Research Article

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Antibiotic susceptibility profiles of *Escherichia coli* strains and fecal contamination in Orontes River, Turkey

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ABSTRACT

This study was to evaluate the level of contamination of surface waters by coliform bacteria and *Escherichia coli*, as well as to detect the fecal pollution and the antibiotic resistance patterns of *E. coli* strains from recovered Asi (Orentes) River in the southern region of Turkey. Total and fecal coliform results were revealed as >1100 MPN/100 mL and 1100 MPN/100 mL for two stations, respectively. A total of 10 *E. coli* isolates were screened for antibiotic susceptibility by the Kirby Bauer method. Resistance to cefotaxime, tobramycin, streptomycin, trimethoprim, cefepime, cefazolin, and ampicillin was determined in 20%, 20%, 40%, 20%, 20%, 20%, and 40%, respectively. These findings point out a widespread distribution of *E. coli* strains produced antibiotic resistance genes in the surface waters in the southern region of Turkey, suggesting an aquatic reservoir for resistance genes. Multiple antibiotic resistance (MAR) rates of %30 of *E. coli* isolates were greater than 0.200. This high index indicates exceeding the permissible water quality limits for human use and consumption and is the primary source of contamination in the discharges from domestic, agricultural, and urban.

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Introduction

Surface water bodies, one of the important sources of drinking water, cover nearly half of the worldwide need. They are more vulnerable to various contamination sites than groundwater protected by the overlying soil parts trapping and attenuating pollutants [1]. The increase in the number of waterborne disease outbreaks like intestinal infections, dysentery, diarrhea, typhoid fever, cholera, and jaundice by contamination of surface freshwater sources constitutes a persistent problem for public health and the environment in developing countries [2, 3]. Thus, biological, chemical, and physical quality parameters of water sources become a major burden for public and regulatory authorities [1, 4]. Biologic diversity in aquatic environments contributes to the sustainability of natural water ecosystems by removing pathogenic contamination, balancing conditions, and playing key roles in nutrient cycling [5]. However, due to

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diseases associated with pathogens carried by water bodies, the monitoring of the biological quality is the main strategy for controlling microbial pollution in terms of public health [6]. The World Health Organization (WHO) guidelines propose checking fecal contamination for assessing human health risks in surface water bodies [7]. The fecal contamination load in surface waters is changed based on anthropogenic/zoogenic origins, discharging of municipal/industrial/domestic wastewater, and agricultural/ storm/ urban/ water runoffs [1].

According to WHO criteria, fecal contamination representing a risk to the health, and environment is investigated by the presence of fecal indicator bacteria (FIB) such as *Escherichia coli* (*E. coli*), total coliforms, or thermotolerant coliforms [2, 8]. Among them, *E. coli* is the most common indicative parameter of fecal contamination in water bodies associated with human health [9]. Although the main source of fecal pollution is human and animal wastes, FIB, which may be reported in many environments, are not directly identify feces sources [8, 10]. Also, the indiscriminate use of antibiotics in agriculture applications and veterinary/human medicine provides the emergence of strains showing greater resistance to various antibiotics in water sources [9].

Beginning in Lebanon and flowing northwards through Syria before entering the Mediterranean Sea in Turkey, the Orontes (Asi) River is a length of 571 kilometers in Western Asia. Asi River, one of the most important rivers in southern Turkey, passes north of Hatay and falls 16 kilometers to the sea south of Samandag.

There has been an advance in the treatment of human and veterinary diseases, increasing feed efficiency, and maintaining good agricultural practices with the discovery of antibiotics. The global dissemination threat to the public health of antibiotic resistance among bacteria due to the developing strategies-based using antibiotics is becoming increasingly common via clinical specimens, foods of animal origin, and aquatic environments [11].

The emergence and dissemination of antibiotic-resistant bacteria (ARB) in water sources such as the Orontes River, which fell from other countries to Turkey, are not only a local and regional problem. Because river water can carry ARB into the Mediterranean basin by crossing borders. This can be caused the variation in the antibiotic resistance profile of bacteria in water and other environments in Turkey. For this reason, the water quality of this kind of river should be continuously monitored for global water safety.

This study aimed to assess the rates of fecal contamination of surface water, and antibiotic resistance profiles among *E. coli* isolated from Orontes River in Hatay, Turkey, and to identify the possible sources of the pollution. It also researched antibiotic resistance profiles of *E. coli* strains isolated from this region when evaluating the risk of potential fecal pollution associated with the Orontes River. Our findings will contribute to a better understanding of how fecal contamination in natural water environments influences environmental and public health.

Material and Methods

Study site description

Asi (Orentes) River which originates from Labweh and Lebanon, locates in the south of Turkey. It pours finally into the Mediterranean Sea in Hatay province by flowing through for about 380 km within three countries (Lebanon, Syria, and Turkey). Due to the discharge of domestic, agricultural, and industrial wastewater in the province directly into the Orontes River without disinfection treatment, the river has been egregiously polluted. Also, it is the main source of fishing and agricultural irrigation. The contamination of enteric pathogens based on household and agricultural wastewater pouring into the river was previously reported [11]. Water samples were collected from two locations (St1; 36°11'23"N, 36°09'05"E) and St2; 36°11'48"N, 36°09'29"E) in which upstream of the Hatay city center and after the passing center. A map of study locations is shown in Fig 1. According to APHA (1998) and WHO (2006) [12, 13], they were collected using a sterile bacteriological sample vial (250 mL) in June (2021). The sample was immediately brought to the lab in an icebox within 2 hours of collection and stored at +4°C in a light-sensitive container until microbiological analyses.

Microbiological analysis

Enumeration of total mesophilic bacteria in surface water, 100 µL of the sample was spread on Plate Count Agar medium, and plates were incubated at 22°C for 72 h and 37°C for 48 h (TS EN ISO 6222-ISO Standards). After incubation, the colony count on agar was noted as colony-forming units per mL (CFU/mL).

The presence of total and fecal coliforms was tested using the most probable number method (MPN) illustrated by Prescott et al. (1996) [14]. The results were expressed as the most probable number/mL comparing them with standard statistical tables. Typical growth of total coliforms (turbidity and bubbles in Durham tube) were monitored in Lauryl Sulfate Tryptose Broth (LST, Merck) at 37°C for 24-48 h. After that, 100 µL from each positive LST broth tube was transferred to an EC (*Escherichia coli*) medium and incubated at 44.5°C for 24-48 h for screening fecal coliform.

EndoC agar differentiated based on lactose fermentation was used for the isolation of *E. coli* strains from the water sample. Typical colonies with metallic green sheen were identified by applying morphological (Gram staining and cell morphology) and standard microbiological (indole, methyl red, Voges Proskauer, citrate, and MUG (4-Methylumbelliferyl β-D-Glucuronide) agar test systems) procedures.

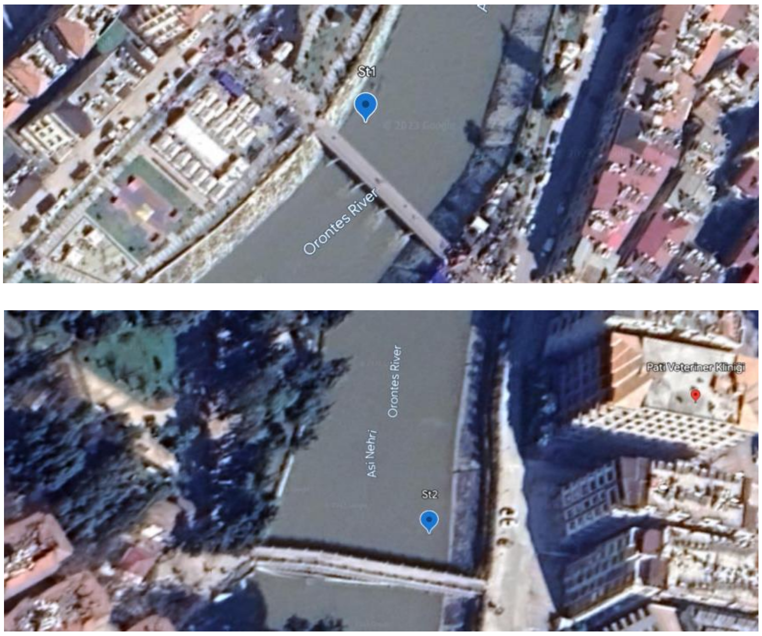

Fig 1 Sampling stations (Google Earth)

Antibiotic susceptibility profile

The susceptibility of *E.coli* isolates was exhibited against the 12 standard antibiotics by disk diffusion method (Bauer et al. 1966) [15] on Mueller-Hinton agar (Oxoid, UK) plate according to the guidelines and recommendations of Clinical & Laboratory Standards Institute (CLSI). The following 12 antibiotics that are important for the human healthcare system were Ampicillin (AMP; 10 µg), Tobramycin (TOB, 10 µg), Cefotetan (CTT; 30 µg), Cefepime (FEP; 30 µg), Cefotaxime (CTX; 30 µg), Chloramphenicol (C; 30 µg), Cefazolin (CZ; 30 µg), Streptomycin (S; 10 µg), Ertapenem (ETP; 10 µg), Tetracycline (TE; 30 µg), Gentamycin (GEN; 30 µg), and Trimetropim (TR; 5 µg) (Bioanalyse). The results were interpreted by the CLSI standard. *E. coli* ATCC 25922 was used for quality control. The multiple antibiotic resistance (MAR) indexes of strains were evaluated based on the formula declared by Krumperman (1983) [16]. Resistance results were analyzed by using the software SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results and Discussion

In the water samples, the concentrations of culturable mesophilic bacteria were enumerated on plate count agar. As summarized in Table 1, the average (n=3) bacteria counts in surface waters were $118x10^3$ and $186x10^3$ CFU/mL at 37°C. These values were numbered $375x10^1$ and $142x10^1$ at 22°C, respectively.

Table 1 Indicator organism measurements in Orontes River water sample

	St1	**St2**
Total bacteria	$118x10^3$ CFU/mL at 37°C $375x10^1$ CFU/mL at 22°C	$186x10^4$ CFU/mL at 37°C $142x10^1$ CFU/mL at 22°C
Total coliform	1100+MPN/100 mL	1100+MPN/100 mL
E. coli	1100 MPN/100 mL	1100 MPN/100 mL

Concentrations of total coliforms in surface waters were >1100 MPN/100 mL, thus this situation indicating bacteriological pollution. Both stations were calculated rather higher values of fecal coliforms, 1100 MPN/100 mL. WHO (2017) [17] and TS 266 (2005) [18] guidelines indicate that total and fecal coliform bacteria (0/100 mL) should not contain in surface water sources that are overused for drinking water and intended for human consumption. Enteric pathogens (*Salmonella* spp., *Shigella* spp., *Vibrio cholera*, and *E. coli*) going into the surface water supply lead to infectious waterborne diseases such as diarrhea, cholera, typhoid fever, and dysentery. These indicator bacteria are usually present in animal and human feces, so our findings are clearly emphasized that fecal coliforms reach the Orontes River through improperly treated sewage and leakage. This might show that the main source of contamination in the Orontes River is domestic sewage and solid residues from surrounding densely populated areas. There are many reports on the existence of fecal coliforms in various aquatic environments in Turkey [19-21]. The highest total coliform and fecal coliform levels were reported to be $13x10^7$ and $14x10^6$ CFU/100 mL for Cırpıcı River; higher than that of our bacterial and fecal pollution results [1]. The detected pollution levels (1100+MPN/100 mL) of Asi River are similar to the results of Avsar (2019) who declared that high coliform contamination in the Karasu River (Sinop, Turkey) was 2400 MPN/100 mL for September [22]. Our coliform results are as high as those determined by Karabas et al. (2017) who highlighted that indicated the uncontrolled discharges with $940x10^3$ CFU/100 mL total coliform value at Alibeykoy Creek [23]. Total coliform levels in Karacaoren I Dam Lake were detected to range from 500 to 100.000 CFU/100 mL and the presence of fecal coliform was also noted [24]. Akkan et al. (2019) reported high fecal coliform pollution (71.66)% in surface water bodies collected from Yaglıdere Stream [25]. Although our coliform contamination rates were lower than those shown by Yalım et al. (2020) and Akkan et al. (2022), our study clearly emphasized the discharge of household and industrial wastewater systems into the surface water without control.

The difference in results between previous studies based on fecal contamination and bacterial load may be explained by changes in water quality, originating from biogeochemical cyclings such as the storage and release of nutrients and physicochemical characteristics in aquatic ecosystems. The sanitary status of the different water bodies such as lakes, rivers, and dams showing different hydraulic characteristics may be changed by seasonal changes, proximity to mineral extraction sites and settlements, and agricultural and industrial activities.

E. coli strains recovered on EndoC plates from the stations were included for further antibiotic susceptible study. A total of 10 bacteria were isolated in equal numbers from the water samples taken from 2 different regions. 5 of them were evaluated as *E. coli*. St1 (3 strains) and St2 (2 strains), respectively. Strains showing Gram-negative bacilli morphological character were identified by biochemical tests (indole, methyl red, Voges Proskauer, citrate; (IMVIC) and MUG test systems). Endo C reactions and results of biochemical tests of *E. coli* strains are shown in Fig. 2.

In addition to bacteriological load, the antibiotic resistance profiles of *E.coli* in water bodies were screened. While the low frequency of resistance to cefotaxime, tobramycin, streptomycin, cefepime, and cefazolin (20%) was determined, *E. coli* strains showed relatively high levels of resistance to Trimetropim and Ampicillin (40%) (Table 2).

Fig 2 Mug test, IMVIC, and Endo C reactions for distinguishing *E. coli* strains; (a: Mug; b: indole; c: citrate; d: methyl red and Voges Proskauer; e: Endo C)

For non-beta-lactam antibiotics, there was a high sensitivity of isolates to tetracycline, gentamycin, and chloramphenicol (100%). None of the *E. coli* strains recovered from river water samples were ESBL producers according to the recommended CLSI standards. By contrast, the obvious spatial homogeneity was exhibited in resistance with 20% cefotaxime, tobramycin, cefepime, and cefazolin. This study indicates that there is no widespread distribution of antibiotic-resistance genes among *E. coli* strains in the water samples of the Asi River.

Table 2 Percentage of total *E.coli* isolates with antibiotic resistance

	Pattern	**Resistance (%)**
Ampicillin	R	40.0±0.5*
Tobramycin	R	20±0.25*
Cefotetan	S	0±0.0*
Cefepime	R	20±0.40*
Cefotaxime	R	20±0.30*
Chloramphenicol	S	0±0.0*
Cefazolin	R	20±0.25*
Streptomycin	R	20±0.15*
Ertapenem	S	0±0.0*
Tetracycline	S	0±0.0*
Gentamycin	S	0±0.0*
Trimethoprim	R	40.0±0.25*

**P*<0.05 compared with the results.

The profile of antibiotic resistance of *E. coli* strains in many freshwater sources of Turkey has been previously reported. Kurekci et al., (2017) showed resistance against extended-spectrum β-lactam antibiotics of *E. coli* strains isolated from the Asi River (Turkey) [11]. For *E. coli* strains recovered from ten rivers in the northern-east coastal region of Turkey, Ozgumus et al. (2009) found resistance to ampicillin, streptomycin, and trimethoprim similar to our susceptibility results [26]. Buyukkaya Kayıs (2022) noted that *E. coli* isolated from 5 different stations in Ataturk Dam in Adıyaman was resistant to chloramphenicol, tetracycline, cefepime, and cefazolin antibiotics contrary to our findings [27]. Mercimek Takcı et al. (2023) pointed to the high-frequency resistance against cefotaxime (62.5%) and streptomycin (50%) antibiotics for *E. coli* strains obtained from Alleben pond (Gaziantep) by contrast with our results [28]. Similarly, Altug et al. (2020) reported the dissemination of high antibiotic resistance in surface water samples, Gulluk Bay [29].

The MAR index ranged from 0.083-0.5 for *E.coli* strains from Asi River water bodies. %30 of *E. coli* isolates showed a MAR index greater than 0.200. A MAR index of 0.200 is represented the difference between low- and high-risk degrees. Those multidrug-resistant bacteria having MAR indices >0.200 indicates the exposure of sewage effluents to high-risk contamination for water bodies with the increased distribution of possibly fecal microorganisms harmful to humans.

Conclusion

This research study provides insights into the abundance of FIB and the antibiotic resistance profile of *E. coli* strains in the surface water sources throughout the Asi River. The high prevalence of fecal indicator bacteria in these surface water sources highlights the risk of fecal contamination from both human and animal origins in built-up and agricultural areas. To prevent surface water contamination, it is necessary to focus more on the projects and public policies that ensured the treatment of wastewater, assuring higher environmental preservation, and reduced risks to public health.

Abbreviations

APHA: American Public Health Association; CFU: Colony Forming Unit; WHO: World Health Organization; LST: Lauryl Sulfate Tryptose Broth; MUG: 4-Methylumbelliferyl β-D-Glucuronide; CLSI: Clinical & Laboratory Standards Institute; MPN: Most Probable Number; *E. coli: Escherichia coli;* ESBL: Extended Spectrum Beta-Lactamase; IMVIC: Indole; Methyl Red; Voges Proskauer; Citrate; MAR: Multiple antibiotic resistance.

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The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
In this work, the laboratory works were conducted by Cevher Karaca and Tahsin Huner. Dr Hatice Aysun Mercimek Takci has supervised their works. The manuscript was edited and finalised by Hatice Aysun Mercimek Takci.

References

1. Paruch, L., et al., Faecal Pollution Affects Abundance and Diversity of Aquatic Microbial Community in Anthropo-Zoogenically Influenced Lotic Ecosystems. Scientific Reports, 2019. *9*(1): p. 1-13.DOI: 10.1038/s41598-019-56058-x
2. Khan, K., et al., Prevalent Fecal Contamination in Drinking Water Resources and Potential Health Risks in Swat, Pakistan. Journal of Environmental Sciences, 2018. 72: p. 1-12. https://doi.org/10.1016/j.jes.2017.12.008.
3. Salamandane, A., et al., High Fecal Contamination and High Levels of Antibiotic-Resistant Enterobacteriaceae in Water Consumed in The City of Maputo, Mozambique. Biology, 2021. *10*(6): p.558. DOI: 10.3390/biology10060558.
4. Salim Dantas, M et al., Impact of Fecal Contamination on Surface Water Quality in The São Francisco River Hydrographic Basin in Minas Gerais, Brazil. Journal of Water and Health, 2020. *18*(1): p. 48-59. DOI: 10.2166/wh.2019.153.
5. Zeglin, L.H., Stream Microbial Diversity in Response to Environmental Changes: Review and Synthesis of Existing Research. Frontiers in Microbiology, 2015. 6: 454. https://doi.org/10.3389/fmicb.2015.00454 (2015).
6. Malla, B., et al., Identification of Human and Animal Fecal Contamination in Drinking Water Sources in the Kathmandu Valley, Nepal, Using Host-Associated Bacteroidales Quantitative PCR Assays. Water, 2018. *10*(12): 1796. https://doi.org/10.3390/w10121796.
7. Genter, F., J. Willetts, and T. Foster, Faecal Contamination of Groundwater Self-Supply in Low- and Middle İncome Countries: Systematic Review and Meta-Analysis. Water Research, 2021. 201: 117350. DOI: 10.1016/j.watres.2021.117350
8. Holcomb, D.A., et al., Human Fecal Contamination of Water, Soil, and Surfaces in Households Sharing Poor-Quality Sanitation Facilities in Maputo, Mozambique. International Journal of Hygiene and Environmental Health, 2020. 226: 113496. https://doi.org/10.1016/j.ijheh.2020.113496
9. Américo-Pinheiro, J.H.P., et al., Iqbal, Monitoring Microbial Contamination of Antibiotic Resistant *Escherichia coli* Isolated from the Surface Water of Urban Park in southeastern Brazil. Environmental Nanotechnology, Monitoring & Management, 2021. 15: 100438. https://doi.org/10.1016/j.enmm.2021.100438.

10. Okotto-Okotto, J., et al., A Longitudinal Study of Long-Term Change in Contamination Hazards and Shallow Well Quality in Two Neighbourhoods of Kisumu, Kenya. International Journal of Environmental Research and Public Health, 2015. 12: p. 4275–4291. DOI: 10.3390/ijerph120404275.
11. Kurekci, C., et al., Characterization of Extended Spectrum β-lactamase (ESBL)-Producing *Escherichia coli* in Asi (Orontes) River in Turkey. Journal of Water and Health, 2017. *15*(5): p. 788-798. DOI: 10.2166/wh.2017.257.
12. APHA, Standard Methods for the Examination of Water and Wastewater, United Book Press, Inc., Baltimore, MD, USA, 1998.
13. World Health Organization, Guidelines for Drinking-Water Quality, Geneva, Switzerland, 2006. https://apps.who.int/iris/bitstream/handle/10665/43428/9241546964_eng.pdf.
14. Prescott L.M., J.P. Harley, and A.K. Donald, Microbiology Laboratory Exercises. W.C. Brown Publishers, New York, 1996.
15. Bauer, A.W., et al., Antibiotic Susceptibility Testing by a Standardized Single Disk Method. American Journal of Clinical Pathology, 1966. 45: p. 493-496. https://asm.org/ASM/media/docs/105bauer.pdf
16. Krumperman, P.H., Multiple Antibiotic Resistance Indexing of *Escherichia coli* to identify High-Risk Sources of Fecal Contamination of Foods. Applied and Environmental Microbiology, 1983. *46*(1): p. 165-170. https://doi.org/10.1128/aem.46.1.165-170.1983
17. World Health Organization, Guidelines for drinking water quality. Geneva, Switzerland, 2017. https://www.who.int/publications/i/item/9789241549950
18. TS266 2005. Sular-insani tüketim amaçlı sular. ICS 13.060. 20. TSE, Ankara. http://infogroup.com.tr/media/files/sular.pdf
19. Buyukkaya Kayis, F., et al., An Investigation of Multiple Antibiotic Resistance and Identification of Bacteria Isolated from Gölbaşı and Azaplı Lakes (Adıyaman). Turkish Journal of Agriculture-Food Science and Technology, 2017. *5*(1): p. 43-47. https://doi.org/10.24925/turjaf.v5i1.43-47.819
20. Hulyar, O., and G. Altug, The Bacteriological Risk Transported to Seas by Rivers; The Example of Çırpıcı River, Istanbul, TR. International Journal of Environment and Geoinformatics, 2020. *7*(1): p. 45-53. https://doi.org/10.30897/ijegeo.704260.
21. Mercimek Takci, H.A., S. Toplar, and M.S. Ozdenefe, Antibiotic and Heavy Metal Resistance of *Escherichia coli* Strains Isolated From the Seve Dam and Konak Pond, Kilis, Turkey. Acta Aquatica Turcica, 2021. *17*(2): p. 290-297. https://doi.org/10.22392/actaquatr.801564
22. Avsar, C., Measurement of Antibiotic Resistance of Microbial Species in the Karasu River (Turkey) Using Molecular Techniques. Water and Environment Journal, 2019. *33*(2): p. 179-191. https://doi.org/10.1111/wej.12388
23. Karabas, N., et al., A Research on Coliform Bacteria in The Golden Horn Estuary (Sea of Marmara, Turkey). Desalination and Water Treatment, 2018. 115: p. 199–206.
24. Yalım, F.B., et al., Karacaören I Baraj Gölü (Burdur) Mikrobiyolojik Kirlilik Düzeyinin Mevsimsel Değişimi. Journal of Limnology and Freshwater Fisheries Research, 2020. *6*(2): p. 120-126. DOI:10.17216/LimnoFish.633769
25. Akkan, T., S. Mehel, and C. Mutlu, Determining The Level of Bacteriological Pollution Level in Yaglıdere Stream, Giresun. Journal of Limnology and Freshwater Fisheries Research, 2019. *5*(2): p. 83-88. https://doi.org/10.17216/limnofish.450722
26. Ozgumus, O.B., et al., Class 1 and Class 2 Integrons and Plasmid-Mediated Antibiotic Resistance in Coliforms İsolated From Ten Rivers in Northern Turkey. Journal of Microbiology 2009. *47*(1), p. 19-27. https://doi.org/10.1007/s12275-008-0206-z.
27. Buyukkaya Kayis, F., Atatürk Baraj Gölünden (Adıyaman) Izole Edilen *Escherichia coli* Bakterilerinde Antibiyotik Dirençlilik Profili. Commagene Journal of Biology, 2022. *6*(1): p. 105-109. https://doi.org/10.31594/commagene.1110770

28. Mercimek Takci, H.A., et al., Microbial Water Quality of Pond Alleben (Gaziantep, Turkey) in Winter and Climatic Changes in The Region. Journal of Water and Climate Change, (in press, 2023), 2023.
29. Altug, G., et al., Antibiotic And Heavy Metal Resistant Bacteria Isolated from Aegean Sea Water and Sediment in Gulluk Bay, Turkey. Johnson Matthey Technology Review, *64*(4): p. 507-525. DOI: 10.1595/205651320x15953337767424.