



Study of Antimicrobial Resistance Pattern of *Escherichia coli* and *Klebsiella* Strains and Multivariate Analysis for Water Quality Assessment of Tigris River, Baghdad, Iraq

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ABSTRACT

The present study aims to assess the pollutant impact from everyday untreated or partially treated industrial wastes, wastewater treatment plants and Baghdad Medical City wastewater discharge into Tigris River, Baghdad, Iraq. Water samples were collected from seven locations of the Tigris River near Baghdad Medical City in November 2018. Morphological characteristics and biochemical methods were used to characterize *Escherichia coli* and *Klebsiella* isolates revealing that the Tigris River accumulate different amounts of antibiotic-resistant *E. coli* and *Klebsiella sp.* isolates and that pattern of resistance is different in each site. *E. coli* was the predominant bacterial contaminant at site 1 which is near sewage of several hospitals in Baghdad Medical City. The influence of different water quality parameters (total dissolved solids, electric conductivity, alkalinity, turbidity, total hardness, calcium, magnesium, sodium, potassium, phosphate ion, nitrate, sulphate, chloride) and heavy metals (Cd, Zn, Co, Cu, and Ni) were investigated at bacterial contaminated site 1. Besides, physiological parameters (pH value and temperature) were applied. The results revealed that these parameters were within Iraqi standards levels with a slight increase in pH and temperature at site 1.

INTRODUCTION

Historically, the Tigris and the Euphrates are the two most significant rivers in Iraq. The Tigris River gains great importance as it is considered the main source of water for Baghdad city, which enters the capital city of Baghdad from the north and exit from the south dividing Baghdad city into the right (Karkh) and left (Rusafa) sections (Alobaidy et al. 2010). For several years the Iraqi rivers were facing problems such as the huge number of dams built in the upstream by Turkey and Iran (Al-Ansari 2013), the floods caused by rains and the discharge of wastewater from the residential and commercial establishments along Tigris River. A little knowledge is currently available on to what extent the biological and chemical pollution affects the Tigris River ecosystems in Baghdad city. Due to the anthropogenic activities, the ecosystems for plants and living organisms are threatened by toxic pollutants, which are passed directly from factories and hospitals into the river without any real treatments (Gadzała-Kopciuch 2004).

One of the major pollutants is antibiotics and antibiotic-resistant bacteria. The heavy use and abuse of antibiotics have resulted in multi-resistant bacteria (Icgen & Yilmaz

2014, Lv et al. 2015, Xu et al. 2017). Although a complete picture of the ecology of the antibiotic-resistant bacteria is still missing, the growing use of antibiotics for medical treatments and in animal production as growth promoters has increased a selective pressure in bacterial populations and the development of antibiotic-resistant bacteria (Titilawo et al. 2015, Zarfel et al. 2013, Zhang et al. 2015). There is a continuous release of antibiotics into the environment from different human activities such as wastewater treatment plants and hospitals effluents, combined sewer overflows, processing plant effluents and agricultural waste (Davies & Davies 2010, Michael et al. 2013). The presence of antibiotics in the Tigris River draws the attention of the researchers due to the risk of spreading antibiotic resistance determinants through microbial communities. The wastewater carries antibiotics and their metabolites, which is excreted with urine and faeces (Harnisz 2013, Le Corre et al. 2012, Leung et al. 2012). In addition, there is a direct inflow of resistant bacteria to the wastewater, especially from the hospital wastewater (Chagas et al. 2011, Korzeniewska 2011). These two major sources of contaminant are released into rivers and lakes directly or from treated sewage. Also, several factories and

hospitals drain their wastewater into the river. As a result, there are many direct and indirect sources which are responsible for the contamination of the Tigris River in Baghdad city (Ibrahim & Asmaa 2017).

Water quality tests stand as an informatics platform to provide valuable data that lead to maintaining a healthy aquatic ecosystem. Some aquatic ecosystems can resist large and different changes in water quality without any detectable effects on the ecosystem, however other ecosystems are more sensitive to small changes in the physical and chemical parameters which can lead to degradation of the ecosystem and affect biological diversity. The changes of physical and chemical water quality are a result of human influences and usually gradual and undetectable due to the invisible adaptations of aquatic ecosystems unless a dramatic shift in ecosystem condition occurs (Stark Jr et al. 2000). The physical and chemical characteristics of a water sample are compared with the World Health Organization (WHO) guidelines or standards to determine water quality. One of the problems is the lack of expert study and regular monitoring of the water quality of most rivers (Robertson et al. 2006, Wickham et al. 2005). It is important to perform river water quality assessment to evaluate the water quality and to detect pollution sources (Sin et al. 2001, Yuan et al. 2011). The rapid population growth and intensive domestic activities, in addition to expanding industrial and agricultural production, which has resulted in the release of large quantities of toxic chemicals, especially heavy metals into rivers worldwide (Srebotnjak et al. 2012, Su et al. 2013). However, toxic metals are not considered in many studies like the major ion chemistry of rivers. Heavy metals may undergo many changes in their speciation due to dissolution, precipitation, sorption, and complexation phenomena during their transport in rivers (Abdel-Ghani & Elchaghaby 2007, Akcay et al. 2003) and this will affect their behaviour and bioavailability (Nicolau et al. 2006, Nouri et al. 2011). A study suggested that the downstream of Tigris River is more seriously polluted by heavy metals than the upstream and midstream sites in Baghdad city (Obaidy 2014). While another study reported that some heavy metals (Cu, Hg, Pb, and Zn) were within the normal limit except Cd ion, which was slightly elevated in Tigris River water samples in Baghdad city (Ibrahim & Asmaa 2017). This work aims to study the profile and prevalence of antibiotic-resistant bacteria in the environment of Tigris River in Baghdad city near Baghdad Medical City and also measures the physical and chemical parameters in Tigris River to understand their effect on the aquatic ecosystem and biological diversity and to investigate whether Tigris River water has been contaminated with heavy metals or not.

MATERIALS AND METHODS

Study sites and collection of water samples: Total 7 river water samples were collected from different locations of the Tigris River in Baghdad city, Iraq in November 2018. Samples were collected from 50 cm below the surface of the Tigris River in 100 mL sterile bottles and 1 m from the edge. All samples were immediately placed on ice and transported back to the laboratory on the same day of collection for further processing.

Physicochemical parameters: Physicochemical parameters including temperature ($^{\circ}\text{C}$) and pH were determined for each Tigris River sample site and recorded using standard methods. This was done with three replication per sample.

Isolation and characterization of bacteria: After the removal of larger particulates by centrifugation at 5000 rpm for 10 minutes, 100 μL and 50 μL from each supernatant were spread on a selective media (MacConkey agar and EMB agar) plates. After 24 hours of incubation at 37°C , the bacterial colonies with distinct colony morphology were selected and analysed for morphological and biochemical characteristics by IMVIC analysis test.

Antimicrobial susceptibility testing: The antibiotic susceptibility test was done by disc diffusion method. All isolates were cultured on MacConkey agar and incubated for 18 hours at 37°C . In the next day, two to three colonies of these organisms were emulsified with normal saline to adjust the inoculum density equal to that of 0.5 MacFarland turbidity standards. Using a cotton swab, each bacterial isolate was spread on Mueller-Hinton agar and left for 10 min at room temperature, and 8 different antibiotics (Amikacin (AK-30 μg), gentamicin (GM-10 μg), Ciprofloxacin (CIP-10 μg), trimethoprim (TMP-10 μg), Amoxicillin (Ax-25 μg), imipenem (IPM-10 μg), Cefotaxime (CTX-30 μg), Azithromycin (AZM-15 μg) discs were placed on the top of the agar and all plates were incubated at 37°C for 24 hrs. After the incubation period, the inhibition zone diameters around the discs were measured using a ruler and then classified according to the standardized table supplied by CLSI guidelines (Wayne 2014).

Chemical analysis: Chemical analysis including the total dissolved solids (TDS), electric conductivity (E.C.), alkalinity (ALK), turbidity (tur) and total hardness (TH), and the major ions (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium, (K^{+}), phosphate ion (PO_4^{-3}), nitrate (NO_3^{-}), sulphate (SO_4^{2-}) and chloride (Cl^{-}) were measured using different methods as listed in Table 1.

Heavy metals analysis: Heavy metals including cobalt, zinc, copper, cadmium, and nickel were determined by

Table 1: Methods used for measuring chemical parameters.

Test	Method
Total Dissolved Solids (TDS)	Gravimetric method (Drying at 103-105 °C)
Electric conductivity (E.C)	Electric conductivity meter
Alkalinity (ALK)	pH meter
Turbidity (tur)	Nephelometer
Total hardness (TH)	EDTA titration method
Ca ²⁺	Atomic absorption spectrophotometer
Mg ²⁺	Atomic absorption spectrophotometer
Na ⁺	Atomic absorption spectrophotometer
K ⁺	Atomic absorption spectrophotometer
PO ₄ ⁻³	Spectrophotometer
NO ₃ ⁻	Spectrophotometer
SO ₄ ²⁻	Turbidity metric method
Cl ⁻	Spectrophotometer

atomic absorption spectrophotometer for water sample in triplicate per sample.

RESULTS AND DISCUSSION

Description of the Study Area

Water samples were taken from 7 sites from Tigris River near the Medical city; Baghdad, Iraq. The total sites distance was

1.975 meters; site 1 was the municipal sewage of Baghdad Medical city and samples were taken from both side of Tigris River as can be seen from Fig 1.

Isolation of *E. coli* and *Klebsiella*

The occurrence of *E. coli* and *Klebsiella* in river water samples varied in different sites. The highest number of bacteria was located at site 1, which indicated that the river water

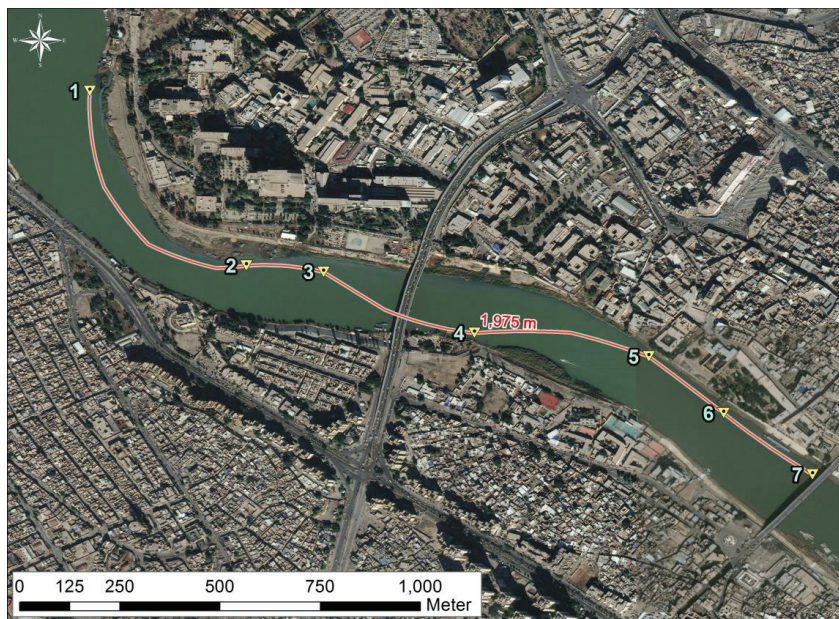


Fig. 1: Description of the study sites. S1: Baghdad Medical city municipal sewage, S2: Medical city hospitals, S3: Medical city hospitals, S4: Residential area, S5: Qishla building, S6: Qishla hour, and S7: Mosque Wazzar.

was severely polluted and suitable for the rapid growth of bacteria (Fig. 2).

The number of *E. coli* and *Klebsiella* at site 1 was significantly higher than the average level of all sampling sites. One possible explanation for this high bacteria abundance was that the water sample at site 1 was collected from the sewage of several hospitals. Site 7 showed a lower number of bacterial isolates. Therefore, it could be speculated that we can see low numbers of bacterial isolates as we go far from sources for river water pollutions.

Profile of Multi-Drug Resistant *E. coli* and *Klebsiella* Isolates from Tigris River

A total of 9 confirmed *E. coli* and *Klebsiella* isolates were profiled for their probable phenotypic resistance to 8 different antimicrobials. All *E. coli* isolates showed a high level

of resistance against amoxicillin as shown in Fig. 3. Varied resistances for other antibiotics were recorded as follows: high levels of resistance equally observed against Gentamicin and Azithromycin. The *E. coli* isolate at S1 was more resistant to different antibiotics than *E. coli* isolate at S7. Conversely, all the *E. coli* isolates were susceptible to Ciprofloxacin and Imipenem. Although wastewater treatment processes reduced bacterial numbers in the sewage and attributed to a partial reduction of microorganism number due to the dilution of treated sewage in river water. The bacterial isolates showed resistance to different antibiotics, which indicates high contamination in the S1 site with antibiotic-resistant bacteria discharged from the sewage of Baghdad medical city hospitals. The pollution of river water is influenced by population density and economic activities, especially sewage effluent. Even with the presence of several water treatment

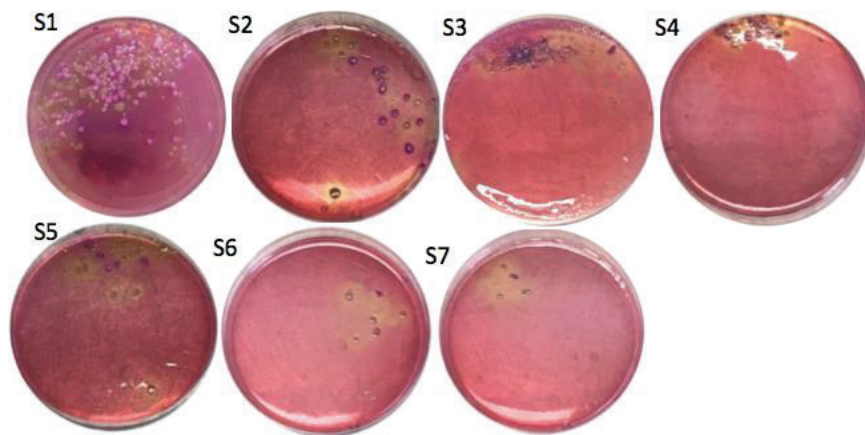


Fig. 2: Heterogenic diversity of the bacteria from Tigris River water samples.

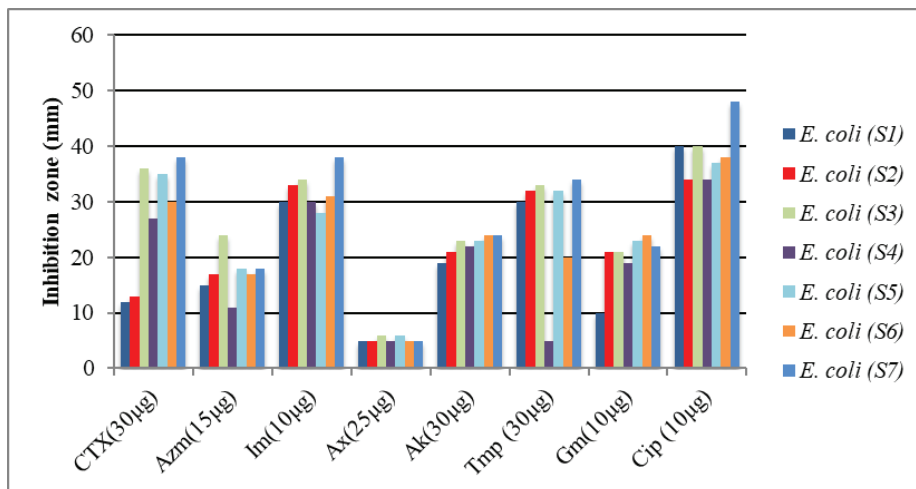


Fig. 3: Antimicrobial resistance of *E. coli* isolated from a river sample.

plants located within Baghdad city, water samples from these plants were contaminated with faecal coliform (Hassan & Mahmood 2018). The presence of coliforms in drinking water is an indicator of water contamination with bacteria or viruses that occur in a faecal matter (Bain et al. 2014). A study showed that upstream of the Tigris River in Baghdad city has the highest total coliform presence (AL-Dulaimi & Younes 2017). The large population, extensive industrial activities and sewage of several hospitals may attribute for contamination of Tigris River with coliform and especially *E. coli*. The comparison between *Klebsiella* isolates was not considered since *Klebsiella* isolates were detected only in two sites from total of seven sites.

The comparison between *E. coli* and *Klebsiella* isolates in S1 and S7 cited in Fig. 4 revealed that *E. coli* and *Klebsiella* isolates at both sites were resistant to amoxicillin and *E. coli* isolates at S1 and S7 were more resistant to different antibiotics than *Klebsiella* isolates which were sensitive to ciprofloxacin, gentamicin, and trimethoprim. This result may be attributed to the *E. coli* resistance characteristics, which indicates that multilateral exchange of genetic material between bacteria of both anthropogenic and en-

vironmental origins is currently occurring and presents a phenomenon of growing importance or river water sample collected near S1 site carried faecal coliforms bacteria from untreated hospital sewage coming from all units of the hospitals, including laboratories, rehabilitation, dialysis, hospitalization, and surgery units, clinics, maternity, laundry, and the cafeteria.

The difference between *E. coli* and *Klebsiella* isolates from S1 showed that *E. coli* are more resistant to selected antibiotics than *Klebsiella*. The obtained result indicates that *E. coli* heavily contaminates the Tigris River. The discharged sewage may also contain antibiotics used as a treatment for patients in these hospitals. A study by Mahmood et al. (2019) confirmed the contamination of water samples in Baghdad city with different antibiotics including fluoroquinolones and B-lactams. The highest antibiotic concentration recorded was ciprofloxacin in the Al-Wihda plant, while amoxicillin was not detected in the same site. Despite the treatment of the municipal sewage, river water may be a good reservoir for antibiotic-resistant microorganisms and plasmid-mediated antibiotic resistance genes. This may pose a public health risk, which needs future evaluation and control.

Table 2: Temperature and pH parameters of the studied area.

Site	S1	S2	S3	S4	S5	S6	S7
Temperature	22	18.1	18.3	18	18.3	18	18.5
pH	8	7	7	7.5	7	7	7

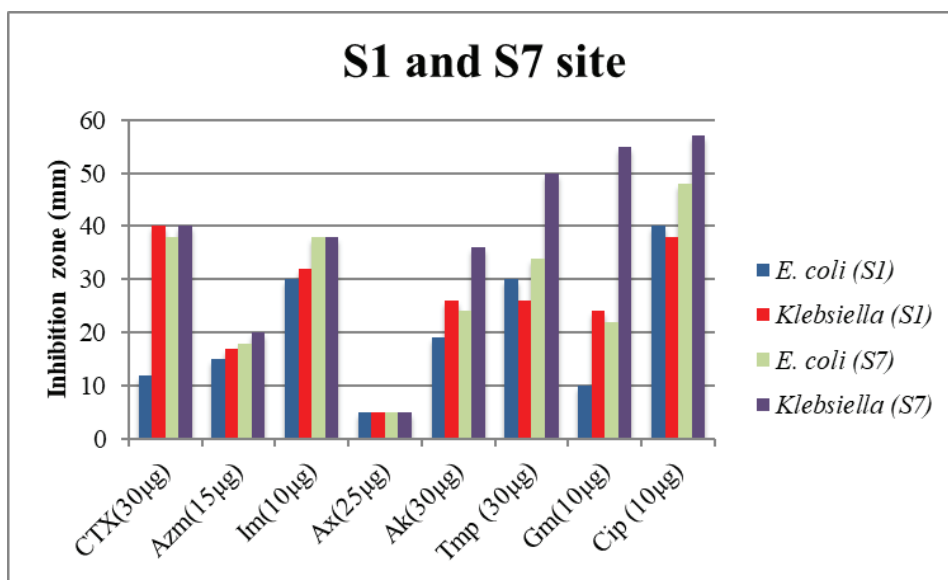


Fig. 4: Antimicrobial resistance of *E. coli* and *Klebsiella* isolated from S1 and S7 river sample.

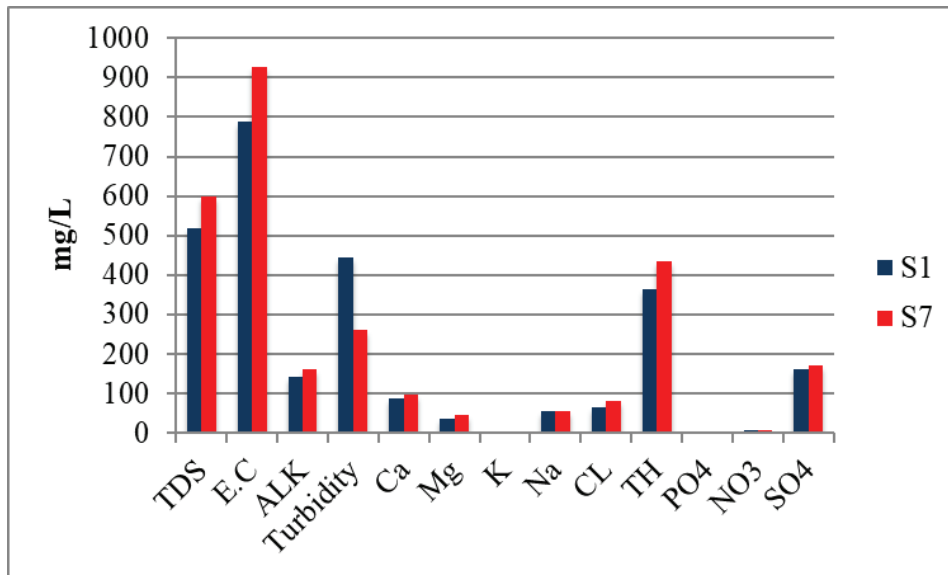


Fig. 5: The concentration of major ions and some environmental parameters in the Tigris Rivers, Baghdad city at site 1 and 7.

Physicochemical Characteristics

The water temperature is an important factor in water quality and the distribution of organisms in an aquatic ecosystem in addition to its role in many metabolisms and other transformations in a water body (Smith 2004). The physicochemical parameters of river water samples are presented in Table 2. The temperature ranged from 18-18.5°C from site 2 to site 7. The highest temperature was noticed at site 1 (22°C), which is near Medical City hospitals. Minor differences in pH were observed between sampling sites. The pH values ranged from 7 to 8, indicating that the Tigris River in Baghdad city is alkaline (pH >7). The highest pH was also recorded at site 1. There are no significant differences in temperature and pH between sampling sites. The Iraqi rivers are characterized by high buffer capacity which explains high pH (Hassan 2004, Abbas 2017).

Environmental Parameters and Metal Concentration

The results in Fig. 5 show different environmental parameters and metal concentrations in water samples of the Tigris River that revealed the difference in turbidity only while there was no significant variation for the other parameters between site 1 and site 7 in this study.

Anthropogenic activity has a great impact on the Tigris River ecosystem. A recent study revealed that middle of Tigris River in Baghdad city (Al-Sarrafa Bridge and Al-Shuhada Bridge) is heavily polluted than upstream of Tigris River (Al-Ani et al. 2019). This study recorded data from February

2017 to February 2018, which agree with our obtained data in November 2018. Due to the increase in rainfall proportion and high water levels in winter in addition to domestic wastes, the turbidity levels increases in river water (Gangwar et al. 2012, Al-Obaidi 2009). In the aquatic ecosystems, electrical conductivity is considered as a good indicator to evaluate total dissolved solids in river water and water purity. The obtained data showed that conductivity in site 1 and site 7 is less than (1500 μ S/cm) which is the Iraqi permissible limits for electrical conductivity (Hassan & Mahmood 2018).

The concentration for each heavy metal was measured and Co, Zn, Cu, Cd and Ni were Nil, 0.007, 0.011, Nil and 0.059 ppm, respectively which indicates that site 1 was not contaminated with heavy metals. The result of heavy metal concentrations in site 1 gives an insight view of Tigris River sample safety at site 1. A study suggested that the site, which is located at the downstream of Tigris River is more seriously polluted by heavy metals than the upstream and midstream sites in Baghdad city (Obaidy et al. 2014).

CONCLUSION

The contamination of the Tigris River in Baghdad city with antibiotic-resistant bacteria is a threat to human health and the river ecosystem. The cause of resistance, or mode of transmission of the antibiotic-resistant gene between pathogenic and environmental bacteria, is unknown. This study indicates that Tigris River in Baghdad city near Baghdad Medical City is a major source of antibiotic-resistant *E. coli*. A vast

quantity of antibiotic-resistant bacteria is discharged to the aquatic ecosystem with hospital sewage. Urgent measures are needed to minimize the effects of releasing wastewaters into water resources. One of the solutions that could minimize the spreading of antibiotic-resistant bacteria to the environment is the preliminary disinfection of hospital sewage before its inflow into the sewage system or Tigris River. This study can provide a platform for defying the currently most popular antibiotic options for human therapy by studying the resistance characteristics of the isolates described in this study. It also appears that drug-resistant *E. coli* is widely distributed in all the rivers sampling sites. Monitoring physiological and chemical parameters in the Tigris River is needed for proper management. Determination of the water quality is essential and comparing the physical and chemical characteristics of a water sample with water quality guidelines. These parameters should be usually at acceptable levels either to humans or aquatic organisms. This study also confers that site 1 is not contaminated with heavy metals.

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