

Original Research Article

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# Identification and isolation of *E. coli* Bacteria from RO Water in the Center of Karbala and its Surrounding Areas

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## 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*,  
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enterobacteriaceae  
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## Introduction

Water, particularly drinking water, is contaminated by a variety of sources, the majority of which are sewage and human waste *E. coli* form 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* (Labella *et al.*, 2021; Luo *et al.*, 2022). 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 (Benladghem *et al.*, 2020). 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 (Chen *et al.*, 2021). 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 (Ragab *et al.*, 2024).

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.

## Materials and Methods

### 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 (Ragab *et al.*, 2024; Zhang *et al.*, 2020). 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 (Giacobassi *et al.*, 2020) 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 (H<sub>2</sub>O<sub>2</sub>) blocked in the paragraph. The appearance of gas bubbles indicates the result (Yakubu *et al.*, 2020).

**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 (Baek *et al.*, 2020) 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 (Brindha & Kuroda, 2022).

**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 (Wang *et al.*, 2021).

**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 and Discussion

### 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.

### 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<sub>2</sub> 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.

### 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).

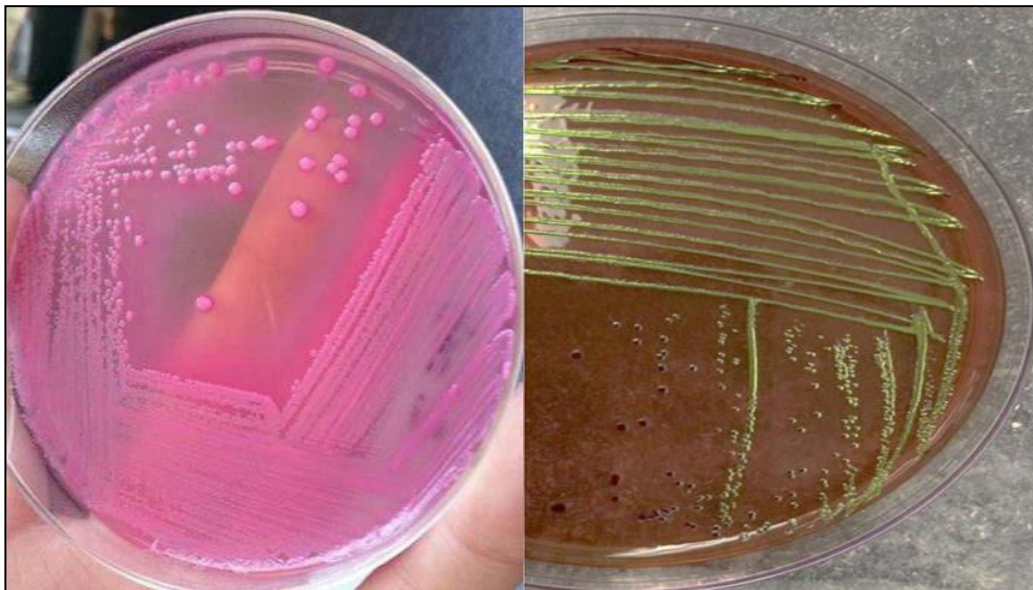
**Table.1** Biochemical test for all *E. coli* isolates

TEST	<i>E.coli</i>
Catalyst	-
Oxidase	-
Indole	-
methyl red	+
Ester consumption	+
motility	-
Glucose fermentation	+
lactose fermentation	+
	+

**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%

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



*E. coli* bacteria are considered natural flora of the human digestive system and usually inhabit the colon (Israr *et al.*, 2022) as a benign symbiont, 3 isolates of *E.coli* 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.coli* 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 (Lee *et al.*, 2021).

Microscopic examination results also showed that *E.coli* cells are short, Gram-negative rods that do not form spores, which is in agreement with Lee *et al.*, (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 (Jaber *et al.*, 2021) 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, H<sub>2</sub>, and O<sub>2</sub> (Abu-Aqil *et al.*, 2023).

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 (Ali and Al-Dahmoshi, 2022). 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 (Szczyka *et al.*, 2020).

Fecal contamination of R.O 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.

### Author Contributions

Alaa Yaqoob Rahi: Investigation, formal analysis, writing—original draft. Z. Abdul Alamir Mezher: Validation, methodology, writing—reviewing.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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