International Journal of Medical Science and Clinical Research Studies

ISSN(print): 2767-8326, ISSN(online): 2767-8342

Volume 05 Issue 02 February 2025

Page No: 354-358

DOI: https://doi.org/10.47191/ijmscrs/v5-i02-29, Impact Factor: 8.188

Identification and Isolation of E. Coli Bacteria from Ro Water in the Center of Karbala and its Surrounding Areas

Alaa Yaqoob Rahi[1], Z. Abdul Alamir Mezher[2]

[1]Department of Science, College of Basic Education, University of Sumer, Iraq

[2]Department of biology, college of education for pure science, university of Karbala, Karbala, Iraq

ABSTRACT

The aim of this research was to find out if the water (Ro) in the majority of Iraqi houses contains E. coli bacteria because of the prevalence of diarrhea and other illnesses among the populace. The goal was accomplished by. The study samples were collected from R. O water filling stations in Karbala, including 40 samples from Karbala Center, 20 samples from Tuwairij District, 20 samples from Al-Hussainiya District, and 20 samples from Ain Al-Tamr District for the purpose of investigating the E. coli bacteria contaminating these waters, where microscopic and cultural examinations and biochemical tests were used. The results of these tests showed that 3 samples of R.O water out of 100 samples, with an isolation rate of 4.5%, gave positive results through their growth on selective and differential media and their fermentation of lactose sugar. On the other hand, the research results showed that the highest percentage of bacteria isolation was from R.O water filling stations in Ain Al-Tamr district with a percentage of 2.5) 2.2%) and the lowest percentage was from R.O water filling stations in Al-Hussainiya district with an isolation percentage of 1.7) 1.5%), while R.O water filling stations in the center of the governorate of Karbala and Twerij district did not record the least contamination with coliform bacteria (0.5 %). Through the research, the results showed that R. O water in some areas is contaminated with E. coli bacteria, which may be the source of human waste, which indicates the inefficiency of these stations.

KEYWORDS: *bacteria, E. Coli, R.O water*

ARTICLE DETAILS

Published On:
22 February 2025

Available on:
https://ijmscr.org/

INTRODUCTION

Water, particularly drinking water, is contaminated by a variety of sources, the majority of which are sewage and human waste. Coliform bacteria are found in human waste, and their presence in drinking water indicates fecal pollution. Urinary tract infections are primarily caused by coliform bacteria. Among the most prevalent infections in the urinary system are gram-negative bacilli. Ninety percent of UTIs are caused by members of the enterobacteriaceae family, mostly E. coli [1,2]. There are two ways that this bacterium can infect humans: exogenous infection, which occurs when the infection originates in the human body, such as the intestines [3].

The antigenic makeup of E. coli is complicated. The three primary categories of antigens include heat-labile and somatic antigens. The former is red (Flageilar antigen) and the latter are heat-labile. According to Wang et al. (2020), exposure to the somatic and capsular antigens is the primary source of the majority of E. coli infections. Additionally, E. Coli possesses numerous virulence factors that aid in infection. Among these include (A. Type I fimbriae Type). toxic necrosis factor, or toxins. hemolytic component. Lipopolysaccharide Enzyme synthesis (cellulose, lipolytic protease, alkaline phosphatase) The iron economy and invasion and serum resistance mechanism [5]. Since these elements facilitate the invasion and dissemination of bacterial cells to host tissue cells, which results in clinical illnesses, they also aid in the colonization of host surfaces by bacteria and the deterioration or destruction of host defenses [6]. The aim of the study: Due to the spread of many diseases and cases of diarrhea among citizens, the study aimed to investigate the presence of E. coli bacteria in the water (Ro) circulating in most Iraqi homes. Investigation of coliform

bacteria in (R.O) water and Diagnosis of coliform bacteria morphologically and physiologically.

METHOD AND MATERIALS

Preparation of specimens

The current study included collecting 100 samples from R.O water filling stations in Karbala Governorate during the period from October 2022 to January 2023, with 30 samples from filling stations in the Karbala center, 20 samples from stations in Ain Al-Tamr district, 20 samples from stations in Al-Hussainiya district, and 20 samples from Twerij district. The samples were kept in special sterile boxes, and then transferred to the laboratory for the purpose of caring for them and diagnosing them, as they were planted in Petri dishes containing nutrient agar medium and MacConkey medium, solid eosin blue medium, using the planning method, and the dishes were incubated at a temperature of 37°C for 10-24 hours for the purpose of diagnosing bacteria growing on the media.

Sterilization

All the prepared and synthetic culture media were sterilized in an autoclave (Model: TM-XD50DV, Jiangxi Binding Medical Equipment Co., LTD**)** at a temperature of (121°C) and a pressure of (15 pounds/inch) for 15 minutes. While the glassware used was sterilized in an electric oven at a temperature of (168°C) for an hour and a half.

Preparation of culture media

Ready culture media, the culture media were prepared according to the manufacturers' instructions as shown in previous studies [6,7]. After preparing the media, they were sterilized using an autoclave, then poured into Petri dishes or test tubes, and then stored at 4°C until use.

Identification of isolated bacteria

Agricultural characteristics: The bacterial colonies were initially identified based on their morphological characteristics in terms of shape, size and color of the colonies. They appeared as pink colonies fermenting lactose on MacConkey medium and bright green on eosin blue medium. **Microscopic properties:** The microscopic characteristics of bacterial cells were studied by performing Gram staining. A single pure colony growing on a nutrient agar medium was taken using a sterile loop, placed on a glass slide with a few drops of sterile water, then the cells were spread and left to dry. They were fixed by passing them over a flame three times quickly and stained with Gram stain. The shape of the cells and their aggregation were observed [8] by examining them under a light microscope.

Biochemical tests

Catalase test: The test was performed by transferring a small amount of bacterial growth grown on the culture medium at 24 hours of age using sterile wooden sticks to the surface of a clean, dry glass slide, then adding a drop of 3% hydrogen peroxide (H2O2) blocked in the paragraph. The appearance of gas bubbles indicates the result [9]. **Oxidase test:** The test was performed by transferring a quantity of bacterial growth using sterile wooden sticks to a filter paper saturated with the prepared reagent. The bacterial colonies turning purple indicates a positive result [10] to detect. **Motility test:** The test was performed by inoculating the tubes containing the medium with the bacterial culture and incubating at a temperature of 37 for 24-48 hours. The spread of growth outside the borders of the stab indicates a positive result [11]. **Indole test:** The test was performed by inoculating tubes containing the culture medium with the bacterial culture and incubating at 37°C for 18-24 hours. Then a few drops of Kovacs reagent were added to each tube with good shaking. The appearance of a red ring at the top of the medium indicates a positive result [12]. **Methyl red test:** The test was performed by inoculating tubes containing the M.R.V.P Medium with the bacterial culture and incubating them at 37°C for 24-48 hours. Then 5 drops of methyl red indicator were added and the tube was shaken. The appearance of the red color in the tube indicates the complete decomposition of sugars and the production of acid.

RESULTS

Isolation and diagnosis of E. coli

The results of the study proved that 3 isolates belonged to E. coli bacteria out of 84 samples taken from water filling stations. R. Then the bacterial isolates were diagnosed by studying the morphological and microscopic characteristics, then the results were confirmed by biochemical tests as follows:

Cultural characteristic

It has pink colonies on MacConkey agar as a result of fermenting lactose sugar, as shown in Figure 1. The results also showed that the bacterial colonies are smooth, circular, and have a green metallic sheen on Eosin methylene blue medium, as shown in (Figure 1) also.

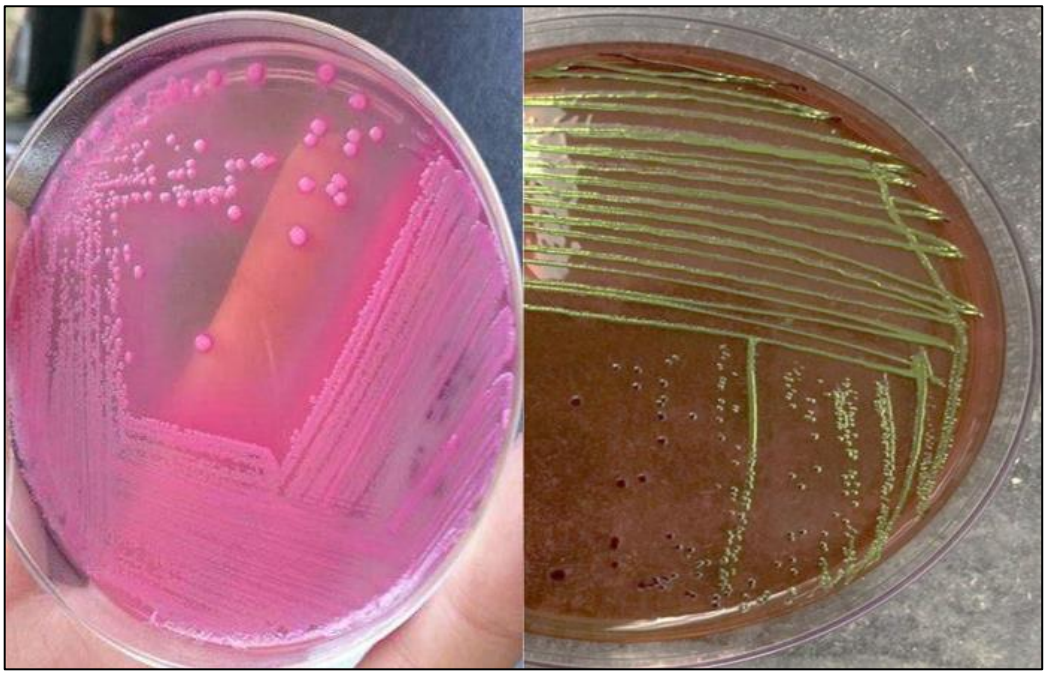

Figure 1. The Colonies of E. coli on MacConkey agar and Eosin methylene blue agar

Microscopic properties

The bacteria are small, Gram-negative rods with a pink color that do not form spores after Gram staining.

Biochemical Test

Catalase test, which is done by forming air bubbles when hydrogen peroxide is added to the bacterial colony placed on glass slides. Motility test, which is done by observing the spread of growth outside the stab limits. Methyl red test, which is done by forming a red color after adding the reagent to the bacterial culture, which is evidence of the complete decomposition of sugars and the production of acid.

Fermentation of glucose, lactose and menthol. E - Positive for indole test by formation of red indole ring as a result of decomposition of tryptophan amino acid and formation of indole ring. Grows on triple sugar iron agar medium and its growth is A/A with production of CO_2 gas. Negative tests, Negative for citrate consumption due to its inability to consume citrate as a sole source of carbon by not changing the color of the medium from green to blue. Negative for oxidase and FoxPro score tests.

Table 1. Biochemical test for all E. coli isolates

TESTES	E-coli
Catalyst	-
Oxidase	-
Indole	-
methyl red	+
Ester consumption	+
motility	-
Glucose fermentation	+
lactose fermentation	+
	+

Distribution of E. coli isolates by geographical location

Three isolates of E. coli bacteria were diagnosed with an isolation rate of 4% out of a total of 100 samples collected from RO water filling stations in karbala Governorate during the period from October 2022 to January 2023, including 300 samples from R.O water filling stations in the center of karbala Governorate, 20 samples from R.O water filling stations in Twerij District, 20 samples from R.O water filling stations in Al-Hussainiya District, 20 samples from R.O water filling stations in Ain Tamr District. The results of the study showed that the highest percentage of bacteria isolation was from R.O water filling stations in Al-Hussainiya District with a percentage of (2.5(2.2%), followed by R.O water filling stations in Ain Tamr District with an isolation rate of

(1.7(1.5%), while R.O water filling stations in the center of Karbala Governorate and Twerij District did not record any contamination with E. coli bacteria (0%) as shown in Table (2).

Table .2. Numbers and percentages of E. coli isolation according to geographical location

location of stations	Total number of samples	Number of positive samples	Percentage%
City Centre Stations	**40**	**0**	**0%**
Twerij district station	**20**	**0**	**0%**
Al-Hussainiya District Station	**20**	**2.5**	**2.2%**
Ain Tamr District Station	**20**	**1.7**	**1.5%**
Total	**100**	**5**	**4%**

DISCUSSION

E. coli bacteria are considered natural flora of the human digestive system and usually inhabit the colon [13] as a benign symbiont, 3 isolates of E.coll bacteria were identified with an isolation rate of 4% out of a total of 100 samples collected from R.O water filling stations in karbala Governorate during the period from October 2022 to January 2023. After the bacterial species under study were initially diagnosed through studying some cultural and microscopic characteristics, E.coll bacteria appeared in the form of pink colonies on MacConkey Agar as a result of fermenting lactose sugar of medium size while having a metallic luster Green on Eosin Blue medium [14].

Microscopic examination results also showed that E.coll cells are short, Gram-negative rods that do not form spores, which is in agreement with Lee and Jeon, (2021). For the chemistry tests, the results in Table (4-1) showed a negative test for oxidase, as the oxidase enzyme test depends on the bacteria possessing cytochrome C oxidase, which is necessary for the process of cellular respiration, as it stimulates the transfer of electrons. Most isolates do not have the ability to produce the oxidase enzyme, and therefore, colon bacteria follow other paths used in the respiration process, which are cytochrome [16] bo3 and cytochrome bd. While the catalase test relied on the presence of the catalase enzyme in bacterial cells that have the ability to produce hydrogen peroxide, including E. coli bacteria, which gave positive results for this test, as hydrogen peroxide is considered toxic to cells, as the enzyme works to decompose hydrogen peroxide into water, H2, and O2) [17].

The ability of E. coli bacteria to give a positive result for the indole test is attributed to the production of the enzyme Tryptophan's, which converts the amino acid tryptophan within the medium components to indole. The use of Kovacs reagent (hydrochloric acid and amyl alcohol) leads to the formation of the red indole party because amyl alcohol does not dissolve in water, but rather works to color the fatty layer in the upper part [18]. All isolates showed a positive result for the methyl red test because the bacteria ferment glucose sugar and the final product is three acids, which increases the acidity of the medium and lowers the pH 4.4, which leads to a change in the color of the reagent to red [19].

CONCLUSIONS

Fecal contamination of R.0 water is indicated by the presence of E. coli bacteria, which may be the cause of human and animal waste. In contrast to other districts, the RO water filling stations in the Ain Al-Tamr district and the core of the Karbala governorate were free of bacterial contamination. Even if the phenotypic investigation may not be enough, more effective tests yield more encouraging outcomes. We suggested Drinking water filling stations should be kept hygienic. The requirement to regularly inspect water filling and desalination facilities in order to identify the sources of bacterial contamination and to pay attention to employees' personal hygiene.

REFERENCES

I. Labella, A., Molero, R., Leiva-Rebollo, R., Pérez-Recuerda, R., & Borrego, J. J. (2021). Identification, resistance to antibiotics and biofilm formation of bacterial strains isolated from a reverse osmosis system of a drinking water treatment plant. *Science of The Total Environment*, *774*, 145718.

II. Luo, L. W., Wu, Y. H., Chen, G. Q., Wang, H. B., Wang, Y. H., Tong, X., & Hu, H. Y. (2022). Chlorine-resistant bacteria (CRB) in the reverse osmosis system for wastewater reclamation: isolation, identification and membrane fouling mechanisms. *Water Research*, *209*, 117966.

III. Benladghem, Z., Seddiki, S. M. L., & Mahdad, Y. M. (2020). Identification of bacterial biofilms on desalination reverse osmosis membranes from the mediterranean sea. *Biofouling*, *36*(9), 1065-1073.

IV. Wang, Y. J., Deering, A. J., & Kim, H. J. (2020). The occurrence of shiga toxin-producing E. coli in aquaponic and hydroponic systems. *Horticulturae*, *6*(1), 1.

V. Chen, Z., Zhu, S., Zhao, L., Wang, X., Liang, M., Mao, D., & Ren, H. (2021). Occurrence of high-risk mcr-1 gene and blaNDM-1 positive superbug in the reverse osmosis filter cartridges of the household water purifiers. *Journal of Hazardous Materials Letters*, *2*, 100011.

VI. Ragab, S., Gouda, S. M., Abdelmoteleb, M., & El-Shibiny, A. (2024). The role of identified and characterized bacteriophage ZCEC13 in controlling pathogenic and multidrug-resistant Escherichia coli in wastewater: in vitro study. *Environmental Technology*, *45*(18), 3544-3558.

VII. Zhang, X., Wang, X., Yang, Q., Jiang, X., Li, Y., Zhao, J., & Qu, K. (2020). Conductometric sensor for viable Escherichia coli and Staphylococcus aureus based on magnetic analyte separation via aptamer. *Microchimica Acta*, *187*, 1-10.

VIII. Giacobassi, C. A., Oliveira, D. A., Pola, C. C., Xiang, D., Tang, Y., Datta, S. P. A., & Gomes, C. L. (2020, December). Sense–analyze–respond–actuate (SARA) paradigm: Proof of concept system spanning nanoscale and macroscale actuation for detection of escherichia coli in aqueous media. In *Actuators* (Vol. 10, No. 1, p. 2). MDPI.

IX. Yakubu, R. O., Lawan, M. K., Kwaga, J. K. P., & Kabir, J. (2020). Isolation, molecular detection and antimicrobial susceptibility profile of Escherichia coli O157: H7 in household-reared small ruminants in Zaria Metropolis, Kaduna state, Nigeria. *Sahel Journal of Veterinary Sciences*, *17*(4), 16-23.

X. Baek, K., Lee, J., Lee, A., Lee, J., Yoon, H. J., Park, H. K., & Choi, Y. (2020). Characterization of intratissue bacterial communities and isolation of Escherichia coli from oral lichen planus lesions. *Scientific Reports*, *10*(1), 3495.

XI. Brindha, S., & Kuroda, Y. (2022). A multi-disulfide receptor-binding domain (RBD) of the SARS-CoV-2 spike protein expressed in E. coli using a SEP-tag produces antisera interacting with the mammalian cell expressed spike (S1) protein. *International journal of molecular sciences*, *23*(3), 1703.

XII. Wang, Y. J., J. Deering, A., & Kim, H. J. (2021). Effects of plant age and root damage on internalization of Shiga toxin-producing Escherichia coli in leafy vegetables and herbs. *Horticulturae*, *7*(4), 68.

XIII. Israr, M., Jadoon, A., Ullah, M. J., Rashid, F., Maroof, L., Qazi, N. U., & Ullah, S. (2022). Prevalance and Antimicrobial Susceptibility Patterns of Salmonella Typhi and Escherichia Coli in Drinking Water of Sub-Division Hassan Khel Peshawar. *Annals of the Romanian Society for Cell Biology*, *26*(01), 1203-1215.

XIV. Lee, H., Han, H., & Jeon, S. (2021). Autonomous internal reflux of magnetic nanoparticle chains in a flow channel for efficient detection of waterborne bacteria. *Analytical Chemistry*, *93*(36), 12237-12242.

XV. Lala, A., Marlina, M., Yusuf, M., Suhendra, R., Maulydia, N. B., & Muslem, M. (2023). Reduction of Microbial Content (Escherichia coli) in Well Water Using Various Processes: Microfiltration Membranes, Aeration and Bentonite Adsorption. *Heca Journal of Applied Sciences*, *1*(1), 24-29.

XVI. Jaber, H., Oubihi, A., Ouryemchi, I., Boulamtat, R., Oubayoucef, A., Bourkhiss, B., & Ouhssine, M. (2021). Chemical composition and antibacterial activities of eight plant essential oils from Morocco against Escherichia coli strains isolated from different Turkey organs. *Biochemistry research international*, *2021*(1), 6685800.

XVII. Abu-Aqil, G., Suleiman, M., Sharaha, U., Riesenberg, K., Lapidot, I., Huleihel, M., & Salman, A. (2023). Fast identification and susceptibility determination of E. coli isolated directly from patients' urine using infrared-spectroscopy and machine learning. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *285*, 121909.

XVIII. Ali, S. A., & Al-Dahmoshi, H. O. (2022). Detection of efflux pumps gene and relation with antibiotics resistance in Uropathogenic Escherichia coli (UPEC) isolated from patients with cystitis. *Iraqi Journal of Science*, 2388-2397.

XIX. Szczuka, A., Chuang, Y. H., Chen, F. C., Zhang, Z., Desormeaux, E., Flynn, M., & Mitch, W. A. (2020). Removal of pathogens and chemicals of emerging concern by pilot-scale FO-RO hybrid units treating RO concentrate, graywater, and sewage for centralized and decentralized potable reuse. *ACS ES&T Water*, *1*(1), 89-100.