Study of the sterilization efficiency of the reverse osmosis water system in the neighborhoods of Babylon Governorate

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Abstract

Background: Individuals requirements accessibility to purification of water for drinking as well as essentials. Illnesses attributed to water-borne pathogens, notably Escherichia coli, are still widespread in underdeveloped areas. Although reverse osmosis (RO) systems are extensively employed as home purification units in Iraq, there has been inadequate research of their microbiological activity under local environments. **Objectives:** In Babylon Governorate, Iraq, this study set out to contrast the bacteriological and physicochemical properties of tap water with RO-treated water and for evaluating the effectiveness of RO systems implemented in households. **Design and Sample:** An analytical comparative cross-sectional study was conducted from October 2025 to May 2026. Two hundred matched water samples (100 tap water and 100 RO water) were collected from 100 houses in Babylon Governorate and tested at Al-Qasim General Hospital. **Results:** *E. coli* was detected in 31.0% of tap water samples and 23.0% of RO water samples. All parameters analyzed (pH, residual chlorine, EC, TDS, TOC, COD and UV absorbance) have been significantly (all $P < 0.001$) decreased upon completing RO treatment. RO water has a high quality of 90.0% compared to tap water 64.0%. **Conclusion:** Domestic RO systems were effective in improving the physicochemical water quality but recurrent bacterial contamination underscores a requirement for routine maintenance, membrane monitoring and continual microbiological surveillance to prevent biofilm formation and assure water safety.

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Introduction

Water is of extreme significance for agricultural production, human health, and the health of ecosystems. Without it life on Earth would not be achievable. Access to clean drinking water was necessary for many reasons ,such as public health, economic prosperity, and protecting the environment ,(Alao et al., 2025)[1]. Groundwater was one of the most significant resources for drinking water for cities and the contamination of these sources was a major issue at present (Abdipour et al., 2025)[2]. All around the house, people use drinking water for things like washing dishes, taking baths, and flushing toilets, in addition to consuming it. Because it was fundamental to human survival, water quality was of the most crucial significance. There must be no chemical, biological, or physical hazards in drinking water for the protection of public health,(Kaynar et al., 2022)[3]. Most people's drinking water comes from surface water sources like rivers, lakes, and streams. Unfortunately, this water was constantly being polluted by harmful chemicals and microbes, which alter the water quality and influences it was environment in an uneven manner based on the concentration of these materials and the number of microbes. This water was also used for agriculture and drinking, so it was already in a precarious ecological balance. Unless there was careful hygiene treatment and monitoring of drinking water sources,(Al-Mayah & Al-Tmemy, 2025)[4], water quality was a key factor in public health, and it was affected by both natural and human factors. Changes in temperature, rainfall patterns and storms and the effects of climate change directly influence the microbiological and chemical stability of the natural water reservoirs and urban drinking water supplies ,(Fira-Mladinescu et al., 2026)[5]. The World Health Organization (WHO) defined safe drinking water as water that did not constitute a significant risk to health over a lifetime of consumption, including different sensitivities among members of the same household. Research by the World Health Organization found that biological contamination of water was responsible for close to 80% of all human diseases in the developing world, (Perveen & Amar-Ul-Haque, 2023)[6].

RO technology greatly alleviated the world's water shortage and was used in about 85% of the world's desalination plants, with a disproportionate share in the Middle East, reflecting the region's innate limitation of fresh water. In reverse osmosis (RO), water molecules in the feedwater can pass through a semipermeable membrane, but salt and other contaminants can be trapped. This process produced a concentrate stream (brine) and clean penetrated water, which can be recycled to improve efficiency of the system. Pressure is used in reverse osmosis (RO) to reverse natural osmosis, in which water with a higher concentration of solutes passes to a lower one ,(Shabib et al., 2025)[7].

And also good quality RO membranes were able to remove more than 99 percent of all dissolved particles, known as a high solute rejection rate ,(Chae et al., 2024)[8].

RO systems are widely used for drinking water treatment and purification. However, the concerns about sterilization efficiency and the potential of microbial contamination inside RO systems still exist. The formation of biofilms in RO systems has a negative impact on the treated water quality and the system performance. Previous studies were mostly oriented towards the assessment of the physicochemical properties of the treated water, while microbiological aspects and biofilm formation, especially under local environmental conditions, were poorly addressed.

Materials And Methods

Study design and Period of study

This is an analytical comparative cross-sectional study to measure the quality of household water and analyze the purification efficiency of reverse osmosis (RO) systems in Babylon Governorate. Water samples were taken from the houses of the selected districts and sent to the laboratories of AL-Qasim General Hospital for analysis. Field sampling and lab analysis were conducted between October 2025 and May 2026.

Study Population and Sample Size

The study population is the households in the Babylon Governorate who use domestic reverse osmosis (RO) to access drinking water.

Water samples from 100 households were collected in pairs using systematic sampling method. Two samples of water were taken from each household before treatment (100 tap water samples) and after treatment (100 RO water samples) making a total of 200 water samples. The paired sampling technique was designed to accurately assess the efficiency of household RO systems in removing physical, chemical and microbiological contaminants .

Methods of calculation of sample size

The sample size for this assessment were determined based on pH differences ($\Delta = 0.4$) with 95% confidence and 80% power and measured one hundred paired water samples.

Sampling and in situ measurements

Paired samples of tap water and reverse-osmosis (RO) water were collected aseptically in sterile containers and a structured questionnaire was given to residents at that same period of time. All sealed containers were immediately

inserted in a insulated, dark, portable cool box to establish a stable cold chain, to assure specimen integrity and to prevent sample contamination. According to the recommended modes of transport targeted at lowering specimen degradation during transit times (Lowe et al., 2020)[9] samples were soon sent to Al-Qasim General Hospital for laboratory evaluation. A physicochemical analysis was then carried out in the field and in the laboratory with calibrated instruments. The collected water samples were analyzed directly for temperature, pH, residual chlorine, electrical conductivity (EC), total dissolved solids (TDS), total organic carbon (TOC), chemical oxygen demand (COD) and UV absorbance. All measurements were done in triplicate under standard operating conditions for analytical accuracy and statistical reliability.

The water temperature was measured in °C with a calibrated multi-parameter digital meter (TDS/EC/Temp) and recorded after the values on the display had stabilized (World Health Organization, 2024)[10] pH and residual chlorine were determined with a calibrated pH/Cl meter on an aliquot of 60 ml . Each sample was fitted with an electrode and stirred gently for 30–60 s to remove any trapped air bubbles and equilibrate. The measured values were compared with the drinking water standards by WHO [20]. pH range of 6.5 to 8.5 and minimum free residual chlorine of 0.5 mg/L. The probes were thoroughly rinsed with de-ionised water between measurements to avoid cross contamination (World Health Organization, 2024; U.S. Environmental Protection Agency, 2024)[11].

Field Sampling and Transport Protocols

A systematic sampling method was used to evaluate the efficiency of the sterilization of Reverse Osmosis systems in Babylon Governorate. Sterile 100 mL polyethylene bottles were prepared for each site, each container uniquely coded to record the sample type (tap water vs. RO water), house number, geographical sector and collection date to ensure strict traceability(U.S. Environmental Protection Agency USEPA, 2016). Water samples were collected during the early morning hours directly from the selected households. The tap was opened for the municipal supply and let run for 2 to 3 minutes as a critical flushing phase to stabilize water temperature and remove stagnant water from the service line. The sterile bottle was then filled directly from the flow, so that no physical contact was made between the tap orifice and the mouth of the container,(USEPA, 2016)[12].

Immediately after, a second sample was taken from the dedicated faucet of the household RO filtration unit, again taking the same aseptic precautions to prevent cross-contamination (World Health Organization WHO, 2024).All collected samples were immediately stored in an insulated cool box at a constant temperature range of 4 °C to 10°C to avoid microbial proliferation or degradation and to protect it from extreme ambient temperatures and direct sunlight,(USEPA, 2016).The samples were sent to the Microbiology Unit at Al-Qasim General Hospital for analysis immediately.

Preparing and Sterilizing Culture Media

MacConkey Broth, MacConkey Agar and Eosin Methylene Blue (EMB) Agar were prepared as per laboratory protocols. The weight of the dehydrated powders was measured by an electronic sensitive balance. For specific 250 ml quantities of distilled water (D.W.) weights of 8.5 g of MacConkey broth, 12.5 g of MacConkey Agar and 9.5 g of EMB Agar were used. The weighed powders were transferred into sterile Erlenmeyer flasks with 250 mL D.W. and agitated well at room temperature to allow uniform suspension of the media components without pre-boiling. The prepared flasks were sterilized by autoclaving at 121°C and 15 psi for 15 min for complete sterilization and complete in-situ dissolution of agar polymers .

Post sterilization cycle, the media were cooled to 45 °C before dispensing inside Biological Safety Cabinet (BSC),(Collee et al., 1996)[13].

The total number of samples in the BSC were prepared using sterile consumables and precision instruments to ensure a sterile working environment(WHO, 2020). The solid agar media were poured into sterile Petri dishes, while the MacConkey Broth was dispensed into sterile 10 mL plastic tubes, 5 mL aliquots, using aseptic techniques like flaming the flask orifice with a Bunsen burner to avoid cross-contamination(Ibo et al., 2020)[14]

Storage and Pre inoculation

The standard formulations for the media required dissolving 34.5 g of MacConkey Broth, 51.5 g of MacConkey Agar, and 37.5 g of EMB Agar per 1000 mL of D.W. which were autoclaved at 121°C and 15 psi for 15 minutes, coded and stored in a refrigerator unit at 4°C for 24 hours before use,(Collee et al., 1996). The Petri dishes were taken out of the refrigerator and kept in an incubator at 37°C for about 20 to 30 minutes for temperature equilibration and to be ready for inoculation. During this pre-heating phase and the subsequent process, the Petri dishes were kept inverted to avoid accumulation of condensation on the surface of the medium.

In diagnostic applications, MacConkey Broth was used for the initial detection of coliform bacteria, MacConkey Agar was used as a selective and differential medium for the isolation of enteric bacteria and EMB Agar for the definitive isolation.

Bacteriological Examination Procedure (MPN Method)

The Most Probable Number (MPN) method was carried out in three consecutive steps:

A. Presumptive Test

Single strength (SS) and double strength (DS) MacConkey purple media were prepared in test tubes with inverted Durham tubes and autoclaved at 121 °C and 15 psi for 15 minutes. For each water sample, three sets of five tubes were inoculated, i.e. 10 mL of water into DS broth for the first set and 1 and 0.1 mL of water into SS broth for the second and third sets, respectively, using sterile pipettes. The tubes were incubated at 37 °C for 24-48 hr. Positive presumptive tests were confirmed by turbidity, gas in Durham tubes and colour change from purple/orange to yellow,(Karki, 2018; Aryal & PhD, 2023)[15]

B. the Confirmed trial

After incubation, the positive tubes were examined for metabolic alterations. The procedure was performed in a BSC to maintain sterility after disinfecting surfaces and hands with 99% ethanol and the use of a Bunsen burner,(Aryal & PhD, 2023)[16]. Using a micropipette, a 0.1 mL aliquot from each positive broth was subcultured onto selective and differential solid media which included MacConkey Agar (for lactose fermenters) and EMB Agar (for identifying *E. coli* through its green metallic sheen). The Quadrant Streaking Method was performed by using sterile, disposable loops to obtain pure colonies, and plate incubation at 37 °C for an additional 24 hours,(Phyo et al., 2019; Akidah et al., 2025)[17] Suspected isolates were confirmed by Gram stain, biochemical profiling (e.g., Indole Test), (Phyo et al., 2019)[18]

Microscopic and Biochemical Identification

The plates (MacConkey and EMB agar) were incubated at 37 °C for 24 h and checked for bacterial growth. Subsequent identification was done based on morphological characteristics of representative colonies. Bacteria were identified by Gram staining according to the chemical composition of their cell wall. Under the microscope, Gram-positive organisms appeared blue and Gram negative appeared red. Biochemical confirmation was mainly done for *Escherichia coli* by performing indole test in tryptone water at 37°C for 24 hours. Positive samples were added with Kovac's reagent which resulted in a red ring confirming the presence of indole and confirms *E. coli* . Negative results indicated the presence of other coliform species such as *Enterobacter spp*, (Collee et al., 1996).

Statistical analysis

This study employed a statistical data analysis using IBM-SPSS version 27 and Microsoft Office Excel 2010 to collect, summarise and analyse the data. A Kolmogorov-Smirnov normality test was performed to test the normal or non-normal distribution of the variables before deciding on statistical approach. Therefore, numerical data were presented descriptively in the form of mean, standard deviation, median, percentiles, and range. In the case of inferential statistics, the Mann-Whitney test was used to compare the average value of two independent groups, assuming a non-normal distribution. In addition, a non-parametric alternative to ANOVA, Kruskal-Wallis, was performed and post hoc testing was conducted to determine if differences among groups were statistically significant. The chi-square test was used to test the relationships among two or more than two categorical variables. A p-value of < 0.05 ($P < 0.05$) was used as the criterion for statistical significance in all analyses.

Results

4.2 The prevalence of bacteria in the water

Table 4.2 showed that 31.0% and 11.0% of the tap water samples were positive for *E. coli* and another bacterial growth respectively, while the remaining 5.0% of the water samples showed mixed bacterial growth.

While the prevalence of *E. coli* in RO water was 23.0%, followed by 11.0% mixed bacteria growth, and 2.0% another bacterial growth.as demonstrated in Table 4.2.

Table 4.2: The prevalence of bacteria in the water

		No.	**%**
Tap water	*E. Coli*	31	31.0
	Another bacterial growth	11	11.0
	Mixed bacteria growth	5	5.0
	Non- growth	53	53.0
RO water	*E. Coli*	23	23.0
	Another bacterial growth	2	2.0
	Mixed bacteria growth	11	11.0
	Non- growth	64	64.0

4.3 The comparison between Tap and RO water regarding the studied parameters

Tap water and RO water were compared in terms of the parameters studied. A comparison of the parameters studied between the Tap and RO water was made.

Tap water vs. RO water comparison (Table 4.3) showed that there was almost the same mean of both water types (22.91±2.80 and 22.89±2.93) with a non-significant difference ($p = 0.886$) between them.

In contrast, pH was significantly lower in RO water (5.61±1.99) compared to tap water (6.90±1.28) with $p < 0.001$. Similarly, Chloride (Cl) levels were significantly reduced in RO water (0.27±0.70 vs. 0.98±1.51, $p<0.001$).

Electrical Conductivity (EC) and Total Dissolved Solids (TDS) showed a marked and significant decrease in RO water (196.01±116.54 vs. 1580.68±1246.58 for EC, and 93.78±153.40 vs. 690.42±148.38 for TDS, both $p < 0.001$).

Furthermore, Total Organic Carbon (TOC) and Chemical Oxygen Demand (COD) are significantly lower in RO water (1.84±4.95 vs. 3.61±1.69 for TOC and 1.35±3.47 vs. 2.55±1.08 for COD, $p < 0.001$).

Last but not least, the Ultraviolet absorbance is also reduced considerably in RO water (0.02±0.05 vs. 0.04±0.03, $p < 0.001$)

Table 4.3: The comparison between Tap and RO water regarding the studied parameters

Parameters	Tap water		RO water		P- value
	Mean± SD	Median	Mean± SD	Median	
Temp	22.91±2.80	22.20	22.89±2.93	22.10	0.886
pH	6.90±1.28	7.10	5.61±1.99	5.50	**<0.001**
CL	0.98±1.51	0.50	0.27±0.70	0.10	**<0.001**
EC	1580.68±1246.58	1405.80	196.01±116.54	79.80	**<0.001**
TDS	690.42±148.38	674.80	93.78±153.40	40.00	**<0.001**
TOC	3.61±1.69	3.07	1.84±4.95	0.74	**<0.001**
COD	2.55±1.08	2.26	1.35±3.47	0.58	**<0.001**
UV	0.04±0.03	0.03	0.02±0.05	0.01	**<0.001**

Discussion

Table 4.2.The prevalence of bacteria in the water

In this study, the prevalence of *E. coli* was 31.0% in tap water and 23.0% in RO water. The result was almost similar with the results of the study carried out in low and middle income countries,(Desye et al., 2024)[19] that the pooled prevalence of *Escherichia coli* isolates in drinking water was 37.94%. In addition, the results indicated that some areas of R. O water in Iraq are contaminated with E. coli bacteria in Karbala city,(Alaa Yaqoob Rahi, 2025)[20]

This was less than the 70.8% (69.8–71.9%) with *E. coli* positive at household POU, and 51.7% (50.3–53.0%) at household POC that was found by.who in their study of findings analysis on 38 nationally representative household surveys conducted between (2014-2021).

The number of *Escherichia coli* in tap water was more than the number in RO water, which could be explained by the fact that the efficiency of water treatment and the risk of contamination during its distribution are not the same.

Normally the tap water is treated by normal treatment procedures such as filtration and chlorination but it still can be contaminated by leakage in pipelines, cross connection with sewage systems or formation of biofilm in the distribution system.

On the other hand, reverse osmosis (RO) systems utilize semi-permeable membranes which are effective in removing most of the microorganisms including bacteria and thus eliminating microbial contamination.

But, the presence of in RO water is due to improper maintenance of RO units, contamination of storage tanks or post treatment handling, which may cause proliferation of bacteria and residual prevalence in RO samples

Table 4.3: Comparison of the studied parameters between Tap and RO water

The present study showed that the tap water had significantly higher levels of PH, CL, EC, TDS, TOC, COD and Ultraviolet as compared to the RO waters at level <0.001.

These findings were consistent with previous studies in Iraq ,(Al-Dulaimi & Younes, 2017)[21] (Muhammad et al., 2021)[22] (Kadhim et al., 2023)[23] which showed that the RO treatment significantly decreases the dissolved solids, salts and organic constituents compared to untreated or municipal tap water .

This could be because tap water usually has higher levels of dissolved minerals, salts, organic substances and residual disinfectants like chlorine that are added during municipal treatment to prevent microbial contamination. These materials

Improving parameters such as EC and TDS as they define the concentration of the dissolved ions of water where organic materials improve the values of the TOC, COD and UV absorbance.
Conversely, reverse osmosis (RO) systems eliminate the majority of dissolved substances, organic material, and contaminants using a semi-porous membrane and
generally limits up to 90-99% of dissolved organic and inorganic compounds, resulting in much lower levels of chemical and physical parameters in RO water.
Hence, the elevated values in tap water are anticipated since it has yet to be purified of natural minerals, treatment chemicals and potential contaminants that may have been added through distribution systems or other environmental sources,(Brooks, 2025)[24]
In this study, the presence of bacteria in water is associated with the presence of high levels of physicochemical parameters. To our knowledge, no study has compared this relationship between tap water and RO water in terms of physicochemical properties. However, it can be compared with a study (which found that DW is not a sterile product and can harbor a large diversity of microorganisms, including pathogens.
The findings demonstrated a significant disparity between the two water sources RO water exhibited a superior quality rate of 90.0%, whereas tap water fell predominantly into the Moderate category at 64.0%.
This gap evidenced the high efficiency of membrane-based filtration in RO systems compared to the centralized plumbing network.Regarding tap water, this result nearly agreed with the findings of(Mudhehe Sekeb and Abdul Kadhim Hadi Al-Sadi ,2024)[25], who attributed the decline in quality within the centralized distribution systems to defects in plumbing and aging infrastructure.

Conclusion

The study demonstrated that the reverse osmosis (RO) systems can be used to enhance the water quality by removing the total chlorine, total dissolved solids (TDS) and dissolved organic compounds which leads to better taste and odor. The efficiency of the removal of the contaminants, however, was greatly dependent on the correct operation and regular maintenance of the system. The pH was found to be within acceptable limits for drinking water; slight differences were noted as a result of the de-mineralization. The microbial analysis revealed that when the sensory characteristics are satisfactory, the lack of adequate maintenance may allow the development of biofilm in the filters and the internal parts, which could reduce the safety of the water. The findings show that sensory perception is insufficient to be used in determining water safety, and that water system performance and continuous monitoring of water quality are essential for maintenance.

Ethical Considerations

The research ethics committee of the College of Health and Medical Technologies, Al-Furat Al-Awsat Technical University, provided the official approval (No. 5888, 15 October 2025). The administrative permission was obtained from Babylon Water Directorate (No. 7/12/482, 22, September 2025). All laboratory procedures and field measurements followed standard protocols and manufacturer's instructions to assure reliability of data.

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