Journal of
Water & Climate Change

IWA PUBLISHING

Journal of Water and Climate Change Vol 14 No 6, 2042 doi: 10.2166/wcc.2023.132

Microbial water quality of pond Alleben (Gaziantep, Turkey) in winter and climatic changes in the region

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ABSTRACT

This study assessed the microbial quality of water sources in winter by microbiological methods and molecular-based quantitative polymerase chain reaction (qPCR) and potential future bacterial loads in the Alleben pond based on the increase in water temperature. Total bacteria, total coliform, and *Escherichia* coli levels using the qPCR were significantly higher in the water samples than those enumerated by culture-dependent microbiological methods. These results showed that our qPCR approach is suitable to estimate the relative abundances of uncultured and cultured indicator microorganisms affected by the water characteristics. Based on our climate data, the daily average rate of 1.8 °C/ increase associated with air temperature for the pond was recorded. As a result of the warming of the water surface, temperature thresholds in winter may breach and, thus, the physicochemical and biological properties of the pond may change by increasing bacterial growth rates. This study is the first analysis to apply combined climate change with molecular approaches to better understand how bacterial concentrations in aquatic environments may change in the future based on air temperature variations for the Gaziantep region. This analysis indicates developing surface water treatment measures and climate adaptation strategies that may reduce bacterial contamination in the Alleben pond.

Key words: antibiotic resistance, methodological approaches, qPCR, warming, water quality

HIGHLIGHTS

- This study analyses the warming of the water surface as the factor affecting the bacterial quality of the pond.
- A large amount of data collection including meteorological air temperature data of the Gaziantep for the past 30 years.
- Bacterial contamination in the Alleben pond exceeds the acceptable levels (0 MPN/100 mL) according to WHO (2017) and TS266 (2005) guidelines.

INTRODUCTION

The microbial contamination of water sources with fecal-derived pathogenic microorganisms (bacteria, virus, and protozoa) and human and/or animal feces may lead to serious public and environmental health problems in many countries (Girones *et al.* 2010; Silva & Domingues 2015). The pathogens in numbers that will result in illness are making difficult to the purification of surface water via desalination. Thus, these water sources, directly used for potable water and industrial application, create an important risk (Panagopoulos 2022a, 2022b; Panagopoulos & Giannika 2022). For this reason, the microbiological safety of waters is essential to protect public health from diseases caused by waterborne pathogens (Silva & Domingues 2015).

Microbial techniques have been conventionally applied to observe changes in the microbial quality of surface and drinking water (Douterelo *et al.* 2014). Culture-dependent methodologies (heterotrophic plate count, membrane filtration, and multiple tube fermentation/most probable number, MPN) have been extensively used for routine microbial quality

monitoring of drinking and surface water, including the detection of fecal and coliform contamination (Allen *et al.* 2004; Sutton 2010; Douterelo *et al.* 2014).

These culture-based techniques that the time (18–96 h) required for confirmation and verification steps only showed <1% of the total microbial diversity of the water samples despite their relative ease of use and low cost (Noble *et al.* 2010; Douterelo *et al.* 2014). Due to this limitation, culture-independent molecular-based approaches have been recently enabled to obtain more detailed results of microbial communities (Douterelo *et al.* 2014).

To determine of waterborne bacterial community without the need for a cultivation step, the rapid and very specific three molecular-based methods are here: the immunological, polymerase chain reaction (PCR), and *in situ* hybridization (ISH) techniques (Rompré *et al.* 2002). The quantitative polymerase chain reaction (qPCR) technique based on quantifying the number of target gene copies of the indicators or pathogens (DNA or RNA) in environmental samples has a high level of specificity and sensitivity without the need for complex cultivation or confirmation step (Girones *et al.* 2010; Ricchi *et al.* 2017; Offenbaume *et al.* 2020). Especially, coliform bacteria are indicators of biological pollution in surface water, groundwater, or supply sources of potable water. The presence of *Escherichia coli*, a member of the coliform group, in water sources, is indicated the pollution of human or animal fecal origin (Acharya *et al.* 2019). The level of total bacteria, total coliform, and *E. coli* in water samples have been determined by the absolute number of bacterial quantities with the qPCR using a calibration curve generated with standard genomic DNA (Ricchi *et al.* 2017).

Climate change, which is a natural phenomenon, has continued to initiate with the industrial revolution and the present day. The effects of climate change with natural and/or human origin vary according to place and time. These effects occurred as a result of events such as changes in temperature and precipitation, ocean currents, sea rise, or fall on the world. Air temperature, the main climate change factor affecting water quality, influences the distribution and vital activities of organisms in water bodies (Fonseca *et al.* 2015).

This article aims to assess the effect of local climate alterations (air temperature) on bacterial contamination and microbial concentration of the surface water source (Alleben pond) for the city of Gaziantep in Turkey. Our approach expects to be replicated for the microbial loads analysis of other surface water sources potentially vulnerable to climate change in the Gaziantep region. So, we hope that our results contribute to the understanding of the probable impact of climate change on the microbial quality of surface water sources in Turkey.

MATERIAL AND METHODS

Sampling

The Alleben pond (37° 04′ 29″ North and 37° 16′ 20″ East) is located approximately 4 km southwest of Gaziantep and at an altitude of 939 m above sea level. It (37° 04′ 29″ North and 37° 16′ 20″ East) with a volume of 2.54 hm^3 is covered about 149 hectares of agricultural land. This pond, which extends from Southeast Taurus to Sof Mountains, is fed by spring, rain, and snow waters. It which was built to prevent flooding is utilized for agricultural irrigation and anthropogenic purposes today. The Alleben stream feeds from its falls into the Kayacık Dam in Oguzeli by flowing through the Gaziantep city center. The contamination of enteric pathogens based on household and agricultural wastewater pouring into the Alleben pond was previously reported (Ozturk 2013).

The water sample was collected from the Alleben pond in the city of Gaziantep, Turkey (Figure 1). The water sample was collected by using a sterile bacteriological sample bottle (250 mL) according to APHA (1998) and WHO (2006). Following collection, the sample was immediately brought to the laboratory in an icebox and stored at +4 °C in a light-sensitive container until microbiological quality analyses.

Microbial water quality analysis

Culture-based techniques

The enumeration of total aerobic bacteria in surface water, 100 μL of the sample was serially diluted (10^{-1}–10^{-5}) and 100 μL of each serial dilution was spread on plate count agar. And plates were incubated at 22 °C for 72 h and 37 °C for 48 h (TS EN ISO 6222). After incubation, the number of colonies on agar was calculated and noted as colony-forming units per mL (CFU/mL).

The MPN method illustrated by Prescott *et al.* (1996) was used to determine the counts of coliform indicator bacteria in the water sample. The sample was inoculated into nine tubes consisting of three tubes for each of the three dilution factors (0.1, 1, and 10 mL). The dilution tubes including Lauryl Sulfate Tryptose Broth (LST, Merck) were incubated at 37 °C for 24–48 h for total coliform. Following incubation, each tube containing an inverted Durham tube was visually investigated for bacterial growth and noted gas

Figure 1 | Map of the sampling area (Google Earth).

production (bubbles). After that, 100 μL from each positive LST broth tube was transferred to an EC (*E. coli*) medium broth tube including a Durham tube and incubated at 44.5 °C for 24–48 h for screening fecal coliform. The number of indicator bacteria was calculated by counting the tubes giving positive reactions and comparing them with standard statistical tables.

Quantitative polymerase chain reaction

Total bacterial DNA from the concentrated sample was extracted with the GF-1 Bacterial DNA Extraction Kit (Vivantis) following manufacturer protocols. The concentration and quality of DNA were detected by using a NanoDrop Multiskan G0 quantification spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), obtaining ranges from 30 to 40 μg/μL. Real-time PCR assays (qPCR) were performed to quantify the number of target genes on a LightCycler® Nano Real-Time PCR Instrument. The primers used for gene amplification are presented in Table 1. The calibration curves for qPCR quantification for 16S rRNA bacterial universal gene, *uidA E. coli* gene, and *lacZ* coliform gene were plotted using the different dilution series of standard *E. coli* ATCC 25922 DNA. Standard DNA was isolated by using the GF-1 Bacterial DNA Extraction Kit (Vivantis) process. Each qPCR process contained 5 μL SYBR Select Master Mix (GoTaq, qPCR Master Mix, 2×), 0.3 μL of 10 mM forward primers and reverse primers each, 2 μL of DNA template, and 2.4 μL of DNase-free deionized water. qPCRs used the following settings: holding at 95 °C for 10 min; 40 cycle amplification under conditions of 95 °C for 10 s with annealing/extension at 52 °C for 16S rRNA bacterial universal gene; 56 °C for *uidA* gene and 50 °C for *lacZ* gene for 15 s, 72 °C for 20 s, finally elongating at 72 °C for 7 min. The thermal profile for the melting curve began at 60 °C with a gradual increase in temperature (0.1 °C/s) to 95 °C. A threshold cycle (C_t) value was detected as the cycle number where fluorescence data crossed the threshold line for all the dilution series of standard DNA. The number of bacterial cells in the surface water sample was estimated from C_t values plotted to different standard DNA dilution standard curves.

Table 1 | Primers used for the detection of total bacteria, total coliform, and *E. coli* in the water sample

Name of gene	The sequence of forward and reverse primers (5′–3′)	Target gene	Product size (bp)	References
16S rRNA	F: 5′-CCTACGGGAGGCAGCAG-3′ R:5′-CTACCAGGGTATCTAATC C-3′	Universal 16S ribosomal RNA for all bacteria	500	Brosius *et al.* (1981)
lacZ3	F: 5′-TTGAAAATGGTCTGCTGCTG-3′ R: 5′-TATTGGCTTCATCCACCACA-3′	β-Galactosidase for coliforms	234	Molina *et al.* (2015)
uidA	F: 5′-TGGTAATTACCGACGAAAACGGC-3′ R: 5′-ACGCGTGGTTACAGTCTTGCG-3′	β-D-Glucuronidase for *E. coli*	162	Bej *et al.* (1991)

Antibiotic resistance profile

MacConkey agar differentiated them based on lactose fermentation used for Gram-negative bacteria from the water sample. Isolated colonies 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. The isolates were tested for susceptibility to the 15 standard antibiotics by the disk diffusion method (Bauer *et al.* 1966) on a Mueller-Hinton agar (Oxoid, UK) plate according to the guidelines and recommendations of the Clinical & Laboratory Standards Institute (CLSI).

The following 15 antibiotics that are important for the human healthcare system were Cefepime (FEP; 30 μg), Cefoperazone (CEP; 75 μg), Cefotaxime (CTX; 30 μg), Ceftazidime (CAZ; 30 μg), Chloramphenicol (C; 30 μg), Ciprofloxacin (CIP; 5 μg), Clindamycin (DA; 2 μg), Erythromycin (E; 15 μg), Gentamicin (G; 10 μg), Imipenem (IMP; 10 μg), Meropenem (MEM; 10 μg), Penicillin G (P; 10 μg), Streptomycin (S; 10 μg), Tetracycline (TE; 30 μg), and Trimethoprim (TR; 5 μg) (Bioanalyse). The susceptibility of strains was interpreted by the CLSI standard results (Table 2). *E. coli* ATCC 25922 was used for quality control. The multiple antibiotic resistance (MAR) indexes of strains were calculated based on the formula declared by Krumperman (1983). Resistance results were analyzed by using the software SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA)

Bacteria identification

DNA extracted from bacteria by using the Bacterial Genomic DNA Extraction Kit (Hibrigen) process was amplificated by the PCR process (initial denaturation at 94 °C for 5 min; denaturation at 94 °C for 35 s, primer annealing at 56 °C for 35 s, and extension at 72 °C for 45 s of 45 cycles; final extension at 72 °C for 5 min) using *uidA* gene-specific primers. The amplified products were verified by running on agarose gel (1%). The nucleotide sequence of the *uidA* gene by using both primers was analyzed at the BM-Labosis (Ankara, Turkey). The gene sequence of strain was compared automatically using the BLAST software (BLAST) against the sequences of bacteria available in databanks (http://www.ncbi.nlm.nih.gov/). The phylogenetic analysis was constructed using a neighbor-joining algorithm.

Air temperature observations

We predict ecosystem changes in the Alleben pond by using air temperature data. Air temperature data sets for Gaziantep were taken from the nearest meteorological station belonging to the Turkish State, Meteorological Service. Data (averages of the daily minimum temperatures) are available from 1990 to 2020 for January by year. As a measure of mean climate conditions, we associated daily minimum pond surface temperatures with air temperatures for January by year.

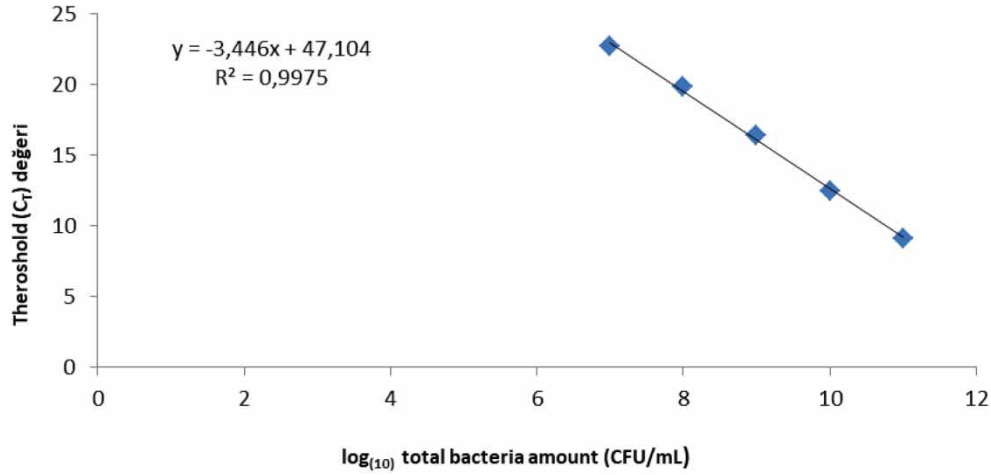
RESULTS

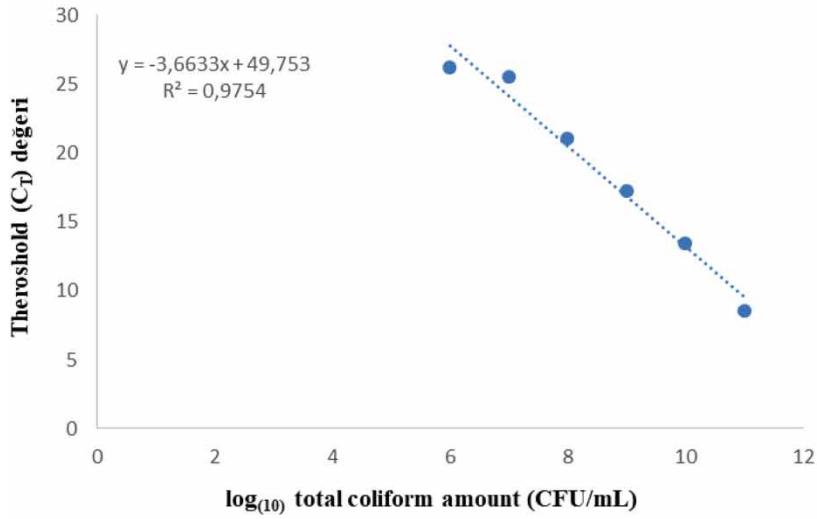
In the water sample, the concentrations of culturable and viable bacterial cells were estimated on the plate on count agar. As summarized in Table 2, the average ($n = 3$) plate counts in surface water were $164.5 \times 10^3 \pm 0.0013$ and $89.5 \times 10^4 \pm 0.002$ CFU/mL at 37 and 22 °C, respectively. The probable counts of total coliform (1,100 + MPN/100 mL ± 0.000) and viable indicator organism *E. coli* (16 MPN/100 mL ± 0.000) are presented in Table 2. These levels were observed to exceed the acceptable levels (0 MPN/100 mL) of surface water according to WHO (2017) and TS 266 (2005) guidelines.

Figure 2 shows that the mean bacterial concentration in the environmental DNA sample amplified by different primer sets was calculated based on the plotted standard curves. The accuracy of qPCR performance was defined by % efficiency and the R^2 value of standard curves. The high accuracy with an overall R^2 value of 0.9975 ± 0.005 and 95.1% efficiency for the total bacteria population was revealed. Also, similar results were recorded for total coliform (R^2 0.9754 ± 0.003 and 87.50%) and *E. coli* (R^2 0.9817 ± 0.005 and 94.10%) standard curves. Similarly, Willis *et al.* (2022) indicate that amplification efficiencies

Table 2 | Indicator organism measurements in the Alleben water sample using conventional and qPCR methods

	Conventional culture-based methods	qPCR
Total bacteria	$164.5 \times 10^3 \pm 0.0013$ at 37 °C/$89.5 \times 10^4 \pm 0.002$ CFU/mL at 22 °C	$125.76 \times 10^4 \pm 0.006$ CFU/mL
Total coliform	1,100 + MPN/100 mL ± 0.000	$302.21 \times 10^3 \pm 0.004$ CFU/mL
E. coli	16 MPN/100 mL ± 0.000	$40.189 \times 10^3 \pm 0.002$ CFU/mL





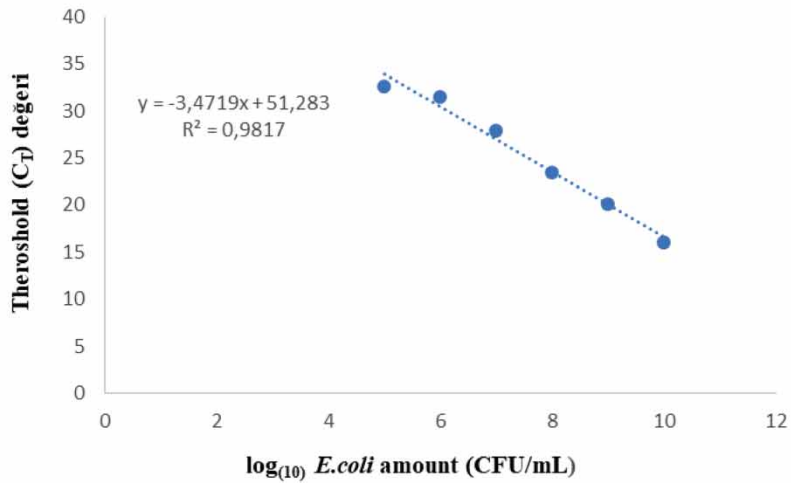


Figure 2 | The plotted standard curves from the real-time PCR using different primer sets.

for qPCR standard curves are ranging from 0.95 ± 0.006 to 0.99 ± 0.008 ($R^2 \geq 0.980$) in a study that monitored 13 recreational water qualities. Total bacterial concentration including both the culturable and non-culturable bacteria determined by the average 16S rRNA gene copies in water was $125.76 \times 10^4 \pm 0.006$ CFU/mL. The amounts of the total coliform and *E. coli* in surface water DNA revealed by the qPCR were $302.21 \times 10^3 \pm 0.004$ and $40.189 \times 10^3 \pm 0.002$ CFU/mL. qPCR values were correlated with culture-based microbial techniques (r: 0.98, $p < 0.01$).

Discrepancies in the counts of culturable-viable and based on gene copies within two methods could be due to the non-culturable bacteria revealed by the qPCR. The standard curve equations are represented in Figure 1, where y is C_t and x is log10 of CFU/mL.

Eight bacterial isolates recovered on MacConkey plates from a water sample were selected for the further antibiotic susceptible study. There was no clear zone of inhibition on MHA plates including Erythromycin, Clindamycin, Penicillin G, Tetracycline, and Trimethoprim standard discs across all bacteria. The strains showed susceptibility to the remaining five antibiotics (Cefepime, Chloramphenicol, Gentamicin, Imipenem, and Meropenem), as well as high-frequency resistance against five antibiotics (Cefoperazone, Cefotaxime, Ceftazidime, Ciprofloxacin, and Streptomycin), which are exhibited ranging from 25 to 62.5% (Table 3). The MAR index ranged from 0.33 to 0.67 for Gram-negative strains from the Gaziantep water source. The bacterial strain showing the highest MAR index was identified by using the BLAST software with 92% sequence similarity. Analysis of the *uidA* gene sequence confirms that the identified strain 1360940 A12 is the type strain of *E. coli* (Figure 3). The GenBank accession number allotted for the *uidA* β-D-glucuronidase gene sequence of the isolate is MZ959605.

Air temperature records showed that the daily average temperatures increased by 1.58 ±0.85 °C during the 30-annual period (Figure 4). The daily average of the highest temperature in January did not change, and the daily average of the lowest temperature increased by about 1.8 ±0.92 °C. Considering the significant changes observed in meteorological parameters in Turkey, the effects of climate change will be observed intensely in Turkey and its surroundings. Akcakaya *et al.* (2015) and Demircan *et al.* (2017) indicated the possible impacts of future climate change with the average air temperature increasing by 1.5–3.7 °C during the 2016–2098 period in Turkey.

DISCUSSION

The use of indicator bacteria has a good strategy to characterize microbial contamination (coliform and fecal coliform) of surface water sources (Truchado *et al.* 2016). Recently, the detection of bacterial pollution in environmental samples has

Table 3 | Percentage of total isolates with antibiotic resistance

	Pattern	Resistance (%)
Clindamycin	R	100 ± 0.0*
Erythromycin	R	100 ± 0.0*
Penicillin G	R	100 ± 0.0*
Tetracycline	R	100 ± 0.0*
Trimethoprim	R	100 ± 0.0*
Cefotaxime	R	62.5 ± 0.25*
Ceftazidime	R	50 ± 0.5*
Streptomycin	R	50 ± 0.45*
Ciprofloxacin	R	37.50.5*
Cefoperazone	R	25 ± 0.5*
Cefepime	S	0 ± 0.0*
Chloramphenicol	S	0 ± 0.0*
Gentamicin	S	0 ± 0.0*
Imipenem	S	0 ± 0.0*
Meropenem	S	0 ± 0.0*

*$P < 0.05$ compared with the results.

Figure 3 | Neighbor-joining tree of the selected *uidA* gene sequence of the genus *E. coli* obtained from the BLAST search of strain 1360940 A12.

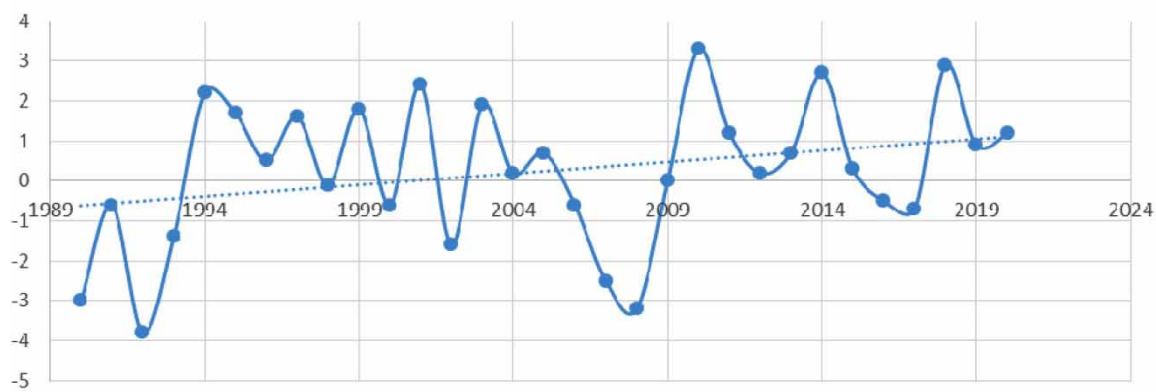


Figure 4 | The variation of the daily minimum average temperatures for January by years.

been both used by growth on a culture medium and by the amplification of the specific gene. However, environmental samples have higher levels of chemical and genetic complexity than those of tissue or pure cultures. Furthermore, other factors including the type of target organism, the number and diversity of bacteria in the sample, the DNA extraction protocol, and qPCR efficiency have limited molecular experiences (Girones *et al.* 2010; Silva & Domingues 2015). For this reason, the

amplification of qPCR equals obtained with the target DNA and quantification standards carefully optimized to reach maximum achievable specificity and sensitivity.

Literature research was defined to comparable results for total bacterial load, fecal and coliform indicator bacteria in the analyses of environmental surface water using culture, and qPCR methods (Noble *et al.* 2010; Krapf *et al.* 2016). Our approach combined with the culture-based and qPCR for the quantification of indicator bacteria from the surface water sample (Gaziantep, Turkey) was successfully performed. This study resulted in observable PCR products for total bacteria load, total coliform, and *E. coli*. In results similar to ours, higher cell loads than those of cultivation-based techniques probably due to the presence of dead or viable, but non-culturable (VBNC) cells were noted by the qPCR method (Ludwig & Schleifer 2000; Converse *et al.* 2012). Especially, the higher levels of *E. coli*, an indicator of fecal contamination, quantified by the qPCR assay were reported than those obtained using the MPN (Ahmed *et al.* 2012; van Frankenhuyze *et al.* 2013). In our study, qPCR *E. coli* counts of the Gaziantep surface water sample were higher (40.189×10^3 CFU/mL) than those acquired by traditional plate counts (16 MPN/100 mL). This finding accords with the result of Truchado *et al.* (2016) who determined that *E. coli* levels quantified by the qPCR assay were higher than those of standard agar plate colony counting methods. Similarly, Ferguson *et al.* (2012) indicated a better correlation between *E. coli* detected by the molecular approach and the presence of fecal indicators than by culture-based methods. In a study of Buckeye Lake beaches, the qPCR results of 14 samples exceeded the current *E. coli* Ohio single sample bathing-water standard (235 CFU/100 mL) (Francy *et al.* 2013). Chern *et al.* (2009) estimated 7.37×10^3 CFU *E. coli* density per 100 mL of marine water from two recreational beaches by the qPCR. Vadde *et al.* (2019) found elevated levels of fecal coliform (>250 CFU/100 mL) at 15 locations in the Tiaoxi River by microbial source tracking (MST)-qPCR assays. Truchado *et al.* (2016) compared two *E. coli* quantification techniques (plate count and qPCR) for irrigation water and fresh produce. *E. coli* levels using the qPCR assay were proved to be significantly higher than those estimated by the plate count in all environmental samples.

Among the most comparable studies, Wu *et al.* (2020) compared the conventional culturable and molecular approaches for fecal indicator quantification during activated sludge wastewater treatment in Indiana, USA. They showed that *E. coli* concentrations were 5.74 and 5.40 $\log_{10}$ CFU/100 mL in primary influent and effluent, and this rate for all samples in secondary and final effluent was below the detection limit (20 CFU/100 mL). But these ratios ranged between 7.23 and 7.14 $\log_{10}$ GC/100 mL in the qPCR measures that no sample was below the detection limit (1.70 $\log_{10}$ GC/100 mL). Our study is in accordance with Dorevitch *et al.* (2017), highlighting that *E. coli* culture data points were fewer ($n = 687$) than qPCR results ($n = 894$) in the nine beaches samples, Chicago. Although our coliform contamination rates were lower than those shown by Dinakaran *et al.* (2022), both studies indicated a similar approach, with a high-throughput molecular detection technique more quickly monitoring surface water samples.

In addition to molecular approaches, previous researches have been carried out to monitor the microbiological quality of water for human consumption using various statistical software packages (Eissa *et al.* 2022; Ismail *et al.* 2022; Nguyen *et al.* 2022).

The qPCR method enables the measuring of low levels of DNA targets in environmental samples. Krapf *et al.* (2016) revealed a lower limit detection of *E. coli* and *Enterococcus faecalis*/100 mL by qPCR analyses, depending upon the culture condition used in a drinking water sample. In another study, Shrestha & Dorevitch (2019) reported that *E. coli* DNA targets in only 1% of recreational Chicago area beach waters amplified. For another study, the levels of *E. coli* contributed to the risk of epidemiological disease in Poland's hot water systems were quantitated around 0.00 genomes/mL (less than 1 genome/mL) by qPCR curve equations (Wolf-Baca & Siedlecka 2019).

Another important aspect of this manuscript is the determination of antibiotic susceptible profiles in Gram-negative bacteria isolated from the Alleben water sample. Although the prevalence of antibiotic-resistant bacteria (ARB) in many freshwater sources in Turkey has been previously demonstrated (Matyar *et al.* 2009; Mercimek Takci *et al.* 2021). This is the first study that indicated the high frequency of resistance in the surface waters of Alleben, Gaziantep. Similarly, Matyar *et al.* (2014) high MAR indices ranged from 0.2 to 0.81, suggesting exposure to antibiotic contamination in Seyhan Dam Lake and River water samples reported. Icgen & Yılmaz (2014) pointed to the resistance of more than 50% of the Kızılırmak River isolates to different types of antibiotics. Similar results were obtained by Nakipoglu *et al.* (2017) who have shown the dissemination of high antibiotic resistance in river water samples. A high percentage of bacterial species isolated from the seawater and sediment samples. Gulluk Bay observed considerable resistance in another study (Altug *et al.* 2020).

Bacteriological and ARB findings in the current study are clearly emphasized to the discharge of household and industrial wastewater systems into the surface water without control. Furthermore, the main source of contamination is the high loading of domestic sewage and solid wastes from surrounding densely populated areas. These anthropogenic factors may affect the

metabolic activities in ecosystems and the biodiversity of aquatic life. The presence of fecal coliforms in aquatic environments observes the contamination of waters with the fecal material of man or other animals.

Climate change, especially temperature, is an important environmental factor that can directly influence physical, chemical, and biological processes (prokaryotic growth) in aquatic ecosystems (Tsai *et al.* 2021). Lakes are marine environments that influence rapidly through the temperature variation between lake surfaces and the surrounding environment. Air temperature causes long-term changes in the thermal structure of lake surface waters (Xie *et al.* 2022). Winter temperature is one of the substantial physical parameters to lake ecosystems that are responded to the changes in annual and daily mean air temperature of the region (Woolway *et al.* 2019).

Our climate change results showed that an average air warming rate of +1.58 °C between 1990 and 2020 may induce rapid warming of daily minimum Alleben pond surface temperature for the next 30 years. Such an increase is predicted to have significant influences on the Alleben pond ecosystem. Warming (1.58 °C) may have been directly linked to exceeding the standard limits in the communities of small-sized thermotolerant coliform bacteria (TCB) that tend to grow faster by affecting the length of the growing season. The high TCB levels in water bodies may be estimated with future temperature changes based on the climate. Due to these critical climate variability effects, appropriate watershed management regulations to improve and maintain water quality are required.

This study, similar to the previous studies (Jayakody *et al.* 2015; Girgibo *et al.* 2023), reported that the effects of temperature increases on the future with the TCB level increase in surface water are directly related. A previous study on bacterial pollution in coastal areas reported similar temperature distributions and an increase in bacterial load and fecal contamination density relating mostly to air warming (Kalkan & Altug 2020). Reitter *et al.* (2021) indicated that high numbers of coliform bacteria are correlated with an average increase of 3.1 °C in air temperature representing the water temperature increase in surface waters. Therefore, we and previous studies emphasize that the higher water temperatures may be occurred more frequently in the near future and challenging safety water production. In a similar climate change study related to increments in the temperature in Portugal, Fonseca *et al.* (2015) indicated that an increase of 1 °C in air daily temperature results in an increase of surface water temperature of 1.1 °C and this leads to an increase of ~2% in bacteria inflow to the water source. As different than our bacterial contamination estimation, Dakhlalla & Parajuli (2019) observed that even small increases in daily temperatures directly cause a decrease in average TCB concentration.

CONCLUSION

This present study evaluated the microbial quality of surface water in the Alleben pond, Gaziantep for human consumption and other associated activities by comparing to the culture-dependent microbiological and culture-independent molecular-based approaches. Our results showed that the qPCR is a suitable and fast approach for the detection and enumeration of bacterial loads in surface water samples. This preliminary research is a good example that emphasized the need to use molecular techniques as additional methods of standard water analyses to achieve more accurate results in a short detection time. But the difference in limits of qPCR quantification for the detection of coliform and fecal coliform loads in various water types may be an important risk. Especially, factors such as the origin of fecal inputs, physicochemical attributes, and microbial community may be directly influenced the decay rates of fecal indicator bacteria in surface water. Therefore, information about the decay rates of enteric microorganisms in different water bodies is crucial for quantitative molecular analysis such as qPCR. Also, our present study in a winter climate reveals that warming of Alleben aquatic ecosystems by 1 °C may shift to microbial diversity consisting of more dominated coliform and fecal coliform bacteria. This is associated with the rapid increase in the concentration of coliforms in surface water sources in the Gaziantep region during the next 30 years. However, further research should be considered on practical solutions to overcome this pollution and control water pollution.

FUNDING

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

AUTHOR CONTRIBUTIONS

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by H.A.M.T., C.K., F.E.S.O., and K.C.G. The first draft of the manuscript was written by H.A.M.T. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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First received 7 March 2023; accepted in revised form 19 May 2023. Available online 31 May 2023