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Molecular Characterization of Antibiotic Resistant *Escherichia coli* Strains Isolated from Tap and Spring Waters in a Coastal Region in Turkey

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A hundred and seventeen antibiotic-resistant *Escherichia coli* strains were isolated from public tap and spring waters which were polluted by fecal coliforms. There were no significant differences between two water sources as to the coliform pollution level ($p > 0.05$). All *E. coli* isolates were detected to be resistant to one or more antibiotics tested. Nearly 42% of the isolates showed multiresistant phenotype. Three (2.5%) of these isolates contained class 1 integron. Sequencing analysis of variable regions of the class 1 integrons showed two gene cassette arrays, *dfrI-aadA1* and *dhfrA17-aadA5*. Resistance to ampicillin, tetracycline or trimethoprim-sulfamethoxazole was transferable according to the results of conjugation experiments. The rate of tetracycline resistance was 15%. *tet*(A)-mediated tetracycline resistance was widespread among tetracycline-resistant *E. coli* isolates. Genotyping by BOX-polymerase chain reaction (BOX-PCR) showed that some of the strains were epidemiologically related. This is the first report on the prevalence and characterization of class 1 integron-containing *E. coli* isolates of environmental origin in Turkey.

Keywords: drinking water, *Escherichia coli*, class 1 integron, tetracycline resistance

Antibiotic resistance has been detected in various aquatic environments including rivers, sewage, ocean water and drinking water (Hermansson *et al.*, 1987; Mezrioui and Baleux, 1994; Ash *et al.*, 2002; Reinthaler *et al.*, 2003; Schwartz *et al.*, 2003). Increased introduction of antimicrobial agents into the environment via medical therapy, agriculture and animal husbandry has resulted in selective pressures on bacterial populations (Col and O'Conner, 1987). Acquisition and transfer of antibiotic resistance and virulence factor genes by the bacteria via horizontal transfer of the resistance (R) plasmids, transposons and integrons are increasing problems in infectious diseases (Leverstein-van Hall *et al.*, 2001). Integrons are capable of mobilizing or integrating gene cassettes encoding antibiotic resistance determinants such as resistance to trimethoprim, aminoglycosides, chloramphenicol or tetracyclines. Class 1 and/or class 2 integrons have been reported in clinical isolates of the *Enterobacteriacea* family (Leverstein-van Hall *et al.*, 2001), in bacteria from food (Sunde, 2005) and also in aquatic environments (Roe *et al.*, 2003). Tetracyclines are broad-spectrum agents, currently using for therapy or prophylaxis for human infections and the prevention and control of bacterial infections in veterinary medicine. They are also used in aquaculture and other plants to control bacterial infection. Several different *tet* genes have been described as conferring resistance to tetracyclines in *E. coli*. The most frequent types of *tet* genes in *E. coli* belong to classes A to E (Chopra and Roberts, 2001).

Fresh water impairment by fecal coliform bacteria is a water-quality issue of national scope and importance. The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogens. *E. coli* is generally accepted as the predominant vehicle for the dissemination of resistance genes and vectors due to its abundance in such environments (Tauxe, 1997). In this study, we examined the phenotypic and genotypic characteristics of antibiotic resistant *E. coli* isolates recovered from public tap and spring waters in Rize and its six counties, a coastal region which contains lots of fresh water sources in Turkey.

Materials and Methods

Sampling site

In Rize province and its six counties on the Black Sea coastal region of northern Turkey (Fig. 1), the local rivers stretching in underdeveloped rural and agricultural areas with intensive cultivation serve as public fresh water after applying the conventional water treatment procedures such as sedimentation and chlorination. However, sewage effluents of the settlements are mostly discharged into these local rivers without applying any wastewater treatment procedures. There are naturally spring water sources in these urbanized areas which are rainy and humid. Thus, here is the richest region for fresh water sources in Turkey. On the other hand, this region is the unique agricultural place for tea plant (*Camellia sinensis*) in Turkey.

Isolation and identification of coliforms

From 36 tap water and 15 spring water in total of 51 drinking water points in Rize region (Fig. 1), 457 water samples were taken for microbiological analysis to detect the presence of coliforms during the years 2000 to 2002. The water samples collected aseptically in sterile glass bottles contain-

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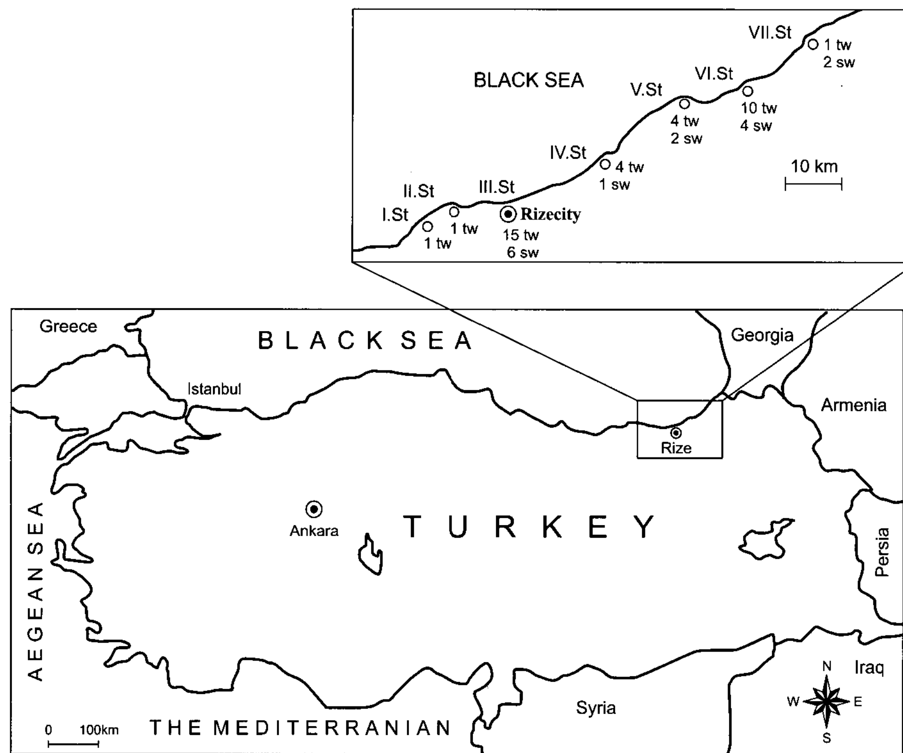

Place	Tap water (tw)	Spring water (sw)	Total no. of site/sampling event
	No. of site/sampling event	No. of site/sampling event	
I. Station	1/5	-	1/5
II. Station	1/4	-	1/4
III. Station	15/175	6/61	21/236
IV. Station	4/30	1/6	5/36
V. Station	4/34	2/9	6/43
VI. Station	10/71	4/26	14/97
VII. Station	1/14	2/22	3/36
Total Station	36/333	15/124	51/457

Fig. 1. Map of Rize province showing tap and spring water stations (St) and the numbers of sampling sites and sampling events.

ing sodium thiosulfate to neutralize the residual chlorine were transported on ice to the Microbiology & Molecular Biology Research Laboratory of Rize University, Rize, Turkey. Samples were processed within 8 h of collection. Coliform bacteria in the water samples were enumerated by a most-probable-number (MPN) multiple-tube fermentation method (Eckner, 1998). Fecal *E. coli* confirmation was achieved by monitoring the acidification and gas production during growth in brilliant green broth (Oxoid, UK) at 44±0.5°C for 24±3 h.

From the fermentation tubes, 117 *E. coli* isolates were included in this study. The identification of *E. coli* was confirmed by performing biochemical tests, as previously described (Brenner, 1986).

Antimicrobial susceptibility testing

The susceptibility tests were carried out by the standard disk diffusion method, and the results were interpreted as described in National Committee for Clinical Laboratory Standards guidelines (now Clinical Laboratory Standards Institute) (NCCLS, 1997). The following antibiotic disks (Oxoid, UK) were used: ampicillin (10 μg), gentamicin (10 μg), netilmicin (30 μg), amikacin (30 μg), tetracycline (30 μg), chloramphenicol (30 μg) and trimethoprim/sulfamethoxazole (1.25 μg/23.75 μg).

Transferability of antibiotic resistance

Transferability of the antibiotic resistance was examined by conjugation assay by using broth mating method (Rice *et al.*, 1990). The transconjugants were selected on Eosine Methylene Blue agar (Oxoid, UK) supplemented with 200 μg/ml of ri-

Table 1. Oligonucleotide primers used for PCR assays in the study

Primer	Target	Sequence (5'-3')	Reference
5'-CS 3'-CS	Class 1 integron variable regions	5'-GGCATCCAAGCAGCAAG-3' 5'-AAGCAGACTTGACCTGA-3'	Lévesque *et al.* (1995)
tet(A)-1 *tet*(A)-2	*tet*(A) gene	5'-GTAATTCTGAGCACTGTCGC-3' 5'-CTGCCTGGACAACATTGCTT-3'	Aarestrup *et al.* (2003)
tet(B)-1 *tet*(B)-2	*tet*(B) gene	5'-CTCAGTATTCCAAGCCTTTG-3' 5'-ACTCCCCTGAGCTTGAGGGG-3'	Aarestrup *et al.* (2003)
tet(C)-1 *tet*(C)-2	*tet*(C) gene	5'-GGTTGAAGGCTCTCAAGGGC-3' 5'-CCTCTTGCGGGAATCGTCC-3'	Aarestrup *et al.* (2003)
BOXA1R	BOX sequences	5'-CTACGGCAAGGCGACGCTGACG-3'	Versalovic *et al.* (1994)

Table 2. Coliform pollution levels in spring and tap water samples

MPN[a] (CFU/100 ml)	No. of fermentative tubes		
	Spring water sample (n=124)	Tap water sample (n=333)	Overall (n=457)
≥240	10	12	22
240	56	43	99
95	2	2	4
23	27	13	40
19	0	0	0
9	0	0	0

[a]MPN, most-probable-number. Chi-square test: $p>0.05$

fampicin to inhibit donor (*E. coli* isolates) and 30 μg/ml of ampicillin, netilmicin, kanamicin, gentamicin, tetracycline, chloramphenicol or 25 μg/ml of trimethoprime-sulfamethoxazole to inhibit recipient *E. coli* K12 strain J53-2 (F^- *met pro* Rif^r). The frequency of transfer was expressed relative to the number of donor cells.

Detection of class 1 integron cassettes

The presence of integrons was examined by PCR with specific primers (Table 1) to amplify the variable regions of class 1 integrons. Reaction composition and cycling parameters were used the methods previously described (Lévesque *et al.*, 1995).

PCR for tetracycline resistance determinants

Tetracycline resistance determinants, *tet*(A), *tet*(B), and *tet*(C) genes were screened by PCR assays by using the primers shown in Table 1. The reaction compositions and the cycling parameters were carried out according to the method as previously described (Aarestrup *et al.*, 2003).

DNA sequencing and analysis

The PCR products were subjected to electrophoresis through 1% agarose containing 0.5 μg/ml ethidium bromide, and then visualized under UV light. After PCR products were purified from the agarose gel by using QIAQuick® *Purification Kits (QIAgen, UK) prior to sequencing,* they were cloned into the pGEM-T Easy vector (Promega, USA) according to the manufacturer's instructions. Recombinant plasmids carrying amplicons of class 1 integrons were sent to Macrogen Inc., Seoul, Korea for sequencing by using the universal oligonucleotide primers, T7 and SP6. Data from sequencing was compared with those available in the GenBank database by using the alignment search tool, BLAST (Altschul *et al.*, 1997), accessible from the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST), and by the multiple sequence alignment program, CLUSTAL W, accessible from the European Bioinformatics Institute website (http://www.ebi.ac.uk/clustalw).

BOX-PCR assay

BOX-PCR fingerprints were obtained with the BOXA1R primer (Table 1). Conditions for PCR were optimized and performed as described (Seurinck *et al.*, 2003). DNA fragments were separated at 60 V for 3 h on 1% agarose gels containing ethidium bromide. Gel was visualized under UV light. Patterns were compared by eye and considered identical when the positions of all bands matched. Differences in band intensity were ignored. All amplified BOX-PCR bands were scored for their presence (1) or absence (0) (data not shown). A similarity matrix was generated by using the Sneath and Sokal (1973) methodology. The Jaccard index citation was used (similarity: $a/a+b$, where a is the homolog bands in two genotypes and where b is the number of non-homolog fragments in two genotypes). The dendogram was generated using the NTSYS-pc with the unweighted pair group method with arithmetic means (UPGMA) tree building method (Rohlf, 1990).

Statistical analysis

The significance of difference between tap water and spring

water samples as to the level of fecal coliform pollution, and also total rate of resistance to antibiotics of the *E. coli* strains isolated from tap and spring water sources were evaluated by chi-square test. P value of $p<0.05$ was considered statistically significant (SPSS Inc., version 10.0, USA).

Results

Fecal coliform pollution

During the sampling period, the water samples from all sampling events displayed intensive coliform contamination (Table 2). Most of the water sources were polluted with the fecal coliform according to the results of fermentative growth in brilliant-green medium at 44±0.5°C (data not shown). There was no significant difference between the number of each categories for most probable number (MPN), 23 to ≥240 CFU /100 ml, in tap water and spring water sources (chi-square test; $p>0.05$) (Table 2). Within 457 water samples collected from all sampling stations during the sampling period, 199 samples (43.51%) were detected to be contaminated by coliform bacteria. Forty-one samples (8.9%) with non-coliform bacteria, and a large part of the samples, 217 (47.4%) were with no growth. Consequently, 117 of the 199 coliforms were detected as *E. coli* which was included in this study. The percentage and number of fecal coliform pollution status in tap and spring water samples are separately shown on Fig. 2A and B, respectively.

Antibiotic susceptibility

From the positive water cultures, 117 *E. coli* strains were isolated. Antibiotic resistance rate was found by using the results of the disk diffusion method. All *E. coli* isolates were detected to be resistant to one or more antibiotics tested. Forty-nine of the strains (41.8%) expressed multiresistant phenotype, defined here as resistance to three or more antibiotics tested. Resistance to ampicillin was prevalent in approximately 55 (47%) of the isolates. Resistance to trimethoprime-sulfamethoxazole (19.6%), amikacin (17.9%), netilmicin (15.3%), gentamicin, (13.6%), tetracycline (12.8%), and chloramphenicol (2.5%) was also detected. We observed that there was no significant difference for the total antibiotic resistance rate of the *E. coli* isolates from tap and spring water sources (chi-square test; $p>0.05$) (Fig. 3).

Carriage of class 1 integron

The forty-nine isolates expressing multiresistant phenotype were directly screened for carriage of class 1 integrons by class 1 integron-specific PCR. Three (nearly 3%) isolates (Rs411b, Rs5a and Rs90a from III, IV, and VII Station, respectively) carried the class 1 integrons (Table 3), which is the first confirmation of class 1 integrons from the environmental bacteria in Turkey.

Class 1 integrons in both of two *E. coli* strains, Rs5a (GenBank accession no. DQ875876) and Rs90a (GenBank accession no. DQ875875), from tap water were 1,586 bp in

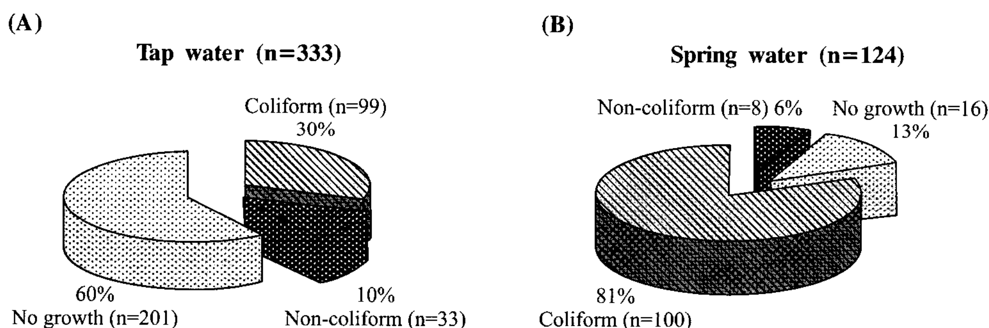

Fig. 2. Results of multiple-tube fermentation assays of tap water (A) and spring water (B) samples.

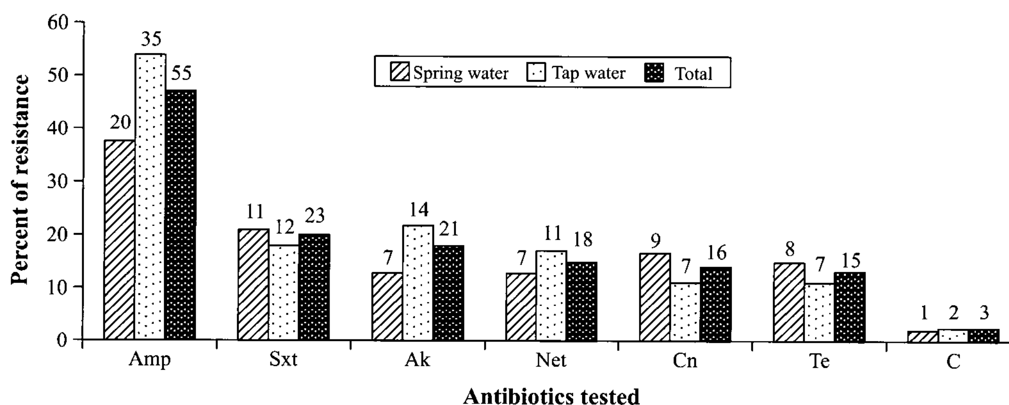

Fig. 3. Comparison of *E. coli* isolates from spring water and tap water regarding total resistance to antibiotics. Chi-square test; $p>0.05$. For abbreviations, see to footnote of Table 3.

size (Fig. 4). The third strain, Rs411b (GenBank accession no. DQ875874) from spring water, harbored a 1,663 bp fragment (Fig. 4). The aligned analysis of amino acid sequencing revealed that the variable region of two of the 1586 bp-integrons contained two gene cassettes, *dhfr1* gene encoding dihydrofolate reductase conferring trimethoprim resistance, and *aadA1* gene encoding aminoglycoside adenyltransferase which confers resistance to aminoglycosides. The sequence analysis of 1,663 bp integron showed that the genetic inserts corresponded to *dfrA17* encoding dihydrofolate reductase conferring resistance to trimethoprime and *aadA5* encoding aminoglycoside 3'-adenylyltransferase conferring resistance to aminoglycosides. According to the results of multiple sequence alignment, CLUSTAL W, nucleotide sequences of two integron cassette arrays detected in this study are completely similar to previously identified gene cassettes that reported from the uncultured bacteria (GenBank accession nos. AY115476 and AY139591) isolated from a wastewater treatment plant (Tennstedt *et al.*, 2003).

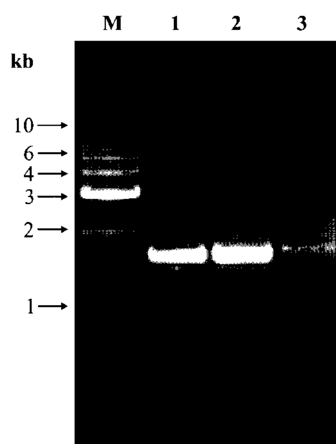

Fig. 4. Class 1 integrons in *E. coli* strains with origin of drinking water. Lane M, 1 kb DNA Ladder (MBI Fermentas, USA); 1, *E. coli* Rs5a; 2, *E. coli* Rs90a; 3, *E. coli* Rs411b.

Nine isolates carrying integron structures and/or transferable antibiotic resistance were analyzed regarding their phenotypic and genotypic properties as listed in Table 3. These strains were isolated among the years 2000 to 2002, mainly from third station in 2001.

No class 1 integron structures were detected in the transconjugants, indicating chromosomally-located class 1 integrons.

Tetracycline resistance determinants

The rate of resistance to tetracycline was 15% among the isolates. We investigated the molecular mechanism of the tetracycline resistance by PCR for *tet*(A), *tet*(B) and *tet*(C) genes which are the most prevalent tetracycline resistance determinants in *E. coli*. Five *E. coli* strains (Rs74, Rs92, Rs134b, Rs171, and Rs268) were resistant to tetracycline via *tet*(A) gene and were also transferable. Two strains (Rs5a and Rs411b) owned *tet*(B)-mediated resistance, but non-transferable resistance mechanism. One strain (Rs450) had both *tet*(A) and *tet*(B) genes. One strain (Rs350) had none of the *tet*(A), *tet*(B) or *tet*(C) genes, but transferable with unknown mechanism, as shown in Table 3. No strains were found to have *tet*(C) gene-mediated tetracycline resistance.

Transferability of antibiotic resistance

Conjugation experiments were carried out to detect the transferability of resistance against the antibiotics used in susceptibility testing. Seven R^+ transconjugants were obtained with the transfer frequency of 6.3×10^{-9} to 10^{-6} (data not shown), suggesting that resistance to ampicillin, tetracycline or trimethoprim/sulfamethoxazole were transferable (Table 3).

BOX-PCR fingerprinting

We genotyped 10 strains carrying integrons and/or transferable antibiotic resistance by BOX-PCR fingerprinting. Analysis yielded patterns of amplification product for all isolates, and these products varied 600 to >6,000 bp, as shown in Fig. 5.

To evaluate the strain diversity of *E. coli* from drinking water sources, dendrograms of BOX fingerprints were constructed by using the UPGMA method of tree building and

Table 3. Epidemiological characteristics of *E. coli* isolates carrying class 1 integron and/or transferable antibiotic resistance

E. coli strain	Date isolated	Water type	Station isolated	Growth at 45°C[a]	Resistance Phenotype[b]	Resistance transferred	Integron (bp/cassette array)[c]	*tet* gene
Rs5a	October 2000	Tap	IV	Yes	Amp Cn Te Sxt	-	1586 / *dhfr1-aadA1*	*tet*(B)
Rs74	February 2001	Tap	III	Yes	Te	Te	(-)	*tet*(A)
Rs90a	February 2001	Tap	VII	Yes	Amp Cn Sxt	-	1586 / *dhfr1-aadA1*	-
Rs134b	March 2001	Spring	IV	Yes	Amp Te	Amp Te	(-)	*tet*(A)
Rs171	May 2001	Spring	III	Yes	Sxt Te	Te	(-)	*tet*(A)
Rs196	June 2001	Spring	VII	Yes	Amp	Amp	(-)	-
Rs268	July 2001	Spring	III	Yes	Amp Te	Amp Te	(-)	*tet*(A)
Rs350	September 2001	Tap	III	Yes	Amp Ak Net Te Sxt	Amp Te Sxt	(-)	-
Rs411b	January 2002	Spring	III	Yes	Amp Cn Net Te C Sxt	Amp Te	1663 / *dfrA17-aadA5*	*tet*(B)
Rs450	February 2004	Tap	IV	Yes	Amp Ak Net TeSxt	Amp Te Ak Net Sxt	(-)	*tet*(A), *tet*(B)

[a]In brilliant green broth
[b]Amp, ampicillin; Te, tetracycline; Ak, amikacin; Cn, gentamicin; Net, netilmicin; C, chloramphenicol; Sxt, trimethoprime-sulfamethoxazole
[c](-), no class 1 integron

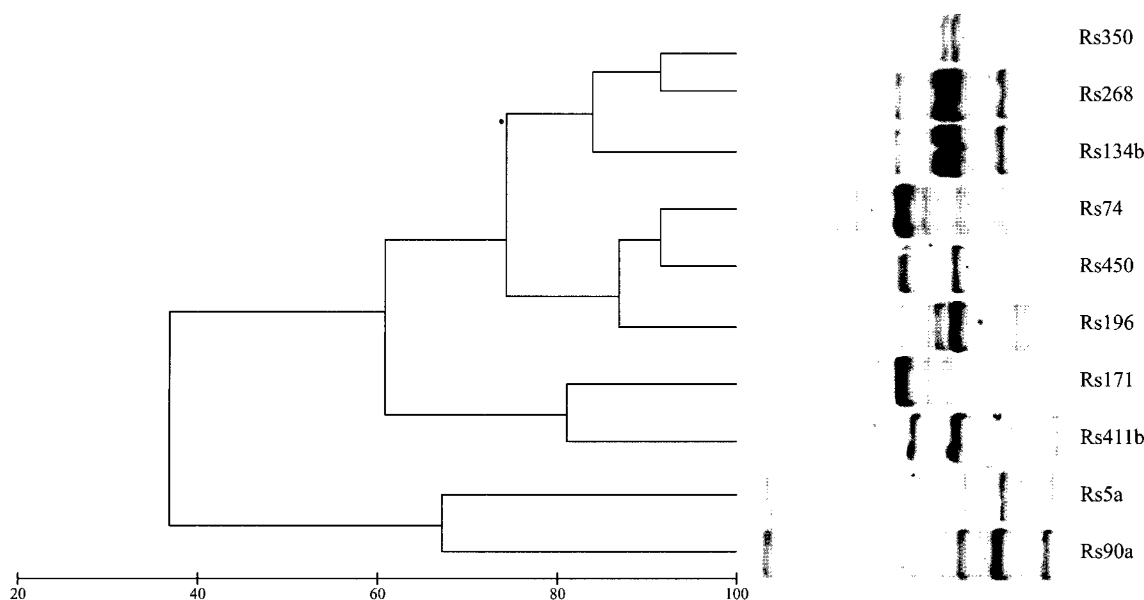

Fig. 5. Clustering of DNA fingerprinting using BOX-primer of bacteria isolated from tap and spring water using the UPGMA method.

significant clusters in each dendrogram were identified (Fig. 5). Pairwise comparison of BOX fingerprints of isolates from the water sources generated similarity scores ranging from 50.0 to 100.0% for the group of water isolates. Two major clusters were obtained, one corresponding to the strains Rs90a and Rs5a and the second to the other strains. There is 100% similarity between Rs5a and Rs90a in one cluster, and between Rs134b, Rs268, Rs350 of the second cluster. However, the lowest genetic similarity was also observed between these two groups (Fig. 5).

Discussion

The quality of water is of vital importance to society. Efficient surveillance and control strategies are crucial for executing a high-quality management of this resource. Rize province has been settled in the northern region of Turkey, and a unique region which contains the richest natural fresh water sources in Turkey. We observed that there was a high fecal coliform counts in every sampling event according to the results of multiple-tube fermentation test, shown on Fig. 1. There was statically no significant difference in each fecal coliform pollution levels, 23 to >240 MPN/100 ml (chi-square test; $p>0.05$). This result presented here reflects that there may be an engineering problem for the water treatment in the region. It has been reported that occurrence of coliform bacteria within a distribution system is a complex interaction of physical, chemical, operational and engineering parameters (Lechevallier *et al.*, 1996). Looking at the emergence period of fecal *E. coli* carrying class 1 integrons and/or transferable antibiotic resistance (Table 3), it can be suggested that the pollution has been probably emerged in year 2001, especially in third station, downtown of Rize province.

Investigations on antibiotic resistance in the aquatic habitat have concerned bacteria of fecal origin because they are used as pollution indicators and may be associated with infectious diseases (Jones *et al.*, 1986). This study describing the screening of antibiotic resistance in *E. coli* isolates shows that there is no significant difference between tap and spring water regarding resistance rate of *E. coli* strains (chi-square test; $p>0.05$). This finding suggests that multi-resistant strains are predominant in both water types.

It has been reported that 40% of the coliforms isolated from a river in Turkey were with multidrug-resistant (Toroglu *et al.*, 2005). We observed that high-levels of resistance to ampicillin (47% of the total isolates) were present in both water types. The high-levels of resistance to ampicillin were in general agreement with that reported by other investigators (Andersen and Sandaa, 1994; Boon and Cattanach, 1999), and has been found to be very common in *E. coli* isolated from the normal human fecal microbiota in Turkey (Ozgumus *et al.*, 2006). The occurrence of coliforms with high-level resistance to ampicillin and to other antibiotics reflects human influence in the environment (Andersen and Sandaa, 1994). Interestingly, resistance to chloramphenicol was detected at relatively low level (3%), probably resulting from limited use of this drug in veterinary or human medicine.

Class 1 integrons were found in three (2.5%) of 117 *E. coli* isolates of fecal origin from public drinking waters. This prevalence was lower than that reported by Roe *et al.* (2003), who showed that 16% of Gram-negative bacteria in irrigation water and sediments contained the class 1 integron. It was already agreement with that reported (Rosser and Young, 1999), who showed that 3.6% of Gram-negative bacteria in an estuarine environment contained the class 1 integron. In addition, our rate is much lower than those reported by Park *et al.* (2003) and Lin *et al.* (2005), who showed that 24% and 58% of multiresistant isolates both with aquatic environments contained the class 1 integron, respectively. All class 1 integron-bearing *E. coli* in this study contained the gene cassettes, *aadA1*, and *aadA5,* conferring resistance

to spectinomycin and streptomycin. Resistance to aminoglycosides, gentamicin and netilmicin tested in the present study was observed in integron-carrying isolates. The *aadA* genes in class 1 integron cassettes observed in the current study are not novel in *E. coli* of environmental origin. Previous works by Tennstedt *et al.* (2003) and White *et al.* (2000) have shown that the *aadA5* gene was widespread in *E. coli* strains of wastewater and clinical origin, respectively.

The results of this study as to the presence of class 1 integron reflect a public health risk because commensal bacteria in aquatic environments may become important reservoirs of antibiotic-resistance genes and transfer the resistance determinants to other bacteria associated with humans. On the other hand, our recent study (Alpay-Karaoglu *et al.*, 2007) on the determinants of the β-lactam resistance from drinking waters in Rize region showed that TEM-1 type β-lactamase genes have been widespread in ampicillin-resistant *E. coli* strains isolated from fresh waters indicating a fecal contamination. Resistance genes carried by the commensal bacteria in such environment are of clinical importance because resistant strains probably originate from the natural environment. They are then carried to a clinical setting by the discharges of carriers such as microbiota of the healthy people under antibiotic selective pressure.

Integron structures have been observed in various environments including in healthy persons (Skurnik *et al.*, 2005), different hospitals in Europe (Martinez-Freijo *et al.*, 1998), various domestic animals (Lanz *et al.*, 2003), bacterial pathogens of plants (Sundin, 2000), fish pathogens (Schmidt *et al.*, 2001), and even in lizards (Waturangil *et al.*, 2003) other than aquatic environments (Rosser and Young, 1999; Roe *et al.*, 2003; Lin and Biyela, 2005). According to the findings of those studies, it can be supposed that there is the link among bacteria from the various environments, reflecting the interchange of antibiotic resistance gene cassettes.

Cernat *et al.* (2002) have reported that all aquatic R^+ strains they isolated from the river water transferred two or more antibiotic resistance markers. Resistance to ampicillin and tetracycline was most frequently observed. On the other hand, Gupta and Ali (2004) have reported from India that *E. coli* isolates from different aquatic environment including rivers in distinct geographical regions expressed high-level heavy metal resistance, especially to mercury. Although we did not investigate the heavy metal resistance in multidrug-resistant strains, resistance to ampicillin, tetracycline or trimethoprim/sulfamethoxazole were transferable. The increased prevalence of transferable resistance may result from the antibiotic selective pressure because of the heavy use of antibiotics for animal feeding or the treatment of infectious diseases. It has been suggested that this pressure may have been exerted in the environment in which the strains were found, the environment subsequently being contaminated by antibiotic resistant bacteria (Linton, 1988). Tetracycline resistance has been used as the key determinant to monitor resistance genes in natural environments. Guardabassi *et al.* (2000) have reported the mechanisms of resistance to tetracycline in *Acinetobacter baumannii* by finding the Tet(A) and Tet(B) determinants in clinical and aquatic strains. Therefore, tetracycline resistance genes, *tet*(A) and *tet*(B), are widespread in drinking water in Rize region. Agersø and Sandvang (2005), have been suggested that soil bacteria in close contact with manure or pigsty environments seemed to have an important role in horizontal spread of plasmid-mediated resistance encoded by class 1 integron gene cassettes and *tet* genes.

A larger percentage of water isolates from urban areas compared to isolates from rural areas has been reported to exhibit resistance to antibiotics, presumably because human isolates were present (Kasper *et al.*, 1990). Parveen *et al.* (1997) has reported that human *E. coli* isolates clustered near isolates obtained from sewage treatment plant effluents and that isolates from animal feces were more similar to nonpoint source isolates. To determine source of fecal pollution, we used BOX-PCR fingerprinting that target BOX sequences, and then concluded that some of the strains had the same PCR patterns which were epidemiologically indistinguishable. However, in Turkey's natural habitats and aquatic environments, we have encountered no studies planned or done for the detection of sources of fecal coliform bacteria. We did not collect the fecal bacteria belonging to the fecal microbiota of the wild or domestic animals to compare the *E. coli* isolated from those samples to our isolates. Thus, we are unable to compare the unknown *E. coli* banding patterns to the known source library of patterns. However, it is very likely that the strains might have originated from various hosts such as humans, domestic animals, and may be from wild animals. Conversion of these data into a dendogram (Fig. 5) reveals the degree of relatedness among the *E. coli* strains from both of tap and spring water samples. The dendogram clearly shows that tap and spring water sources were possibly contaminated by the same sources of fecal coliforms. Particularly, strains Rs5a and Rs90a are much more similar to each other than the other strains Rs134b, Rs268, and Rs350. This may be because of contaminated by different fecal source.

Numerous factors might have involved for the release of resistant strains in the environment. Insufficient sanitation facilities (Al-Jebouri and Al-Meshhadani, 1985), indiscriminate use of antibiotics (Amyes *et al.*, 1992) and lack of well- managed sewerage systems are important contributing factors. Consequently, water from contaminated sources is used for drinking purpose by the people in Rize region, and the resistant bacteria are thus transmitted to humans. The data presented here can be used to address the question of whether drinking water sources or rivers used as fresh water source are significant reservoirs for spread of transferable resistance genes and the receptacle for the antimicrobial resistance determinants. We believe that surveillance programs can provide valuable information for the persistence and mobility of resistance genes between community and clinical settings.

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trol in PCR for class 1 integron.

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