



Prevalence of Gram-Negative Bacteria in Drinking Water in Mosul City and Their Resistance to Chlorine and Antibiotics

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ABSTRACT

This study aimed to assess the prevalence of Gram-negative bacteria in drinking water supplied to different areas of Mosul city and to evaluate their resistance to chlorine and selected antibiotics. Drinking water samples were collected from 17 locations representing both sides of the city. Residual chlorine concentrations were measured, and bacterial contamination was determined using the membrane filtration method. The results revealed that all samples were contaminated with Gram-negative bacteria at varying levels depending on the sampling site. The highest level of contamination was recorded in the Nahrawan area (240 CFU/100 mL), while the lowest was observed in the Sumer area (3 CFU/100 mL). Residual chlorine concentrations in the analyzed samples ranged from 0 to 1.0 mg/L. Bacterial isolates identified using the VITEK® 2 Compact system included *Escherichia coli* and *Klebsiella pneumoniae* (Enterobacteriaceae), *Aeromonas hydrophila* (Aeromonadaceae), and *Acinetobacter baumannii* (Moraxellaceae). All isolated bacteria exhibited resistance to chlorine concentrations up to 0.8 mg/L, whereas complete sensitivity was observed at a concentration of 1.0 mg/L, with increased susceptibility at higher concentrations. Antibiotic susceptibility testing demonstrated that all isolates were sensitive to seven antibiotics but showed resistance to penicillin, ampicillin-sulbactam, and amoxicillin-clavulanate.

Keywords: Gram-negative bacteria, drinking water, chlorine, antibiotics

INTRODUCTION

Water pollution is a global challenge that significantly affects developing countries, where access to clean and safe drinking water is limited, the World Health Organization (WHO) estimates that approximately 785 million people worldwide lack access to clean water, resulting in approximately 3.4 million deaths annually from waterborne diseases, water pollution-related incidents continue to occur in developed countries, despite their typically more efficient water treatment systems (WHO, 2022). There is growing concern about contaminants in drinking water, which pose serious challenges to water treatment technologies (USAPA,2023) .

Chlorine is commonly used as a disinfectant in water treatment plants to eliminate pathogenic microbes including bacteria viruses and parasites ensuring that water is safe for human consumption however the increasing prevalence of chlorine resistant bacterial strains has raised questions about the effectiveness of chlorine in maintaining water quality bacterial resistance to chlorine and drinking water is a significant public health issue this occurs when microorganisms acquire the ability to survive and grow in the presence of chlorine which normally disrupts the microorganisms cellular functions this resistance is a complex process that is influenced by multiple factors including the genetic potential of the bacteria environmental conditions chlorine concentration and exposure time some bacteria such as *Escherichia coli*, *Salmonella*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, have demonstrated increased tolerance to normal doses of chlorine, especially when water quality is deteriorating or when chlorine concentrations are insufficient (Zhu *et al.*, 2020).

The consequences of bacterial resistance to chlorine in drinking water are serious, as it poses a risk of waterborne diseases outbreaks including those caused by antibiotic-resistant microbes to in response to this threat, current research focuses on understanding the mechanisms underlying chlorine resistance as well as exploring alternative disinfection methods or new strategies to improve water safety (Zhao *et al.*,2025) .

Antibiotic- resistant bacteria in drinking water pose a growing threat to public health because contaminated water can provide a direct pathway for the spread of resistant microorganisms to human's antibiotic-resistant bacteria in drinking water can cause difficult to treat infections for long hospital stays increase health care costs and lead to increased mortality (CDC,2024). human activities such as the widespread use of antibiotics in healthcare agriculture and livestock production Accelerate the emergency spread of resistance strains although wastewater treatment plants can affectively remove many microorganisms, they are not always designed to eliminate antibiotic resistant bacteria or antibiotic residues present in wastewater (Nasrollahian *et al.*, 2024).

improving wastewater treatment procedures creating more effective sterilizing technology and enforcing stronger laws on the use of antibiotics in agriculture in Healthcare or some of the measures being taken to solve this issue furthermore preventing the spread of antibiotic resistant bacteria in drinking water requires public health policies that support improved wastewater management enhanced antibiotics stewardship and less than antibiotic contamination of the environment (Duarte *et al.*,2022).

MATERIALS AND METHODES

Sample collection and measurement of residual chlorine concentration:

To gather the necessary drinking water samples 17 locations covering areas on either side of Mosul city were chosen. A Lovibond chlorine screening apparatus (Tintometer/ GmbH) was used to assess the samples residual chlorine concentration prior to collection. After that samples were collected in sterile circumstances using 100 milliliters sterile glass vials filled with five milliliters of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) to eliminate any remaining chlorine. The necessary information was recorded in samples transferred to the Laboratory within a period not exceeding three hours (CDC, 2024) .

Bacteriological examination:

The bacteriological examination used membrane filtration method, which is ideal for examining water samples with low bacterial content. Sample filter papers were cultured on m-Endo agar (HI media/India) to isolate Gram-negative bacteria. They were incubated at 35°C for 24 hours, after which the number of growing colonies was counted and the number of bacteria per 100 ml of sample was determined (Kemper *et al.*, 2023). Bacteria were then isolated from the growing colonies and identified microscopically using Gram stain and purified on MacConkey medium (Scharlau/ Spain). The bacteria were then identified using the Vitek2 compact system.

Effect of different chlorine concentrations test on bacteria isolated from drinking water:

Different chlorine concentrations (8, 4, 2, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, and 0.2 mg/L) were prepared using distilled water as a solvent. The bacterial suspension was then prepared in saline solution and compared to a McFarland 0.5 tube, which corresponds to an average bacterial count of 1.5×10^8 cells/ml. Plates containing Mueller-Hinton Agar (Scharlau/ Spain) culture medium were inoculated with bacterial suspension using cotton swab. After soaking, 6 mm holes were made in the culture medium and 100 microliters of each previously prepared chlorine concentration were placed in them. After incubation, the diameters of inhibition were measured, and the results were recorded.

Antibiotic susceptibility test:

Ten antibiotics were used in this test: Trimethoprim 10µg\disc (TPM), Ciprofloxacin 10µg\disc (CIP), Ceftriaxone 10µg\disc (CRO), Penicillin 10µg\disc (P), Azithromycin 15 µg\disc (AZM), Piperacillin 100 µg\disc (PRL), Doxycycline 10 µg\disc (DO), Amoxicillin-Clavulanate 30 µg\disc (AMC), Ampicillin-Sulbactam 25 µg\disc (AM), and Levofloxacin 5 µg\disc (LEV). The Kirby-Bauer disk diffusion susceptibility method was used to conduct this test (CLSI, 2021).

RESULTS AND DISCUSSION

(Table 1) shows the number of Gram-negative bacteria contaminating drinking water samples taken from different regions in Mosul using the membrane filtration method Fig. (1). It also shows the percentage of residual chlorine in each sample. The results indicate that all samples under study were contaminated with Gram-negative bacteria at different levels depending on the location where the sample was taken. According to World Health Organization guidelines, the number of bacteria in drinking water should be zero per 100 ml (WHO, 2022).

Table 1: Bacterial counts and chlorine concentrations of drinking water samples from Mosul city regions.

Sample collection region	No. of G-bacteria / 100 ml	Residual chlorine mg/L	contamin ation result	Sample collection region	No. of G-bacteria / 100 ml	Residual chlorine mg/L	contamin ation result
Al-Iqtisadiyeen	9	0.2	+	Wadi Hajar	14	0.4	+
Al-Risala	4	1.0	+	Al-Mamoun	10	0.2	+
Al-Shifa'a	12	0.2	+	Mosul Al-Jadida	22	0.2	+
Al-Dandan	9	0.8	+	Al-Nahrawan	240	0.0	+
Al-Rashidiya	9	0.6	+	Al-Bakr	6	0.2	+
Al-Arabi	11	0.8	+	Al-Mithaq	19	0.2	+
Al-Kahira	8	0.2	+	al-Kanisah	14	0.2	+
Somar	3	0.8	+	Al harmate	9	0.2	+
Al-Wahda	9	0.8	+				

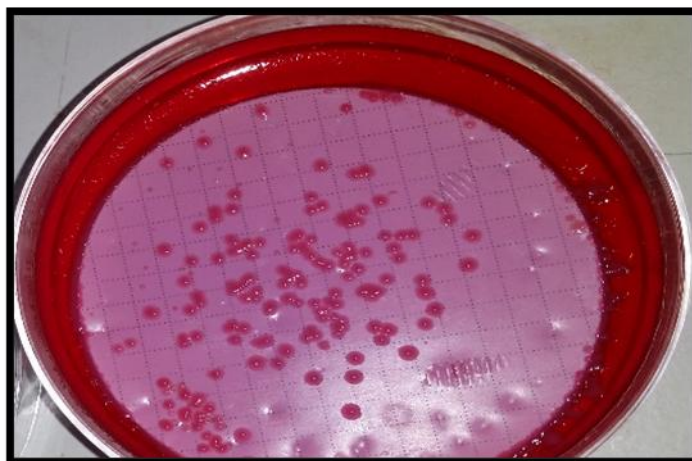


Fig. 1: Bacterial colonies growing on filter paper in m-Endo agar medium

The most contaminated samples were the Nahrawan region sample (240 CFU/100 ml) and the least in the Sumer region sample (3 CFU /100 ml). The contamination values in the samples of the other regions ranged between these two values. The contamination of all the samples with the pathogenic bacteria studied is a clear indicator of the failure of the sterilization processes of drinking water and thus the risk associated with the consumption of this water through diseases that can be caused by the contaminated bacteria. The contamination of drinking water samples may be attributed to inefficient sterilization in water treatment projects, or to breaks or damage in the distribution drink water networks, which may lead to the mixing of sewage with them.

Returning to table 1, we note that the concentrations of residual chlorine in the studied samples were different the lowest of which was in the Nahrawan region (0 milligrams/ L) which explains the high level of contamination in this sample the samples from the other regions had residual chlorine concentrations ranging from (0.2 - 1.0) milligrams/ L. According to international regulations for the amount of residual chlorine in drinking water these quantities are deemed acceptable (CDC, 2024). The subsequent findings of the current investigation indicated that the emergence of contamination may suggest the ineffectiveness of chlorine non sterilization or the resistance of bacteria to the residual chlorine in drinking. water samples.

(Table 2) shows the types of Gram-negative bacteria that were isolated from the contaminated drinking water samples in the current study and identified by using the VITEK® 2 Compact system Fig. (2), which were represented by *Escherichia coli* and *Klebsiella pneumoniae*, which belong to the Enterobacteriaceae, *Aeromonas hydrophila*, which belongs to the Aeromonadaceae, and *Acinetobacter baumannii*, which belongs to the Moraxellaceae. All of these types are pathogenic bacteria, and their presence in drinking water is of high risk.

Escherichia coli is usually used as a key indicator of water contamination of human origin, and its presence is associated with the risk of several intestinal diseases, most notably food poisoning and diarrhea (Ferro *et al.*,2024). *Klebsiella pneumoniae* causes respiratory infections, urinary tract infections, and sepsis, especially in immunocompromised individuals. its presence in drinking water contaminated with human feces has been linked to several hospital-acquired infections (Loudermilk *et al.*,2022). *Aeromonas hydrophila* is an opportunistic bacterium that contributes to several diseases, including food poisoning, skin and wound infections, respiratory infections, and even sepsis. It is more dangerous in immunocompromised individuals or those with chronic diseases, this bacterium is a water pollutant, especially in areas that lack effective water disinfection standards (Singh *et al.*,2025).

Acinetobacter baumannii is a multidrug-resistant bacterium that can cause a range of serious illnesses, including food poisoning, respiratory infections, urinary tract infections, sepsis, and surgical infections, especially in immunocompromised individuals pose, Its presence in drinking water is a public health threat due to its ability to easily survive in non-sterile environments, Its presence in

drinking water has been reported in several regions and has been associated with the spread of infections in hospitals and communities with contaminated drinking water (Dekic *et al.*, 2019; Carvalheira *et al.*, 2021)

Table 2: Illustrate dominate bacteria isolated from drinking water and their families.

Bacterial types	Family
<i>Escherichia coli</i>	Enterobacteriaceae
<i>Klebsiella pneumoniae</i>	Enterobacteriaceae
<i>Aeromonas hydrophila</i>	Aeromonadaceae
<i>Acinetobacter baumannii</i>	Moraxellaceae

The figure displays four sequential screenshots of a VITEK Compact system microbiology report. Each report includes patient information, organism identification, analysis time, and source details.

- Report 1:** Patient ID: 351, Lab ID: 351. Selected Organism: *Escherichia coli*. Analysis Time: 5.83 hours. Status: Final. Bionumber: 0407610740526600.
- Report 2:** Patient ID: 396, Lab ID: 396. Selected Organism: *Klebsiella pneumoniae ssp pneumoniae*. Analysis Time: 3.85 hours. Status: Final. Bionumber: 6607734353561010.
- Report 3:** Patient ID: 386, Lab ID: 386. Selected Organism: *Acinetobacter baumannii*. Analysis Time: 6.02 hours. Status: Final. Bionumber: 0241010303500352.
- Report 4:** Patient ID: 391, Lab ID: 391. Selected Organism: *Aeromonas hydrophila*. Analysis Time: 6.02 hours. Status: Final. Bionumber: 1425703350500231.

Fig. 2: Examples of results for diagnosing bacteria isolated from drinking water using the VITEK® 2 Compact system

(Table 3) illustrate how the four bacterial species under study were affected by varying chlorine concentrations. It is observed that all of these bacteria were resistant to chlorine concentrations up to 0.8 mg/L and became sensitive at 1 mg/L. As indicated by the diameters of inhibition Fig. (3), their sensitivity rose with increasing concentration.

Table 3: Effect of different chlorine concentrations on the studied bacteria (inhibition diameter mm).

chlorine con. mg/L	Bacterial types			
	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Aeromonas hydrophila</i>	<i>Acinetobacter baumannii</i>
8	41(S)	30(S)	35(S)	28(S)
4	30(S)	22(S)	22(S)	20(S)
2	26(S)	18(S)	17(S)	17(S)
1	15(S)	16(S)	14(S)	12(S)
0.9	6(S)	6(S)	6(S)	6(S)
0.8	6(R)	6(R)	6(R)	6(R)
0.7	6(R)	6(R)	6(R)	6(R)
0.6	6(R)	6(R)	6(R)	6(R)
0.5	6(R)	6(R)	6(R)	6(R)
0.4	6(R)	6(R)	6(R)	6(R)
0.3	6(R)	6(R)	6(R)	6(R)
0.2	6(R)	6(R)	6(R)	6(R)

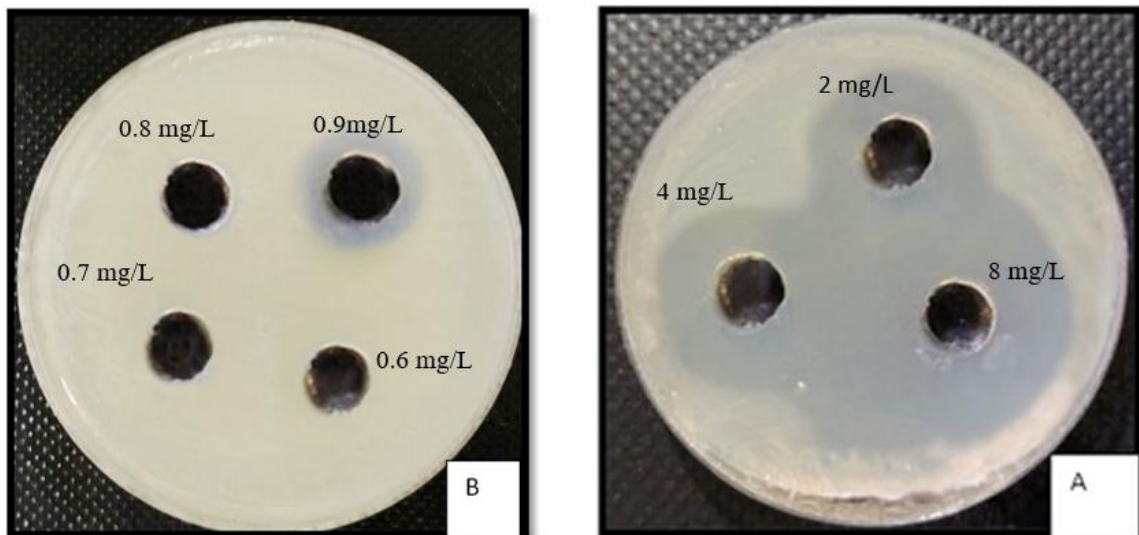


Fig. 3: Effect of chlorine on *E. coli*, A. (8, 4, 2) mg/L concentrations. B. (0.9, 0.8, 0.7, 0.6) mg/L concentrations.

The effect of chlorine depends primarily on its concentration in water. At high concentrations, chlorine is effective in killing bacteria by affecting their cell membranes and biological processes. The difference in effect between bacterial species also reflects the differences in the composition of cell membranes and their ability to resist toxic agents (Zhang *et al.*,2022). Chlorine resistance can arise through multiple mechanisms, including the formation of protective biofilms on pipe surfaces, which protect bacterial cells from the disinfectant, or through genetic mutations that confer a greater ability to withstand oxidative stress caused by chlorine, in addition, excessive use or incorrect dosage of chlorine in water treatment can contribute to the selection of resistant strains, making it difficult to control bacterial contamination in water systems (Honeker *et al.*,2019).

Returning to the results of the current study, Table (1), we find that all the values of residual chlorine in the studied drinking water samples, except for the Risala area, ranged between (0.2-0.8 mg/L), which explains the presence of the bacteria isolated in the current study in the polluted drinking water samples, as they are resistant to these concentrations according to the results shown

in (Table 3). This suggests that the chlorination of drinking water in areas of Mosul city may require re-evaluation to achieve effective residual chlorine concentrations capable of effectively eliminating microorganisms, enhancing the efficiency of the disinfection process and ensuring the safety of water for human consumption.

Chlorine is commonly used as a disinfectant to eliminate pathogens, its effectiveness may be affected by the presence of biofilms-organized bacterial communities coated with a self-produced polymeric matrix, biofilms can form on surfaces exposed to chlorine, providing a protective environment for bacteria and potentially reducing the antimicrobial efficacy of chlorine, recent research has highlighted the role of microplastics in facilitating the growth of antibiotic-resistant bacteria. Microplastics act as surfaces to which bacteria adhere and form biofilms, protecting them from antibiotics and disinfectants such as chlorine, and the biofilm formed on microplastic surfaces was significantly stronger than on other materials (Gross *et al.*, 2025). Understanding how chlorine disinfection affects these microorganisms is critical for developing effective water treatment strategies that protect public health. (Zhao *et al.*, 2025), The relationship between bacterial resistance to antibiotics and sensitivity to chlorine is complex and influenced by various factors, including bacterial species, environmental conditions, and exposure history (Gross *et al.*, 2025).

(Table 4) shows the results of the antibiotic sensitivity test of four studied bacterial isolate, it is noted that all bacterial species showed sensitivity to seven types of antibiotics, while they were resistant to P, AM, and AMC antibiotics (Figure 4), which reflects their possession of resistance mechanisms against them. These bacterial species usually produce beta-lactamase enzymes that degrade the beta-lactam ring in antibiotics, rendering them ineffective. In addition, they possess other mechanisms such as efflux pumps that effectively expel antibiotics from inside the cell, which reduces their concentration inside the cells and mitigates their antimicrobial effects for microbes, the ability of these bacteria to form biofilms, which provides a protective environment that reduces the penetration of antibiotics and enhances resistance (Bhandari *et al.*,2022).

Table 4: Results of antibiotic sensitivity tests for the bacteria under study isolated from drinking water

Antibiotics	Bacteria types			
	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Aeromonas hydrophila</i>	<i>Acinetobacter baumannii</i>
TMP	22(S)	32(S)	21(S)	24(S)
CIP	32(S)	38(S)	35(S)	32(S)
CRO	21(I)	20(I)	15(S)	21(S)
P	6(R)	6(R)	6(R)	6(R)
AZM	18(S)	52(S)	21(S)	17(S)
PRL	12(R)	21(I)	22(S)	15(S)
DO	18(S)	17(S)	15(S)	16(S)
AMC	6(R)	10(R)	6(R)	11(R)
AM	6(R)	6(R)	6(R)	6(R)
LEV	25(S)	32(S)	32(S)	27(S)

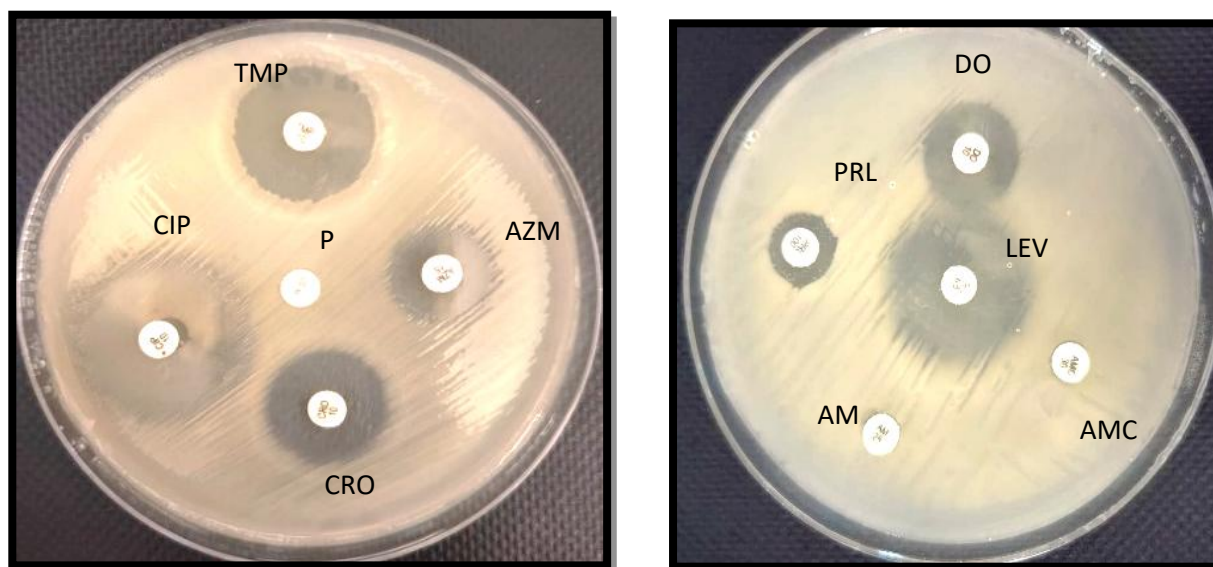


Fig. 4: Sensitivity of *Klebsiella pneumoniae* bacteria to antibiotics

The presence of antibiotic-resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) in drinking water poses significant public health risks, as it can lead to the spread of antibiotic-resistant infections, wastewater treatment plants play a pivotal role in the spread of antibiotic-resistant bacteria, studies have shown that these facilities may contain mobile genetic elements carrying resistance genes at various stages of water treatment, the persistence of these elements suggests that wastewater treatment processes may not be sufficient to eliminate ARBs, allowing their continued presence and potential spread in natural water bodies. (Klvanova *et al.*, 2024).

CONCLUSIONS

The study confirmed that all tested drinking water samples in Mosul were contaminated with Gram-negative bacteria, specifically the genera *E. coli*, *K. pneumoniae*, *A. hydrophila*, and *A. baumannii*. These bacteria exhibited high resistance to chlorine, maintaining their viability and growth up to a residual concentration of 0.8 mg/L. This explains their persistence within the treated water networks, as complete bacterial activity was only eradicated at a concentration of 1 mg/L.

A direct correlation was observed between increasing chlorine concentration and inhibition efficiency. This confirms that traditional disinfection methods (at low concentrations) are insufficient to guarantee public health safety. Therefore, it is necessary to re-evaluate the effectiveness of current disinfection processes and maintain effective and stable chlorine concentrations within the network to mitigate the risk of disease outbreaks

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انتشار البكتيريا السالبة لصبغة كرام في مياه الشرب لمدينة الموصل ومقاومتها للكلور والمضادات الحيوية

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الخلاصة

اجري هذا البحث بهدف التحري عن البكتيريا السالبة لصبغة كرام وبيان مدى انتشارها في مياه الشرب المجهزة للمناطق المختلفة في مدينة الموصل وتحديد مقاومتها لكل من الكلور والمضادات الحيوية، جمعت لهذا الغرض عينات مياه الشرب من سبعة عشرة موقعا تمثل احياء جانبي مدينة الموصل وتم قياس تركيز الكلور المتبقي في هذه العينات واستخدمت طريقة الترشيح بالأغشية للكشف عن التلوث فيها.

اظهرت النتائج ان جميع العينات قيد الدراسة كانت ملوثة بالبكتيريا السالبة لصبغة كرام وبدرجات متفاوتة حسب موقع اخذ العينة، وكانت أكثر العينات تلوثا هي عينة حي النهروان (240 خلية/100 مل) واقلها في عينة حي سومر (3 خلية/100 مل) وتدرجت قيم التلوث في عينات الاحياء الاخرى بين هاتين القيمتين. وكانت قيم تراكيز الكلور المتبقي في العينات المدروسة (صفر - 1.0 ملغم/لتر). وتبين ان انواع البكتيريا السالبة لصبغة كرام التي تم عزلها وشخصت بنظام VITEK® 2 Compact System من عينات مياه الشرب الملوثة تمثلت ببكتيريا *Escherichia coli* و *Klebsiella pneumoniae* التي تعود الى العائلة المعوية Enterobacteriaceae وبكتيريا *Aeromonas hydrophila* التي تعود لعائلة Aeromonadaceae وبكتيريا *Acinetobacter baumannii* التي تعود لعائلة Moraxellaceae.

اظهرت نتائج اختبار تأثير تراكيز الكلور المختلفة على الأربعة أنواع من البكتيريا المدروسة ان جميع هذه البكتيريا كانت مقاومة لتراكيز الكلور لحد تركيز 0.8 ملغم/لتر مما فسر ظهورها في عينات مياه الشرب الملوثة واصبحت حساسة للكلور في التركيز 1 ملغم/لتر وازدادت حساسيتها مع زيادة التركيز. وبينت نتائج اختبار حساسية هذه البكتيريا تجاه المضادات الحيوية ان جميعها اظهرت حساسية تجاه سبعة انواع من المضادات بينما كانت مقاومة تجاه المضادات Penicillin و Ampicillin-sulbactam و Amoxicillin-Clavulanate.

الكلمات المفتاحية: البكتيريا السالبة لصبغة كرام، مياه الشرب، الكلور، المضادات الحيوية.