



Multidrug Resistance Patterns of *Escherichia coli* from Wastewater in Baghdad

Wid Maan AL-Bayati¹ and Hiba Qasim Hameed^{2*}

^{1,2}Department of Biology, College of Education Pure Sciences (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.

*Corresponding Author

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Abstract

Hospital wastewater is believed to be a leading source of antibiotic resistant bacteria, which presents a risk to both the ecosystem and public health. The current study endeavored to isolate and identify *Escherichia coli* from the wastewater of multiple hospitals in Baghdad City, Iraq, and characterize its antibiotic resistance patterns. The study resulted in identifying, by biochemical means using the VITEK system, a total of 50 *E.coli* isolates. Antibiotic susceptibility tests for 11 antibiotics were conducted on each isolate via the Kirby–Bauer (KB) disk diffusion method. Overall, all isolates showed considerable resistance to the following antibiotics: erythromycin and clarithromycin (100%), amoxicillin (86%), cefoxitin (72%), ceftriaxone (66%), and tetracycline (56%). Conversely, gentamicin (6%) and meropenem (16%) exhibited the lowest resistance patterns among all isolates. The Multiple Antibiotic Resistance (MAR) index indicated that 44 isolates (88%) exceeded 0.2, signifying that a high number of isolates were contaminated or exposed to antibiotics.

Keywords: *Escherichia coli*; hospital wastewater; antibiotic resistance.

1. Introduction

Water is essential for all life forms, but it is also a major vector for environmental and public health problems worldwide, primarily due to the contamination of freshwater sources by the direct release of untreated (i.e., sewage that contains high concentrations of toxic chemicals and disease-causing germs) directly into freshwater sources (such as rivers, lakes, and coastal areas) without proper treatment before discharge ^{1,2}.

A great number of research studies indicate that waste from hospitals can lead to the dispersion of bacterial pathogens and antibiotic resistance genes in the environment³. Waste from hospitals is particularly dangerous as it contains hazardous waste and large amounts of bacteria resistant to antibiotics, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Salmonella typhi* ^{4,5}.

Antibiotic resistance genes (ARGs) are a serious threat to public health and the environment due to their ability to be transferred between different bacterial species via horizontal gene transfer (HGT) mechanisms⁶. The transfer of ARGs between species promotes the spread of antibiotic resistance and makes the treatment of bacterial infections more complicated ⁷.

The rising incidence of Antibiotic Resistance is a serious issue of public health related to the Contamination of drinking and surface water by antibiotics, which resulted from over-prescription and/or improper use of antibiotics. This has generally resulted due to the increased use of antibiotics with respect to Aquaculture, Combined Sewer Overflows, HealthCare, Industrial Discharge, Agriculture, Livestock, and Domestic Wastewater sources ⁸.

Escherichia coli is included with Enterobacteriaceae based on its classification as a Gram-negative and rod-shaped, facultatively anaerobic, non-spore-forming bacterium, which can either have flagella or not⁹. Past research shows biofilm production in *E. coli* is one of the most important virulence contributors to its pathogenic characteristics as well as increasing antibiotic resistance¹⁰. The excessive use of antibiotics in hospitals and the incorrect disposal of wastewater have been responsible for the development of antibiotic-resistant bacteria and their spread to other community and environmental settings¹¹. The Tigris River in Iraq is the primary source of water for many communities in the country. Local studies have indicated that it is heavily polluted and unfit for drinking, in addition to being contaminated with resistant bacterial isolates, especially near hospitals. *E. coli* bacteria have been detected as a common indicator of water quality deterioration^{12,13}.

This research aimed to isolate and identify multidrug-resistant *Escherichia coli* (MDR) from hospitals wastewater in Baghdad, and evaluate their antibiotic resistance patterns. Understanding the prevalence of MDR-resistant bacteria in hospital wastewater can contribute to improved environmental pollution monitoring and public health protection. This study also aligns with the SDG of the UN global goals, particularly Goal 3 (Good Health and Well-being) and Goal 6 (Clean Water and Sanitation), which emphasize the importance of providing safe water and reducing sources of microbial pollution in the environment.

2. Materials and Methods

2.1. Collection of bacterial isolates

Twenty wastewater samples from Baghdad hospitals were collected in sterile, sealed 100 ml containers between 20 August 2025 and 12 October 2025. The samples were transported to the laboratory within two hours of collection, and then serial dilutions (10^{-1} – 10^{-6}) to all samples, and 100 microliters were taken from each dilution by micropipette and cultured on MacConkey agar medium by L-shaped spreader. The cultures were incubated for 24 hours at 37°C. The isolates that were taken from the initial culture were repeated on another MacConkey plate to confirm purity and also cultured on EMB medium.

2.2. Identification of isolates

2.2.1. Gram staining

After Gram staining, all bacterial isolates were examined microscopically. Smears were viewed using a light microscope with the 40× objective and the oil immersion lens (100×) using the method described by¹⁴.

2.2.2. Biochemical tests

Identification was accomplished by conducting biochemical tests, including the catalase test, oxidase discs test, Simmons citrate test, methyl red test, and Voges Proskauer test, according to standard methods as outlined in¹⁵.

2.2.3. Identification using the VITEK system

E. coli isolates were identified via the VITEK system. There are 2 parts to the VITEK system: a computer and an analytical instrument that contains test cards with 48 micro-wells, which are filled with dehydrated culture media and colorimetric indicators for testing. The VITEK system automatically collects and records the color changes of the wells due to bacterial growth and utilizes this information to identify the isolates using biochemical reaction techniques.

2.3. Antibiotic susceptibility testing

To carry out antibiotic susceptibility testing of *E. coli* isolates, a number of specified antibiotics were used as contained in **Table 1** using the methodology known as the Kirby-Bauer disk diffusion method, established by¹⁶. The testing commenced when individual colonies were isolated overnight at 37°C on MacConkey agar. The following day, an inoculum preparation was achieved by transferring 3–5 separate colonies to a clean, sterile 5 mL tube filled with sterile saline solution and mixed with a vortex mixer to create a homogenous bacterial suspension. The

turbidity of this suspension was adjusted according to the 0.5 McFarland standard, which relates to an approximate concentration of 1.5×10^8 CFU/mL.

The sterile cotton swab was inserted into the bacterial suspension and was rolled several times around the edge of the tube. A sterile forceps was used to wipe off excess liquid from the outside of the swab. Once dried, the swabs were used to streak the Mueller-Hinton Agar plates in all four directions. The Mueller-Hinton agar plates had been rotated approximately 60°C after each of the four accesses to ensure an equal number of bacteria were inoculated on the agar surface. There were two plates made for each isolate. The four Mueller-Hinton agar plates that had been inoculated were allowed to sit for approximately 10 minutes prior to adding the 5-6 antibiotic disks located in the center of the agar plate. To ensure that all the excess liquid had been absorbed by the antibiotic disks on the surface of the agar, the disks were placed on top of the agar with sterile forceps and pressed down on the top surface of the agar plates. The plates were incubated for 24 hours at 37°C. After the incubation period, the diameters of the zones of inhibition (measured in mm) around the antibiotic disks were measured with a flat ruler, and the results were assessed by reference to the Clinical and Laboratory Standards Institute's results guidelines¹⁷.

Table 1. Antibiotics used in the study.

Antibiotic	Concentration
Meropenem	10 µg
Gentamicin	10 µg
Ciprofloxacin	5 µg
Ceftriaxone	10 µg
Ceftazidime	30 µg
Aztreonam	30 µg
Amoxicillin	10 µg
Cefoxitin	30 µg
Erythromycin	10 µg
Clarithromycin	5 µg
Tetracycline	30 µg

2.4. Multiple Antibiotic Resistant Index Value (MAR)

All *E.coli* isolates recovered from wastewater were tested against all of the antibiotics included in this study to calculate the MAR value. The MAR value was calculated by the ratio of a certain type of bacterial isolate's resistance to a group of antibiotics by comparing the number of Antibiotic Resistance Types (ARTs) to the total number of ARTs in the study.

So when calculating the MAR value ,

MAR Index = a/b

Where a = the number of ARTs to which an isolate has a resistance and b = the number of ARTs used in the study.

In this study, it is noted that a MAR value that is >2 indicates a high concentration of contamination from an environmental source of antibiotics, while a MAR value <2 indicates a low level of environmental contamination from antibiotics¹⁸.

3. Results

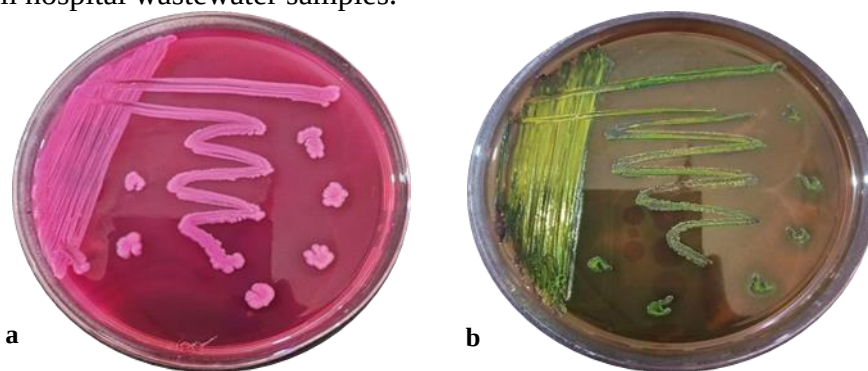
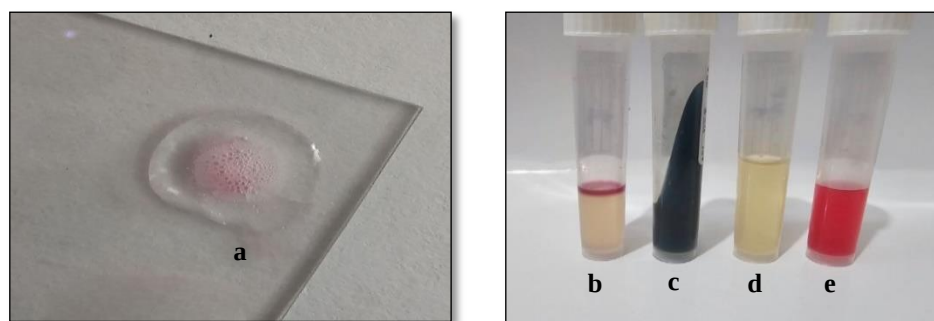
3.1. Isolation and identification of bacteria

After collecting samples and culturing them on preferential and differentiating media (**Table 2, Figures 1-3**), the bacterial isolates were microscopically examined, biochemically tested, and identified using the VITEK system. From the 50 isolates of *E. coli* received, the remaining bacterial and non-classified isolates that gave uncertain biochemical test results were excluded. All the identification findings have been compiled into **Table 2**.

Table 2. Tests and results used for the identification of *Escherichia coli* isolates.

No.	Test	Result
1	MacConkey agar	Appearance of pink, smooth, and shiny colonies
2	Eosin Methylene Blue (EMB) agar	Appearance of metallic green, shiny colonies
3	Microscopic examination	Presence of pink, rod-shaped cells
4	Indole test	Positive; formation of a red indole ring
5	Methyl Red test	Positive; development of red color
6	Catalase enzyme	Positive; formation of gas bubbles
7	Oxidase enzyme	Negative; no color change of colonies
8	Voges–Proskauer test	Negative; no color change of the medium
9	Citrate utilization test	Negative; no color change of the medium
10	VITEK system	Positive; all isolates confirmed as <i>E. coli</i>

This comprehensive identification approach confirmed precise validation of *E.coli* isolates recovered from hospital wastewater samples.

**Figure 1.** Growth of *Escherichia coli* on (a) MacConkey agar and (b) Eosin Methylene Blue (EMB) agar.**Figure 2.** Morphological appearance of bacterial cells under the light microscope at 100× magnification after Gram staining, presence pink, rod-shaped cells.**Figure 3.** Biochemical tests used for the identification of bacterial isolates, including: a. Catalase test b. Indol test c. Citrate utilization d. Voges-Proskauer test e. Methyl red test.

3.2. Antibiotic susceptibility testing

The result showed that 100% of the *E.coli* isolates taken from wastewater were resistant to erythromycin and clarithromycin. 86% of isolates were resistant to amoxicillin; 72% were resistant to cefoxitin; 66% were resistant to ceftriaxone; and 56% were resistant to tetracycline. Other antibiotic resistances are as follows: 28% to aztreonam, 42% to ceftazidime, and 26% to ciprofloxacin. The lowest levels of resistance were found with the meropenem (16%) and gentamicin (6%) (**Table 3** and **Figure 4**).

Table 3. Percentage distribution of antibiotic susceptibility patterns among *Escherichia coli* isolates.

Antibiotic	Sensitive isolates	Resistant isolates	Intermediate isolates	Total
Meropenem	41 (82%)	8 (16%)	1 (2%)	50 (%100)
Gentamicin	22 (44%)	3 (6%)	25 (50%)	50 (%100)
Ciprofloxacin	14 (28%)	13 (26%)	23 (46%)	50 (%100)
Clarithromycin	0 (0%)	50 (100%)	0 (0%)	50 (%100)
Ceftriaxone	15 (30%)	33 (66%)	2 (4%)	50 (%100)
Ceftazidime	18 (36%)	21 (42%)	11 (22%)	50 (%100)
Erythromycin	0 (0%)	50 (100%)	0 (0%)	50 (%100)
Aztreonam	22 (44%)	14 (28%)	14 (28%)	50 (%100)
Amoxicillin	3 (6%)	43 (86%)	4 (8%)	50 (%100)
Cefoxitin	9 (18%)	36 (72%)	5 (10%)	50 (%100)
Tetracycline	19 (38%)	28 (56%)	3 (6%)	50 (%100)

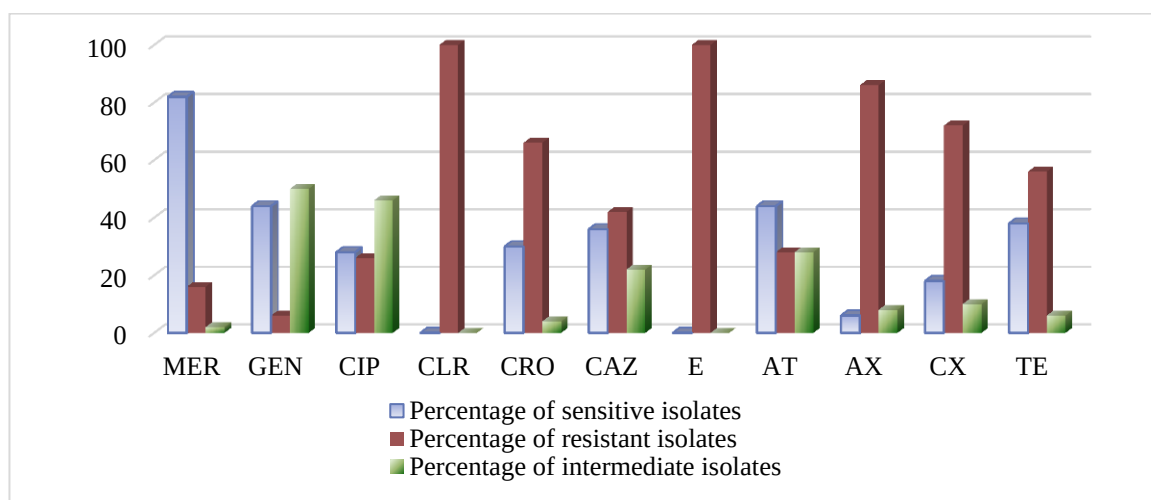


Figure 4. The representation of antibiotic susceptibility testing results. MER: Meropenem, GEN: Gentamicin, CIP: Ciprofloxacin, CLR: Clarithromycin, CRO: Ceftriaxone, CAZ: Ceftazidime, E: Erythromycin, AT: Aztreonam, AX: Amoxicillin, CX, TE: Tetracycline.

3.3. Multiple Drug Resistance (MDR)

Isolates of *E.coli* originating from hospital wastewater primarily were found exhibiting multidrug resistance (MDR) as evidenced by a high rate of antibiotic resistance among these isolates. Six of the isolates demonstrated resistance to two different classes of antibiotics (12%), eight to three classes of antibiotics (16%), seven to four classes of antibiotics (14%), seven to five classes of antibiotics (14%), and ten to six classes of antibiotics (20%); two to seven classes of antibiotics (4%); two to eight classes of antibiotics (4%); and seven to nine classes of antibiotics (14%), and only one isolate resulted in a total of ten antibiotic resistances, as shown in **Figure 5**. The results clearly demonstrate the presence of multidrug-resistant *E.coli* in the wastewater setting and that there is significant selective pressure on this organism due to the frequency of antibiotic exposure within a hospital environment.

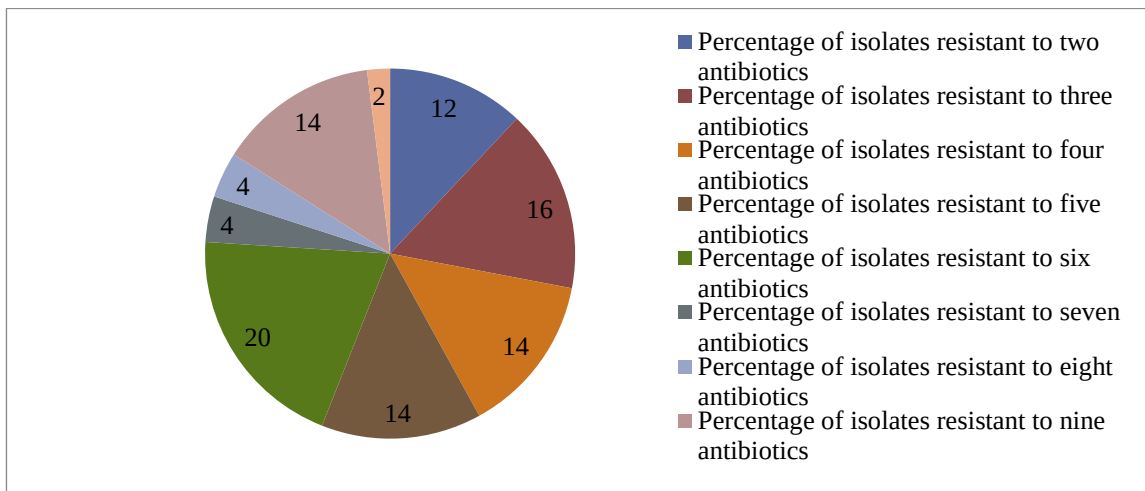


Figure 6. Percentage distribution of multidrug resistance (MDR) patterns among *Escherichia coli* isolates.

Table 4. Number of multidrug-resistant bacterial isolates, number of antibiotics to which resistance was observed and MDR percentage.

No. of MDR isolates	No. of antibiotics resisted	MDR (%)
6	2	12%
8	3	16%
7	4	14%
7	5	14%
10	6	20%
2	7	4%
2	8	4%
7	9	14%
1	10	2%

3.4. Determination of the Multiple Antibiotic Resistance (MAR) Index

The MAR index was calculated for all isolates, as illustrated in **Figure 7**.

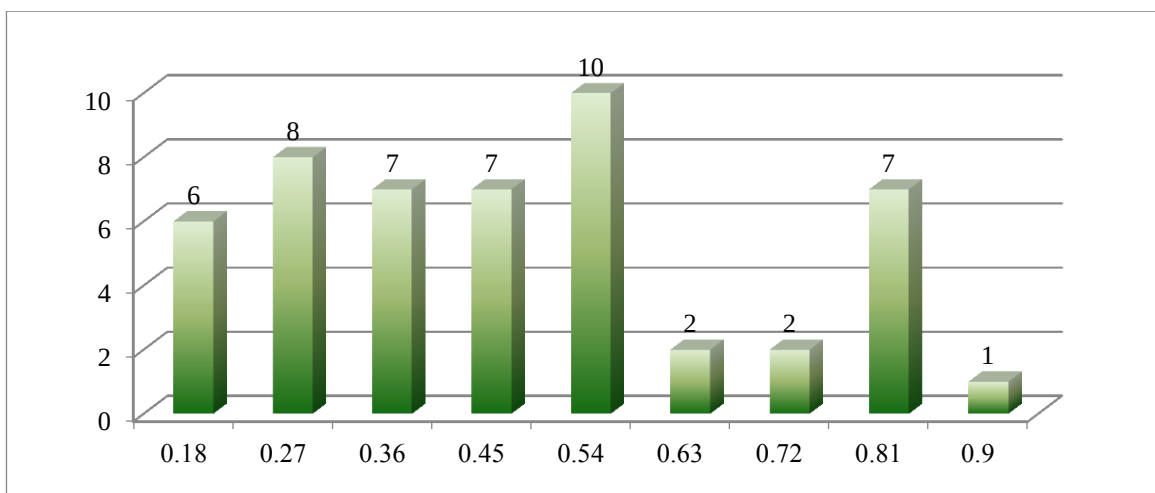


Figure 7. Distribution of MAR index values among *Escherichia coli* isolates..

4. Discussion

The goal of this research was to assess the antibiotic susceptibility and resistance patterns of *E. coli* collected from hospital sewage. Considerable variability was seen among the different antibiotic susceptibility and resistance rates noted for the *E.coli* isolates tested. This variability could be attributed to the overuse of antibiotics in hospitals and improper disposal of unprocessed hospital wastewater. Contaminated hospital wastewater contains large amounts of

medical waste, harmful chemicals, and many microorganisms, thereby creating an environment conducive to the development and persistence of multidrug-resistant bacterial strains^{19,20}.

The current findings on resistance rates for *E. coli* and meropenem indicate a rate of 16%, which is comparable to the findings of ²¹ with a resistance rate of 21.25% but lower than those of ²² at 44.4%.

Regarding gentamicin, the current study indicates that 6% of *E.coli* isolates were resistant and agrees with the results reported by ²³ and ²⁴ with resistance rates of 7% and 6%, respectively. However, our results partially coincide with ²⁵, who found that 18% of *E.coli* isolates were resistant to gentamicin, whereas there was a significant discrepancy when compared to the findings of ²¹, who showed an even higher resistance rate for this antibiotic of 56.25%.

This study showed that the resistance to ciprofloxacin was 26%, while ²⁶ had reported a ciprofloxacin resistance rate of 30% and ²⁷ was 17.5% of the isolates. However, there was a very strong contrast with the research finding of ²⁸, which showed that all of the isolates were 100% susceptible to ciprofloxacin.

The current study, like previous studies^{26,28}, reported a total (100%) resistance of all isolates to erythromycin; both of those studies reported a resistance rate of 98% and 88.8%, respectively, while ²⁹ reported a 76% erythromycin resistance among their isolates.

The result of this study also showed the same total (100%) resistance of all isolates to clarithromycin. This high level of resistance is likely due to the inherent resistance to macrolides (e.g., erythromycin) found in Gram-negative bacteria. This is caused by two factors: 1) Gram-negative bacteria have a lower permeability of their outer membranes, and 2) these bacteria contain efflux pumps that prevent the penetration of the antibiotic³⁰.

The level of resistance to ceftriaxone observed in this study (66%) is comparable to that from ³¹ which reported a higher level of resistance (78%), and partially consistent with ²², a higher level of resistance (82.2%). However, the level of resistance to the ceftriaxone in this study is significantly higher than what was reported by ²⁷ which is 23.5%.

The resistance rate of 42% against ceftazidime in the current study is consistent with the findings of ³², and ³³, who both report a resistance rate of approximately 50%, while it was considerably greater than that reported by ²⁶ at 88%.

Aztreonam produced similar resistance rates (28%) when compared to ³⁴ (26%) and ³⁵ (34%), with the exception of ³², who showed a higher level of resistance (64%).

The resistance rate to amoxicillin observed in this study (86%) was similar to the resistance rates reported by ²⁸ (100%) and ³¹ (93%) but was higher than that of ²⁴ (42%). The resistance to cefoxitin (72%) was greater than the resistance rates reported by ³⁶ (53.5%) and ³⁷ (47.6%), although it was less than the 100% reported by³⁸.

The resistance rate to tetracycline in this study is 56%, and this result is consistent with the findings of ^{37,39} who reported resistance rates of 52.4% and 66.3%, respectively. In contrast, another study reported a higher resistance rate of 94%, which exceeded the rate shown in the current study²⁵.

Conclusively, the findings represent that most of the isolates of *Escherichia coli* collected have exhibited multi- and broad-spectrum resistance to antibiotics. The increasing incidence of resistance to a wide range of antibiotics by microorganisms, clinical infections, disease and death caused by resistant bacteria are increasingly common and is a major concern facing modern medicine.³⁴.

In addition, most of the isolates had a High MAR index, with 44 isolates (88%) showing a MAR index value of >0.2, indicating that these microorganisms are likely to have been sourced from a highly contaminated and high-risk environment. The results of this study are consistent with previous studies^{18,40,41}, which showed the presence of a high proportion of *E. coli* isolates exhibiting MAR indices of >0.2 in their results (81%, 33–94%, and 94%, respectively).

Despite the important findings of this study, some limitations should be noted. The isolation and identification of bacteria based on traditional phenotypic methods, and the study focused on

Escherichia coli as a key indicator of bacterial contamination in hospital wastewater.

The results of this study indicate some research gaps that warrant future studies, including the use of molecular techniques such as PCR to detect genes responsible for antibiotic resistance, in addition to studying other microorganisms present in wastewater and evaluating the efficiency of wastewater treatment systems to reduce the spread of antibiotic-resistant bacteria in the environment.

5. Conclusion

One of the major sources of multidrug-resistant *E. coli* bacteria is hospital wastewater. High levels of resistance to multiple commonly prescribed antibiotics have been identified in the isolates studied, including but not limited to erythromycin, amoxicillin, cefoxitin, ceftriaxone, and tetracycline. The study showed that the majority (88%) of the isolates tested in this research contained a Multiple Antibiotic Resistance (MAR) index of > 0.2 . This indicates that these isolates are highly likely to have been contaminated with multiple antibiotics from the surrounding environment at some point during their life cycle.

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Conflict of Interest

The authors declare that they have no conflicts of interest.”

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References

1. Amin M, Al-Mohannadi A, Al-Ansari M, Hassan M. Wastewater from hospitals in developing countries: occurrence, risks, and challenges of treatment technologies. *Appl Microbiol.* 2024;3,60:1–12.
2. Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I, Fatta-Kassinos D. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Sci Total Environ.* 2013;447:345-60. <https://doi.org/10.1016/j.scitotenv.2013.01.032>
3. Pandey A, Afsheen AF, Tiwari SK. Isolation and characterization of multidrug resistant cultures from wastewater. *J Pharm Biomed Sci.* 2011;13(7): 1-7.
4. Anitha J, Jayraaj IA. Isolation and identification of bacteria in biomedical wastes. *Int J Pharm Pharm Sci.* 2012;4(5):386–388.
5. Shahzad A, Mian AH, Ul Haq I, Khan MA, Matiullah, Ali K, Hamid T. The emergence of different bacterial pathogens in hospital wastewater samples and their antibiotic resistance pattern. *Mater Circ Econ.* 2021;3:28. <https://doi.org/10.1007/s42824-021-00046-y>
6. Zhang S, Wang Y, Song H, Lu J, Yuan Z, Guo J. Dissemination of antibiotic resistance genes in aquatic environments: a review. *Sci Total Environ.* 2018;627:1627–1639.
7. Duarte AC, Rodrigues S, Afonso A, Nogueira A, Coutinho P. Antibiotic resistance in drinking water: old and new strategies to remove antibiotics, resistant bacteria, and resistance genes. *Pharmaceuticals.* 2022;15(4):393. <https://doi.org/10.3390/ph15040393>.
8. Obaid WA. Comprehensive phenotypic and genotypic analysis of multidrug-resistant and virulent *Enterococcus* spp. from untreated hospital wastewater and river water in Baghdad, Iraq. *Res Sq.* 2025; preprint. <https://doi.org/10.21203/rs.3.rs-7649505/v1>
9. Naidoo N, Zishiri OT. Presence, pathogenicity, antibiotic resistance, and virulence factors of *Escherichia coli*: a review. *Bacteria.* 2025;4:16. <https://doi.org/10.3390/bacteria4010016>
10. Hamady DR, Ibrahim SK. The study on ability of *Escherichia coli* isolated from different clinical

- cases to biofilm formation and detection of *csgD* gene responsible for curli production. *Biochem Cell Arch.* 2020;20(2):5553–5557.
11. Zhang C, Qiu S, Wang Y, Hao R, Li X, Shi Y, Hu X, An D, Li Z. Higher isolation of NDM-1 producing *Acinetobacter baumannii* from hospital sewage in Beijing. *PLoS One.* 2013;8:e64857. <https://doi.org/10.1371/journal.pone.0064857>.
 12. Abdulsattar B, Abdulsattar JO, Habeeb K. Study of antimicrobial resistance pattern of *Escherichia coli* and *Klebsiella* strains and multivariate analysis for water quality assessment of Tigris River, Baghdad, Iraq. *Nat Environ Pollut Technol.* 2020;19(3):1051–1058. <https://doi.org/10.46488/nept.2020.v19i03.050>.
 13. Mahdi ZM, Al-Saadi HM, Al-Mayali HK. Detection of resistance genes in waterborne *Escherichia coli* isolates from Baghdad wastewater. *J Water Health.* 2024;22(1):55–66.
 14. Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA. *Jawetz, Melnick & Adelberg's Medical Microbiology.* 26th ed. New York: McGraw-Hill; 2013. p.245–248.
 15. Tille PM. *Bailey & Scott's Diagnostic Microbiology.* 14th ed. St. Louis (MO): Elsevier; 2017. 1115 p.
 16. Hudzicki J. Kirby–Bauer disk diffusion susceptibility test protocol. *Am Soc Microbiol.* 2009.
 17. Clinical and Laboratory Standards Institute. M100: Performance standards for antimicrobial susceptibility testing. 35th ed. Wayne (PA): Clin Lab Stand Inst.; 2025.
 18. Isaiah DO, Otokunefor K, Agbagwa OE. Multiple antibiotic resistance indexing and molecular identification of *Escherichia coli* isolated from clinical and nonclinical sources in Port Harcourt Metropolis, Nigeria. *Pan Afr Med J.* 2025;51:11. <https://doi.org/10.11604/pamj.2025.51.11.38524>.
 19. Joshi DC, Prasad S, Sharma A, Joshi N. Pathological contaminants in wastewater from hospital waste: implications and mitigation strategies. In: *Emerging Contaminants in Water and Wastewater: Sources and Substances.* Cham: Springer Nature Switzerland; 2025. p. 43-85. https://doi.org/10.1007/978-3-031-82579-8_3.
 20. Manaia CM, Rocha J, Scaccia N, Marano R, Radu E, Biancullo F, Cerqueira F, Fortunato G, Iakovides IC, Zammit I, Kampouris I. Antibiotic resistance in wastewater treatment plants: Tackling the black box. *Environ Int.* 2018;115:312-24. <https://doi.org/10.1016/j.envint.2018.03.044>
 21. Altameemi Z, Talib A, Fakhry S. Detection of antibiotic-resistant *Escherichia coli* in the Euphrates River, Iraq. *bioRxiv [Preprint].* 2025. <https://doi.org/10.1101/2025.08.30.673253>
 22. Kumar G, Balakrishana K, Mukhopadhyay C, Eshwara VK, Kadam SB. Characterization and comparative analysis of antimicrobial resistance in *Escherichia coli* from hospital and municipal wastewater treatment plants. *J Water Health.* 2024;22(12):2274–2284. <https://doi.org/10.2166/wh.2024.126>.
 23. Gaspar CM, Csiszter LT, Lăzărescu C, Tîrban I, Pentea M, Butnariu M. Antibiotic resistance among *Escherichia coli* isolates from hospital wastewater compared to community wastewater. *Water.* 2021;13(24):3449. <https://doi.org/10.3390/w13233449>.
 24. Dagher LA, Joeman D, Kharroubi SH, Jaafer H, Kasem I. Nationwide assessment of water quality in rivers across Lebanon by quantifying fecal indicator densities and profiling antibiotic resistance of *Escherichia coli*. *Antibiotics.* 2021;10(9):1083. <https://doi.org/10.3390/antibiotics10091083>
 25. Wu D, Ding Y, Yao K, Gao W, Wang Y. Antimicrobial resistance analysis of clinical *Escherichia coli* isolates in neonatal ward. *Front Pediatrics.* 2021;9:670470. <https://doi.org/10.3389/fped.2021.670470>.
 26. Sadeq HS, Waheeb MQ. Phenotypic characterization of *Escherichia coli* and determination of their sensitivity to antibiotics. *Microb Sci Arch.* 2024;4(3):17–23. <https://doi.org/10.47587/msa.2024.4302>.
 27. Karumangnye P, Rugaika A, Mlei K, Machunda R. Antibiotic resistance patterns of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from hospital wastewater. *Appl Microbiol.* 2023;3(2):867–882. <https://doi.org/10.3390/applmicrobiol3030060>.
 28. Al-Qatan MAR, Al-Khayyat MZ, Saeed HK. Isolation of antibiotic-resistant bacteria from sewage water in Mosul City. *Biochem Cell Arch.* 2019;19(1):1415–1418.
 29. Tufa KF, Birhanu AG. Antimicrobial resistance profile of *Escherichia coli* isolated from hospital and industrial wastewater systems. *Environ Health Insights.* 2025;19:11786302251339254. <https://doi.org/10.1177/11786302251339254>.
 30. Venter H, Mowla R, Ohene-Agyei T, Ma S. RND-type drug efflux pumps from Gram-negative bacteria: molecular mechanism and inhibition. *Front Microbiol.* 2015;6:377. <https://doi.org/10.3389/fmicb.2015.00377>.
 31. Khatlab A, Nizam DA, Alamir DL. Isolation of *Escherichia coli* from effluents of some Damascus

- University hospitals and determination of their susceptibility to beta-lactam antibiotics. Damascus Univ J Basic Sci. 2023;39(3):112–126.
32. Davidova-Gerzova L, Lausova J, Sukkar I, Nesporova K, Nechutna L, Vikova K, Svobodova M, Soukupova M, Palenikova J, Kaspar J, Dotejska M. Hospital and community wastewater as a source of multidrug-resistant ESBL-producing *Escherichia coli*. Front Cell Infect Microbiol. 2023;13:1084085. <https://doi.org/10.3389/fcimb.2023.1184081>.
 33. El Garraoui Q, Logman A, Amouat FE, Hasnaou S, Soras N, Logman S. Antimicrobial resistance and molecular detection of extended-spectrum β -lactamase-producing *Escherichia coli* in municipal wastewater in Marrakech. PLoS One. 2025;20(6):e0325816. <https://doi.org/10.1371/journal.pone.0325816>
 34. Sader HS, Mendes RE, Arends SR, Carvalhaes CG, Castanheira M. Antimicrobial activities of aztreonam-avibactam and comparator agents tested against Enterobacterales from European hospitals analysed by geographic region and infection type (2019–2020). Europ J Clin Microbiol Infect Dis. 2022;41(3):477–87. <https://doi.org/10.1007/s10096-022-04400-z>.
 35. Abdelgaleil RR, Ibrahim HA, Ahmed DS, Ahmed AB. Multidrug-resistant *Escherichia coli* in wastewater sources: a comparative study and identification of resistance hotspots. BMC Microbiol. 2023;23:298. <https://doi.org/10.1186/s12866-025-04244-5>.
 36. Amador PP, Fernandes RM, Prudêncio MC, Barreto MP, Duarte IM. Antibiotic resistance in wastewater: occurrence and fate of Enterobacteriaceae producers of class A and class C β -lactamases. J Environ Sci Health A. 2015;50(1):26–39. <https://doi.org/10.1080/10934529.2015.964602>
 37. Endalamaw K, Tadesse S, Asmare Z, Rebede D, Erkihun M, Abela B. Antimicrobial resistance profile of bacteria from hospital wastewater at two specialized hospitals in Bahir Dar City, Ethiopia. BMC Microbiol. 2024;24(1):525. <https://doi.org/10.1186/s12866-024-03992-8>
 38. Mataseje LF, Niu YD, Tyler SD, Daignault S, Zhand T, Low DE. Characterization of cefoxitin-resistant *Escherichia coli* isolates from recreational beaches and private drinking water in Canada between 2004 and 2006. Antimicrob Agents Chemother. 2009;53(7):3126–3130. <https://doi.org/10.1128/AAC.01330-08>
 39. Seguni NZ, Kimera ZI, Msafiri F, Mgya FX, Joachim A, Mwingwa A, Matee MI. Multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolated from hospital sewage flowing through community sewage system and discharging into the Indian Ocean. Bulletin of the National Research Centre. 2023 May 11;47(1):66. <https://doi.org/10.1186/s42269-023-01039-4>.
 40. Hussein IA, Talib AA, Fakhry SS. Prevalence and antimicrobial susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* isolated from the Tigris River, Baghdad, Iraq. Iraqi J Sci. 2025;66(12): 5408–5423. <https://doi.org/10.24996/ij.s.2025.66.12.%g>.
 41. Adegoke AA, Madu CE, Aiyegoro OA, Stenström TA, Okoh AI. Antibigram and beta-lactamase genes among cefotaxime resistant *E. coli* from wastewater treatment plant. Antimicrob Resist Infect Cont. 2020;9(1):46. <https://doi.org/10.1186/s13756-020-0702-4>