Annals of Microbiology, **57 (2)** 281-288 (2007)

Investigation of antibiotic resistance profile and TEM-type β-lactamase gene carriage of ampicillin-resistant *Escherichia coli* strains isolated from drinking water

Sengul ALPAY–KARAOGLU[1], Osman Birol OZGUMUS[1]*, Elif SEVIM[1], Fetiye KOLAYLI[2], Ali SEVIM[1], Pinar YESILGIL[1]

[1]Department of Biology, Faculty of Arts and Sciences, Rize University, 53100 Rize; [2]Department of Medical Microbiology, Faculty of Medicine, Kocaeli University, 41900 Kocaeli, Turkey

Received 8 January 2007 / Accepted 30 April 2007

Abstract - Fifty-five ampicillin-resistant (Amp^r) *Escherichia coli* strains were isolated from 51 drinking water points in Rize region containing abundant fresh water sources in Turkey during the years 2000 to 2002 and from January to February 2004. The large number of organisms (nearly 57%) exhibited resistance to three or more antibiotics commonly used in human and veterinary medicine. These strains displayed a multiresistant phenotype. Nearly half of the strains (27%) expressed resistance to ceftazidime, but these strains were not an extended-spectrum β-lactamase-producer according to the results of double-disk synergy test. All isolates were then screened for the carriage of TEM-type β-lactamase gene (bla_{TEM}) by polymerase chain reaction. TEM-type β-lactamase genes were found in six (11%) isolates. Sequence analysis showed TEM-1 type genes. However, isoelectric focusing analysis did not confirm the production of TEM-1 type β-lactamase except for one strain. Conjugation experiments showed that resistance to ampicillin, tetracycline or trimethoprim/sulfamethoxazole was transferable in six (11%) isolates. Emergence of transferable antibiotic resistance and bla_{TEM-1} gene in *E. coli* strains from public drinking waters possesses a significant public health risk.

Key words: ampicillin-resistant *Escherichia coli*, bla_{TEM-1} gene, drinking water.

INTRODUCTION

Antibiotic resistance has been observed in various aquatic environments including rivers, coastal areas, domestic sewage, surface waters, sediments, lakes, polluted ocean water by sewage and drinking water (Mezrioui and Baleux, 1994). Increased introduction of antimicrobial agents into these environments via medical therapy, agriculture and animal husbandry has resulted in new selective pressures on natural bacterial populations (Col and O'Connor, 1987; Du Pont and Steele, 1987). It was reported that this has exacerbated the problem of controlling microbes in a disease setting and has caused a resurgence of bacterial diseases worldwide due to the acquisition and transfer of antibiotic resistance genes and virulence factors (Tomasz, 1994).

The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogens. The ability of the resistant bacteria and resistance genes to move from one ecosystem to another is documented by the various cases in which transmission of resistant bacteria has been demonstrated between animals and humans. The inclusion of the certain growth promoters in animal feed has been recognized as a cause for the selection of the resistance genes in the commensal microflora of animals. They are transmitted to humans via the food chain (Kruse, 1999). Similarly, drinking and bathing water could represent a source for the acquisition of resistant bacteria in humans. However, further studies are necessary to validate this hypothesis. *Escherichia coli* has been generally accepted as the predominant vehicle for the dissemination of resistance genes and vectors due to its abundance in such environments (Tauxe, 1997).

β-Lactam antibiotics account for approximately 50% of global antibiotic consumption (Livermore, 1998). Resistance to β-lactam antibiotics such as penicillins and cephalosporins is commonly mediated by the β-lactamase enzymes. *Escherichia coli* strains also become resistant to ampicillin by plasmid-mediated class A β-lactamase enzymes such as TEM-1. They tend to mutate to extended-spectrum activity (Livermore, 1995). Epidemiological findings indicate that TEM-1 is the most common plasmid-mediated β-lactamase between clinical Gram-negative bacteria (Bush and Jacoby, 1997; Sirot *et al.*, 1997; Hoffmann *et al.*, 2006; Mendonca *et al.*, 2006).

Rize region has been settled in Northern coastal area which has the richest natural fresh water sources in Turkey. In this study, we aimed to investigate the carriage of bla_{TEM} genes and the frequency of antibiotic resistance in Amp^r *E.*

* Corresponding author. Address: Biyoloji Bolumu, Fen-Edebiyat Fakultesi, Rize Universitesi, Milli Piyango Egitim Kampusu, 53100 Rize, Turkiye;
Phone: +90 464 2236126 ext 1220; Fax: +90 464 2235376; E-mail: microbsman@yahoo.com.

coli strains isolated from public drinking waters in Rize city and its six counties.

MATERIALS AND METHODS

Sampling sites. Fifty-five water samples were collected in sterile glass bottles from 51 different sampling sites in Rize city and six coastal counties, Iyidere, Derepazari, Cayeli, Pazar, Ardesen and Findikli, during the years 2000 to 2002 and from January to February 2004. These settlements are located in the Black Sea coastal region of Northern Turkey (Fig. 1). The distance between each cities varies from 8 to > 20 km as seen in Fig. 1. This area is therefore the richest region for fresh water in Turkey. It is also rainy and humid.

Isolation of Amp^r *Escherichia coli* strains. The water samples were transported on ice to the Microbiology & Molecular Biology Research Laboratory of Rize University and were processed within 8 h of collection. Water samples were inoculated into the lactose peptone water (Oxoid, England) to detect facultative anaerobic growth. Confirmation of coliform growth was achieved by monitoring the acidification and gas production during growth in lactose peptone water. To isolate Amp^r *E. coli* strains, 100 µL of two-fold serial dilutions of bacterial cultures were spread onto the surface of Eosine Methylene Blue (EMB) agar (Merck, Germany) containing ampicillin (Fischer, USA) at 40 µg/mL; according to the criteria of Clinical and Laboratory Standards Institute (CLSI, 2003), members of the *Enterobacteriaceae* family which are capable of growing in concentration of ≥ 32 µg/mL of ampicillin are regarded as resistant to ampicillin. At least five presumptive *E. coli* colonies on EMB agar were picked up by a sterile toothpick and replica plated on a fresh medium containing 40 µg/mL of ampicillin. The presence of *E. coli* was confirmed by demonstration of indole production from tryptophan in tryptone water (Merck) and appearance of colony morphology on EMB agar, as previously described by Brenner (1986). After identification to the species level, organisms were stored in 20% of glycerol at -30 ºC until next use.

Antimicrobial susceptibility testing. The susceptibilities of the *E. coli* isolates and their transconjugants to the antibiotics were determined by the standard disk diffusion method as described in CLSI (2003) guidelines. The results were interpreted by using the breakpoints in the same guidelines (CLSI, 2003). The following antibiotic disks (Oxoid) were used in susceptibility testing: sulbactam/ampicillin (10 µg /10 µg), cefazolin (30 µg), cefuroxime (30 µg), ceftazidime (30 µg), gentamicin (10 µg), amikacin (30 µg), netilmicin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg) and trimethoprim/sulfamethoxazole (1.25 µg/23.75 µg).

The double-disk synergy test (DDS). For the detection of an extended-spectrum β-lactamase (ESBL) enzyme, the DDS tests (Jarlier *et al.*, 1988) were performed as a standard disk diffusion test (CLSI, 2003) on Mueller-Hinton

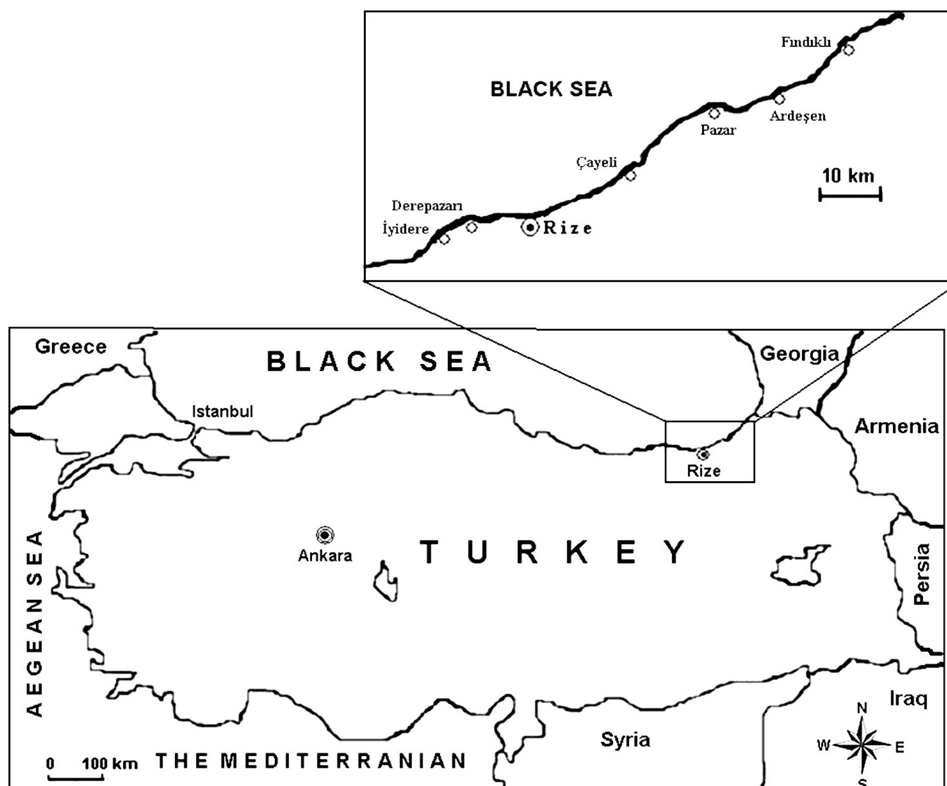

FIG. 1 - Map of Rize region showing sampling places.

agar (Difco, USA). A potentiation of the zones of ceftazidime disk by amoxicillin/clavulanic acid (20 μg/10 μg) (Oxoid) was indicative of the possible presence of an ESBL. *Escherichia coli* J53-2 (pUD18 encoding for SHV-3 extended-spectrum β-lactamase), kindly provided from Dr. George A. Jacoby, Lahey Hitchcock Clinic, Massachussets, USA was used as positive control for DDS test.

Isoelectric focusing (IEF). The analytical IEF was performed by the method with slight modifications described by Matthew *et al.* (1975) by using a Mini IEF cell system (Bio-Rad, USA) in the Laboratory of Medical Microbiology Department in Kocaeli University. Bacteria grown on Luria-Bertani (LB) agar (1% tryptone, 0.5% sodium chloride, 0.5% yeast extract, 1.5% agar, pH 7.4) were harvested and transferred into 0.1 M of phosphate buffer (pH 7.0). Enzymes were extracted from the bacteria by freezing and thawing. The analytic IEF was performed on polyacrylamide gel containing 40% of ampholines (pH 3-10), and then electrophoresed at a constant 1 watt for one hour. The gel was stained with 500 μg/mL of a chromogenic substrate, nitrocefin, to make the β-lactamase bands visible, and then photographed. The β-lactamases TEM-1 (pI 5.4) and SHV-2 (pI 7.6) were used as the reference bands of known β-lactamases.

Conjugation assay. Conjugation assays were performed by broth mating method (Rice *et al.*, 1990) with slight modifications. Equal volumes of cultures of the Amp^r *E. coli* strains as donor and *E. coli* K12 strain J53-2 (F^- *met pro* Rif^r) as the recipient, grown with agitation in LB broth were mixed and incubated without shaking at 35 ºC for 18 h. Transconjugants were selected on EMB agar supplemented with 150 μg/mL rifampin (Hoecst, Germany) to inhibit donor strains and 50 μg/mL ampicillin, 10 μg/mL tetracycline (Sigma, USA) or 25 μg/mL trimethoprim/sulfamethoxazole (Roche, Germany) to inhibit the recipient.

PCR assay. To prepare templates for the TEM-specific PCR, Amp^r *E. coli* strains and their transconjugants were inoculated into 3 mL of LB broth and incubated at 37 ºC for 20 h with shaking. Cells from 1.5 mL of the overnight culture were harvested by centrifugation at 13000 *x g* for 3 min. After decanting the supernatant, the pellet was re-suspended in 500 μL of sterile deionised water. The cells were lysed by heating to 95 ºC for 10 min, and cellular debris was removed by centrifugation. A 1-μL of supernatant was used as template for PCR amplification of TEM genes with the intragenic oligonucleotide primers, previously designed by Arlet and Philippon (1991); OT1 (5'-TTGGGTGCACGAGTGGGTTA-3') and OT2 (5'-TAATTGTTGCCGGGAAGCTA-3'), to amplify a 504-bp fragment (see results Fig. 4). PCR amplifications were carried out on a DNA thermal cycler (Eppendorf, USA), and performed in a volume of 50 μL containing DNA template (100-200 ng), 10 pM of each primer (OT1/OT2 or OT3/OT4), 20 μM of each deoxynucleotide triphosphate (dATP, dCTP, dGTP and dTTP) (Promega, USA), 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1% Triton-X-100 and 1 U *Taq* DNA polymerase (Promega). The PCR program consisted of an initial denaturation step at 94 ºC for 10 min followed by 26 cycles of DNA denaturation at 94 ºC for 2 min, primer annealing at 60 ºC, primer extension at 72 ºC for 2 min and a final extension at 72 ºC for 7 min. After the last cycle, the products were stored at 4 ºC. The PCR products were then analysed in 2% agarose gel containing 0.5 μg/mL of ethidium bromide (Sigma) and visualized under UV light.

Sequencing of bla_{TEM} genes. TEM-type β-lactamase genes in the strains were amplified with the sequencing primers, OT3 (5'-ATGAGTATTCAACATTTCCG-3') and OT4 (5'-CAATGCTTAATCAGTGAGG-3'), previously designed by Arlet *et al.* (1995), to obtain the complete β-lactamase gene. Reaction mixture and cycling parameters were carried out as mentioned above. Polymerase chain reaction products were purified from the agarose gel by using QIAQuick® Purification Kits (QIAgen, UK). Purified and cleaned amplicons were sent together with the same primer for sequencing to the Macrogen Inc., Seoul, Korea.

Sequence analysis. Data of putative proteins of nucleotide sequence was compared with those available in the GenBank database by using the alignment search tool BLAST program (Altschul *et al*., 1997) accessible on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Amp^r *Escherichia coli* isolates and antibiotic resistance patterns

We isolated Amp^r *E. coli* strains in each sampling events during the study period. The number and frequency of the strains isolated from the sampling sites are shown in Fig. 2. The highest number of the strains was found in two sites, 20 strains in 21 sampling sites in Rize city and 11 strains in 14 sampling sites in Ardesen. We concluded that the main cause of the coliform pollution might have resulted from the domestic activities. One of the most important characteristics of Rize region is only area where tea plant is grown in Turkey. Drinking water sources in the region are obtained from the local rivers stretching in underdeveloped rural areas and agricultural areas with intensive cultivation. Some of the domestic sewage inputs discharge into these local streams. Thus, all the negativities associated with the presence of domestically polluted streams prompted us to investigate for the antibiotic resistance traits of *E. coli* from the public drinking waters.

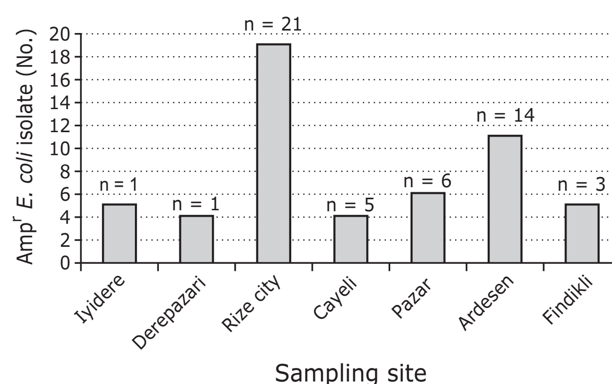

FIG. 2 - Number of Amp^r *Escherichia coli* strains isolated from 51 sampling sites (n) in Rize region.

TABLE 1 - Resistance frequency of 55 Amp^r *Escherichia coli* strains from sampling sites in Rize region

Sampling site	*Escherichia coli* isolates (n = 55)	No. of resistant organisms to antibiotics*									
		CZ	CXM	CAZ	SAM	AK	NET	CN	TE	SXT	C
Iyidere	24a, 24b, 24c, 24d, 24e	0/5	2/5	0/5	5/5	1/5	1/5	0/5	0/5	0/5	0/5
Derepazari	23a, 23b, 23c, 23d	0/4	2/4	0/4	4/4	0/4	1/4	0/4	0/4	0/4	0/4
Rize city	1a, 1b, 1c, 1d, 1e, 1f, 1g**, 2, 3, 4a**, 4b, 4c, 4d, 4e, 5a, 5b, 5c, 6, 7, 8	6/20	7/20	3/20	19/20	4/20	5/20	3/20	5/20	5/20	1/20
Cayeli	20, 21, 22a, 22b**	0/4	1/4	2/4	3/4	2/4	2/4	0/4	3/4	1/4	0/4
Pazar	18, 19a**, 19b**, 19c, 19d**, 19e	2/6	3/6	1/6	6/6	2/6	3/6	0/6	1/6	1/6	1/6
Ardesen	11, 12a, 12b, 12c, 12d, 13, 14, 15, 16, 17a, 17b	0/11	9/11	6/11	11/11	4/11	3/11	4/11	0/11	0/11	0/11
Findikli	9a, 9b, 9c, 10a, 10b	1/5	3/5	2/5	4/5	1/5	2/5	3/5	1/5	2/5	0/5

* CZ, cefazolin; CXM, cefuroxime; CAZ, ceftazidime; SAM, sulbactam/ampicillin; AK, amikacin; NET, netilmicin; CN, gentamicin; TE, tetracycline; SXT, trimethoprim/sulfamethoxazole; C, chloramphenicol.; ** *E. coli* isolate carrying *bla*$_{TEM-1}$ gene.

Most investigations on antibiotic resistance in aquatic habitat have concerned bacteria of faecal origin because they are used as pollution indicators or may be associated with infectious diseases (Jones *et al*., 1986). Antibiotic-resistant bacteria (Al-Ghazali *et al.*, 1988; Pathak *et al.*, 1993) and antibiotics (Halling-Sørensen *et al*., 1998) are discharged in various amounts into the environment as a result of indiscriminative use of antibiotics in medical, veterinary, and agricultural practices. River waters are the main receptacle for these pollutants because they receive the sewage of urban effluents resulted from human activities. The resistance to ampicillin in *E. coli* and coliforms via β-lactamases has been previously demonstrated in different aquatic environments such as sewage, sludge, irrigation waters, rivers and drinking waters in varied ranges between 11% and 96% (Boon and Cattanach, 1999; Papandreou *et al.*, 2000; Reinthaler *et al.*, 2003; Roe *et al.*, 2003). Toroglu *et al*. (2005) also reported that 49% of Gram-negative enterobacteria isolated from a Turkish river were found to be β-lactamase producers.

Multiple antibiotic resistance phenotypes have been used to make a distinction between human and nonhuman sources of *E. coli* (Wiggins *et al.*, 1999; Harwood *et al.*, 2000). A large number of Amp^r *E. coli* isolates (nearly 57%) were multidrug-resistant (regarding resistant strains to three or more antibiotics excluding ampicillin) as listed in Table 1. One of the isolates (*E. coli* strain 1g) from Rize city showed resistance to all antibiotics except for amikacin, while one (*E. coli* strain 5c) from Rize city was sensitive to 10 antibiotics tested (Table 2). There was no clear resistance pattern common in all isolates. However, sulbactam-ampicillin in nine isolates, cefuroxime/sulbactam-ampicillin in seven isolates and amikacin/netilmicin/sulbactam-ampicillin in five isolates were predominant patterns in approximately half of the isolates (Table 2). All isolates were resistant to, at least, one or more β-lactam antibiotics tested other than ampicillin. Resistance to non-β-lactam antibiotics was at variable rates ranging 4 to 31% (Fig. 3). A high frequency of antibiotic resistance to sulbactam-ampicillin (95%), cefuroxime (49%), netilmicin (31%), ceftazidime (27%) amikacin (25%) and gentamicin and trimethoprim/sulfamethoxazole (16%) and a low frequency of chloramphenicol (4%), cefazolin (15%) and tetracycline (18%) were found (Fig. 3). The frequency of the resistance to

TABLE 2 - The predominant antibiotic resistance profiles of 55 Amp^r *Escherichia coli* isolates and their *bla*$_{TEM-1}$ gene carriage

Pattern of antibiotic resistance*	*Escherichia coli* isolate
CAZ	9a
SAM	1b, 6, 4c, 4d, 4e, 23c, 23d, 24a, 24b
SAM CXM	2, 5b, 13, 14, 23b, 24c, 24e
SAM CN	12d
SAM CAZ	16, 18, 20
SAM TE	1c
SAM CZ	3
SAM CZ CXM	1f
SAM SXT CXM	7
AK NET SAM	4b, 19a**, 19b**, 22a, 24b
TE SXT SAM	8
SAM CXM CAZ	10b, 15, 17b
AK SAM CXM	12a
TE SAM CAZ	21
NET SAM CXM	23a
CN TE SXT SAM	1a, 9b
CN SAM CZ CXM	1d**
CN SAM CXM CAZ	12b
AK NET SAM CXM	12c
NET SAM CXM CZ	19c
C SXT SAM CXM	19d**
TE SAM CZ CXM	19e
AK NET SAM CZ CXM	1e
AK NET TE SXT SAM	4a**
CN NET SXT SAM CXM	10a
AK NET SAM CZ CXM CAZ	5a
CN AK NET SAM CXM CZ	9c
CN AK NET SAM CXM CAZ	11, 17a
AK NET TE SXT SAM CXM	22b
CN NET TE C SXT SAM CZ CXM CAZ	1g**
Sensitivity to all antibiotics except ampicillin	5c

* See footnote to Table 1; ** *E. coli* isolate carrying *bla*$_{TEM-1}$ gene.

cefuroxime, ceftazidime, sulbactam-ampicillin and gentamicin was relatively high in the strains from two sampling sites (Rize city and Ardesen) in comparison to the ones from the other sites (Table 1). Roe *et al.* (2003) reported that the resistance to ampicillin was prevalent in approximately 11% of the *E. coli* strains from irrigation water and sediments in the United States. They observed the resistance to tetracycline (9%), kanamycin (2%) and gentamicin (0.3%). However, in the current study, the frequency of the resistance to tetracycline and gentamicin were found to be higher than the authors' results (Fig. 3).

The resistance frequency of cefuroxime and ceftazidime, broad- and extended-spectrum cephalosporins was found to be higher than the cefazolin, a broad-spectrum cephalosporin. About half of the isolates resisted to ceftazidime (Table 1). Ceftazidime resistance led us to think that these strains might be the ESBL-producers. However, no ESBL activity was detected in those strains by DDS test. We suppose that this resistance phenotype may probably result from a secondary change to the permeability which becomes a barrier to the entrance of an extended-spectrum cephalosporin into the bacterial cell.

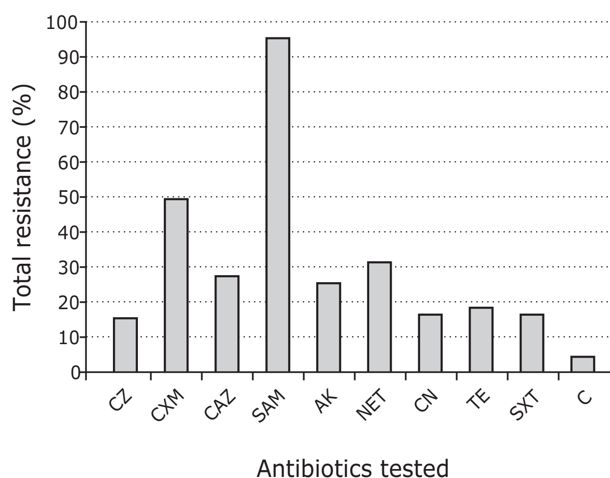

FIG. 3 - Frequency of total antimicrobial resistance in 55 Amp^r *Escherichia coli* isolates. For the abbreviations, see footnote to Table 1.

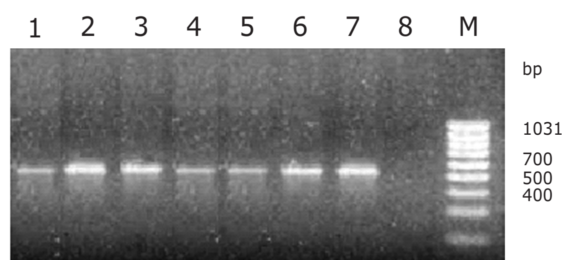

FIG. 4 - PCR analysis of *bla*$_{TEM}$ genes of the strains with the primer pairs OT1/OT2. M, DNA Ladder 100 bp (MBI Fermentas, USA); 1, *E. coli* 1g; 2, *E. coli* 4a; 3, *E. coli* 19a; 4, *E. coli* 19b; 5, *E. coli* 19d; 6, *E. coli* 22b; 7, positive control (pUC18); 8, negative control (*E. coli* ATCC 25922).

The chloramphenicol-resistant strains were found only in two settlements, Rize city and Pazar (Fig. 1). The resistance frequency of chloramphenicol was found as 4% (Fig. 3). Misra *et al.* (1979) only found 1% of the resistant *E. coli*. In previous studies (Tamanai-Shacoori *et al.*, 1995; Krcmery *et al.*, 1989) carried out in various aquatic environments the resistance rate in coliform bacteria has been found in far higher rates (17% to 60%). The low rate of resistance in the present study reflects a decline as a result of the decreased use of chloramphenicol. In addition, resistance to chloramphenicol has been found rather low in Gram-negative bacteria from retail fish in Turkey (Matyar *et al.*, 2004).

bla$_{TEM}$ genes in Amp^r *Escherichia coli* isolates

Six (11%) of 55 Amp^r strains from three neighbouring sites (Rize city, Cayeli and Pazar) gave positive amplicons for *bla*$_{TEM}$ gene by PCR. A 504-bp amplicon was obtained from all six isolates (Fig. 4).

We guessed that TEM-1 gene-carrying strains might have been the hyperproducers of TEM-1 penicillinase. However, results of the IEF analysis strongly refuted this suggestion except for *E. coli* strain 4a (Table 3). Overproduction of TEM-1 penicillinase in *E. coli* has been reported to result in increased resistance to a wide range of β-lactams and β-lactam/β-lactamase inhibitor combinations (Martinez *et al.*, 1987) and widespread among Turkish *E. coli* isolates of clinical (Ozgumus *et al.*, 2002) and faecal flora origin (Ozgumus *et al.*, 2006). The enzyme extracts of five TEM gene-carrying strains did not focus at pI 5.4 in analytical IEF analysis carried out in triplicate (Table 3). However, PCR assays repeatedly revealed that six strains were positive for the *bla*$_{TEM}$ gene. Moreover, sequence analysis of the PCR products showed *bla*$_{TEM-1}$ gene. BLAST analysis showed that putative amino acid sequence of *bla*$_{TEM}$ genes were 100% identical to the other *bla*$_{TEM-1}$ genes in the GenBank (http://www.ncbi.nlm.nih.gov/) (data not shown). This contrariness may be explained by the insufficiency of the gene expression due probably to the presence of a weak or repressed promoter of the structural genes of *bla*$_{TEM}$ or no gene expression. On the other hand, it may be a bacterial adaptation to the natural environment which is under less antibiotic pressure in comparison to a clinical environment.

Recently, retrieved *bla*$_{TEM}$ sequences from aquatic environments have been found identical or very similar to β-lactamase gene sequences previously characterized from clinical isolates. This suggests that aquatic ecosystem is a reservoir of *bla* sequences (Henriques *et al.*, 2006). Moreover, enterobacterial resistance genes encoding β-lactamase activities have been detected by PCR from biofilms in wastewater, surface water and drinking water (Schwartz *et al.*, 2003).

Interestingly, no *bla*$_{TEM}$ gene was found in *E. coli* 1d by PCR, but detected an enzyme focused at pI 5.2, indicating that this strain had an inhibitor-resistant TEM (IRT) β-lactamase gene (*bla*$_{IRT}$). We think that this gene is probably located on bacterial chromosome because no transferable resistance phenotype was observed for this strain (Table 3).

Transferable antibiotic resistance

Resistance to ampicillin, trimethoprim/sulfamethoxazole or tetracycline was found to be transferable in six (11%) isolates (Table 3) by conjugation experiments. However, no

TABLE 3 - Properties of the Amp^r *Escherichia coli* isolates harbouring self-transmissible R plasmid and *bla*$_{TEM-1}$ gene

E. coli isolate	Antibiotic resistance phenotype*	Antibiotic resistance phenotype of R$^+$ transconjugants**	Place	Date	Sequence analysis***	pI****
1c	SAM TE	AMP SAM TE	Rize city	July 2001		ND
1d	SAM CZ CXM CN	-	Rize city	September 2001	*bla*$_{IRT}$	5.2
1g	SAM CZ CXM CAZ TE CN NET SXT C	AMP SAM CZ CXM CAZ CN TE	Rize city	January 2002	*bla*$_{TEM-1}$	(-)
4a	SAM TE AK NET SXT	AMP SAM TE SXT	Rize city	September 2001	*bla*$_{TEM-1}$	5.4
9c	SAM CZ CXM CN AK NET	AMP SAM CZ CXM	Findikli	June 2001		ND
19a	SAM AK NET	-	Pazar	February 2004	*bla*$_{TEM-1}$	(-)
19b	SAM AK NET	-	Pazar	February 2004	*bla*$_{TEM-1}$	(-)
19d	SAM CXM C SXT	-	Pazar	February 2004	*bla*$_{TEM-1}$	(-)
21	SAM CAZ TE	AMP SAM TE	Cayeli	March 2001		ND
22b	SAM CXM AK NET TE SXT	AMP TE AK NET SXT	Cayeli	February 2004	*bla*$_{TEM-1}$	(-)

* See footnote to Table 1; ** -, no transconjugation; *** *bla*$_{IRT}$, inhibitor resistant TEM β-lactamase gene; **** ND, not done; (-), no enzyme band detected by IEF assay.

bla$_{TEM}$ gene was detected in three (1c, 9c and 21) of six strains and their transconjugants by PCR. Although we could not determine the exact molecular mechanisms, such kind of resistance to ampicillin is likely to be commonly mediated by chromosomally induced AmpC β-lactamases (Livermore, 1995). On the other hand, mutations causing decreases in permeability will also augment the resistance of *E. coli*.

Antibiotic resistance traits in some of the TEM gene-carrying strains (1d, 19a, 19b and 19d) were not transferable, leading us to think that the TEM genes have probably been located on bacterial chromosome.

High rates of multiple antibiotic resistant *E. coli* isolates have been found in drinking waters in the United States. Some of these resistances such as resistance to ampicillin and tetracycline have been found transferable by conjugation mechanism (Walia *et al.*, 2004). Findings from India indicate the similar rates for the transferability of antibiotic resistance in the rural drinking water systems. Authors reported that 15.3% of the antibiotic resistant bacteria were able to transfer their resistance determinants via conjugation (Gaur *et al.*, 1992). Our results seem to be almost concordant with the authors' results.

The frequency with which these multiresistant organisms are found suggests that characterization of resistance genes should provide information about reservoirs for antibiotic resistance in the environment. However, we conclude that further investigations are required concerning the effects of bacteria carrying transferable antibiotic resistance traits released from drinking water systems on the natural ecosystem and on humans.

In conclusion, it was very curious and worrying phenomenon for public health that we isolated multidrug-resistant *E. coli* strains carrying *bla*$_{TEM-1}$ gene from "the public drinking waters". We think that occurrence of these organisms in drinking waters could possibly be used an alternative indicator for faecal contamination of human origin. These data raise an important question about the impact of *E. coli* carrying *bla*$_{TEM-1}$ gene as potential reservoir of resistance genes in drinking water. To address this question, further molecular epidemiological investigations should be committed to screen for these genes in various aquatic environments. An evolutionary bacterial response to the various environmental stresses such as residual antimicrobial agents and polluting substances from industrial facilities on natural bacterial populations should regularly be monitored to obtain useful and conceivable data to take the strict precautions of bacterial resistance to antimicrobial agents used in medical, veterinary or agricultural purposes.

Acknowledgements

This work was supported by the research fund of Karadeniz Technical University, Trabzon, Turkiye (Project numbers 2004.125.003.01 and 20.111.004.4) and also in part by the Scientific and Technical Research Council of Turkiye, Directorate of Human Resources Development (Project number B.02.1.BAK.0.09.00.00/4771-1634). We wish to thank Prof. Murat Erturk (Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkiye) for correction of English.

REFERENCES

Al-Ghazali M.R., Jazrawi S.F., Al-Doori Z.A. (1988). Antibiotic resistance among pollution indicator bacteria isolated from Al-Khair River, Baghdad. Water Res., 22: 641-644.

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., and Lipman D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res., 25: 3389-3402.

Arlet G., Philippon A. (1991). Construction by polymerase chain reaction and use of intragenic DNA probes for three main types of transferable b-lactamases (TEM, SHV, CARB). FEMS Microbiol. Lett., 82: 19-26.

Arlet G., Brami G., Décrè D., Flippo A., Gaillot O., Lagrange P.H., Philippon A. (1995). Molecular chracterisation by PCR-restriction fragment length polymorphism of TEM β-lactamases. FEMS Microbiol. Lett., 134: 203-208.

Ausubel F.M., Brent R., Kingston R.E., Moore D.D., Seidman J.G., Smith J.A., Struhl K. (1995). Short Protocols in Molecular Biology, 2nd edn., John Willey & Sons, New York.

Boon P.I., Cattanach M. (1999). Antibiotic resistance of native and faecal bacteria isolated from rivers, reservoirs and sewage treatment facilities in Victoria, South-Eastern Australia. Lett. Appl. Microbiol., 28: 164-168.

Brenner D.J. (1986). Facultatively anaerobic Gram-negative rods. In: Krieg N.R., Holt J.G., Eds, Bergey's Manual of Systematic Bacteriology, Vol. 1, Williams & Wilkins, Baltimore, pp. 408-516.

Bush K., Jacoby G.A. (1997). Nomenclature of TEM β-lactamases. J. Antimicrob. Chemother., 39: 1-3.

CLSI - Clinical and Laboratory Standards Institute (2003). Performance standards for antimicrobial disk susceptibility tests, 8th edn., Approved standard M2-A8., CLSI, Wayne, Pa, USA.

Col N.F., O'Connor R.W. (1987). Estimating worldwide current antibiotic usage: Report of task force I. Rev. Infect. Dis., 9: S, 232-S243.

Du Pont H.L., Steele J.H. (1987). Use of antimicrobial agents in animal feeds: Implications for human health. Rev. Infect. Dis., 9: 447-460.

Gaur A., Ramteke P.W., Pathak S.P., Bhattacherjee J.W. (1992). Transferable antibiotic resistance among thermotolerant coliforms from rural drinking water in India. Epidemiol. Infect., 109: 113-120.

Halling-Sørensen B., Nors Nielsen S., Lanzky P.F., Ingerslev F., Holten Lützhøft H.C., Jørgensen S.E. (1998). Ocurrence, fate and effects of pharmaceutical substances in the environment-A review. Chemosphere, 36: 357-393.

Harwood V.J., Whitlock J., Withington V. (2000). Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: Use in predicting the source of fecal contamination in subtropical waters. Appl. Environ. Microbiol., 66: 3698-3704.

Henriques I., Moura A., Alves A., Saavedra M.J., Correia A. (2006). Analysing diversity among β-lactamase encoding genes in aquatic environments. FEMS Microbiol. Ecol., 56: 418-429.

Hoffmann H., Sturenburg E., Heesemann J., Roggenkamp A. (2006). Prevalence of extended-spectrum β-lactamases in isolates of the *Enterobacter cloacae* complex from German hospitals. Clin. Microbiol. Infect., 12: 322-330.

Jarlier V., Nicolas M.H., Fournier G., Philippon A. (1988). Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis., 10: 867-878.

Jones J.G., Gardener S., Simon B.M., Pickup R.W. (1986). Antibiotic resistant bacteria in Windermere and two remote upland tarns in the English Lake district. J. Appl. Bacteriol., 60: 443-453.

Krcmery V., Bajizukova A., Langsadl L., Kotuliakova M., Sobotova O. (1989). Evaluation of the resistance of *Enterobacteriaceae* strains to antibiotics-comparison of strains from clinical material versus environment. J. Hyg. Epidemiol. Microbiol. Immun., 33: 299-304.

Kruse H. (1999). Indirect transfer of antibiotic resistance genes to man. Acta Veter. Scand., 92 (suppl.): 59-65.

Livermore D.M. (1995). β-Lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev., 8: 557-584.

Livermore D.M. (1998). β-Lactamase-mediated resistance and opportunities for its control. J. Antimicrob. Chemother., 41(Suppl. D): 25-41.

Martinez J.L., Cercenado E., Rodriguez-Creixems M., Vicente-Perez M.F., Delgado-Iribarren A., Baquero F. (1987). Resistance to β-lactam/clavulanate. Lancet: 1473.

Matthew M., Harris A.M., Marshall J.M., Ross G.W. (1975). The use of analytic isoelectric focusing for detection and identification of β-lactamases. J. Gen. Microbiol., 88: 169-178.

Matyar F., Dincer S., Kaya A., Colak O. (2004). Prevalence and resistance to antibiotics in Gram negative bacteria isolated from retail fish in Turkey. Ann. Microbiol., 54: 151-160.

Mendonca N., Louro D., Castro A.P., Diogo J., Canica M. (2006). CTX-M-15, OXA-30 and TEM-1-producing *Escherichia coli* in two Portuguese regions. J. Antimicrob. Chemother., 57: 1014-1016.

Mezrioui N., Baleux B. (1994). Resistance patterns of *Escherichia coli* strains isolated from domestic sewage before and after treatment in both aerobic lagoon and activated sludge. Water Res., 28: 2399-2406.

Misra D.S., Kumar A., Singh I.P. (1979). Antibiotic resistance and transfer factor (R+) in *Escherichia coli* isolated from raw sewage. Indian J. Med. Res., 70: 559-562.

Ozgumus O.B.,Tosun I., Aydin F., Kilic A.O., Erturk M. (2006). Carriage of mobilizable plasmid-mediated β-lactamase gene in ampicillin-resistant *Escherichia coli* strains with origin of normal fecal flora. Turk. J. Med. Sci., 36: 307-314.

Ozgumus, O.B., Tosun, I., Aydin, F., Kilic, A.O. (2002). Non-conjugative plasmids encoding TEM-type b-lactamase in clinical isolates of *Escherichia coli.* Infek. Derg., 16: 329-333.

Papandreou S., Pagonopoulou O., Vantarakis A., Papapetropoulou M. (2000). Multiantibiotic resistance of Gram-negative bacteria isolated from drinking water samples in Southwest Greece. J. Chemother., 12: 267-273.

Pathak S.P., Bhattacherjee J.W., Ray P.K. (1993). Seasonal variation in survival and antibiotic resistance among various bacterial populations in a tropical river. J. Gen. Appl. Microbiol., 39: 47-56.

Reinthaler F.F., Posch J., Feierl G., Wust G., Haas D., Ruckenbauer G., Mascher F., Marth E. (2003). Antibiotic resistance of *Escherichia coli* in sewage and sludge. Water Res., 37: 1685-1690.

Rice L.B., Willey S.H., Papanicolaou G.A., Medeiros A.A., Eliopoulos G.M., Moellering R.C. Jr., Jacoby G.A. (1990). Outbreak of ceftazidime resistance caused by extended-spectrum β-lactamases at a Massachusetts chronic care facility. Antimicrob. Agents Chemother., 34: 2193-2199.

Roe M.T, Vega E., Pillai S.D. (2003). Antimicrobial resistance markers of Class 1 and Class 2 integron bearing *Escherichia coli* from irrigation water and sediments. Emerg. Infect. Dis., 9: 822-826.

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.

Sirot D., Recule C., Chaibi E.B., Bret L., Croize J., Chanal-Claris C., Labia R., Sirot J.A. (1997). Complex mutant of TEM-1 β-lactamase with mutations encountered in both IRT-4 and extended spectrum TEM-15, produced by an *Escherichia coli* clinical isolate. Antimicrob. Agents Chemother., 41: 1322-1325.

Tamanai-Shacoori Z., Arturo M., Pommepuy M., Mamez C., Cormier M. (1995). Conjugal transfer of natural plasmids between *Escherichia coli* strains in sterile environmental water. Cur. Microbiol., 30: 155-160.

Tauxe R.V. (1997). Emerging foodborne diseases: An evolving public health challenge. Emerg. Infect. Dis., 3: 425-434.

Tomasz A. (1994). Multiple-antibiotic-resistant pathogenic bacteria: A report on the Rockfeller University Workshop. North. Eng. J. Med., 330: 1247-1251.

Toroglu S., Dincer S., Korkmaz H. (2005). Antibiotic resistance in Gram negative bacteria isolated from Aksu River in (Kahramanmaras) Turkey. Ann. Microbiol., 55: 229-233.

Walia S.K., Kaiser A., Parkash M., Chaudhry G.R. (2004). Self-transmissible antibiotic resistance to ampicillin, streptomycin, and tetracycline found in *Escherichia coli* isolates from contaminated drinking water. J. Environ. Sci. Health Part A Tox. Hazard. Subst. Environ. Eng., 39: 651-662.

Wiggins B.A., Andrews R.W., Conway R.A., Corr C.L., Dobratz E.J., Dougherty D.P., Eppard F.R., Knupp S.R., Limjoco M.C., Mettenburg J.M., Rinehardt J.M., Sonsino J., Torrijos R.L., Zimmerman M.E. (1999). Use of antibiotic resistance analysis to identify nonpoint sources of fecal pollution. Appl. Environ. Microbiol., 65: 3483-3486