

Research Article

Isolation and identification of pathogenic bacteria from drinking tap water and Tigris River water sources in Baghdad

Susan Abdul Raheem Hasan* 

Biology Department, College of Education for Pure Science (Ibn Al-Haitham)/ University of Baghdad, Baghdad, Iraq

Bushra Kadhim Shakir

Quality Control Department, Baghdad Water Authority, Baghdad, Iraq

*Corresponding author. E-mail: sazan.a.h@ihcoedu.uobaghdad.edu.iq

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Abstract

Water is a resource and a crucial aspect of living and surviving. In Iraq, the Tigris River is one of the most critical water sources. The present study aimed to provide an insight analysis of some water quality parameters including the microbial content of drinkable tap water and river water. Ten Water samples (T1- T10) in triplicate were collected from sampling sites -Site I (Tap water) from home water taps, supplied by the Water Filtration Station/ Al Karama Project/ Al-Karkh> 10 from Site II (R1- R10) River water from Tigris River (around or near the Water Filtration Station/ Al Karama Project) every week (from September to half of November 2022), then were immediately placed in sterile bottles and transported to Microbiology lab for the analysis. Site I, turbidity was (0.7- 6) NTU. Free Chlorine availability was 0.5- 3.5 mg/L>CFU/ mL ranged from 0 to 40 in Total Plate Count (TPC);membrane filtration method (MFM) was unsatisfactory (T1, T8,T9), other samples were satisfactory. Site II, coliform result was 5200- 9200 CFU/ 100mL;Lauryl Tryptose broth (11000–49000) MPN/100mL;Brilliant Green Bile broth (6900–17000 MPN/100mL); EC-MUG (*E. coli* medium with 4-methylumbelliferyl-β-D-glucuronide) was4900- 22000 MPN/100mL and EC-Broth was 4900- 22000 MPN/100mL. m-Endo Agar LES was +ve for all samples. All Tigris River water samples , were contaminated with coliform bacteria: *E. coli*, *C. freundii*, and *Sphingomonas paucimobilis* (sample R2) as non-coliform. Site I were drinkable and reliable, corresponding to Iraqi and WHO typical parameters, while Site II was under standardization.

Keywords: Coliform, Non-coliform, Tap water analysis, Tigris River, Water contamination

INTRODUCTION

Effective strategies and policies are needed to manage and implement solutions for the significant water pollution problem. It is imperative to oversee the implementation of these strategies and policies to achieve the desired results (United Nations, 2023). Effective management of water supplies is vital in sustainable development, particularly in increasing population growth, industrialization, and agricultural activities. In nature, most of these pollutants are organic and commonly occur in very small intensities starting from parts per trillion to parts per billion (Rodriguez-Narvaez *et al.*, 2017). Emerging contaminants indicate synthetic or natural chemicals and microbes not typically checked in the circumstances of ecologies. Despite this lack of monitoring, they possess the potential to be released

into an environment that results in recognized or presumed harmful impacts on environmental and anthropological health.

Researchers have recently studied surface, subsurface, drinking water, and wastewater micropollutants (Kumar *et al.*, 2022). The presence and extent of fecal pollution are crucial in evaluating water quality and the risk of human infection (World Health Organization, 2021). The Gram-negative, rod-shaped bacterium known as a coliform can ferment lactose into gas and acid with relative ease within 24 or 48 hours at $35 \pm 2^\circ$ C. Typically, these bacteria are classified into four distinct genera within the Enterobacteriaceae family, namely *Escherichia coli*, *Citrobacter freundii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae* (Halkman and Halkman, 2014; Li *et al.*, 2021).

Coliform bacteria are usually applied as indicators of microorganisms' water contamination, which steer society's well-being safety observation (Some *et al.* 2021). Water samples are examined for *E. coli*, a type of bacteria that usually lives in human bowels and warm-blooded mammals. This bacterium is an essential indicator of fecal coliform contamination, as it is the most probable cause of Urinary Tract Infections (UTI) (Hasan *et al.*, 2021). Interpreting the examination for coliform bacteria can be problematic since some bacteria are recovered from soil and surface fresh water that might not be of intestinal origin. As a result, detecting coliform bacteria can be challenging, although not proof of fecal contamination, and may indicate a failure in treatment or water ingress into the distribution system (Bai *et al.*, 2022).

There are over 45,000 infant fatalities annually due to polluted water and inadequate sanitation, and approximately eighty percent of diseases and nearly third of deaths in developing world are triggered by water consumption that is contaminated. WHO states around six hundred million instances of dysentery and diarrhea (Singh *et al.*, 2021). Human waste or sewage in surface water might pose a threat since it carries pathogenic bacteria (Denchak, 2023). It is quite risky to drink, swim, and bathe in water; water quality monitoring aims to determine whether a water source is suitable for human consumption. In addition, aquatic plants and animals are understandably alarmed when sewage is present since it upsets the oxygen balance (Sharma and Sharma, 2012). Water quality assessment has been performed to determine if a water source is suitable for a man's living purposes and use. Guidelines, criteria, or the highest permissible level of water quality characteristics are used to determine whether water is suitable or polluted with microbes (WHO, 2021). In water, fecal coliform bacteria are observed, which signifies that the water may be contaminated by fecal matter from warm-blooded animals. To create parameter-based methods for evaluating water quality, as well as for fundamental and applied studies in aquatic microbial ecology, bacterial count in feces is essential; the existence of *Klebsiella*, *Clostridium perfringens*, and fecal streptococci shows the pollution of water. *Proteus* spp., *Shigella* spp., *Salmonella enteritidis*, *Salmonella typhimurium*, and *Salmonella typhi* are the most common types of bacteria in wastewater (Some *et al.*, 2021).

Water characteristics and quality assessments for several distinct water samples have been investigated concurrently and reported. The objective of this study was to perform a thorough investigation of the problem of bacterial contamination in water, explicitly identifying the causative bacteria to determine the most prevalent genera and examining possible reasons for their occurrence in both the Tap water Site I and Tigris River water (Site II) in Baghdad city for two consecutive months and

additionally to delve into the antibiotic sensitivity of these bacteria.

MATERIALS AND METHODS

Samples collection

Two distinct water sources were used to collect the samples: Site I (T1- T10) in Baghdad's city tap water system in the Al-Karkh district (Water Filtration Station/ Al-Karama Project) (33.356938, 44.356539) and Site II (R1- R10)- Tigris River (near Al Karama Project) (33.359582, 44.355351) (Fig. 1). From each site, ten samples (in triplicate) were collected separately to be analyzed within different periods (one sample a week) from September to half of November 2022). 100 mL of each sample were collected weekly, ; three replicate samples (from T1- T10, at site I and from R1- R10 at site II, separately) from each site were assumed to be drinking and Tigris River waters that were taken from the same place for different periods randomly and the mean value were considered. Once collected, the samples were immediately placed in designated sterile bottles with caps and transported to the microbiology lab. To ensure the results were as accurate as possible, samples were initiated for microbiological inspection within two hours of arrival to prevent contamination or unexpected changes. This allowed us to move on to the next step seamlessly; sterile distilled water samples were utilized as the negative control (Some *et al.*, 2021).

Water samples analysis

The techniques employed to detect and quantify organisms that served as indicators and various bacterial communities included Membrane filtering (MF), Total plate count agar (TPC), and Multiple-tube fermentation (MTF). The MTF approach is widely regarded as the predominant method for assessing the coliform index in water samples (Sites I and II) due to its accuracy, reliability, and ease of use; a sequence of tests involves presumptive and confirmatory, and the third is the completed test (Cayabo *et al.*, 2021). The free chlorine concentrations were measured using a portable free chlorine meter (LPCL-A10 from Labtron, UK), while turbidity was assessed with a portable turbidity meter (Tub 430T from WTW/Xylem). These methods were employed following the manufacturers' guidelines as supplementary measures to ensure water quality (LeChevallier *et al.*, 1981)

Drinking Water Samples (Site I)

The microbiological contamination of the drinking water (Site I, T1-T10) was analyzed using MF and TPC measures. These methods enabled the recognition and quantity of indicator microbes, such as bacteria found in tap water. MPN indicator was applied to assess the



Fig. 1. Study area and sampling locations: - SI: Baghdad's city tap water system in the Al-Karkh district (Water Filtration Station/ Al-Karama Project) (33.356938, 44.356539), and SII: Tigris River (near Al-Karama Project) (33.359582, 44.355351).

degree of pollution, described as MPN/ 100 mL (Balogun *et al.*, 2017). The initial test conducted was TPC for enumerating the total count of microbial populations in samples (Site I, T1- T10); besides finding coliforms by MF method, m-endo Agar LES media was used (Arifan *et al.* 2019; Sarni *et al.*, 2023). The growth of bacterial colonies should be limited to a certain range 30 to 300. For media preparation, all items were sterilized, agreeing to the guidelines of the manufacturer, in an autoclave ($121^{\circ}\text{C} \setminus 15 \text{ min}$) (Dhafin *et al.*, 2023). For microbial analysis, 1 mL of the sample was poured into a petri dish and incubated at $35.5 \pm 0.5^{\circ}\text{C}$ for 48 hrs. The number of colonies that appeared in white to yellowish color was calculated in CFU/mL and recorded for colony growth analysis (Lisafitri *et al.*, 2024).

To detect coliform, utilized the MF or membrane filtration method. A 100 mL water sample was collected in a sterilized 250 mL container with sodium thiosulfate to eliminate residual chlorine effects. Next, the sample was filtered through a sterile filtration unit with 0.45mm pores and a vacuum pump. The membranes were detached with sterilized forceps in aseptic conditions and positioned inverted to m-Endo Agar Les, eliminating any might-presented air bubbles. Petri dishes were placed in the incubating machine ($35 \pm 0.5^{\circ}\text{C}$ for 24 hrs.) to calculate indicator bacterial populations (Al-Dulaimi and Younes, 2017).

Tigris River water (Site II) samples

For all samples (R1- R10, site II), the Total Plate Count method was used first and the MPN method was used to detect total and fecal coliform by inoculating serials fermentation tubes via five tubes for every dilution. To detect all lactose fermenter bacteria, presumptive test results indicated the presence of coliform bacteria in tubes that produce gas, and Lauryl Tryptose Broth

(LTB) was used to quantify total and fecal coliform bacteria (Rice *et al.*, 2012). Water samples were added to fermentation tubes holding 10 ml of sterile LTB, and then the tubes are incubated at $35 \pm 0.5^{\circ}\text{C}$ for 48 hours. Presumptive test was used to find tubes that generated gas in Durham's tubes, while the negative tubes were re-incubated for 48 hours (Humudat *et al.*, 2020; Abu-Sini *et al.*, 2023). Bacterial cultures from a positive tube were inoculated on Brilliant Green Broth (BGB) and then incubated ($35 \pm 0.5^{\circ}\text{C} \setminus 24 \text{ hrs.}$) as the confirmatory test; in the presence of lactose broth, colonies generate gas. For record MPN confirmatory test for bacterial colonies that grew in the positive tube were sub-cultured on m-Endo Agar Les at the same incubation conditions (Some *et al.*, 2021).

Coliform Identification

In addition to all previous media that confirmed the existence of coliform bacteria, additional steps were used as CLED (cystine–lactose–electrolyte-deficient) and Chromogenic agar media by subculture one of the single pure colony to these media, and the incubation period was 24 hrs. at 37°C . The VITEK2 system, GN card (BioMérieux), was the identification and conformational step, according to the manufacturer's directions (Hasan *et al.*, 2021).

Antibiotic resistance in water coliforms

According to the manufacturing company (Biome Rieux, United States of America), a pure single colony was selected from each sample for an antimicrobial sensitivity test using the VITEK2 system to detect coliform antibiotic resistance (Hasan *et al.*, 2021).

RESULTS AND DISCUSSION

The analysis of 10 tap water (Site I) samples (T1 to T10) from the drinking water system in Baghdad city

(Water filtration station/ Al Karama project) revealed some parameters and coliform and non-coliform detection. The number of CFU/ mL ranged from 0 to 40; as a result, for the TPC method, sample number T6 had zero colonies of bacteria and that may be related to its low turbidity value (1.5 NTU) and high amount of Cl_2 (3.5 mg/L). This indicated that it was the best positive result for the absence of such contamination in the drinking water. Similarly, all other nine samples had a CFU/mL below 50 (Table 1), suggesting they were safe and drinkable water sources (Hammadi *et al.*, 2021).

Coliform bacteria, classified as Gram-negative rods, possess the unique ability to thrive in both aerobic and anaerobic environments. These organisms are known for their robust lactose fermentation process, which produces high levels of acid and gas; this reaction occurs at a temperature of $35\pm 2^\circ\text{C}$ and typically takes 24-48 hours to complete (Maalah and ALazzawi, 2023). Total Coliforms are a form of bacteria frequently present in human feces and the natural environment and are regarded as a general indicator of water pollution. They have been utilized for many years to gauge drinking water quality due to their ease of detection and enumeration (Tominaga and Ishii, 2020).

Membrane filtration method (MFM), the growth of colonies was recorded as present (existence of coliform) or absent (non-coliform). The data showed that the presence was a -ve result; at the same time, the absence was a +ve result (Table 1). The evaluation is widely used to determine water quality concerning the wellness of ecosystems, the protection of human exposure, and potable water (Al Fatlawy *et al.*, 2023). The water samples were tested for coliform bacteria; samples T1 (22 CFU/mL), T8 (40 CFU/mL), and T9 (11 CFU/mL) were unsatisfactory, while the other seven were satisfactory and drinkable, indicating that the test failed the water quality validation, suggesting they were not free from coliform and fecal contaminants, compatible to standards for Iraq and WHO (0 CFU/ 100 mL) (Iraqi Criteria, 2015; *World Health Organization*, 2012). MFM uses m-Endo Agar LES to enumerate coliforms in drinking water whenever a metallic sheen develops when lactose is fermented by coliforms, resulting in the production of aldehydes, while Lactose-nonfermenting bacteria create colorless, transparent colonies (Rompré *et al.*, 2002). Discrete colonies thus formed can be easily transferred to confirmation media. The result of identification on agar media (CLED, chromogenic, besides m-Endo Agar LES) indicated the presence of some coliform genera, followed by the conformation for identification by the Vitek2 system. CLED agar is a valuable growth medium that aids in isolating and differentiating urinary and fecal pathogens and contaminants. Cystine plays a crucial role in promoting the formation of cystine-dependent dwarf colonies. Also, bacteria can be differentiated according to their fermentation capabilities

when lactose exposure. During the fermentation of lactose, yellow colonies are produced, owing to acid production. Furthermore, microorganisms with decarboxylate L-cystine cause an alkaline reaction and form deep blue colonies, allowing specific bacterial characteristics identification through changes in the medium's color (Mackey and Sandys, 1966; Karah *et al.*, 2020). Enterobacteria Chromogenic Agar is a specialized medium that distinguishes *E. coli* from other Enterobacteria. This innovative plate can also quantify the presence of both *E. coli* and enterobacteria. *E. coli* colonies exhibit a distinct dark blue-green hue, making them readily identifiable. Meanwhile, the growth of other bacteria is suppressed, and if they grow, they will appear as colorless colonies (Fallon *et al.*, 2002; Cho *et al.*, 2020).

Vitek2 system result indicated that the bacterial isolates were *E. coli* and *Citrobacter freundii* in samples T1 and T8, but only T9 were *Citrobacter freundii*. Coliform bacteria commonly be part of some genera in Enterobacteriaceae: *Citrobacter freundii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *E. coli*, and *Klebsiella pneumoniae* (Tominaga and Ishii, 2020).

The result of turbidity for samples (Site I) was (0.7- 6) NTU; the highest rate was for sample T8, which could be attributed to its highest TPC (40). While in sample T10, the turbidity was 5 NTU, and that could be related to other water contents and fungi, dust and dirt, and other nonliving contaminants; the free chlorine availability should be above 0.5 to 5 mg/L, so the negative samples (T1, T8 and T9) revealed the lowest Cl_2 level as 0.5 mg/L, according to Iraqi and WHO standards (0.5- 5 mg/L) (Iraqi Criteria, 2009; *World Health Organization*, 2018). As reported by Al Fatlawy *et al.* (2023) the turbidity was 8.7 NTU, and they mentioned that this value was higher than the permissible level recommended (1- 5 NTU); their result coordinated with two samples in the present study for turbidity values.

Tigris River water samples (Site II)

Tigris River water (Site II), for all samples (R1- R10), results for TPC ranged 5200- 9200/ CFU/ 100mL for all dilutions, the considerable range was from 30 to 300; thus, the first dilution, 0.1, was eliminated because it was over the range, third and fourth below the range (less than 30 per 1mL; while the 0.01 dilution was chosen because it was within the permissible level (30- 300 CFU/ 100mL) (Iraqi Criteria, 2015). Fecal coliform bacteria were ≥ 59000 CFU using TPC in Al-Gharraf River, as mentioned by Al-Mayah and Rabee (2018), and their result was within the range of this study. A bacteriological and coliform examination for total coliform count was done using the MPN method for all ten Tigris River samples (R1- R10) within different time periods. The highest average coliform colony counts by MPN method were observed in Tigris River water that ranged from

11000–49000 MPN/100mL, in LTB broth as a primitive test, while in the second test (confirmative BGB test for coliform), they were 6900–17000 MPN/ 100mL; EC-MUG as another confirmative test for *E. coli* existence (4900- 22000 MPN/100mL), and EC-Broth for fecal coliform confirmation of their presence (4900- 22000) MPN/100mL. Finally, the complementary test (m-Endo Agar LES) was the last coliform contamination examination step as a +ve or -ve growth indicator (Table 2). It was found that all 10 samples (Site II, R1- R10) were contaminated with coliform bacteria, especially *E. coli*. The presence of indicator organism isolates for bacterial contamination was confirmed by the growth ability on m-Endo Agar LES, followed by a subculture for pure single colonies on CLED and Chromogenic agar media. Further complete identification of coliform isolates was made by using the VITEK2 system. According to its analysis data, the result was *E coli* and *Citrobacter freundii* for all samples (R1, R3- R10) except sample R2, which revealed the presence of the genus *Sphingomonas paucimobilis* as non-coliform bacteria which can ferment lactose and propagate on tested and selected specific coliform media (De Vries *et al.*, 2017). *Escherichia coli* is a G-ve bacteria that predominantly resides in digestive tract of humans. However, it is imperative to recognize that not all strains of *E. coli* are innocuous, as specific variants can cause a wide range of illnesses. The pathogenic strains include enterotoxigenic, enteropathogenic, enterohemorrhagic, and enteroinvasive *E. coli*. Notably, enterotoxigenic *E. coli* can be found in the fecal matter of both humans and cattle (Hasan *et al.*, 2021). Discharging feces into water sources can result in contamination, which may transmit pathogenic bacteria to humans via tap water. If not treated appropriately, such contamination may lead to illness. Therefore, it is essential to take the necessary

steps to ensure the quality of water sources and to implement proper treatment methods to prevent the spread of harmful bacteria (Kristanti *et al.*, 2022)

Citrobacter species are bacteria commonly found in various environments, including intestinal tracts of humans and animals, alongside the soil, sludge, sewage, and water. They are non-spore-forming, facultatively anaerobic, rod-shaped, and Gram-negative. In a study by Jabeen *et al.*, in 2023, it was reported that these bacteria have also been identified in vegetables (Jabeen *et al.*, 2023; Islam *et al.*, 2024). Medical disorders that affect newborns, infections of the urinary tract, and serious underlying diseases such as cancer, diabetes, hypertension, respiratory diseases, besides nosocomial infections, are often associated with the detection of some of *Citrobacter* spp., particularly *C. freundii*, *C. braakii*, and *C. koseri* (Mollasalehi and Esmailli, 2023; Jabeen *et al.*, 2023). *C. freundii*, a common freshwater fish pathogen, restricts aquaculture development. As an Enterobacteriaceae genus, it causes health issues in humans, animals, and fish, making co-occurrence challenging (Pan *et al.*, 2021).

Sphingomonas paucimobilis is an infrequent source of bacteremia that has the potential to impact vigorous and immunocompromised persons. Public-attained diseases caused by *Sphingomonas* are greatest repeated than nosocomials. It is noteworthy to emphasize that *S. paucimobilis* is an unusual pathogen, and its identification could be challenging in clinical settings (Sagar, 2024).

Sphingomonas paucimobilis, formerly acknowledged as *Pseudomonas paucimobilis*: rod-shaped Gram-ve bacterium that has been recognized as a potential source of infections in community and hospital environments. The microbe is thought to exist naturally in soil and water, including the water supply within medical facilities

Table 1. Tap water samples (Site I, T1- T10) showing TPC test, MFM method, bacterial growth genus, turbidity, and free chlorine as environmental parameters (Mean values of triplicate of each sample).

No. of sample	TPC/ 1mL/48hr	MFM Coliform / 100mL/24hr	Bacterial growth	Turb NTU	Cl ₂ mg/L
T1	22	P	<i>E. coli</i> & <i>Citrobacter freundii</i>	3.1	0.5
T2	4	A	-	2.3	1.0
T3	2	A	-	3.3	1,0
T4	12	A	-	2.8	2.0
T5	15	A	-	3.4	1.0
T6	Zero	A	-	1.5	3.5
T7	15	A	-	0.7	1.5
T8	40	P	<i>E. coli</i> & <i>Citrobacter freundii</i>	6.0	0.5
T9	11	P	<i>Citrobacter freundii</i>	3.0	0.5
T10	8	A	-	5.0	2,5

T: tap water sample; TPC: total plate count; MFM: membrane filtration method; Turb: turbidity; Cl₂: free chlorine; *E.*: *Escherichia*; Standard values: TPC (0- 50 CFU/mL), MFM (0 CFU/ 100 mL) (Iraqi Criteria, 2015; *World Health Organization*, 2012); Turb (1 – 5 NTU); Cl₂ (0.2- 0.5 mg/L (Iraqi Criteria, 2009; *World Health Organization*, 2018)).

Table 2. TPC, MPN results for Tigris River water (Site II, R1- R10) for dilutions (0.1, 0.01, 0.001, 0.0001) per 100 mL in all tested samples with the last complementary m-Endo Agar LES and the turbidity parameter (Mean values of triplicate of each sample).

No. of sample	TPC CFU/ 100mL	LTB		BGB		EC-MUG		EC-Broth		turbidity NTU	m-Endo Agar
		combination of positive	MPN/ 100 mL	combination of positive	MPN/ 100 mL	combination of positive	MPN/ 100 mL	combination of positive	MPN/ 100 mL		
R1	310,6500*,13,3	5-4-0-0	13000	5-4-0-0	13000	5-3-0-0	7800	5-4-0-0	13000	30	+
R2	312,6600*,17,5	5-4-1-0	17000	5-4-1-0	17000	5-4-0-0	13000	5-4-0-0	13000	30	+
R3	309,5200*,11,5	5-3-1-0	11000	5-2-1-0	6900	5-2-0-0	4900	5-2-0-0	4900	20	+
R4	315,6500*,12,8	5-4-1-0	17000	5-4-1-0	17000	5-3-1-0	11000	5-3-1-0	11000	25	+
R5	310,5600*,8,2	5-5-2-0	49000	5-4-1-0	17000	5-3-1-0	11000	5-3-1-0	11000	20	+
R6	310,5700*,16,7	5-3-2-0	14000	5-3-2-0	14000	5-2-1-0	6900	5-3-1-0	11000	35	+
R7	305,5400*,8,3	5-4-1-0	17000	5-4-1-0	17000	5-3-0-0	7800	5-3-0-0	7800	35	+
R8	308,9200*,21,6	5-5-2-0	49000	5-4-1-0	17000	5-3-1-0	11000	5-3-1-0	11000	35	+
R9	312,7500*,18,4	5-5-1-0	33000	5-5-1-0	33000	5-4-0-0	13000	5-4-0-0	13000	32	+
R10	302,6200*,8,2	5-4-3-0	27000	5-4-2-0	22000	5-4-2-0	22000	5-4-2-0	22000	25	+

* TPC CFU/ 100mL for all dilutions,; TPC: Total Plate Count; LTB: Luryl Tryptose Broth; BGB: Brilliant Green Bile Broth; EC-MUG: *E. coli* medium with 4-methylumbelliferyl-β-D-glucuronide; EC-Broth: *E. coli* broth; m-Endo Agar LES: modified Endo Agar Lawrence Experimental Station; MPN: Most Probable Number. The standard values: TPC; LTB; BGB; EC-MUG; EC-Broth; m-Endo Agar LES (30- 300 CFU/ 100mL) (Iraqi Criteria, 2015).

(Rinawati and Kumalawati, 2022).

Sphingomonas paucimobilis has been found in a fouled reverse osmosis membrane used to produce drinking water of superior quality. *Sphingomonadaceae*, also known as sphingomonads, are a part of the Proteobacteria group and are known for their adaptability in terms of physiology and metabolism. Among the Sphingomonadaceae, *Sphingomonas* is a widely spread genus that is one of the primary colonizers of membranes and is still dominant in the developing membrane biofilm (De Vries *et al.*, 2017). The turbidity was (20-35) NTU; this turbidity may be attributed to some heavy metals and dust particles in river water (Nsief *et al.*, 2012).

The increase in multidrug resistance bacteria can be attributed to the extensive and inappropriate use of antimicrobial agents. *E. coli*, *Citrobacter* spp., and *Sphingomonas* spp. (Hasan *et al.*, 2021; Islam *et al.*, 2024). Resistance to antibiotics in *Citrobacter freundii* causes a risk to community healthiness (Monte *et al.*, 2024).

All the isolates' genera, *E. coli*, and *C. freundii*, were analyzed for antibiotic susceptibility. Findings indicated two *E. coli* isolates (tap water, Site I) were sensitive to all antibiotics (ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ceftazidime, Cefepime, aztreonam, imipenem, meropenem, Amikacin, gentamycin, tobramycin, ciprofloxacin pefloxacin, minocycline, colistin, rifampicin, and trimethoprim/ sulfamethoxazole). The susceptibility of *C. freundii* isolates to the same antibiotics was also sensitive to all of them. The Other researchers' results revealed that *E. coli* was sensitive to ciprofloxacin, gentamycin, with levofloxacin. In contrast, ceftazidime, azithromycin besides amoxicillin/clavulanic acid, the remaining isolates were resistant to them (Abu-Sini *et al.*, 2023), and this result wasn't much in this study.

If treated drinking water tests positive for coliform bacteria, treatment failure could result. Water used for medicine compounding that contains *Escherichia coli* that poses antibiotic-resistant, a serious threat to the health of everyone who consumes it, especially those with impaired immune systems (Abu-Sini *et al.*, 2023).

For Tigris River water (Site II), all isolated *E. coli* bacteria were sensitive to all antibiotics excluding one isolate (resist ticarcillin), minocycline, and trimethoprim/ sulfamethoxazole. AST results for *C. freundii* also showed sensitivity for all antibiotics except one isolate resist minocycline. Antibiotics are still used to prevent and cure aquatic infections caused by *C. freundii*, notwithstanding their rise in prevalence. The prolonged use of antibiotics can lead to undesirable effects such as drug resistance and residue, and *C. freundii* has been found to have several resistance genes (Zhou *et al.*, 2019). It is critical to find a way to protect aquatic products from *C. freundii* infections, and one way to do that is to cre-

ate a vaccine or other treatment that is both effective and safe for the environment. Vaccines have emerged as a powerful tool for illness prevention in farmed animals, including fish (Pan *et al.*, 2021). *Sphingomonas paucimobilis* shows resistance to aztreonam only. *S. paucimobilis* clinical isolates' resistance to amikacin, ciprofloxacin, and imipenem was 0%, which agrees with the current study but showed resistance to cefepime, ceftazidime (5%, 10%) that our isolate was sensitive to them (Cheong, 2017). Analysis of microorganisms in the Tigris River water shows that Water becomes unfit for human drinking and use due to faecal contaminations.

Conclusion

The present study analyzed the existence of total bacteria, coliform bacteria, and other bacterial content in both tap water (Site I) and the Tigris River (Site II). Most sensitive bacteria isolated from the water samples were found to be environmental isolates rather than clinical in origin. While all tap water samples (Site I, T1-T10) analyzed were found to be within acceptable quality ranges and safe to drink, a few samples were considered unsatisfactory. In contrast, Tigris River water (Site II, R1- R10) did not meet the standard quality requirements for drinking water. Some coliform was detected in the tap water samples, showing the need for more accurate and efficient water treatment. It is recommended that regular water quality inspections be performed to reduce the possibility of infection by microorganisms and other parameters, especially *E. coli* and other index microorganisms.

Conflict of interest

The authors declare that they have no conflict of interest.

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