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Potential Public Health Significance of Faecal Contamination in South-western Coastal Area in Istanbul, Turkey

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The prevalence of faecal coliform bacteria and to detect *Escherichia coli* via the *uidA* gene, to identify the associated faecal pollution sources and to predict faecal contamination in an active seaside area of the Marmara Sea. Systematic microbiological testing and PCR/DGGE analysis of subsequent isolates. Most probable number (MPN) analysis was performed to monitor the *E. coli* population in seawater over a period of 24 months (January 2009-December 2010) and the genetic heterogeneity was determined using *uidA* PCR/DGGE analysis of subsequent isolates. The prevalence of total coliform bacteria ranged from a mean MPN value of 10^1 -10^4 per 100 ml. In this study, in addition to *E.coli* other enteric bacteria species were also detected at Stations 4 and 5. It could be concluded that post bloom waters contain secondary metabolite compounds exhibiting anti-bacterial activity against *E.coli*. Analysis of *uidA* gene using BLAST analysis showed a temporal and spatial genetic homogeneity in *E. coli* marine populations. We concluded that the enteric bacteria levels observed at the stations sampled, are a likely consequence of the discharge of untreated wastewater to the Ayamama Stream and Kucukcekmece Lagoon on the coastal line. It is interesting to note that there were no remedial activities, even though the anthropogenic pollutants were above levels considered a threat to human health. The high density of faecal coliform bacteria and prevalence of *E. coli* in the area under study suggests a potential public health risk of water-borne outbreaks.

Key words: Faecal coliform bacteria, *Escherichia coli*, *uidA* gene, Istanbul, Turkey.

Recreational waters are susceptible to a variety of sources of microbial pollution, which can contain pathogenic microorganisms that cause gastrointestinal, upper respiratory tract, ear, eye, nasal cavity and skin infections [1,2]. These microorganisms may come from point source discharges, such as raw sewage, storm water, combined sewer overflows, effluents from wastewater treatment plants and industrial sources. Non-point source discharges, such as agriculture, forestry, wildlife and urban run-off, can also impair water quality[3]. Discriminating between these different sources of faecal pollution in order to develop remediation strategies has been a perennial challenge for water quality managers [4].

Faecal coliforms are Gram negative bacilli able to ferment lactose at elevated temperatures and include species such as *Escherichia coli*. The presence of antibiotic resistant coliforms in water samples is a strong indicator of faecal pollution from animal and/or human sources. Traditionally, detection and enumeration of bacterial pathogens have been largely based on the use of selective culture and standard biochemical methods. More recently, the use of the polymerase chain reaction (PCR) has provided rapid and highly sensitive methods for the specific detection of pathogenic microorganisms in the aquatic environment [5,6]. Studies focusing on the detection of *E.coli* have

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also resulted in the development of numerous PCR primer sets suitable for further DGGE analysis of diversity. These include genes encoding 16S rRNA, 23S rRNA, β-D-glucuronidase and others. However, with the exception of one segment of *uidA* [7], none have been evaluated for their potential to differentiate *E.coli* communities with regard to environmental water quality. The *uidA* gene, encoding β-D-glucuronidase, has been used for detecting *E.coli* in several environmental studies[6,8].

The aims of this study were to detect *E.coli* via the *uidA* gene, to identify the associated faecal pollution sources and to predict faecal contamination in an active seaside area of the Marmara Sea. The most probable number (MPN) analysis was performed to monitor the *E. coli* population seawater over a period of 24 months (January 2009-December 2010) and the genetic heterogeneity was determined using *uidA* PCR/ DGGE analysis of subsequent seawater isolates. Environmental factors associated with poor water quality locations and sources in this coastal area were observed and inferences made.

MATERIALS AND METHODS

Study area and water samples

As the largest city in Turkey, Istanbul's coastal area is used, directly or indirectly, by over 12 million people for fishing, transportation and recreation; therefore, the monitoring of microbiological quality and related risk assessment is of particular significance for public health. The present study analysed the prevalence and isolation of faecal coliform bacteria, with an emphasis on *E. coli*, from the coastal region.

Water samples were obtained monthly from seven stations along the south-western coast of Istanbul for a period of two years starting in January 2009 through December 2010 (Figure 1, Tables 1 and 2).

Monitoring stations were selected at locations that were either highly urbanized or where the coastal ecosystem was heavily deteriorated [10,11,12]. All stations were located near the coast, for Universal Transverse Mercator (UTM) coordinates see Table 1. The first station (Atakoy) was located near a harbor and is exposed, in particular, to anthropogenic pollutants carried by the Ayamama Stream and Atakoy wastewater treatment plant. The second station (Yesilkoy), located at 1 km off the coast outside of the gulf, can be considered unaffected from the presence of cyanobacterial and algal blooms. Station 3 (Menekse) was selected as a region which is used as a public beach during summer months. The 4th and 5th Stations were selected in areas of the Kucukcekmece Lagoon, which is connected to the Sea of Marmara via a narrow channel, representing the lagoon ecosystem and its connection with the sea. Station 6 (Avcilar) represents the fast sea transport central harbor in Istanbul. Station 7 (Zeytinburnu) was selected point of influence of domestic and industrial settlements (factory wastes etc) which is particularly influenced by seasonal rainfall.

Pre-noon water samples were collected approximately 30 cm below the water surface with a bacteriologic sampler (500 ml) in sterilized dark glass bottles (APHA 2005). Samples were analysed under standard laboratory conditions and processed within 2 h of collection. The physical parameters assessed were temperature, pH, salinity and dissolved oxygen (*in situ*) using a multiprobe model (Hanna Company, HI 9828).

Isolation and Identification of Bacterial Isolates

Multiple-Tube Fermentation Technique (five replicate) (9221) / Standard Total Coliform Fermentation Technique (Lauryl Sulfate Broth (Merck 1.10266)) was used to estimate the prevalence of faecal coliform bacteria and for the isolation of *E. coli* (9221B) [13]. Standard water and wastewater methods were applied for the analysis (9221) [13]. Lactose-fermenting colonies were further characterized by replica-plating on Eosin methylene blue agar (EMB, Merck 1.01347) and enteric chromagar (HiMedia, MV1353) and incubated at 37°C overnight. *E.coli*, pink colonies, was selected on the plate and inoculated into *E.coli* chromagar (HiMedia, MV1353) at 37°C overnight. For the identification of *Enterobacteriaceae* species, in particular *E.coli*, colonies were selected and confirmed by IMVIC tests (9221F) [13].

DNA extraction from *E. coli* isolates

For each station that provided positive matches to *E.coli* by IMVIC test, a single pink *E.coli* colony was randomly chosen for molecular confirmation by DGGE and sequencing analysis. Where no *E.coli* could be detected, a randomly

selected enteric bacteria colony was selected. Single colonies were picked from *E.coli* chromagar, re-suspended in 467 µm TE (pH 8) buffer. Proteinase K (3 µl, 20 mg/ml) and SDS (30 µl, 10%) were added and incubated at 37°C for 1 hour. Following the addition of an equal volume (500 µl) of phenol: chloroform:alcohol isoamyl (25:24:1), samples were centrifuged for 5 minutes at 16,000 rpm. The top aqueous phase was transferred to a fresh 2 ml tube and an equal volume of phenol:chloroform:alcohol isoamyl (25:24:1) added, prior to centrifugation at 16,000 rpm for 5 minutes. The DNA was purified with two rounds of phenol: chloroform: alcohol isoamyl extraction, followed by chloroform: alcohol isoamyl extraction. DNA was precipitated using 2.5 volumes of ice-cold 95% ethanol and 0.1 volume of 3M sodium acetate followed by incubation at 22°C for 20 minutes. After centrifugation at 16,000 rpm for 30 minutes, DNA pellets were washed twice with cold 70% ethanol, and then centrifuged at 16,000 rpm for 20 minutes. The ethanol was removed and pellet was air dried. The pellet resuspended in 40 µl, 10 mM Tris-HCl (pH 8.0) and stored at -20°C[14].

After DNA extraction was completed, the DNA concentration of the 13 samples was determined with NanoDrop Spectrophotometer (NanoDrop® ND-1000, Thermo Scientific) and ranged between 65 and 113 ng/µl.

PCR and DGGE

A 166 bp fragment of the *uidA* gene was amplified from suspected *E. coli* isolates using primers UAL 1939 (5'-TATGGAATTTC GCCGATTTT-3') and UAR 2105 (5-TGTTTGCC TCCCTGCTGCGG-3') (Bej *et al.* 1991). A GC clamp was attached to the 50 end of primer UAL-1939, leading to UAL-1939GC (Farnleitner *et al*. 2000). The PCR reaction mixture was prepared as follows: 1 × PCR buffer, 3 µM $MgCl_2$, 200 µM of each deoxynucleoside triphosphate (Promega Corporation, Wisconsin), 0.5 µM of each primer, 1 U of Taq DNA polymerase (Promega Corporation, Wisconsin) and DNA template, to a final volume of 50 µl. All amplification reactions were performed using a Rapid Cycler (Idaho Technology, Idaho). The PCR cycling conditions were a touchdown approach of 30 cycles as follows: genomic DNA was denatured at 94°C for 5 min, followed by 5 cycles of 92°C for 1 min, 65°C for 1 min, and 72°C for 1 min 30 sec, 5 cycles of 92°C for 1 min, 55°C for 1 min, and 72°C for 1 min 30 sec, and 20 cycles of 92°C for 1 min, 50°C for 1 min, and 72°C for 1 min 30 sec with a final extension step of 72°C for 7 min. A 2-min incubation at 72°C was added to the end of PCR program. A positive control of *E. coli* and nuclease-free water as a negative control was included in every procedure.

Visualisation of amplified DNA

PCR-amplified DNA was visualised using 1.5% agarose gel electrophoresis. The products of each PCR reaction for each environmental isolate were run in a 0.5 TAE buffer. The gels were stained in ethidium bromide solution (0.5 × TAE buffer with a final concentration 0.5 mg/l EtBr), PCR products were visualized with a UV transilluminator to confirm the presence of appropriately sized amplicons. DGGE was performed using an Ingeny PhorU-2 system 15 µL of PCR product was applied directly onto an 8% w/v polyacrylamide gel (acrylamide /N,N'-methylene bisacrylamide, 37:1, w/w) in 1 × TAE buffer (40 mmol/L Tris pH 7.4, 20 mmol/L NaAcetate, 1 mmol/L Na_2 EDTA). A 48 to 68% linear denaturing gradient was formed using 20% and 80% denaturants (100% denaturant being 7 mol/L urea and 40% v/v formamide). Electrophoresis was performed at a constant voltage of 100V and a temperature of 60 °C for 16 hrs. Following electrophoresis, gels were stained for 1 hr in Milli-Q water containing 1 µg/mL Ethidium Bromide then de-stained in Milli-Q water for 1 hr, visualised on a UV transilluminator (Syngene GeneGenius) and photographed using the Syngene GeneSnap software. Bands of interest were excised and incubated in 30 µL of DNA water at 4°C overnight prior to sequencing. The sequencing reactions were carried out by LGC AGOWA (Germany). Sequence data were verified using Sequencher version 4.1, compared to the GenBank nucleotide database using BLAST searchers, and aligned in BioEdit to reference sequences downloaded from GenBank.

RESULTS

Physico-chemical parameters showed typical seasonal variations (See Table 2**).** The mean sea surface temperature values varied between 8.6°C and 27.9°C over the sampling period, the highest (29.3ºC) and lowest 8.3ºC temperatures being recorded in August 2010 and March 2009, respectively, at the fifth station. Mean surface

Table 1. Location of stations

Stations	Station's codes	UTM coordinate (35 T)
1	Atakoy	0657417 - 4537372
2	Yesilkoy	0654500 - 4535733
3	Menekse	0649406 - 4537938
4	Channel	0649097 - 4539098
5	Kucukcekmece lagoon	0646803 - 4538521
6	Avcilar	0644637 - 4537159
7	Zeytinburnu	0659240 - 4538569

temperature did not differ considerably among the sites ($p<0.001$). Salinity fluctuated between 17 – 21 ppt during the sampling period. Unlike temperature, the ranges observed in salinity and pH over the sampling period did not show any sudden or large changes that are likely to impact on bacterial growth or survival.

The prevalence of total coliform bacteria ranged from a mean MPN value of 10^1 -10^4 per 100 ml; of particular relevance, some station's coliform counts exceed the limits set by the World Health Organization for recreational water (200 faecal coliforms per 100 ml). According to the microbiological standards used by the Department of Environmental Protection in Turkey, recreational beaches containing an *E.coli* count of <20/100 ml are graded as "good".

Our data demonstrate that during the sampling period, *E. coli* was endemic in the coastal area around the southwestern part of Istanbul. *E. coli* was detected in all stations sampled during the monitoring period (Table 2, Figure 2). In particular, at the second, fourth and fifth stations, *E.coli* was not observed in some *winter* months and in the *August*, presumably due to its location and negative effects of seasonal physical parameters, such as temperature (Figure 2). Likely sources of faecal contamination can be identified for all of the sampling stations, such as Ayamama Stream, Kucukcekmece Lagoon. The continuous seeding of these coastal waters from such sources, suggests that *E.coli* and other enteric bacteria can now be considered as established inhabitants of the local microbiological community, regardless of their transient presence as individuals cells.

As a facultative aerobe, *E. coli* can survive under aerobic as well as microaerophylic conditions. Sea water, depending on weather conditions and anthropogenic affects, can be aerobic or anaerobic, thus making the anthropogenic waste water the most likely reservoir of *E. coli*. As can be seen in Figure 2, the banding pattern observed following amplification with the *uidA* marker showed that enteric bacteria species

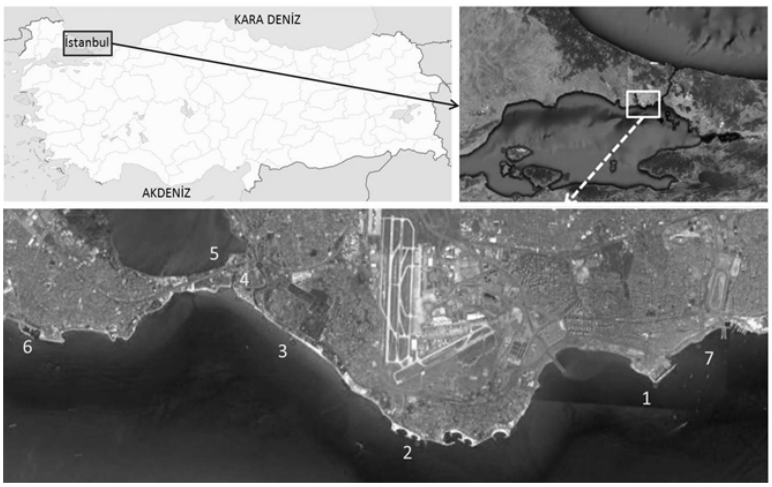

Fig. 1. Study area (adapted by Sivri *et al*.[9])

Table 2. Monthly variations of temperature (T°C), pH and total coliform bacteria (TCB)(MPN/100 ml), size of *uidA* fragment presence (166 bp) and absence (No Positive Colonies N/A) *E.coli* or different enteric bacteria species (>550 bp) at the sampling stations

M	T°C	pH	BC	St 1	St 2	St 3	St 4	St 5	St 6	St 7
Jan09	9.2	8.1	TCB	$14*10^2$	$11*10^3$	$43*10^1$	$21*10^2$	$24*10^2$	$15*10^2$	$43*10^1$
			uidA	166 bp	N/A	166 bp	166 bp	166 bp	166 bp	166 bp
Feb09	8.9	8.2	TCB	$14*10^3$	$14*10^3$	$23*10^1$	$14*10^3$	$14*10^3$	$23*10^1$	$93*10^1$
			uidA	166 bp	N/A	166 bp	166 bp	166 bp	166 bp	166 bp
Mar09	8.7	8.2	TCB	$93*10^1$	$21*10^2$	$9*10^1$	$75*10^1$	$11*10^3$	$24*10^1$	$93*10^1$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Apr09	12.4	8.0	TCB	$24*10^2$	$11*10^1$	$43*10^1$	$43*10^2$	$11*10^3$	$43*10^1$	$24*10^1$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
May09	16.5	7.9	TCB	$11*10^2$	$21*10^1$	$23*10^1$	$24*10^1$	$11*10^2$	$15*10^1$	$23*10^1$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Jun09	21.4	8.0	TCB	$14*10^3$	$11*10^3$	$43*10^1$	$21*10^3$	$24*10^2$	$15*10^2$	$43*10^1$
			uidA	166 bp	166 bp	166 bp	>550 bp	>550 bp	166 bp	166 bp
Jul09	24	8.1	TCB	$15*10^2$	$24*10^2$	$15*10^1$	$14*10^2$	$24*10^2$	$24*10^1$	$9*10^1$
			uidA	166 bp	N/A	166 bp	166 bp	166 bp	166 bp	166 bp
Aug09	26.7	8.0	TCB	$23*10^2$	$24*10^1$	$34*10^1$	$39*10^1$	$14*10^2$	$23*10^2$	$9*10^1$
			uidA	>550 bp	166 bp	166 bp	166 bp	>550 bp	166 bp	166 bp
Sep09	21.4	7.9	TCB	$75*10^2$	$23*10^1$	$43*10^2$	$14*10^3$	$15*10^3$	$43*10^1$	$9*10^1$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Oct09	17.5	7.8	TCB	$75*10^1$	$24*10^1$	$46*10^2$	$21*10^2$	$9*10^2$	$93*10^1$	$9*10^1$
			uidA	>550 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Nov09	14	8.1	TCB	$23*10^2$	$11*10^3$	$23*10^1$	$21*10^1$	$23*10^2$	$21*10^2$	$23*10^1$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Dec09	11	8.0	TCB	$11*10^2$	$7*10^1$	$9*10^2$	$11*10^3$	$15*10^3$	$9*10^1$	$43*10^1$
			uidA	166 bp	N/A	166 bp	166 bp	166 bp	166 bp	166 bp
Jan10	10.7	8.1	TCB	$11*10^3$	$93*10^2$	$24*10^1$	$27*10^2$	$11*10^2$	$21*10^2$	$43*10^1$
			uidA	166 bp	N/A	166 bp	166 bp	166 bp	166 bp	166 bp
Feb10	9.6	8.2	TCB	$14*10^3$	$11*10^3$	$9*10^1$	$11*10^3$	$14*10^3$	$23*10^1$	$93*10^1$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Mar10	8.6	8.3	TCB	$93*10^2$	$21*10^2$	$9*10^2$	$75*10^1$	$13*10^3$	$23*10^1$	$93*10^1$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Apr10	14.2	8.0	TCB	$24*10^2$	$23*10^1$	$43*10^1$	$43*10^1$	$14*10^3$	$43*10^1$	$43*10^1$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
May10	17.3	8.0	TCB	$75*10^1$	$23*10^1$	$23*10^1$	$75*10^1$	$14*10^3$	$23*10^1$	$23*10^1$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Jun10	24.3	7.9	TCB	$24*10^3$	$93*10^2$	$42*10^1$	$93*10^1$	$11*10^2$	$21*10^3$	$43*10^1$
			uidA	166 bp	166 bp	166 bp	>550 bp	>550 bp	166 bp	166 bp
Jul10	25.8	7.9	TCB	$15*10^1$	$11*10^2$	$15*10^1$	$11*10^2$	$11*10^2$	$24*10^1$	$9*10^1$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Aug10	27.9	7.9	TCB	$24*10^1$	$29*10^1$	$43*10^1$	$39*10^1$	$11*10^2$	$21*10^2$	$93*10^1$
			uidA	166 bp	N/A	166 bp	>550 bp	>550 bp	166 bp	166 bp
Sep10	22.0	8.0	TCB	$93*10^2$	$43*10^1$	$43*10^1$	$11*10^3$	$15*10^3$	$4*10^1$	$9*10^1$
			uidA	>550 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Oct10	18.7	8.0	TCB	$75*10^1$	$24*10^1$	$46*10^2$	$21*10^2$	$9*10^2$	$93*10^1$	$9*10^1$
			uidA	>550 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Nov10	15.6	8.2	TCB	$43*10^2$	$15*10^3$	$43*10^1$	$21*10^2$	$21*10^2$	$15*10^2$	$11*10^2$
			uidA	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp	166 bp
Dec10	10.7	8.1	TCB	$15*10^3$	$7*10^1$	$9*10^2$	$11*10^3$	$15*10^3$	$93*10^1$	$43*10^1$
			uidA	166 bp	N/A	166 bp	166 bp	166 bp	166 bp	166 bp

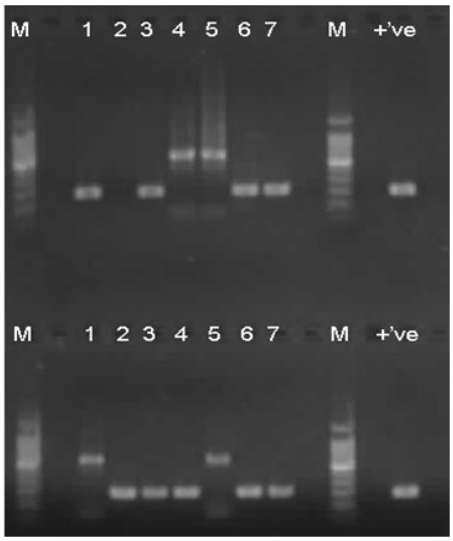

Fig. 2. Amplification of β-D-glucuronidase gene fragment (*uidA, 166 bp*) from *E. coli* isolates using primer pair UAL-1939GC and UAL-2105 (M: 100-bp ladder (Promega) Lane +'ve : positive *E. coli* strain. Upper side denotes Stations 1 to 7 isolated in August 2010, bottom side, isolates 1 to 7 isolated in August 2009. Station 2 in August 2010 failed to generate any positive colonies for *E.coli* (see Table 2 and text for details), and a random, non-pink colony forming enteric isolate was analysed instead.

Fig. 3. Denaturing Gradient Gel Electrophoresis analysis of *uidA* gene fragments amplified from *E.coli* isolates sampled during April 2010 from stations 1 to 7 (indicated). Bands were excised and sent for sequencing to reveal 100% identity to characterized *E.coli* strains including K12 and O104:H4.

distinct from *E.coli*, were often present at the first, fourth and fifth stations when *E.coli* was absent (as shown by the larger fragments amplified using the *uidA* primers).

Molecular analysis via DGGE and sequencing confirmed the presence of *E.coli* when indicated by IMVIC testing. All *E.coli* isolates provided *uidA* gene fragments identical in size and sequence (Figures 3), with 100% shared identity to *E.coli* strains including K12, O104:H4, KO11FL, O103:H2, O26:H11 and O7:K1 (data not shown). When no *E.coli* was detected in a sample (St2: Jan09, Feb09, Jul09, Dec09, Jan10, Aug10, Dec10; St1: Aug09, Oct09, Sep10, Oct10; Sts 4 and 5: Jun09, Jun10, Aug10) and a random enteric bacteria was selected for molecular analysis, the distinctive 166 bp *E.coli uid*A fragment was not successfully amplified, adding further validation to the IMVIC testing results. In place, either no fragment was amplified at all, or a larger band of approximately 550 bp was amplified.

DISCUSSION

Public and environmental health protection requires safe swimming and bathing waters, which must be free of pathogenic bacteria. Among the pathogens transmitted by water bodies, enteric pathogens are the ones most frequently encountered. Consequently, sources of faecal pollution in water and its associated microbiological properties must be strictly regulated [15]. Indicator organisms are globally used as a warning of possible contamination and as an index of water quality deterioration [16,17].

When the results of total faecal coliform bacteria were examined, it was found in many cases that the abundance of *E.coli* was higher than acceptable. It was determined that the bacterial population increased during spring and early-summer periods. However, as the environmental conditions began to change and the temperature began to increase, changes in bacterial genera were also observed. Indeed, the diversity and the number of organisms also changed with respect to seasonal changes in temperature. Water temperature may cause changes in bacterial mortality and rate of multiplication [18]. In particular, at the second station, *E.coli* was not observed at the colder times of year. However, as noted by Solic and Krstulovic [19]

increased temperature and salinity is detrimental to the survival of faecal coliform in the presence of sunlight [20]. For this reason, during the spring period *E. coli* was detected in all stations, but at a lesser extent during the summer, particularly August (Table 2).

On the other hand, from this study it could be concluded that the reason for the increase in the enteric bacteria levels at stations 4 and 5, is the continual seeding by untreated wastewater sourced from the Kucukcekmece Lagoon and human facilities occurring along the coastal line. Previous studies have shown that excessive growth of cyanobacteria, referred to as blooms, represent a serious threat to this area [9,10,11]. Freshwater cyanobacteria are acknowledged synthesisers of biologically active and structurally diverse secondary metabolites [21,22,23]. These compounds exhibit activity against organisms including viruses, bacteria and other algal species [24]. In this study, in addition to *E.coli* other enteric bacteria species were also detected at Stations 4 and 5. In June 2010 and August 2010, the chlorophyll-*a* concentration was measured 94,2 µg/l and 181,4 µg/l respectively at the same stations by Sivri *et al.*[9]. It could be concluded that post bloom waters contain secondary metabolite compounds exhibiting anti-bacterial activity against *E.coli*. Previous work has shown that enteric bacteria species do not survive easily under cyanobacterial bloom pressure [25,26,27].

According to Lasaide *et al.* [6] because of the indigenous nature of *E. coli* considerations must be made about the importance of this bacterium as an indicator of faecal contamination, and the necessity of knowing the source of contamination if public health is to be protected. In this study, for the first time, the prevalence of faecal coliform bacteria and *Escherichia coli* in south-western coast in Istanbul were determined with *uidA* gene. In addition, a comprehensive snapshot of the dynamics of the *E.coli* composition in south-western side in Istanbul was obtained by PCR- DGGE based analysis and sequencing. In our study, only one gene was studied, the gene coding for β-D-glucuronidase. Analysis of *uidA* gene using DGGE analysis and BLAST results showed temporal and spatial genetic homogeneity in *E. coli* marine populations.

It light of these observations, it is clear that the samples collected during this study were exposed to a significant pollution load. The results reveal that the area around Istanbul faces bacteriological pollution and the existing pollution level in this area is near the criterion specified for aquaculture, fishery and recreational activity, posing a significant threat to human health. Government authorities need to take effective measures (e.g. proper sanitation facilities and wastewater treatment) to reduce pollution of the coastal area to avoid serious consequences for healthcare management within local communities.

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