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Occurrence of antimicrobial resistance, integrons and virulence genes in *Escherichia coli* isolated from drinking water in Van, Turkiye: a cross-sectional study

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Abstract

Background *Escherichia coli* is one of the recommended freshwater indicator organisms for faecal contamination and can be associated with the pool of genes encoding antibiotic resistance. The aim of this study was to investigate the molecular characterisation of antibiotic resistance and to identify the virulence genes in *E. coli* isolated from drinking water samples collected for the purpose of monitoring microbial drinking water quality.

Methods The *E. coli* isolates ($n=244$) from municipal tap water samples collected in Van province in Turkiye over a five-month period in 2018 were examined in this study. Antimicrobial susceptibility testing was performed using the standard disc diffusion method with 12 antibiotics. Integron content, the resistance genes such as bla_{TEM}, bla_{SHV}, bla_{CTX-M} groups, *tet*A-E genes, *qnrA, qnrB, qnrS, qepA,* and *aac(6')-Ib-cr* were screened and the virulence genes (*afa*, *sfa*, *fimA*, *hly*, *iroN*, *aer*, *cnf*, *iutA* and *pap*) were assessed by specific PCRs. The fingerprinting of the isolates harbouring the integron was evaluated using dendogram analysis of the band patterns obtained from the enterobacterial repetitive intergenic consensus (ERIC) PCR.

Results Ampicillin, tetracycline, streptomycin, nalidixic acid and trimethoprim/sulfamethoxazole were the most common resistances. MAR index of 18.03% of the isolates was greater than 0.2. The two isolates produced ESBL. Thirty seven (15.16%) isolates carried the class 1 or class 2 integrons harboured the gene cassettes such as *aadA*, *sat*, *estX* and unnamed protein gene. Thirty two (21.47%) isolates were found to carry bla_{TEM}, 5 (3.33%) isolates carried bla_{SHV} and 2 (1.3%) isolates carried the bla_{CTX-M} group genes. *tet*A (81.25%) and *tet*B (91.66%) were present in tetracycline-resistant isolates. *qnrS* (29.92%), *qnrB* and *aac(6')-Ib-cr* (3.84%) were present in the nalidixic acid resistant isolates. Most of the isolates carried the virulence genes such as *fimA*, *iutA*, *iroN*, *aer* and *sfa*. Isolates belonged to 65.57% B1, 15.98% A, 9.42% D and 9.01% B2 phylogroups. A statistically significant difference was found between the phylogenetic groups of the isolates and the integron content ($p<0.001$). Fingerprinting showed the 25 different phylotypes in four clusters.

Conclusions Molecular epidemiological findings in such a five-month period in the 11 sampled districts in the city of Van appearently showed us that the tap waters in public use was continuously contaminated by the antibiotic resistant and virulent *E. coli* isolates. This could pose a serious public health risk and, thus need the serious precautions for the infrastructures.

Keywords *Escherichia coli*, MAR index, Virulence gene, Integron, Phylogenetic analysis

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Introduction

Waterborne infections are a major cause of death and morbidity worldwide. Therefore, antibiotic-resistant bacteria in drinking water have received increasing attention in recent years [1]. In addition to their use in humans, antibiotics are also used in animal husbandry and agriculture, resulting in the spread of antibiotic resistant microorganisms to the environment, causing water and soil pollution [2, 3].

According to World Health Organization (WHO) research, 80% of the world's diseases and 50% of the world's child deaths are caused by poor quality drinking water. There are more than 50 diseases caused by poor drinking water quality [4]. The presence and extent of faecal contamination is an important factor in assessing the microbiological quality of drinking water and the risk of human infection from exposure to that water. *E. coli* is the most commonly used biomarker of faecal contamination in water [5].

The environment, including animal and human intestinal microbiota and water resources, are natural habitats and reservoirs of antibiotic resistant bacteria and resistance genes [6]. Antibiotic resistant bacteria selected in the intestines of treated humans and/or animals are transferred between these ecosystems via fecal discharges [7]. These fecal bacteria can then exchange resistance genes with other bacterial populations through horizontal transfers. The spread of antibiotic resistance genes is facilitated when they are carried by genetic structures that are often associated with each other, such as plasmids, transposons, and resistance integrons [8].

Integrons have the ability to carry or integrate gene cassettes that code for resistance to antibiotics. Integrons play an important role in the distribution of antibiotic resistance, especially in Gram negative pathogens [9]. While antimicrobial resistance is usually carried by plasmids through horizontal gene transfer, these plasmids can also carry virulence factors [10]. *E. coli* isolates have some gene regions responsible for virulence factors that can encode adhesins, toxins, siderophores and hemolysins. When considering the relationship between virulence and AMR in the environment, the presence of integrons that are positively correlated with selected virulence genes is shown, without considering antibiotic resistance genes and integrons alone [11].

In recent years, antibiotic resistant *E. coli*, particularly extended spectrum beta lactamase (ESBL) producers, have been attracting attention and are now considered a serious threat to human health [12]. ESBLs are beta lactamases with an extended range of activity that have arisen from mutations or have been transmitted through horizontal gene transfer. They are found in several classes, such as TEM, SHV and CTX-M. All these enzymes are usually found on plasmids and can therefore be transferred more efficiently [13].

Another marker that can be used as molecular indicators of anthropogenic changes in aquatic environments is tetracycline resistance. Tetracycline accumulates along the food chain and causes toxicity in the microbial community, thus having negative effects on ecosystems and promoting the development and spread of antibiotic resistance [14].

The quinolone group of antibiotics is a group of antibiotics widely used as prophylactics and growth promoters in both human and veterinary medicine. Therefore, aquatic environments may contain a high prevalence of microorganisms carrying quinolone residues. Plasmid mediated quinolone resistance genes (PMQR) together with ESBL and carbapenemase genes are likely to be carried by the same plasmids that are horizontally transmitted to other organisms in the aquatic environment by horizontal transmission [15].

In this study, integron content, the genes encoding for the antibiotic resistance and virulence factors were analysed at the molecular level in *E. coli* isolates obtained from samples collected for drinking water quality monitoring in Van province.

Methods

Study period and area

One hundred and six drinking water samples (municipal tap water) were collected between January and May 2018 from areas such as mosque, primary school, shelter, health centre, water tank and house, from districts and locations geographically representative of the province of Van city (Fig. 1) in the eastern region of Turkiye to assess the microbiological water quality.

Water quality counts and determinations were made in the collected drinking water samples and *E. coli*, coliform and *Enterococcus* counts per 100 mL were calculated. Three randomly selected *E. coli* isolates from each site were included in the study (Table 1).

Drinking water sampling and bacterial isolation

Water samples were analyzed according to the procedures outlined in the standard method of *TS EN ISO 9308–1* water quality counting and detection of *E. coli* and coliform bacteria by the membrane filtration method [16]. A total of 244 *E.coli* isolates were obtained and, the bacterial cultures with 20% glycerol were stored at −80 °C for further use.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested with Kirby-Bauer disk diffusion method on Mueller–Hinton Agar plates (Merck, Germany), according to the guidelines of Clinical and Laboratory Standards Institute [17]. Twelve antibiotic disks (Oxoid,



Fig. 1 Map of the province of Van in Turkiye, showing the location of the source of the water samples. **A**, Turkiye; **B**, The city of Van next to Lake Van; **C**, Sampling districts around the city of Van

UK) were tested: ampicillin (10 µg), amoxicillin/clavulanic acid (20 µg/10 µg), amikacin (30 µg), cefuroxime (30 µg), ceftazidime (10 µg), meropenem (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), streptomycin (10 µg), gentamicin (10 µg), nalidixic acid (30 µg), trimethoprim/sulfamethoxazole (25 µg). *Escherichia coli* ATCC 25922 was used as the quality control isolate in susceptibility tests.

Screening of extended spectrum beta lactamases (ESBLs) production

Bacterial isolates were tested for ESBL enzymes using the Double-Disc Synergy Test (DDST) method [18]. ESBL production was tested using ceftazidime (30 µg), cefotaxime (30 µg) and amoxicillin/clavulanic acid (20 µg/10 µg) discs used for the screening test [17]. A positive result was confirmed by zone expansion on the amoxicillin/clavulanic acid disc.

Multiple antibiotic resistance index

The multiple antibiotic resistance (MAR) index (MARI) is a quantitative measure used to assess the level of bacterial resistance to multiple antibiotics [19]. The MARI was calculated by dividing the number of isolates showing resistance to antibiotics (a) by the total number of antibiotics tested (b).

$$\mathrm{MARI} = \mathrm{a/b}$$

A MARI above 0.2 indicates that multiple antibiotic resistance has been acquired [20].

PCR assays

Total DNA was extracted from the isolates as template for polymerase chain reaction (PCR) by boiling method [21], and stored at −20°C. All isolates were screened for integrase (*intI1* and *intI2*), the variable regions (5'CS/3'CS for class 1 and hep51/hep74 for class 2 integrons), beta lactamases (bla_{TEM}, bla_{SHV} and $bla_{\mathrm{CTX\text{-}M}}$ groups) and virulence genes (*afa, sfa, fimA, hly, iroN, aer, cnf, iutA* and *pap*) in ampicillin-resistant isolates, tetracycline resistance genes (*tet* A to *E*) in tetracycline-resistant isolates and quinolone resistance genes (*qnrA, qnrB, qnrS, qepA, aac(6')-Ib-cr*) in nalidixic acid resistant isolates. Data for the oligonucleotide primer sequences, reaction conditions and the cycle parameters are given in Table 2. PCR conditions are described in the supplementary material. The PCR products were electrophoresed in a 1% agarose gel containing 0.5 µg mL-1 ethidium bromide (Sigma, USA) and visualised under UV light.

Phylogenetic grouping

Phylogenetic groups (A, B1, B2 and D) of *E. coli* isolates obtained from drinking water samples were determined by multiplex PCR using specific primers for the *chuA, yjaA* and TspE4.C2 genes, reaction conditions and the cycle parameters as previously described [22].

Statistical analysis

Statistical analyses were performed using IBM SPSS version 25.0 (SPSS Inc., Chicago, Illinois, USA). In the tables, categorical variables are presented as number (n) and percentage (%). The chi square (pearson chi-square) test was used for the comparison of categorical variables. $p < 0.05$ was considered statistically significant.

ERIC-PCR fingerprinting and data analysis

Enterobacterial repetitive intergenic consensus (ERIC) PCR fingerprinting was used to determine clonal relatedness of

Table 1 Sources and pollution levels of tap water samples over a five-month period in 2018

Sampling Period	Sampled District of Van City	Sampling Site (sampling number, *n*)	Range of Bacterial Contamination Level (CFU/100ML)	*E. coli* Isolates Included in the Study
Jan 23-Jan 31	Edremit	Kurubas (district center) (*n*=1),	*E. coli*: 4 Coliform: 4 Enterococci: 0	3
	Baskale	Primary school (*n*=2)	*E. coli*: 1-6 Coliform: 3-67 Enterococci: 0	4
	Ercis	Mosque (*n*=4)	*E. coli*: 1-520 Coliform: 0-42 Enterococci: 0	10
Feb 6- Feb 28	Ozalp	Mosque (*n*=1)	*E.coli*:2 Coliform:38 Enterococci: 0	2
	Ercis	House (*n*=1)	*E.coli*:11 Coliform:42 Enterococci:5	3
	Caldiran	Mosque (*n*=1)	*E.coli*:150 Coliform:960 Enterococci: 0	3
	Muradiye	Mosque (*n*=1)	*E.coli*:2 Coliform:14 Enterococci: 0	1
	Baskale	Mosque (*n*=2), Health care center (*n*=1)	*E. coli*: 1-7 Coliform: 2-12 Enterococci: 0-4	7
	Tusba	Primary school (*n*=1)	*E. coli*: 1 Coliform: 3 Enterococci: 0	1
Mar 7- Mar 28	Caldiran	Mosque (*n*=4), Health care center (*n*=1)	*E. coli*: 1-3 Coliform: 3-94 Enterococci: 0-6	8
	Muradiye	Mosque (*n*=1), Primary school (*n*=1)	*E. coli*: 1-9 Coliform: 15-21 Enterococci: 0	4
	Gurpinar	Mosque (*n*=4), Primary school (*n*=1)	*E. coli*: 24-47 Coliform: 3-75 Enterococci: 0	15
	Ercis	Kockopru (district center) (*n*=1), Mosque (*n*=2), Water tank (*n*=2)	*E. coli*: 1-140 Coliform: 4-100 Enterococci: 2-310	12
	Gevas	Primary school (*n*=1), Health care center (*n*=1)	*E. coli*: 3 Coliform: 20 Enterococci:4	3
	Edremit	Primary school (*n*=1)	*E. coli*: 10 Coliform: 37 Enterococci: 0	3
	Ipekyolu	Mosque (*n*=1), Primary school (*n*=1)	*E. coli*: 4-6 Coliform: 14-15 Enterococci: 0	6
Apr 2-Apr 25	Tusba	Primary school (*n*=6)	*E. coli*: 1-34 Coliform: 9-47 Enterococci: 0-17	12
	Ipekyolu	Mosque (*n*=3), Primary school (*n*=2)	*E. coli*: 1-46 Coliform: 6-73 Enterococci: 0-2	13
	Bahcesaray	Ulubeyli village (*n*=1), Mosque (*n*=1), Primary school (*n*=1)	*E. coli*: 1-5 Coliform: 14-65 Enterococci: 3-5	5

Table 1 (continued)

Sampling Period	Sampled District of Van City	Sampling Site (sampling number, *n*)	Range of Bacterial Contamination Level (CFU/100ML)	*E. coli* Isolates Included in the Study
	Gevas	House (*n*=2), Mosque (*n*=2), Primary school (*n*=1)	*E. coli*: 2-57 Coliform:11-87 Enterococci: 0	14
	Muradiye	Primary school (*n*=1)	*E. coli*: 28 Coliform:62 Enterococci: 0	3
	Baskale	House (*n*=3)	*E. coli*: 1-27 Coliform:33-53 Enterococci: 0-8	5
	Ozalp	House (*n*=1), Health care center (*n*=2)	*E. coli*: 3-94 Coliform:17-61 Enterococci: 1-35	9
	Caldiran	Hankoy (district center) (*n*=1), Mosque (*n*=2), Primary school (*n*=2)	*E. coli*: 1-3 Coliform:29-619 Enterococci: 0-1	8
	Ercis	Districts center (*n*=1)	*E. coli*: 4 Coliform:62 Enterococci: 0	3
	Catak	Primary school (*n*=1)	*E. coli*: 9 Coliform:15 Enterococci: 0	3
May 9-May 23	Ercis	Mosque (*n*=14), Primary school (*n*=2), Toilet (*n*=1)	*E. coli:* 1-180 Coliform: 0-110 Enterococci: 0-26	34
	Baskale	House (*n*=2), Primary school (*n*=2)	*E. coli:* 6-44 Coliform: 9-37 Enterococci: 0	14
	Catak	Primary school (*n*=2)	*E. coli:* 4-70 Coliform: 47-80 Enterococci: 38-79	5
	Caldiran	House (*n*=1), Mosque (*n*=2), Primary school (*n*=1)	*E. coli:* 7-84 Coliform: 0-56 Enterococci: 0-79	12
	Ozalp	Mosque (*n*=1)	*E. coli:* 4 Coliform: 31 Enterococci: 0	3
	Saray	Mosque (*n*=1)	*E. coli:* 3 Coliform:0 Enterococci: 0	3
	Edremit	Health care center (*n*=1)	*E. coli:* 2 Coliform:0 Enterococci: 2	2
	Ipekyolu	Mosque (*n*=2)	*E. coli:* 3-51 Coliform: 32-53 Enterococci: 3-10	3
	Gevas	Primary school (*n*=3)	*E. coli:* 18-48 Coliform: 4-15 Enterococci: 0-13	8
Total		106		244

the isolates. For ERIC-PCR, the primers ERIC1R (5'-ATG TAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'- AAG TAAGTGACTGGGGTGAGC G-3') were used [23]. The reaction conditions and cycle parameters used were as described by Ardakani and Ranjbar [24]. The obtained band profiles of the isolates were analyzed using the GelCompar II software system (version 6.5; Applied Maths, Sint-Martens-Latem, Belgium). The dice correlation coefficient was

Table 2 The oligonucleotide primer sequences and the properties used in PCR experiments

Group of Primer	Target Gene	Sequence (5′ → 3′)	Product Size (bp)	References
Integrons	*intI1 integrase*	GGTCAAGGATCTGGATTTGG ACATGCGTGTAAATCATCGTC	500	[53]
	intI2 integrase	CACGGATATGCGACAAAAAGGT GTAGCAAACGAGTGACGAAATG	740	[53]
	Class 1 integron	GGCATCCAAGCAGCAAG AAGCAGACTTGACCTGA	variable	[54]
	Class 2 integron	GATGCCATCGCAAGTACGAG CGGGATCCCGGACGGATGCACGATTTGTA	variable	[55]
Beta Lactams	bla_{TEM}	ATGAGTATTCAACATTTCCG CCAATGCTTAATCAGTGAGG	857	[56]
	bla_{SHV}	TTATCTCCCTGTTAGCCACC GATTTGCTGATTTCGCTCGG	796	[57]
	bla_{CTX-M}	TTTGCGATGTGCAGTACCAGTAA CGATATCGTTGG TGGTGCCATA	544	[58]
	Group 1 bla_{CTX-M}	GCGTGATACCACTTCACCTC TGAAGTAAGTGACCAGAATC	260	[59]
	Group 2 bla_{CTX-M}	TGATACCACCACGCCGCTC TATTGCATCAGAAACCGTGGG	341	[59]
	Group 8 bla_{CTX-M}	CAATCTGACGTTGGGCAATG ATAACCGTCGGTGACAATT	207	[60]
	Group 9 bla_{CTX-M}	ATCAAGCCTGCCGATCTGGTTA GTAAGCTGACGCAACGTCTGC	293	[60]
Tetracyclines	*tetA*	GTAATTCTGAGCACTGTCGC CTGCCTGGACAACATTGCTT	917	[60]
	tetB	CTCAGTATTCCAAGCCTTTG ACTCCCCTGAGCTTGAGGGG	396	[60]
	tetC	GGTTGAAGGCTCTCAAGGGC CCTCTTGCGGGAATCGTCC	589	[61]
	tetD	AAACCATTACGGCATTCTGC GACCGGATACACCATCCATC	787	[62]
	tetE	AAACCACATCCTCCATACGC AATAGGCCACAACCGTCAG	278	[62]
Quinolones	*qnrA*	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516	[63]
	qnrB	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	469	[63]
	qnrS	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	428	[64]
	qepA	AACTGCTTGAGCCCGTAGAT GTCTACGCCATGGACCTCAC	596	[65]
	aac(6′)-Ib-cr	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	482	[66]
Virulence Genes	*fimA*	GTTGTTCTGTCGGCTCTGTC ATGGTGTTGGTTCCGTTATTC	447	[67]
	pap1	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	328	[68]
	pap2	GCAACAGCAACGCTGGTTGCATCAT AGAGAGAGCCACTCTTATACGGACA	336	[68]
	sfa	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	410	[68]
	aer	TACCGGATTGTCATATGCAGACCG AATATCTTCCTCCAGTCCGGAGAAG	602	[68]
	cnf	AAGATGGAGTTTCCTATGCAGGAG CATTCAGAGTCCTGCCCTCATTATT	498	[68]

Table 2 (continued)

Group of Primer	Target Gene	Sequence (5′ → 3′)	Product Size (bp)	References
	hly	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177	[68]
	afa	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	[68]
	iutA	GGCTGGACATCATGGGAACTGG CGTCGGGAACGGGTAGAATCG	300	[69]
	iroN	AAGTCAAAGCAGGGGTTGCCCG GACGCCGACATTAAGACGCAG	665	[70]
Phylogenetic Groups	*chuA*	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	279	[22]
	yja	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	211	[22]
	TspE4.C2	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	152	[22]

used for similarity calculation, and the unweighted pairwise grouping mathematical analysis (UPGMA) method was used for cluster analysis (optimization 1.0, tolerance 1.0). Isolates with band similarities above 90% were accepted as the same clone [25].

Sequencing and bioinformatic analysis

The PCR products were purified on Expin™ GeneAll columns (South Korea) and sent to Macrogen Inc. (Amsterdam, The Netherlands) for sequencing by using their own primers. The nucleotide sequences were compared with those available in the GenBank database by using an alignment search tool available on the website [26].

Results

Antimicrobial susceptibility testing and detection of ESBL

The highest resistance rates of the isolates were found to ampicillin (n =149, 61.06%), tetracycline (n =48, 19.67%), streptomycin (n =36, 14.75%), trimethoprim/sulfamethoxazole (n =34, 13.93%) and nalidixic acid (n =26, 10.65%). The lowest resistance rates were observed to gentamicin (n =3, 1.22%) and meropenem (n =2, 0.81%). No isolates were resistant to amikacin (Fig. 2). In double disk synergy tests, 2 isolates (n =2, 0.81%) (914–3 from Caldiran, 1249 from Ercis) of 244 isolates collected in the last 2 periods were found to produce ESBL.

MAR index

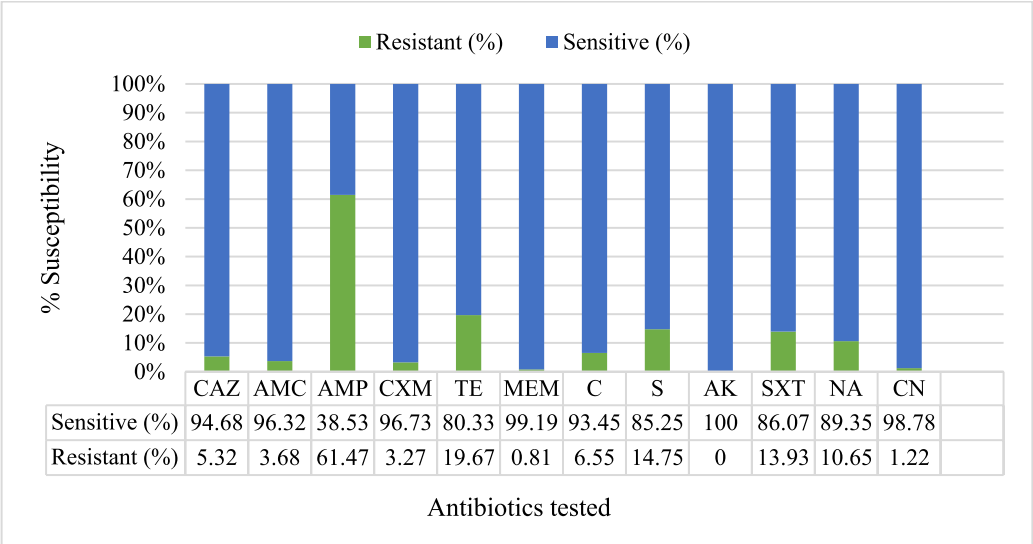
According to MAR index calculations, high multidrug resistance (MDR) to at least three different antibiotic classes were detected in 44 out of 244 isolates (18.03%), which had a MARI greater than 0.2 (Fig. 3), while 200 (81.96%) isolates had a value less than 0.2. It was determined that the majority of the isolates with MAR index above 0.2 were isolated from Gurpinar district. The highest MAR index of 0.58 was reported only for the three isolates (1260–3 from Ercis,1296–1 from Ozalp,1508–1 from Ercis) isolated in the last period.

PCR assays

PCRs for the integrase gene revealed that 37 (15.16%) of the isolates carried class 1 integrons, and one (n =1, 0.4%) carried both class 1 and class 2 integrons. Partial PCR amplification of the variable regions of the integrons showed that 14 class 1 integrons and one class 2 integron carried gene cassettes ranging 700 to1000 bp and >2500 bp, respectively (Table 3). İntegron-containing isolates were concentrated in Ercis and Gurpinar districts. Empty integrons were defined as those in which the integrase gene was detected but no amplification of the variable regions. DNA sequence analysis of the variable regions of the integrons showed the gene cassettes such as *aadA*, *sat*, *estX* and unnamed protein gene (Table 3).

The bla_{TEM} (n =32, 21.47%) and bla_{SHV} (n =5, 3.3%) and $bla_{\text{CTX-M}}$ (n =2, 1.3%) groups were detected in ampicillin-resistant isolates (n =149). Two isolates (914–3 from Caldiran, 1249 from Ercis) carried the $bla_{\text{CTX-M}}$, and when subgroups were investigated, both of them contained both $bla_{\text{CTX-M}}$ Group 1 and $bla_{\text{CTX-M}}$ Group 2. Identified $bla_{\text{CTX-M}}$ group genes were not sequenced for allelic variants.

One of the virulence genes, *fimA*, was found to be positive in all ampicillin-resistant isolates, and the *iutA* and *iroN* genes were found to be positive at a very high rate of 34% and 30%, respectively. Among the virulence genes, the *aer* gene was positive in 12.6% of the isolates, while the *sfa* gene was positive in 9.3% of the isolates. The *cnf* gene was positive in five samples, the *hly* gene was positive in three samples and the *afa* gene was positive in only one sample.



	CAZ	AMC	AMP	CXM	TE	MEM	C	S	AK	SXT	NA	CN
Sensitive (%)	94.68	96.32	38.53	96.73	80.33	99.19	93.45	85.25	100	86.07	89.35	98.78
Resistant (%)	5.32	3.68	61.47	3.27	19.67	0.81	6.55	14.75	0	13.93	10.65	1.22

Fig. 2 Percentage of antimicrobial susceptibility of the *E. coli* isolates. CAZ, ceftazidime; AMC, amoxicillin/clavulanic acid; AMP, ampicillin, CXM, cefuroxime; TE, tetracycline; MEM, meropenem; C, chloramphenicol; S, streptomycin; AK, amikacin; SXT, trimethoprime/sulfamethoxazole; NA, nalidixic acid; CN, gentamicin

Of the tetracycline-resistant isolates ($n = 48$), 39 (81.25%) were found to carry the *tetA* gene and 44 (91.66%) the *tetB* gene, 72.91% ($n = 35$) of the isolates carried both *tetA* and *tetB* genes. No isolate carrying any of the other tetracycline resistance genes (*tetC*, *tetD*, *tetE*) was found.

When the nalidixic acid resistant isolates ($n = 26$) were analysed, it was found that 7 (26.92%) carried the *qnrS* gene and one (3.84%) carried the *qnrB* and *aac(6')-Ib-cr* genes. No isolates were found to carry any of the other quinolone resistance genes (*qnrA* and *qepA*). High levels of resistance and virulence genes were found in isolates

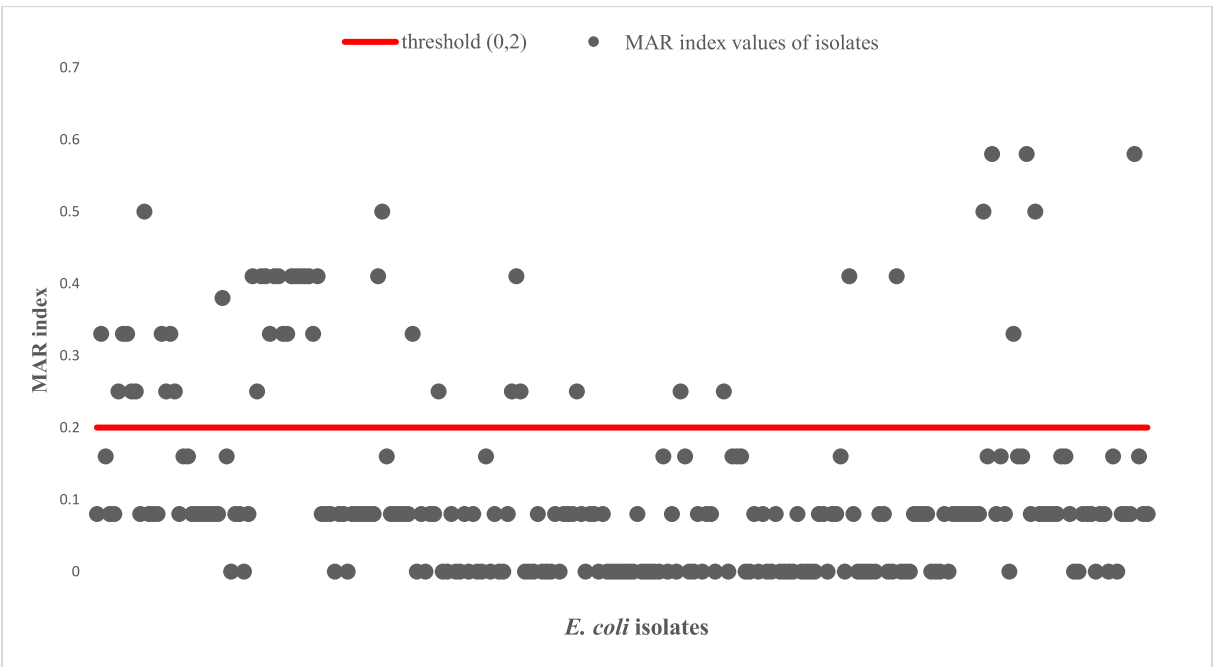


Fig. 3 A scatter plot showing the MARI of *E. coli* isolated from drinking water samples. The isolates situated above the threshold line (red) are referred to as highly resistant isolates

Table 3 Epidemiological properties of integron-containing *E. coli* isolates

E. coli Isolate	Sampling Site	Sampling Location	Isolation Date	Antibiotic Resistance Profile	Integrase Gene	Integron Size (~bp)	Gene Casette	GenBank Accession number	Antimicrobial Resistance Gene	Virulance Genes	Phylogenetic Group	MAR Index
208-2	Primary School 3	Ercis	31.01.2018	CAZ, AMP, NA	*intI1*	-	EI*		bla_{SHV}	*fim*	B1	0,25
210-1	Mosque 2	Ercis	31.01.2018	AUG, AMP, TE, MEM, S, SXT	*intI1*	-	EI		bla_{TEM}	*fim, iutA, iroN, aer*	A	0,5
210-2	Mosque 2	Ercis	31.01.2018	AMP	*intI1, intI2*	2500	ND**		bla_{TEM}	*fim, iutA, iroN*	B1	0,08
213-1	Mosque 3	Ercis	31.01.2018	AMP, S, SXT	*intI1*	1000	*estX*,unnamed protein gene	PV636551 PV636552	bla_{TEM}	*fim, iutA, iroN, aer, sfa*	B2	0,25
213-2	Mosque 3	Ercis	31.01.2018	AMP, TE, S, SXT	*intI1*	-	EI		bla_{TEM}	*fim, iutA, iroN, aer, sfa*	D	0,33
213-3	Mosque 3	Ercis	31.01.2018	AMP, S, SXT	*intI1*	-	EI		bla_{TEM}	*fim, iutA, iroN, aer, sfa*	B2	0,25
597-1	Primary School 6	Gurpinar	12.03.2018	AMP, TE, S, SXT	*intI1*	-	EI		-	*fim*	D	0,25
597-2	Primary School 6	Gurpinar	12.03.2018	AUG, AMP, CXM, TE, SXT	*intI1*	-	EI		-	*fim, iutA, iroN*	D	0,41
597-3	Primary School 6	Gurpinar	12.03.2018	AUG, AMP, TE, S, SXT	*intI1*	-	EI		bla_{TEM}	*fim*	D	0,41
669-1	Mosque 10	Gurpinar	19.03.2018	AMP, TE, S, SXT	*intI1*	1000	*estX*, unnamed protein gene	PV636553 PV636554	bla_{TEM}	*fim*	D	0,33
669-2	Mosque 10	Gurpinar	19.03.2018	AMP, TE, C, S, SXT	*intI1*	-	EI		bla_{TEM}, *tetA, tetB*	*fim*	D	0,41
669-3	Mosque 10	Gurpinar	19.03.2018	AMP, TE, C, S, SXT	*intI1*	700	*sat*	PV636555	*tetA, tetB*	*fim, iutA, iroN*	D	0,41
670-1	Mosque 11	Gurpinar	19.03.2018	AMP, TE, S, SXT	*intI1*	-	EI		*tetA, tetB*	*fim*	D	0,33
670-2	Mosque 11	Gurpinar	19.03.2018	AMP, TE, S, SXT	*intI1*	-	EI		bla_{TEM}, *tetA, tetB*	*fim, iutA, iroN*	D	0,33
670-3	Mosque 11	Gurpinar	19.03.2018	AMP, TE, C, S, SXT	*intI1*	1000	*estX*, unnamed protein gene	PV636556 PV636557	bla_{TEM}, *tetA, tetB*	*fim, iutA, iroN, aer*	D	0,41
671-1	Mosque 12	Gurpinar	19.03.2018	AMP, TE, C, S, SXT	*intI1*	1000	*estX*,unnamed protein gene	PV636558 PV636559	bla_{TEM}, *tetA, tetB*	*fim*	D	0,41
671-2	Mosque 12	Gurpinar	19.03.2018	AMP, TE, C, S, SXT	*intI1*	700	*sat*	PV636560	bla_{TEM}, *tetA, tetB*	*fim*	D	0,41
671-3	Mosque 12	Gurpinar	19.03.2018	AMP, TE, C, S, SXT	*intI1*	700	*sat*	PV636562	bla_{TEM}, *tetA, tetB*	*fim*	B1	0,41
672-1	Mosque 13	Gurpinar	19.03.2018	AMP, TE, C, S, SXT	*intI1*	700	*sat*	PV636561	bla_{TEM}, *tetA, tetB*	*fim, iutA, iroN*	D	0,41
672-2	Mosque 13	Gurpinar	19.03.2018	AMP, TE, S, SXT	*intI1*	1000	*aadA*	PV636563	bla_{TEM}, *tetA, tetB*	*fim*	B1	0,33
672-3	Mosque 13	Gurpinar	19.03.2018	AMP, TE, C, S, SXT	*intI1*	1000	*sat*	PV636564	bla_{TEM}, *tetA, tetB*	*fim*	B1	0,41

Table 3 (continued)

E. coli Isolate	Sampling Site	Sampling Location	Isolation Date	Antibiotic Resistance Profile	Integrase Gene	Integron Size (~bp)	Gene Casette	GenBank Accession number	Antimicrobial Resistance Gene	Virulance Genes	Phylogenetic Group	MAR Index
703-1	Health Care Center 2	Caldiran	21.03.2018	-	*intI1*	-	EI		-	*fim*	B1	0
719-2	Mosque 14	Ercis	21.03.2018	AMP	*intI1*	-	EI		-	*fim, iutA, iroN*	A	0,08
722-2	Mosque 15	Ercis	21.03.2018	AMP	*intI1*	-	EI		-	*fim, iutA*	B1	0,08
722-3	Mosque 15	Ercis	21.03.2018	AMP	*intI1*	-	EI		-	*fim, iutA, iroN*	B1	0,08
745-1	Water Tank 1	Ercis	23.03.2018	AMP, TE, S, SXT, NA	*intI1*	1000	*aadA*	PV636565	*qnrS, aac(6')-Ib-cr, bla*$_{TEM}$ *tetA, tetB*	*fim, iutA, iroN*	D	0,41
745-2	Water Tank 1	Ercis	23.03.2018	AMP, TE, C, S, SXT, NA	*intI1*	1000	*aadA*	PV636566	*tetA, tetB*	*fim, iutA, aer, sfa*	A	0,5
745-3	Water Tank 1	Ercis	23.03.2018	AMP, TE	*intI1*	-	EI		*bla*$_{TEM}$, *tetA, tetB*	*fim*	B1	0,16
746-1	Water Tank 2	Ercis	23.03.2018	TE	*intI1*	-	EI		-	*fim*	B2	0,08
773-3	Mosque 16	Ipekyolu	26.03.2018	TE, S, SXT, NA	*intI1*	-	EI		*tetA, tetB*	*fim*	B1	0,33
1038-2	Health Care Center 4	Ozalp	11.04.2018	AMP, S, SXT	*intI1*	1000	*sat*	PV636567	-	*fim, iutA, iroN, aer*	B1	0,25
1224-1	Primary School 25	Catak	9.05.2018	AMP, TE, S, SXT	*intI1*	-	EI		*bla*$_{TEM,}$ *tetA, tetB*	*fim*	A	0,33
1233-1	Primary School 27	Baskale	09.05.18	AMP, TE, C, NA	*intI1*	-	EI		*qnrS, qnrB, bla*$_{TEM,}$ *tetA, tetB*	*fim*	B1	0,25
1260-1	Toilet 1	Ercis	9.05.2018	AMP, TE, C, S SXT, NA	*intI1*	-	EI		*qnrS, bla*$_{TEM,}$ *tetA, tetB*	*fim, iroN*	A	0,33
1260-3	Toilet 1	Ercis	9.05.2018	AMP, TE, C, S, SXT, NA, CN	*intI1*	-	EI		*qnrS, bla*$_{TEM,}$ *tetA, tetB*	*fim, iroN*	A	0,5
1252-2	Primary School 30	Ercis	9.05.2018	AMP, TE, SXT, NA	*intI1*	-	EI		*bla*$_{TEM,}$ *tetA, tetB*	*fim*	B1	0,58
1508-1	Mosque 47	Ercis	23.05.2018	AMP, TE, C, S, SXT, NA, CN	*intI1*	-	EI		*bla*$_{TEM,}$ *tetA*	*fim, iutA, iroN, aer*	B1	0,33

CAZ Ceftazidime, *AMC* Amoxicillin/clavulanic acid, *AMP* Ampicillin, *CXM* Cefuroxime, *TE* Tetracycline, *MEM* Meropenem, *C* Cloramphenicol, *S* Streptomycin, *AK* Amikacin, *SXT* Trimethoprime/sulfamethoxazole, *NA* Nalidixic acid, *CN* Gentamicin

**EI* Empty integron

***ND* Not detected

Raw data of integron DNA sequence of gene cassettes used in this study is described in supplementary material

Table 4 Phylogenetic group distribution of isolates with/without integron

E. coli Isolates	Number and (%) of Isolates in Phylogroups				
	A	B1	B2	D	*p* value
with integron (*n*=37)	6 (16.21%)	14 (37.83%)	3 (8.10%)	14 (37.83%)	**<0.001***
without integron (*n*=207)	33 (15.94%)	146 (70.53%)	19 (9.17%)	9 (4.34%)	
Total	**39 (15.98%)**	**160 (65.57%)**	**22 (9.01%)**	**23 (9.42%)**	

*Pearson chi-square test, *$p<0.05$ was considered statistically significant

containing the integron in samples isolated from Gurpinar and Ercis districts.

Phylogenetic grouping

Phylogenetic analysis of 244 drinking water *E. coli* isolates revealed the presence of four phylogroups: B1, A, D and B2 and the following distribution: phylogroups 65.57% B1 ($n=160$), 15.98% A ($n=39$), 9.42% D ($n=23$) and 9.01% B2 ($n=22$) (Table 4). The results of the analyses related to the comparison of the phylogenetic groups of the isolates with the presence of integron were given. A statistically significant difference was found between the phylogenetic groups and the presence of integron ($X^2=31.914$, $p<0.001$). The isolates from phylogenetic groups B1 and D were statistically significantly more likely to contain integrons than the other isolates in groups A and B2 ($p<0.001$).

ERIC PCR fingerprinting

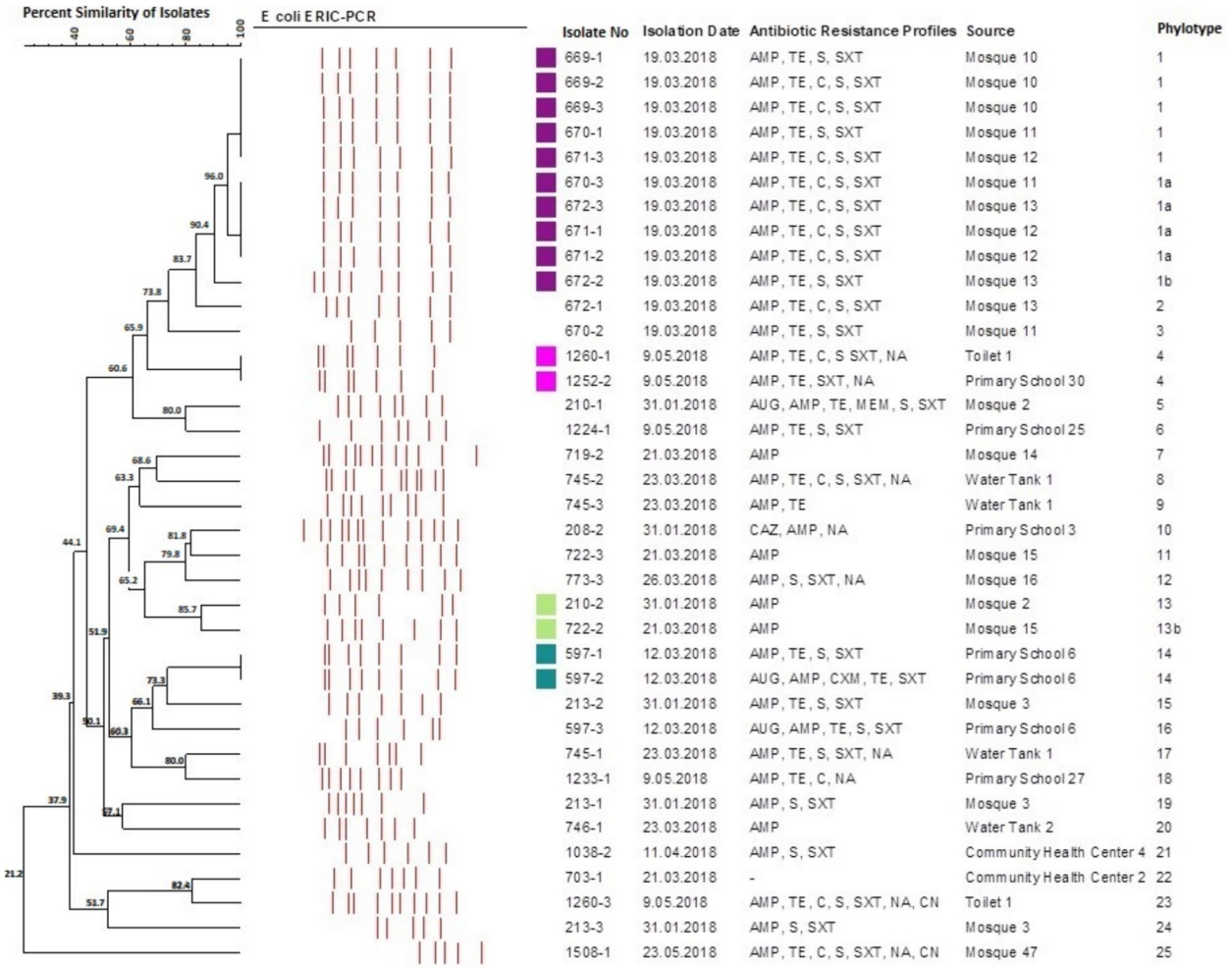
Clonal similarity was investigated among 37 integrase gene positive *E. coli* isolates phylotyped by the ERIC PCR method (Fig. 4). Among 37 *E. coli* isolates, 25 different phylotypes were detected and the isolates were grouped into 4 clusters (tolerance 1.0, optimisation 1.0, cut-off 85%). Sixteen out of 37 *E. coli* isolates were in one of the clusters and the clustering rate was 43.2%. The largest cluster was phylotype1 with 10 isolates (669–1, 669–2, 669–3, 670–1, 670–3, 671–1, 671–2, 671–3, 672–2, 672–3) isolated from Gurpinar district, phylotype 4 (1260–1, 1252–2), phylotype 13 (210–2, 722–2) and phylotype 14 (597–1, 597–2) clusters with two isolates each were isolated from Ercis and Gurpinar districts, respectively. When the molecular epidemiological data of the isolates carrying the integrase gene were examined, it was seen that the majority of the isolates showed heterogeneous distribution. It was determined that the isolates in the four clonally close clusters had high antibiotic resistance. However, although these isolates had different antibiotic resistance profiles and were taken from different sample sources, it was determined that they were isolated from Ercis and Gurpinar districts.

Discussion

Antibiotic resistance represents a significant public health concern on a global scale. However, its environmental impact has been overshadowed by the preponderance of clinical studies. Surface water is highly susceptible to anthropogenic contamination, rendering it an optimal environment for the acquisition and dissemination of antibiotic resistance [27]. It is now virtually impossible to identify a region in the world where antibiotics have not been detected in surface water. Antibiotic resistance has been identified in a range of aquatic environments, including rivers, wastewater, seawater and drinking water [28, 29].

The acquisition of antibiotic resistance is facilitated by the antibiotics themselves, which can be transferred by mobile elements [30]. *Escherichia coli* is an opportunistic pathogen that thrives in aquatic environments and acts as a vector for the horizontal transfer of antibiotic resistance genes. The World Health Organization [12]. has established guidelines for drinking water quality that consider the presence of *E. coli* in water systems as evidence of faecal contamination.

An examination of the standards set in terms of public health reveals that total coliforms, fecal streptococci, *E. coli* and sulfite-reducing anaerobes should not be present in drinking and utility waters [31]. The principal objective of a microbiological examination of water is to ascertain the presence of pathogenic microorganisms. For this purpose, the coliform group of microorganisms are employed as indicators of microbiological contamination in water [32]. In the study conducted in 2007 to examine the microbiological quality of drinking and utility water in the Van Region in terms of public health, water samples were taken from a variety of sources, including wells, streams, springs, fountains, taps and tanks in both the city centre and surrounding districts. The majority of the samples were found to be non-compliant with the standards in terms of coliform group microorganisms. The presence of the coliform group of microorganisms was not detected in samples taken from storage waters. However, the highest levels of



Isolate No	Isolation Date	Antibiotic Resistance Profiles	Source	Phylotype
669-1	19.03.2018	AMP, TE, S, SXT	Mosque 10	1
669-2	19.03.2018	AMP, TE, C, S, SXT	Mosque 10	1
669-3	19.03.2018	AMP, TE, C, S, SXT	Mosque 10	1
670-1	19.03.2018	AMP, TE, S, SXT	Mosque 11	1
671-3	19.03.2018	AMP, TE, C, S, SXT	Mosque 12	1
670-3	19.03.2018	AMP, TE, C, S, SXT	Mosque 11	1a
672-3	19.03.2018	AMP, TE, C, S, SXT	Mosque 13	1a
671-1	19.03.2018	AMP, TE, C, S, SXT	Mosque 12	1a
671-2	19.03.2018	AMP, TE, C, S, SXT	Mosque 12	1a
672-2	19.03.2018	AMP, TE, S, SXT	Mosque 13	1b
672-1	19.03.2018	AMP, TE, C, S, SXT	Mosque 13	2
670-2	19.03.2018	AMP, TE, S, SXT	Mosque 11	3
1260-1	9.05.2018	AMP, TE, C, S SXT, NA	Toilet 1	4
1252-2	9.05.2018	AMP, TE, SXT, NA	Primary School 30	4
210-1	31.01.2018	AUG, AMP, TE, MEM, S, SXT	Mosque 2	5
1224-1	9.05.2018	AMP, TE, S, SXT	Primary School 25	6
719-2	21.03.2018	AMP	Mosque 14	7
745-2	23.03.2018	AMP, TE, C, S, SXT, NA	Water Tank 1	8
745-3	23.03.2018	AMP, TE	Water Tank 1	9
208-2	31.01.2018	CAZ, AMP, NA	Primary School 3	10
722-3	21.03.2018	AMP	Mosque 15	11
773-3	26.03.2018	AMP, S, SXT, NA	Mosque 16	12
210-2	31.01.2018	AMP	Mosque 2	13
722-2	21.03.2018	AMP	Mosque 15	13b
597-1	12.03.2018	AMP, TE, S, SXT	Primary School 6	14
597-2	12.03.2018	AUG, AMP, CXM, TE, SXT	Primary School 6	14
213-2	31.01.2018	AMP, TE, S, SXT	Mosque 3	15
597-3	12.03.2018	AUG, AMP, TE, S, SXT	Primary School 6	16
745-1	23.03.2018	AMP, TE, S, SXT, NA	Water Tank 1	17
1233-1	9.05.2018	AMP, TE, C, NA	Primary School 27	18
213-1	31.01.2018	AMP, S, SXT	Mosque 3	19
746-1	23.03.2018	AMP	Water Tank 2	20
1038-2	11.04.2018	AMP, S, SXT	Community Health Center 4	21
703-1	21.03.2018	-	Community Health Center 2	22
1260-3	9.05.2018	AMP, TE, C, S, SXT, NA, CN	Toilet 1	23
213-3	31.01.2018	AMP, S, SXT	Mosque 3	24
1508-1	23.05.2018	AMP, TE, C, S, SXT, NA, CN	Mosque 47	25

Fig. 4 Dendrogram based on the ERIC-PCR patterns and epidemiological data of integron-containing *E.coli* isolates. (Coloured squares show the four clusters with the highest similarity among 25 different phylotypes as a result of ERIC-PCR analysis)

these microorganisms were found in stream waters, followed by well waters [33]. The present study shows that tap water samples taken from Van city over a five-month period apparently contained high levels of *E. coli*, total coliform and enterococci.

The antibiotic resistance rates of the *E. coli* isolates included in our study were found to be highly resistant to antibiotic groups such as beta-lactams, tetracyclines, sulfonamides and quinolones. It was found that the isolates with high MAR index were mostly isolated in March in the 3rd period, and the isolates were obtained from Ercis and Gurpinar districts. *E. coli* from human samples or from open and closed water sources such as streams, rivers and ponds have been reported the high resistance to many groups of antibiotics. It is a worrying situation that we detect such a high resistance rates to the beta-lactams clinical use in *E. coli* isolates even in a short period of five months. Odontor et al. [34], has reported that drinking water sources were contaminated with *E. coli* with high MAR index, which posed a potential risk. Similarly, in our study, the number of isolates with high MAR index was determined to be at a rate that cannot be ignored, suggesting that it may cause an increase in contamination in drinking water, especially in months with high precipitation such as March.

Antibiotic resistance has often been linked to integrons. This system is an important factor in the acquisition of antibiotic resistance genes by Gram-negative bacteria. They are found in many different environments over different evolutionary time periods and are important for their ability to move between species and lineages. In studies of antibiotic resistance and integron content of isolates from different water sources (well, tap water, stream, river basin and lagoon), it was found that there were 40–80% resistant isolates and these isolates contained 7–80% integron. Later, when sequence

analysis was performed, the resistance gene family was identified in gene cassettes [35–38]. In this study, *E. coli* isolates from tap water samples were resistant to more than one antibiotic and 15.16% ($n = 37$) were found to contain class 1 and class 2 integrons. DNA sequence analysis showed *aadA, sat, estX* and *unnamed protein gene* genes as previously determined [39]. The high rate of the integron content of *E. coli* isolates not only in drinking water but also in the other water sources indicates that *E. coli* isolates of the environmental origin may be a important reservoir for the genetic elements coding for the resistance traits to many groups of antibiotics, and those integrons can play an important role in the transmission of this resistance traits.

Tetracycline antibiotics are one of the most commonly used groups of antibiotics in both veterinary and clinical settings for treatment and growth promotion in poultry [14]. Studies show that tetracycline concentrations are high in animal manure, wastewater treatment plants, soil and water environments. In our study, because of the high rate of tetracycline resistance, the *tetA-E* genes were analysed and the *tetA* and *tetB* genes were found to be quite high. In environmental water and animal studies with bacteria isolated from samples from poultry farms, drinking water reservoirs and rivers, 15–60% *tetA* and 18–20% *tetB* genes were detected. In parallel with our results, no isolates carrying *tetC, tetD* or *tetE* genes were found in the studies. We consider that the results show the high use of tetracycline in many areas, leading to an increase in tetracycline resistance in enteric bacteria from the environment [38, 40–42].

Quinolones are broad-spectrum antibiotics commonly used in human and veterinary medicine to treat bacterial infections [43]. Farmers often use them to prevent infection or as an alternative to good agricultural practice and as growth promoters in livestock [44]. Although quinolone resistance in *Enterobacteriaceae* is mainly thought to be due to point mutations in the quinolone resistance determining regions (QRDRs) of the type II topoisomerase genes (*gyrA, gyrB* and *parC, parE*), there are increasing reports of plasmid-mediated quinolone resistance (PMQR) determinants [15]. In many studies investigating the dynamics of AMR transmission from contaminated external environments such as poultry, soil, surface water and canal water, plasmid-mediated quinolone genes have been examined in isolated bacteria and PMQR genes have been found to be positive in rates of up to 90% [15, 45]. Although the presence of *gyrA* or *parC* mutations and resistance level to other quinolones and/or fluoroquinolones other than nalidixic acid was not examined, we believe that our nine PMQR-containing isolates may confer high resistance to quinolones and/or fluoroquinolones. Quinolone resistance can also be induced by mutations in efflux pump gene regulators. In addition to target mutations, ciprofloxacin resistance can also be conferred by plasmid-mediated quinolone resistance (PMQR) genes and efflux pumps such as OqxAB [46]. It has been reported that fluorouinolone resistance is caused by interaction between PMQR genes and mutations in both *gyrA* and *parC* genes, whereas mutation in only one gene can explain quinolone resistance, and accumulation of PMQR genes and mutations in the quinolone resistance determining region confer high resistance to fluoroquinolones. [47].

The role of the environment in the emergence and spread of antimicrobial resistance is increasingly recognised. Information on the virulence of resistant bacteria in environmental systems is limited. Recent studies on the relationship between AMR and virulence have gained considerable importance. Different virulence genes in *E. coli* isolates from surface water, dry and wet samples has been reported [48, 49]. In these studies, as in our study, *fim* genes were found to be quite common. In our study, ten different virulence genes were evaluated, all virulence genes were found to be positive except for one isolate, all isolates carried the *fimA* gene, 34% carried the *iutA* and *iroN* genes, and 30% carried the *aer* and *sfa* genes. It is our contention that future studies of bacterial pathogenesis would benefit from an investigation of virulence factors and antibiotic resistance in isolates of drinking water origin. This approach would facilitate a more comprehensive understanding of the mechanisms underlying bacterial survival in challenging conditions in both environmental and clinical settings. Phylogrouping is a method used to trace microbial sources in drinking water, particularly by analysing faecal samples from selected host species [50]. In the present study, phylogenetic group B1 was predominant in our isolates. However, a relationship between the phylogenetics and virulence of the bacteria has been found and it has been reported that virulent isolates generally belong to group B2 and to a lesser extent to group D, while commensal isolates belong to groups A and B1 [51]. It is noteworthy that the present study revealed a statistically significant association between phylogenetic groups B1 and D and the presence of integrons ($p < 0.001$). A simple method such as ERIC PCR was used to provide information about the genetic similarity of bacterial strains, as it is simpler, faster and cheaper than pulsed field gel electrophoresis (PFGE) or multi-locus sequence typing (MLST) [52]. According to the ERIC-PCR fingerprint results (Fig. 4), the largest cluster was phylotype 1, in which 10 isolates containing integrons from Gurpinar districts belonged to phylogenetic group D.

Conclusions

This study provides detailed molecular epidemiological data on *E. coli* isolated from human drinking water. The fact that the isolates carrying integron, antibiotic resistance and virulence genes were obtained from Ercis and Gurpinar and the fact that this district is the most populous district of Van province in terms of population may be one of the reasons for such high contamination. The results of our study indicate that the molecular epidemiology of antimicrobial resistance in bacteria that cause contamination of public drinking water should be investigated. It is thought that the transmission of such virulent bacteria to humans may lead to an important public health problem. Human-associated aquatic environments are a reservoir for horizontal gene transfer. Even this study, conducted over a short period of five months, demonstrated that virulent *E. coli* isolates of human origin can persistently contaminate drinking water. We believe that continuous monitoring of drinking water for antibiotic resistant and virulent coliforms is important for public health and the data obtained will contribute to the improvement of water treatment facilities and infrastructures. Prevention of contamination of drinking water sources with animal and human wastes, regular technical inspections of sewage and water distribution systems, regular surveillance of water sources for the presence of MDR *E. coli* by microbiological tests will contribute significantly to the prevention of contamination of drinking water with MDR *E. coli.*

Abbreviations

AMR	Antimicrobial resistance
DDST	Double-disc synergy test
ERIC-PCR	Enterobacterial repetitive intergenic consensus polymerase chain reaction
ESBL	Extended-spectrum beta lactamase
EUCAST	European committee on antimicrobial susceptibility testing
MARI	Multiple antibiotic resistance index
MDR	Multiple drug resistance
PMQR	Plasmid mediated quinolone resistance
QRDR	Quinolone resistance determining region
WHO	World health organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-04046-9.

Supplementary Material 1.

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Authors' contributions

Erva RAKICI: Research, Experimental studies, Verification, Writing-Original Draft, References, Writing-Review & Editing. Elif AYDIN: Research, Writing-Original Draft, Writing-Reviewing& Editing. Elif Seren TANRIVERDİ: Dendogram analysis, evaluation and interpretation. Osman Birol OZGUMUS: Project management, Conceptualization, Formal analysis, Writing-Original Draft, Writing-Revision & Editing.

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Data availability

The datasets generated and/or analysed during the current study are available in the [National Center for Biotechnology Information (NCBI), https://www.ncbi.nlm.nih.gov/nucleotide/] repository, [PV636551, PV636552, PV636553, PV636554, PV636555, PV636556, PV636557, PV636558, PV636559, PV636560, PV636561, PV636562, PV636563, PV636564, PV636565, PV636566 and PV636567]".

Declarations

Ethics approval and consent to participate

Not applicable. The necessary approval for this study was obtained from the Van Provincial Directorate of Health (No: 18670582–604.02). This study was approved by the Ethics Committee for Non-Interventional Research of the Van Training and Research Hospital (Date: 03/05/2018, Decision number: 2018/08).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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