Iraqi Journal of Biotechnology, 2025, Vol. 24, No. 2, 193-203

Characterization of New Antibiotic Resistant *Escherichia coli* Isolates from Water Treatment Plants Using *16S rRNA* Gene Sequencing

[1,2]Heba R. Fadhil, [2]Jasim M. Awda, [3]Mohammed Taha

[1,2] Department of Food Science , College of Agricultural Engineering Sciences / University of Baghdad
[3]Environment, Water and Renewable Energy Directorate /ministry of science& Technology

Received: June 6, 2024 / Accepted: July 30, 2024 / Published: July 5, 2025

Abstract: The World Health Organization has identified antibiotic resistance among bacteria as one of the most serious dangers to human health. This present study focuses on the isolation and identification of antibiotic-resistant *Escherichia coli* using *16S rRNA* gene sequencing based on molecular techniques from water samples. Between September and December 2023, we collected fifty aseptic water samples from various water treatment plants in Baghdad city. A number of biochemical and molecular tests confirmed the isolation of *Escherichia coli* from the samples. Isolates has been placed into nutrient broth and cultured them on various agar media, such as MacConkey agar and EMB agar. For biochemical analysis, we used Gram's staining, string techniques, and Vitek. Using the Kirby-Bauer disk diffusion technique, we tested the antibiotic sensitivity of 45 *Escherichia coli* isolates to fourteen commonly prescribed antibiotics. The data indicates a significant variation in the percentage of antibiotic resistance among E. coli isolates across different regions. In some regions, such as medical cities, the resistance rate was very high for almost all antibiotics. Other regions, like Al-Rustaimiya Station, also exhibit a high resistance rate. We amplified and compared 16S rRNA gene sequences to the NCBI sequence database using primers. A study of the partial 16S rRNA sequence revealed that two new strains, HHWW and HS, were 100% similar to *E. coli* and had a lot in common with species in the genus *Escherichia*.

Keywords: *PCR, 16S rRNA , Multi drug resistance, Escherichia coli*.

Corresponding author: (Email: jasim.awda@coagri.uobaghdad.edu.iq).

Introduction

Escherichia coli is a group of bacteria commonly found in the intestines of humans and animals. While most species are harmless, some can cause severe illness. The emergence of antibiotic-resistant *E. coli* in drinking water sources poses a significant public health threat. These resistant strains can be difficult to treat with conventional antibiotics, increasing the risk of treatment failure and complications (1, 2).

Contamination of drinking water with *E. coli* can originate from various sources, including inadequately treated wastewater, agricultural runoff, and faulty distribution systems (3, 4). This contamination is a growing concern as *E. coli* readily develops resistance to antibiotics due to factors like the overuse of antibiotics in agriculture and medicine and the ability of these bacteria to acquire and share resistance genes through horizontal gene transfer(5).

A range of studies have explored the prevalence and characteristics of antibiotic-resistant *Escherichia coli* in various water sources. (6,7). Researchers discovered high levels of antibiotic resistance in *E. coli* from drinking (8, 9) and identified specific sources as high-risk (10). Similarly, researchers found antibiotic-resistant *E. coli* in irrigation water and vegetables(11), using 16S rRNA gene sequencing to identify and characterize these isolates.

Water sources with the same sequence types and clones found in both sources suggest cross-contamination (12, 13).

The World Health Organization (WHO, Geneva, Switzerland) highlights antimicrobial resistance (AMR) as one of the top ten risks to global health, supporting the "One Health" approach's focus on immediately coordinating efforts to improve the environment, animal health, and human health (14). Currently, the World Health Organization (WHO, Geneva, Switzerland) estimates that AMR causes 1.27 million global deaths annually (15) and projects that this number will rise to 10 million by 2050(16).

The study of bacterial genetics is useful in finding and identifying bacteria (17).

The goal of this study is to use polymerase chain reaction (PCR) to look at the nucleotide sequence of the 16S rRNA gene of pathogenic E. coli from water treatment plants.

By comparing isolates from various sources or outbreaks, we can use the nucleotide sequence results from this research to understand the diversity of pathogenic *E. coli* and track the spread of contamination.

Methodology

Ethical approve

College of Agricultural Engineering Sciences / University of Baghdad , Baghdad, Iraq.

Issue number D.A.Z, 23.8.2023.

Sampling

Between October and December 2023 in Baghdad, we collected 50 water treatment plant samples, ten from each of the drinking water treatment plants (Al Wathba, Al Wahda, and Al Karama) and the waste water treatment plants like the Al-Rustamiya wastewater treatment plant, including influent, effluent, and various stages of the treatment process. Each site used a 500-mL sterile bottle with a cap and a label. Each water sample had a volume of approximately 250 mL, and we immediately transported it to the laboratory for further processing within two hours. The Ministry of Science and Technology's Environment, Water, and Renewable Energy Directorate's laboratories conducted each bacteriological experiment. The process entails isolating and identifying *E. coli* isolates. Water samples were analyzed to isolate and identify Escherichia coli isolates using the multiple-tube fermentation technique (18, 19), After culturing on MacConkey agar and Eosin Methylene Blue Agar from nutritional broth and incubating for 24 hours at 37°C (20, 21), the results were confirmed by biochemical testing. The protocol (BioMerieux, Marcy L'Eyoily, France) also used the Vitek-2 Compact for phenotype identification and confirmation. The results automatically came out in the form of a schedule. Before using the *E. coli* isolates, the isolates were stored at -80 oC in nutrition broth with 15% glycerol. (22).

Antibiotic-sensitivity testing

Agar diffusion tests were performed to determine the antibiotic susceptibility of *E. coli* isolates. For the Clinical Laboratory Standards Institute (23), one to three colonies of fresh *E. coli* isolates were mixed to grown overnight with two milliliters of a 0.9 percent sterile sodium chloride solution. This was done to bring the turbidity of the bacteria suspensions up to the 0.5 ± 0.05 McFarland standard. The antibiotic discs had 15 µg of Azithromycin, 30 µg of Chloramphenicol, 15 µg of Meropenem, 30 µg of Ceftrazidime, 10 µg of ampicillin, 15 µg of ciprofloxacin, 10 µg of gentamicin, 30 µg of amoxicillin-clavulanic acid, and 10 µg of imipenem. then left the plates to sit at 36 °C for 16 hours to observe changes in resistance, intermediate, and susceptibility with the diameter of the inhibition zone.

Molecular characterization

DNA extraction was performed using the Wizared kit (Promega, USA), following the manufacturer's recommendations (24). frozen universal primers for the LacZ3 and 16SrRNA genes were obtained from (tables 1) (25), to detect the galactosidase gene and make sure the diagnosis of *E. coli* isolates,lyophilized primers were dissolved in nuclease-free water to achieve a final concentration of 100 pmol/l as a stock solution. Using the Go Tag Green Kit, the master mix was prepared for each PCR by calculating the required amount of each reaction component. The PCR process involved thoroughly mixing the additives and adding them to small PCR tubes at a volume of 23 l. Next, individually were added 2 ul of extracted DNA from each sample to each tube, resulting in a total of 25 ul, and placed these tubes inside the Bio-Rad thermocycler (USA). The PCR program for the LacZ3 gene and 16 SrRNA genes is similar (26, 27). (Table 2).

Table (1): The product size, primer sequence, and targeted gene.

Primer name	Primer Sequence	Gene	Amplicon size
lacZ3	F: 5- TTGAAAATGGTCTGCTGCTG -3	β-galactosidase	243bp
	R: 5- TATTGGCTTCATCCACCACA -3		
16SrRNA	*27-F* AGAGTTTGATCCTGGCTCAG	16S ribosomal RNA	1500bp
	1492-R GGTTACCTTGTTACGACTT		

Table (2): The PCR program for the LacZ3 gene and 16 SrRNA genes.

Stage	Temperature	Time	Cycle
Denaturation	95C°	5 min	one
Denaturation	95 C°	30 Sec	Thirty cycles
Annealing	60 C°	30 Sec	
Elongation	72 C°	45 Sec	
Elongation	75C°	7 min	one

With gel electrophoresis, a 2% agarose gel, and a 100-bp ladder DNA marker from Erlangen, Germany (Peqlab), The target sequence amplicons were found.

Gene sequencing of 16S rRNA gene

Specialized software was used to analyze the PCR products of that the Macrogen Corporation of Korea sent for to be sequenced for two of the isolates using an automated DNA sequencer.
The phylogenetic tree was constructed using the neighbor-joining method.

Statistical Analysis

The Statistical Analysis System (SAS) (28) tool was used to determine the impact of various components on the research parameters. This investigation used the chi-square test to compare statistically significant percentages (0.05 and 0.01 likelihood).

Results and discussion

The study's findings indicated that the *E. coli* (forty-five isolates) isolated from the water samples were 100% positive. In the Durham tube on MacConkey broth media, all the *E. coli* isolates displayed a change in color from bright green to cloudy green, along with the presence of gas. All of the isolates in the EMBA medium showed a change in color to metallic green. The addition of the kovach reagent to the pepton water buffer medium results in the formation of a red ring (Figure 1).

Figure (1): *Escherichia coli* isolates on Eosin Methylene Blue agar(EMBA) and a red ring on the pepton water buffer media.

Every E. *coli* isolate yielded positive results from the distinctive biochemical tests (IMViC test, TSI, and motility), with the exception of the Voges-Proskauer (VP) and citrate utilization tests.

The data in Table 3 shows the number of *E. coli*-positive isolates found in water samples collected at several stations, but it does not differentiate between drinking and waste water.

Table (3): The quantity and percentage of *E. coli* positive isolates from the drinking water station.

Station name	No. of isolates	Positive No. (%)
Al- Karama	10	22.2% (10/45)
Al Wathba	10	22.2% (10/45)
Al-Wahda	6	13.3(6/45)
Al-Rustamiya	10	22.2% (10/45)
Medical city	9	20%(9/45)

It is important to note that the presence of *E. coli* in water indicates fecal contamination and can pose a serious health risk. (29), While the percentages in the table may seem small, any *E. coli* presence is a cause for concern. The discharge of sewage wastewater and manure effluents, which contain various bacterial pathogens, is one of the main causes of aquatic resource decay (30, 31). Fecal bacteria can live for a long time in water, which makes them a good way to find out what kinds of pathogens might be in surface water (32 , 33). The results show that there is a lot of *E. coli* isolate contamination at the study sites, which means that people should be careful when using the water from these sites for drinking, farming, fishing, and other purposes. This discovery highlights how the water treatment plant's *E. coli* concentration is a suitable indicator for tracking the water quality.

Antibiotic resistant

The isolates of *E. coli* from drinking water treatment plants were very resistant (between 20% and 100%) to antibiotics like TE, Amp, E, CAZ, and CTX. They were less resistant (between 0% and about 27%) to antibiotics like ATM, MEM, CN, Cl, IPM, AAM, and AUG. Ciprofloxacin and cefotriaxone sensitivity are 100% in the Al-Wahda plant.

Al-Karama plants exhibit a resistance rate of 100%, while Al-Wathba plants demonstrate a resistance rate of 73% and 80%, respectively.

In Figure 2, the *E. coli* isolates can be seen from wastewater treatment plants in a medical city are very sensitive (778% to 100%) to ATM, CN, C, IPM, ASM, AG, and CIP. However, they are only moderately resistant (33% to 44%) to MEM. The isolates are also highly resistant (44% to 100%) to TE, E, AM, CTX, CAZ, and MEM.

There was 100% resistance to antibiotics (AAM, E, TE, CTX) and high resistance (35%–70%) in Al-Rustymia wastewater treatment plants (CN, CAZ, CIP, CRO, IPM, AG). Only MEM demonstrates 100% sensitivity, whereas ATM and AZM show less resistance (10%) to 15%. The p-value for each antibiotic is statistically significant (p-value < 0.01), which means there is a very low probability that the observed resistance occurred by chance.

Escherichia coli is exhibiting a rising trend in AR emergence and dissemination (34, 35). The transfer of resistance across bacteria facilitates the development and establishment of AR, a significant concern that diminishes the therapeutic effectiveness of antibiotics against infections (36, 37). The association between the frequency of AR patterns in aquatic settings and *E. coli* isolates has been the subject of several studies (38, 39).

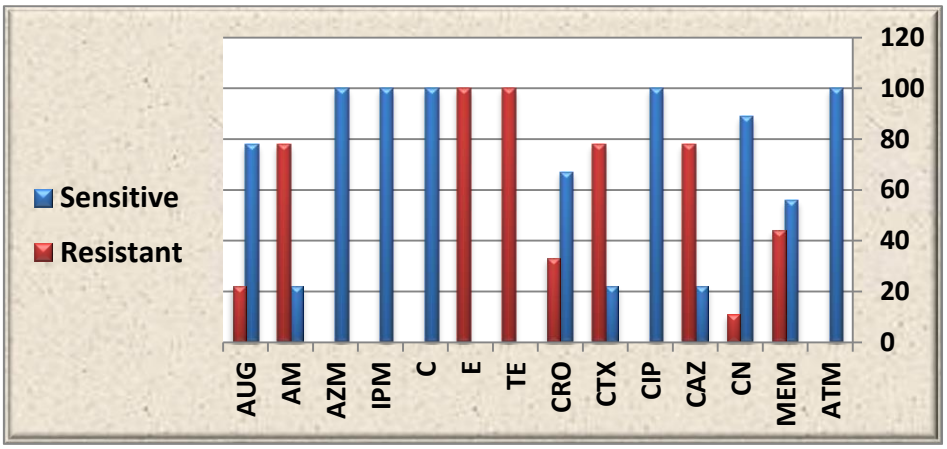

Figure (2): The percentage of antibiotic resistance exhibited by *E. coli* bacteria isolated from wastewater at Medical City Hospital

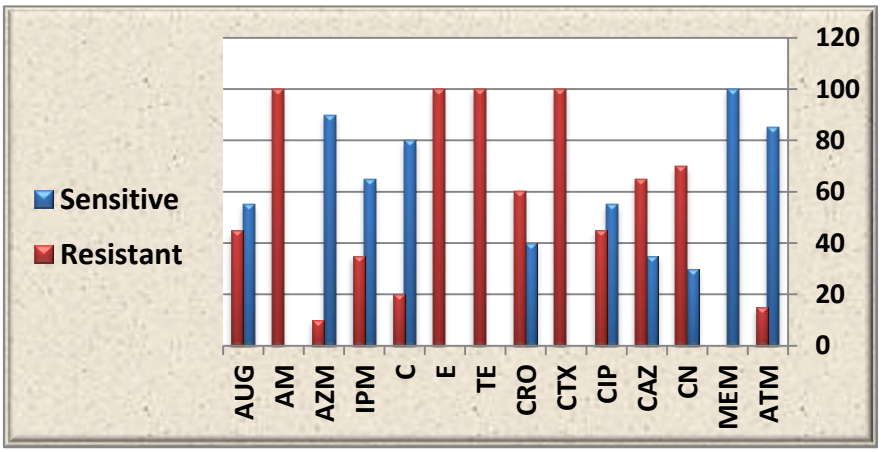

Figure (3): The percentage of antibiotic resistance exhibited by *E. coli* bacteria isolated from wastewater at Al-Rustamiya wastewater treatment plant.

The data show a significant difference in the percentage of antibiotic resistance in *E. coli* isolates between different regions. In some regions, such as medical cities, the resistance rate was very high for almost all antibiotics. Other regions, like Al-Rustaimiya Station, also exhibit a high resistance rate.

The results of this study show that there is a widespread prevalence of antibiotic resistance among *E. coli* isolates in different regions (40). It also shows that there is a significant difference in the resistance rate between different regions. These results raise serious concerns, as they indicate that many infections caused by *E. coli* may be difficult or impossible to treat with antibiotics. (41, 42).

lacZ3 and 16SrRNA genes detection

All the positive *E. coli* samples in this study had 243 base pairs (bp) for the lacZ3 gene and 1500 bp for the 16S rRNA gene bands (Figures 4,5, 43, and 44). the 16S rRNA was used to gene sequence because it is present in all prokaryotes, including almost all bacteria, and its function remains constant over time (45).

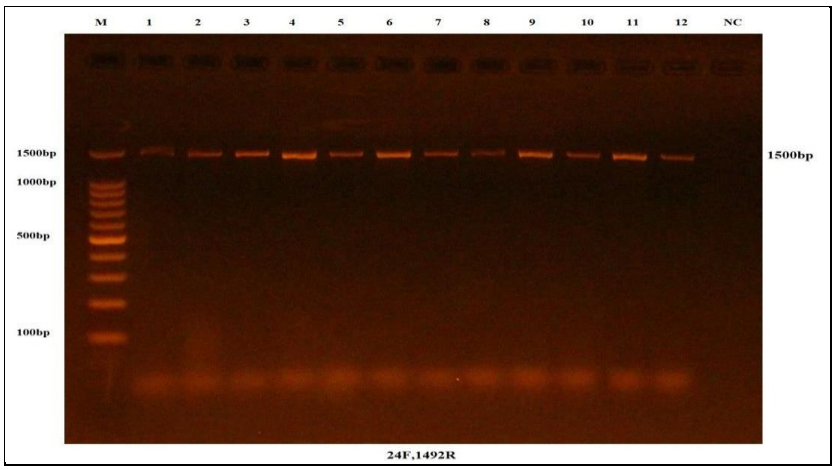

Figure (4): Results of the amplification of 27*F,1492R genes* of bacterial species were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-12resemble 1500 bp PCR products.

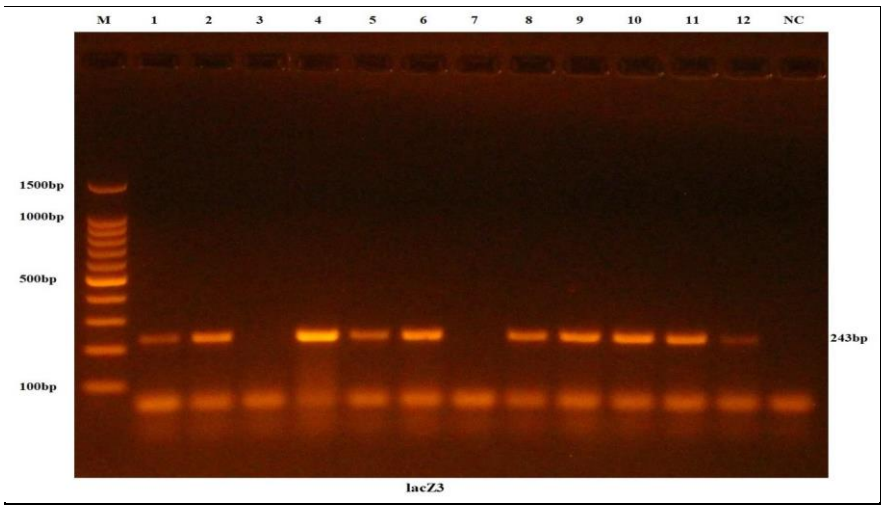

Figure (5): Results of the amplification of *lacZ3 genes* of bacterial species were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-12resemble 243 bp PCR products.

Sequencing of 16S rRNA Gene

The 16S rRNA gene, which has both hypervariable and highly conserved sections, is present in at least one copy in bacterial species. Recent years have shown a growing interest in using 16S rRNA gene sequences to distinguish novel bacteria from strains(46).

It has developed into a valuable tool for identifying bacteria (47). One of the greatest directives of microbiology is Bergey's Manual of Systematic Bacteriology, which uses 16sRNA as a tool for bacterial identification (48).

Phylogeny, which determines the relationships between bacteria, has gained significance as a method to identify an unknown genus or species.(49)

Escherichia coli-like organisms pose significant challenges for species identification when determined by visual and biochemical traits. The 16S rRNA region sequencing method is nevertheless a good one for identifying species. Geneious software was used to

compare the 16S rRNA gene it to the type strains identified through 16S rRNA gene sequencing at GenBank.

Phylogenetic trees, a type of branching diagram, illustrate the evolutionary relationships between different organisms. This study's tree illustrates the relationships between different strains of (*E. coli*). The genetic distance between SKLAN and YZEC is 0, and these strains split off pretty early on the tree, so HS probably comes from a different branch (Figure 6).

Based on the cluster diagram described, can say that PP463717.1 is likely closely related to some of the other *E. coli* strains in the diagram. However, Genbank has the identified isolate's partial 16S rRNA gene sequence under accession numbers PP463717 and PP463722. Deposit number PP463717 refers to the isolate (HHWW), and deposit number PP463722 refers to the isolate (HS) that was registered in GenBank.

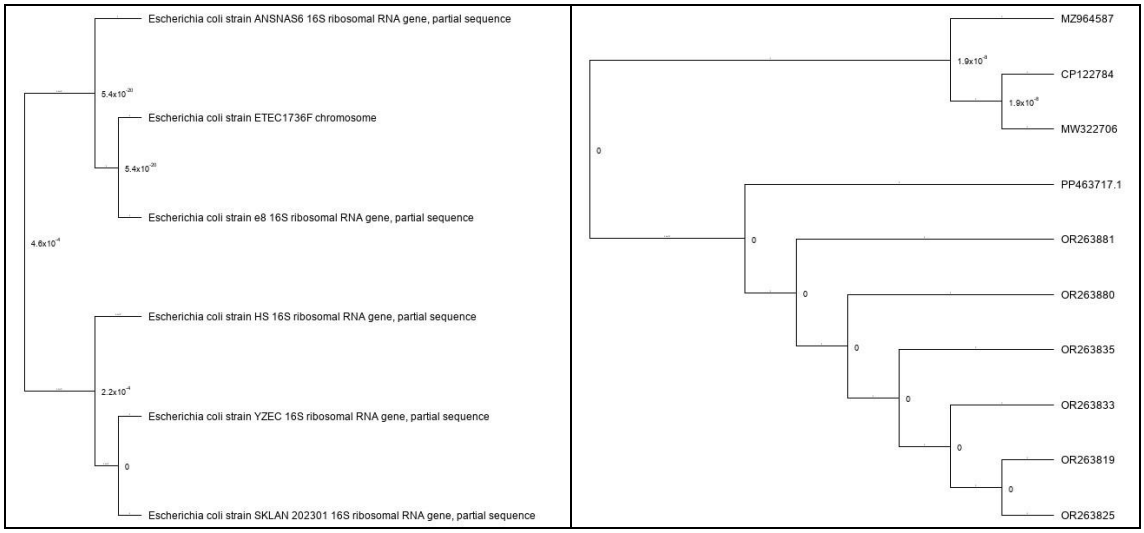

Figure (6): a phylogenetic tree, which is a type of branching and cluster diagram of *Escherichia coli* isolates. [The genetic tree on the left indicates the isolate (HS) and the genetic tree on the right indicates the isolate (HHWW)].

Based on a partial 16S rRNA sequence alignment and a phylogenetic tree, The two strains, HHWW and HS, were very similar to *E. coli* (100%).

The 16S rRNA gene is particularly interesting because it can identify genus and species of organisms that don't match standard biochemistry profiles, strains those commercial systems don't identify well or likely enough, or taxa that aren't typically associated with human diseases (50).

Conclusion

The prevalence of antibiotic-resistant *E. coli* strains in water treatment plants and apartial 16S rRNA sequence alignment showed that the two new strains (HHWW and HS) were 100% similar to *E. coli* and were closely related to other species in the genus *Escherichia*. We also conclude that the existence of antibiotic-resistant *E. coli* in water treatment plants poses a significant public health risk, particularly if the resistance profile includes routinely prescribed medications.

References

1. Odonkor, S. T. and Addo, K. K. (2018). Addo, I. Y. (2018). Prevalence of multidrug-resistant Escherichia coli isolated from drinking water sources. International journal of microbiology, 2018.
2. Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. AIMS microbiology, 4(3): 482.
3. Daly, M.; Powell, J.; O'Connell, N.H.; Murphy, L. and Dunne, C.P. (2023). Antimicrobial Resistance Is Prevalent in E. coli and Other Enterobacterales Isolated from Public and Private Drinking Water Supplies in the Republic of Ireland. Microorganisms 11(5): 1224.
4. Bortolotti, D.; Trapella, C.; Bragonzi, A.; Marchetti, P.; Zanirato, V.; Alogna, A., *et al*., (2019). Conjugation of LasR quorum-sensing inhibitors with ciprofloxacin decreases the antibiotic tolerance of P. aeruginosa clinical strains. Journal of Chemistry.
5. Mukhlif, S.S.; Husseinm, Z.T.; Taha, M.; Taen, M.M. and Rustoom, H. Q. (2019). Prevalence of Panton-Valentine Leukocidin (PVL) Gene among Staphylococcus Aureus Isolated from Environmental Surface of working area in Microbiology Laboratory, REVISTA AUS 26.4
6. Gonçalves, V.D.; Meirelles-Pereira, F.; Cataldo, M.; Fonseca, B.D.O.; Nogueira, B.A.; Olivella, J.G.B., *et al*. (2019). Detection of multidrug-resistant Enterobacteriaceae isolated from river waters flowing to the Guanabara Bay and from clinical samples of hospitals in Rio de Janeiro, Brazil. Biomedica , 39: 135–149
7. Collignon, P. J. and McEwen, S. A. (2019). One health—its importance in helping to better control antimicrobial resistance. Tropical medicine and infectious disease, 4(1): 22.
8. AL-Jubouri, S. S. and Shami, A. M. (2022). Molecular Detection of Cephalosporin Resistance Genes in Escherichia coli Isolated from Urinary Tract Infections in Baghdad Hospitals. Iraqi journal of biotechnology, 21(2): 145-152.
9. Hala M.A. (2024) .The antibacterial activity of glycolipopeptide produced from Lactoococcus lactis HN21 aginst some clinical pathogens in combined with some standard antibiotics. Iraqi Journal of Agricultural Sciences, 55(Special Issue), 12-24.
10. AL-Lami, R. A.; Al-Hayanni, H. S. and Shehab, Z. H. (2022). Molecular investigation of Some beta-lactamase genes by PCR and DNA sequencing techniques in clinical Escherichia coli. Iraqi Journal of Science, 63(10): 4205-4212.
11. Hammadi, A. H.; Yaseen, N. N. and Al-Mathkhury, H. J. (2015). Molecular Detection of Some β-lactamases Genes in Uropathogenic Escherichia coli. Iraqi Journal of Science, 1925-1931.
12. Srinivasan, R.; Karaoz, U.; Volegova, M.; MacKichan, J.; KatoMaeda, M.; Miller, S., *et al*. (2015). Use of 16SrRNA Gene for Identification of a Broad Range of Clinically Relevant Bacterial Pathogens. PLOS ONE. 10(2): 1-22.
13. Adnan, M.; Aziz, I. H. and Al-Deresawi, M. S. (2014). Molecular detection of Proteus mirabilis using PCR technique among urinary tract infection patients. Iraqi journal of biotechnology, 13(2), 35-47.
14. WHO (2020). Antimicrobial Resistance. Geneva: WHO.
15. Ranjbar, R. and Alam, M. (2023). Antimicrobial Resistance Collaborators (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Evidence Based Journals*, *27*: 16 - 16.
16. Gaze, W.H. and Depledge, M. (2017). Antimicrobial Resistance: Investigating the Environmental Dimension-Frontiers 2017: Emerging Issues of Environmental Concern Chapter 1.
17. Hussein, Z. T.; Mukhlif, S. S.; Taha, M. and Ali, S. A. (2023). Identification and Characterization of Pseudomonas aeruginosa 16S rRNA Gene Isolated from Contaminated Soil with Oil Residues. IOP Conf. Series: Earth and Environmental Science, 1215(2023): 012001
18. Skof, A.; Koller, M.; Baumert, R.; Hautz, J.; Treiber, F.; Kittinger, C., *et al*. (2024). Comparison of the Antibiotic Resistance of *Escherichia coli* Populations from Water and Biofilm in River Environments. Pathogens (Basel, Switzerland), 13(2): 171.

19. Al-Kareem, A. (2023). Isolation and Diagnosis of Vancomycin-Resistant Escherichia coli from Different Water Sources. Egyptian Journal of Aquatic Biology and Fisheries, 27(2): 585-590.
20. Mahdi, Z.M.; Hatif, Z.H.; Younis, M.H. and Allah, R.A.A.A. (2023). Detection of Resistance Genes (Blandm and Blavim) of Escherichia Coli in the Aquatic Environment. Iraqi Journal of Science, 6193-6203.
21. Tambi, A.; Brighu, U. and Gupta, A. B. (2023). Methods for detection and enumeration of coliforms in drinking water: a review.
22. Rashid, A. M. and Al Ameer, A. K. (2023). Detection of Escherichia coli From Urinary Tract by Using by VITK 2 System. Journal of Survey in Fisheries Sciences, 10(3S): 1564-1568.
23. Clinical and Laboratory Standards Institute (2021). Performance standards for antimicrobial susceptibility testing. CLSI supplement M100, 106-112.
24. García-Alegría, A. M.; Anduro-Corona, I.; Pérez-Martínez, C. J.; Guadalupe Corella-Madueño, M. A.; Rascón-Durán, M. L. and Astiazaran-Garcia, H. (2020). Quantification of DNA through the NanoDrop Spectrophotometer: Methodological Validation Using Standard Reference Material and Sprague Dawley Rat and Human DNA. International journal of analytical chemistry.
25. Abu-Sini, M.K.; Maharmah, R.A.; Abulebdah, D.H. and Al-Sabi, M.N.S. (2023). Isolation and Identification of Coliform Bacteria and Multidrug-Resistant Escherichia coli from Water Intended for Drug Compounding in Community Pharmacies in Jordan. *Healthcare*, 11(3): 299.
26. Molina, F.; Lopez-Acedo, E.; Tabla, R.; Roe, I.; Gomez, A. and Rebollo, J.E. (2015). Improved detection of Escherichia coli and coliform bacteria by multiplex PCR. BMC Biotechnol, 15: 48
27. AL-Nasrawi, M. M. and AL-Hashimy, A. B. (2020). Molecular study of some virulence genes of Escherichia coli isolated from women with urinary tract infection in AL-Najaf City. Iraqi journal of biotechnology, 3(19).
28. SAS. (2018). Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.
29. Sugiah, S.; Mutmaina, G. N.; Mamay, M. and Nurisani, A. (2023). "Isolation and identification of escherichia coli in well water located in Garut Regency", Science Midwifery, 11(1): 195-201.
30. Adefisoye, M.A. and Okoh, A. I. (2016) "Identification and antimicrobial resistance prevalence of pathogenic Escherichia coli strains from treated wastewater effluents in Eastern Cape, South Africa," Microbiology Open, 5(1): 143–151.
31. Agustina, A. C. (2021). Analyze the health care system and the Identifikasi Escherichia coli dari Depo Air Minum Isi Ulang di Kota Semarang. Life Science, 10(1): 23–32.
32. Nurliyana, M. R.; Sahdan, M. Z.; Wibowo, K. M.; Muslihati, A.; Saim, H.; Ahmad, S. A., *et al*. (2018). The detection method of Escherichia coli in water resources: A review. In Journal of Physics: Conference Series, 995: 012065.
33. Berendes, D.; Knee, J.; Sumner, T.; Capone, D.; Lai, A.; Wood, A., *et al*. (2019). Gut carriage of antimicrobial resistance genes among young children in urban Maputo, Mozambique: Associations with enteric pathogen carriage and environmental risk factors. PLoS One., 14(11): e0225464.
34. Bobate, S.; Mahalle, S.; Dafale, N. A. and Bajaj, A. (2023). Emergence of environmental antibiotic resistance: Mechanism, monitoring and management. Environmental Advances, Volume 13.
35. Catalano, A.; Iacopetta, D.; Ceramella, J.; Scumaci, D.; Giuzio, F.; Saturnino, C., *et al*. (2022). Multidrug Resistance (MDR): A Widespread Phenomenon in Pharmacological Therapies. Molecules (Basel, Switzerland), 27(3): 616.
36. Baron, S. A.; Diene, S. M. and Rolain, J. M. (2018). Human microbiomes and antibiotic resistance. Human Microbiome Journal, 10: 43-52.
37. Berendes, D.; Knee, J.; Sumner, T.; Capone, D.; Lai, A.; Wood, A., *et al*. (2019). Gut carriage of antimicrobial resistance genes among young children in urban Maputo, Mozambique: Associations with enteric pathogen carriage and environmental risk factors. PLoS One, 14(11): e0225464.
38. Ben, Y.; Fu, C.; Hu, M.; Liu, L.; Wong, M. H. and Zheng, C. (2019). Human health risk assessment of antibiotic

resistance associated with antibiotic residues in the environment: A review. Environmental research, 169: 483-493.

39. Wright, G. D. (2010). Antibiotic resistance in the environment: a growing concern. International journal of medical microbiology, 290(6): 349-354.
40. O'Neill J. (2016). Tackling drug-resistant infections globally: final report and recommendations. London: Review on Antimicrobial Resistance.
41. Thomas, C. and Tampé, R. (2020). Structural and Mechanistic Principles of ABC Transporters. Annual review of biochemistry, 89: 605–636.
42. Imran, M.; Jha, S. K.; Hasan, N.; Insaf, A.; Shrestha, J.; Shrestha, J., *et al*. (2022). Overcoming multidrug resistance of antibiotics via nanodelivery systems. Pharmaceutics, 14(3): 586.
43. Dinakaran, D. R.; Shanmugam, H.; Nambi, I. M. and Doble, M. (2022). Comparative analysis of molecular and conventional methods for bacteriological water quality assessment in drinking water resources around Chennai. Water Practice and Technology, 17(3): 708-718.
44. Hasan, M. H.; Abdulla, S. M. and Ulaiwi, A. H. (2022). Isolation and molecular detection of some virulence associated genes in avian pathogenic *E. coli*.
45. Love, C. J.; Masson, B. A.; Gubert, C. and Hannan, A. J. (2022). The microbiota-gut-brain axis in Huntington's disease. In International Review of Neurobiology, 167: 141-184.
46. Priyanka, T.; Kantha, D. A.; Vinuprakash, K. C. and Harsh, T. (2019). Isolation, characterization and molecular identification of bacteria from commercial sources using 16S rRNA sequencing for domestic waste water treatment. International Journal of Innovative Technology and Exploring Engineering, 8: 2278-3075.
47. Srinivasan, R.; Karaoz, U.; Volegova, M.; MacKichan, J.; Kato-Maeda, M.; Miller, S., *et al*. (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PloS one, 10(2), e0117617.
48. Johnson, J. S.; Spakowicz, D. J.; Hong, B. Y.; Petersen, L. M.; Demkowicz, P.; Chen, L., *et al*. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nature communications, 10(1): 5029.
49. Church, D. L.; Cerutti, L.; Gürtler, A.; Griener, T.; Zelazny, A. and Emler, S. (2020). Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. Clinical microbiology reviews, 33(4): 10-1128.
50. Ranjan, R.; Rani, A.; Metwally, A.; McGee, H. S. and Perkins, D. L. (2016). Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. Biochemical and biophysical research communications, 469(4): 967–977.