria had a higher MAR index than that of coliform bacteria. This may be due to several factors: natural resistance to some antibiotics; receiving resistance genes from other environmental bacteria more easily; greater selection for resistance; or exposure to additional antibiotics used on fish farms.

In addition to bacterial resistance to antibiotics, the prevalence of ARGs in Gram-negative bacteria from aquatic ecosystems has previously been studied (Nawaz et al. 2006, Balta et al. 2010, Boran et al. 2013). However, the occurrence of these resistance determinants in bacteria isolated from rainbow trout farms and their aquatic environments in Turkey is ambiguous. Tetracycline is the most frequently used antibiotic in veterinary medicine and in aquaculture worldwide (Miranda 2012). Sandalli et al. (2010) reported that bacteria isolated from rivers of the Black Sea region of Turkey had *tetA* and *tetB* resistance genes among tested *tet* genes. DePaola & Roberts (1995) isolated and characterized tetracycline-resistant Gram-negative bacteria from catfish and reported that these isolates harbored either the *tetD* or *tetE* gene. DePaola et al. (1988) indicated that the majority of tetracycline-resistant *A. hydrophila* strains from catfish contained either *tetA* or *tetE*. Nawaz et al. (2006) indicated that *tetE* is the dominant tetracycline determinant followed by *tetB* in aeromonads isolated from catfish. Balta et al. (2010) surveyed the presence of *tetA* or *tetB* in 44 oxytetracycline-resistant *Yersinia ruckeri* strains isolated from rainbow trout *Oncorhynchus mykiss* grown in commercial fish farms in Turkey and found that 16 strains had *tetA* and 2 had *tetB*. In that study, among *tet* genes in coliform bacteria, *tetA* was the most prevalent, followed by *tetD*, *tetB*, and *tetC*. Pathogenic fish bacteria had a different gene frequency distribution than coliform bacteria: *tetD* > *tetB* > *tetA* > *tetC*. In the present study, all 3 sulfonamide resistance genes were found in the bacteria isolated from rainbow trout in fish farms. In previous studies, *sul* genes were not found to occur in equal frequencies, and in most of the experiments the pattern of gene frequency distribution was *sul2* > *sul1* > *sul3* (Blahna et al. 2006, Frank et al. 2007) and *sul1* > *sul2* > *sul3* (Boran et al. 2013). Although samples from the present study had different frequencies than that in previous reports, the gene frequency distribution was *sul2* > *sul1* > *sul3* in coliform bacteria and *sul1* > *sul2* > *sul3* in pathogenic fish bacteria.

Beta lactams, broad-spectrum antibiotics, are used extensively in the aquaculture industry. TEM, SHV, PSE, OXA, and CTX-M are beta lactam resistance genes (Ishida et al. 2010). These genes were reported in different geographical locations and aquatic environments (Briñas et al. 2002, Boran et al. 2013). In the present study, among beta lactam genes, *ampC* was the most prevalent resistance gene in coliform and fish pathogenic bacteria, followed by $bla_{\text{CTX-M1}}$, $bla_{\text{TEM-OT12}}$, $bla_{\text{TEM-OT34}}$, and bla_{PSE}.

The overuse of antibiotics in aquaculture causes a selective pressure that creates a reservoir of drug-resistant bacteria and transferable resistance genes in fish pathogens and other bacteria in the aquatic environment. From this reservoir, resistance genes may disseminate by horizontal gene transfer and reach human pathogens, or drug-resistant pathogens from the aquatic environment may affect humans directly (Heuer et al. 2009).

Several antibiotic resistance determinants associated with mobile genetic elements have been identified with regard to aquaculture (Rosser & Young 1999). In the present study, plasmid frequencies in coliform and fish bacterial pathogens were 30.81% and 12.5%, respectively. Also, among transferable resistance genes, *ampC* was the most transferable, followed by $bla_{\text{CTX-M1}}$ and *tetA* in coliform bacteria. *Enterobacter sakazakii*, the only coliform fish disease agent, contained *ampC* and $bla_{\text{CTX-M1}}$ transferable resistance genes. In the present study, 34.86% of *E. coli* isolates having plasmids were able to transfer their plasmids to the recipients. Although some of the bacteria had plasmid and antibiotic resistance, they did not transfer their antibiotic resistance gene to recipient *E. coli*. This may indicate chromosomal mediated resistance rather than plasmid mediated resistance.

The ecological consequences of polluted aquatic environments may be, along with the spread of antibiotic-resistant microorganisms, genetic pollution with resistance determinants that are foreign to the indigenous bacteria, resulting in an altered distribution of resistance determinants. The prevalence of ARGs in the aquatic environment may reduce the efficiency of antibiotics in treating fish diseases.

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LITERATURE CITED

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic logical alignment search tool. J Mol Biol 215: 403–410

Aoki T (1992) Present and future-problems concerning the development of resistance in aquaculture. In: Michel C, Alderman D (eds) Chemotherapy in aquaculture: from theory to reality. Office International des Epizooties, Paris, p 254–262

Arlet G, Philippon A (1991) Construction by polymerase chain reaction and intragenic DNA probes for three main types of transferable β-lactamases (TEM, SHV, CARB). FEMS Microbiol Lett 82:19–25

Atwill ER, Lewis DJ, Bond RF, Pereira GMC, Huerta M, Ogata SB (2007) Protocol consideration for monitoring fecal coliform and in Northern California Estuaries. University of California, Davis School of Veterinary Medicine and University of California Cooperative Extension, Sonoma County, Santa Rosa, CA

Austin B, Austin DA (2007) Bacterial fish pathogens: diseases of farmed and wild fish, 4th edn. Praxis Publishing, Chichester

Balta F, Sandalli C, Kayis S, Ozgumus OB (2010) Molecular analysis of antimicrobial resistance in *Yersinia ruckeri* strains isolated from rainbow trout (*Oncorhynchus mykiss*) grown in commercial fish farms in Turkey. Bull Eur Assoc Fish Pathol 30:211–219

Bhattacherjee JW, Pathak SP, Gaur A (1988) Antibiotic-resistance and metal tolerance of coliform bacteria isolated from gomati river water at Lucknow City. J Gen Appl Microbiol 34:391–399

Blahna MT, Zalewski CA, Reuer J, Kahlmeter G, Foxman B, Marrs CF (2006) The role of horizontal gene transfer in the spread of trimethoprim-sulfamethoxazole resistance among uropathogenic *Escherichia coli* in Europe and Canada. J Antimicrob Chemother 57:666–672

Boran H, Terzi E, Altinok I, Capkin E, Bascinar N (2013) Bacterial diseases of cultured Mediterranean horse mackerel (*Trachurus mediterraneus*) in sea cages. Aquaculture 396-399:8–13

Briñas L, Zarazaga M, Sáenz Y, Ruiz-Larrea F, Torres C (2002) Betalactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. Antimicrob Agents Chemother 46:3156–3163

Cernat R, Balotescu C, Ivanescu D, Nedelcu D and others (2007) Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains isolated from drinking and recreational, salmaster waters. Int J Antimicrob Agents 29:S274

CLSI (Clinical and Laboratory Standards Institute) (2011) Performance standards for antimicrobial susceptibility testing. Twenty-first informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA

Costello MJ, Grant A, Davies IM, Cecchini S, Papoutsoglou S, Quigley D, Saroglia M (2001) The control of chemicals used in aquaculture in Europe. J Appl Ichthyol 17: 173–180

DePaola A, Roberts MC (1995) Class-D and Class-E tetracycline resistance determinants in gram-negative bacteria from catfish ponds. Mol Cell Probes 9:311–313

DePaola A, Flynn PA, Mcphearson RM, Levy SB (1988) Phenotypic and genotypic characterization of tetracycline-resistant and oxytetracycline-resistant *Aeromonas hydrophila* from cultured channel catfish (*Ictalurus punctatus*) and their environments. Appl Environ Microbiol 54:1861–1863

Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP, Raoult D (2000) 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J Clin Microbiol 38:3623–3630

Frank T, Gautier V, Talarmin A, Bercion R, Arlet G (2007) Characterization of sulphonamide resistance genes and class 1 integron gene cassettes in Enterobacteriaceae, Central African Republic (CAR). J Antimicrob Chemother 59:742–745

Frerichs GN (1984) Isolation and identification of fish bacterial pathogens. Institute of Aquaculture, University of Stirling

Halling-Sørensen B, Nielsen SN, Lanzky PF, Ingerslev F, Holten Lützhøft HCH, Jørgensen SE (1998) Occurrence, fate and effects of pharmaceutical substances in the environment — a review. Chemosphere 36:357–393

Heuer H, Smalla K (2007) Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. Environ Microbiol 9:657–666

Heuer OE, Kruse H, Grave K, Collignon P, Karunasagar I, Angulo FJ (2009) Human health consequences of use of antimicrobial agents in aquaculture. Clin Infect Dis 49: 1248–1253

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey's manual of determinative bacteriology. Lippincott Williams & Wilkins, Baltimore, MD

Ishida Y, Ahmed AM, Mahfouz NB, Kimura T, El-Khodery SA, Moawad AA, Shimamoto T (2010) Molecular analysis of antimicrobial resistance in gram-negative bacteria isolated from fish farms in Egypt. J Vet Med Sci 72:727–734

Kayis S, Capkin E, Balta F, Altinok I (2009) Bacteria in rainbow trout (*Oncorhynchus mykiss*) in the southern Black Sea region of Turkey — a survey. Isr J Aquacult Bamid 61:339–344

Kerrn MB, Klemmensen T, Frimodt-Møller N, Espersen F (2002) Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance. J Antimicrob Chemother 50:513–516

Krumperman PH (1983) Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol 46: 165–170

Kusuda R, Kawai K (1998) Bacterial diseases of cultured marine fish in Japan. Fish Pathol 33:221–227

Lévesque C, Piche L, Larose C, Roy PH (1995) PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob Agents Chemother 39:185–191

Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10: S122–S129

Matyar F (2012) Antibiotic and heavy metal resistance in bacteria isolated from the Eastern Mediterranean Sea Coast. Bull Environ Contam Toxicol 89:551–556

Matyar F, Gulnaz O, Guzeldag G, Mercimek HA, Akturk S, Arkut A, Sumengen M (2014) Antibiotic and heavy metal resistance in Gram-negative bacteria isolated from the Seyhan Dam Lake and Seyhan River in Turkey. Ann Microbiol 64:1033–1040

Miranda CD (2012) Antimicrobial resistance associated with salmonid farming. In: Keen Pl, Montforts MHHM (eds) Antimicrobial resistance in the environment. Wiley-Blackwell, Hoboken, NJ, p 423–451

Nawaz M, Sung K, Khan SA, Khan AA, Steele R (2006) Biochemical and molecular characterization of tetracycline-resistant *Aeromonas veronii* isolates from catfish. Appl Environ Microbiol 72:6461–6466

Ng LK, Martin I, Alfa M, Mulvey M (2001) Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probes 15:209–215

Nguyen HNK, Van TT, Nguyen HT, Smooker PM, Shimeta J, Coloe PJ (2014) Molecular characterization of antibiotic resistance in *Pseudomonas* and *Aeromonas* isolates from catfish of the Mekong Delta, Vietnam. Vet Microbiol 171:397–405

Olesen I, Hasman H, Aarestrup FM (2004) Prevalence of β-lactamases among ampicillin resistant *Escherichia coli* and *Salmonella* isolated from food animals in Denmark. Microb Drug Resist 10:334–340

Ozturk RC, Altinok I (2014) Bacterial and viral fish diseases in Turkey. Turk J Fish Aquat Sci 14:275–297

Rice LB, Willey SH, Papanicolaou GA, Papanicolaou GA and others (1990) Outbreak of ceftazidime resistance caused by extended-spectrum β-lactamases at Massachusetts Chronic Care Facility. Antimicrob Agents Chemother 34: 2193–2199

Romero J, Feijoó CG, Navarrete P (2012) Antibiotics in aquaculture — use, abuse and alternatives. In: Carvalho ED, David GS, Silva RJ (eds) Health and environment in aquaculture. InTech, Rijeka, p 159–198

Rosser SJ, Young HK (1999) Identification and characterization of class 1 integrons in bacteria from an aquatic environment. J Antimicrob Chemother 44:11–18

Sáenz Y, Briñas L, Domínguez E, Ruiz J, Zarazaga M, Vila J, Torres C (2004) Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal and food origin. Antimicrob Agents Chemother 48:3996–4001

Saladin M, Cao VT, Lambert T, Donay JL and others (2002) Diversity of CTX-M β-lactamases and their promoter regions from *Enterobacteriaceae* isolated in three Parisian hospitals. FEMS Microbiol Lett 209:161–168

Sandalli C, Ozgumus OB, Sevim A (2010) Characterization of tetracycline resistance genes in tetracycline resistant *Enterobacteriaceae* obtained from a coliform collection. World J Microb Biot 26:2099–2103

Sapkota A, Sapkota AR, Kucharski M, Burke J, McKenzie S, Walker P, Lawrence R (2008) Aquaculture practices and potential human health risks: current knowledge and future priorities. Environ Int 34:1215–1226

Schwartz T, Kohnen W, Jansen B, Obst U (2003) Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water and drinking water biofilms. FEMS Microbiol Ecol 43:325–335

Su HC, Ying GG, Tao R, Zhang RQ, Fogarty LR, Kolpin DW (2011) Occurrence of antibiotic resistance and characterization of resistance genes and integrons in *Enterobacteriaceae* isolated from integrated fish farms in South China. J Environ Monit 13:3229–3236

Van TTH, Chin J, Chapman T, Tran LT, Coloe PJ (2008) Safety of raw meat and shellfish in Vietnam: an analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. Int J Food Microbiol 124: 217–223

White PA, McIver CJ, Rawlinson WD (2001) Integrons and gene cassettes in the Enterobacteriaceae. Antimicrob Agents Chemother 45:2658–2661

Xu J, Xu Y, Wang H, Guo C and others (2015) Occurrence of antibiotics and antibiotic resistance genes in a sewage treatment plant and its effluent-receiving river. Chemosphere 119:1379–1385

Zhang XX, Zhang T, Fang HHP (2009) Antibiotic resistance genes in water environment. Appl Microbiol Biotechnol 82:397–414

Zühlsdorf MT, Wiedemann B (1992) *Tn21*-specific structures in gram negative bacteria from clinical isolates. Antimicrob Agents Chemother 36:1915–1921

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