

Original article

Occurrence of *Escherichia coli* as an Indicator of Pollution in Groundwater Wells in Tobruk City, Libya

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ABSTRACT

The issue of water scarcity has become increasingly severe, particularly in arid and semi-arid regions like those in the Arab world, where water resources are limited. Tobruk, a city facing such challenges, also grapples with water shortages due to the limited capacity of its desalination plant. This study aims to detect the presence of *E. coli* as an indicator of pollution in groundwater wells in the city of Tobruk. Water samples were collected from groundwater wells in the city of Tobruk during the summer (June - July) in the year 2025. A total of 17 samples were collected from wells in different areas of the city, and the presence of *E. coli* bacteria was discovered using the method: The multiple fermentation tube method, endorsed by both the US Environmental Protection Agency (US-EPA) and the American Public Health Association, which will be employed for *E. coli* detection. This method provides both qualitative and quantitative analysis, identifying the type and number of bacteria in a sample. Out of the seventeen analyzed samples, bacterial growth was detected in thirteen samples (76.5%), while four samples (23.5%) showed no detectable bacterial growth. *E. coli* was isolated from seven samples (41.2 %), making it the most prevalent microorganism identified in the present study. The relatively high proportion of *E. coli* -positive samples suggests significant fecal input, potentially originating from sewage leakage, surface runoff, or improper waste disposal practices in the studied areas. Mixed bacterial growth was observed in six samples (35.3%), where *E. coli* co-occurred with Coliform bacteria, *Klebsiella* spp., or *Proteus* spp. The presence of polymicrobial contamination is environmentally significant, as it reflects multiple contamination sources and prolonged exposure to organic pollution. Single bacterial isolates were identified in seven samples (41.2%), including *Acinetobacter calcoaceticus*, *Serratia odorifera*, *Proteus* spp., *Citrobacter freundii*, and *Pseudomonas* spp. These organisms are widely recognized as environmental and opportunistic bacteria capable of surviving in aquatic ecosystems enriched with organic matter. Overall, the results indicate that well depth is an important but insufficient factor on its own in determining groundwater microbiological safety. Structural integrity of wells, hydrogeological settings, proximity to pollution sources, and land-use practices collectively influence the occurrence and distribution of microbial contaminants. The findings confirm that several groundwater sources in the study area are microbiologically compromised, with varying degrees of *E. coli* contamination. Moreover, the presence of other bacterial species in *E. coli*-negative samples highlights the necessity of comprehensive microbiological assessment rather than reliance on a single indicator organism. Continuous monitoring, improved sanitation management, and appropriate water treatment measures are essential to ensure public health protection.

Introduction

Water is a renewable gift from God Almighty and is essential to life as we know it. Water that is acceptable for human consumption for both drinking and cooking is referred to as safe or potable water. A strong, healthy society must have safe, drinkable water, but 1.1 million people worldwide still lack access to clean water for home use and drinking. Because drinkable water offers many beneficial services to both humans and the ecosystem, its significance can never be overstated. When used appropriately, water both directly and indirectly boosts a nation's GDP [1]. Water scarcity presents a significant challenge for arid and semi-arid regions worldwide. The combination of high evaporation rates and limited rainfall creates specific difficulties for these areas in securing sufficient water resources to sustain their industries, agriculture, and populations [2].

In Libya, water is derived from four main sources, with groundwater accounting for nearly 95% of the nation's water supply. The other sources include surface water, such as rainfall and reservoirs, along with desalinated seawater and recycled wastewater. Groundwater serves as the primary source of drinking and irrigation water across the African continent [3]. Tobruk, a city facing such challenges, also grapples with water shortages due to the limited capacity of its desalination plant. Mohamed Rizk, the director of the Seawater Desalination Plant in Tobruk, has expressed concerns about a looming

water crisis linked to the aging equipment at the plant. He emphasized the criticality of the plant as Tobruk's primary water source [4]. Consequently, the city has turned to groundwater as an alternative source to mitigate this shortfall.

Groundwater is defined as water stored in the ground; generally, water accumulates underground due to rain. Groundwater will either be renewed by continuous or periodic rain, or it will not be renewed and instead will be confined in geological rock formations as a result of rainwater during ancient times [5]. Groundwater is an important natural water source that serves as a reliable and convenient option for residential use [6], and roughly one-third of the world's population utilizes groundwater for drinking purposes [7]. Groundwater is a valuable natural water reservoir that can be regarded as a safe and accessible water source for residential use [8]. Groundwater has a lot of advantages such as high quality, hardly affected by seasons, storage without spatial limitations, and more available quantities compared to surface water. However, it constitutes only 30% of all freshwater on the Earth. The demand for fresh groundwater is increasing consistently due to the augmentation of global population and incessant economic growth, and the loss of surface water value due to pollution. Despite these advantages, groundwater has some disadvantages, such as high mineral content, the possibility of land subsidence, high cost of extraction, and the risk of saltwater intrusion [9].

In most cases, groundwater is cleaner than surface water, as it is usually protected against the risk of pollution by the soils and rock layers. Hence, groundwater is used as drinking water in most countries. However, rising world population, changes in land use, and rapid industrialization have heavily affected the quality of groundwater due to the leakage of pollutants underground, which has put groundwater at risk [10]. Due to the rapid growth of population, urbanization, and industrialization, there is increasing pressure on land, water, and the environment. The concentration of population in urban areas has led to an increase in buildings, roads, vehicles, and factories. These human activities result in several effects, including water waste and storm drains, smoke and dust, and solid waste, which contribute to severe water and air contamination. Evaluating and mapping groundwater pollution is a crucial aspect, as the physical, chemical, and microbiological properties of groundwater determine its suitability for agricultural, industrial, and domestic uses [11].

Libya faces one of the world's most severe water shortage problems. Rainfall is scarce, except in the narrow coastal strip. The country has increasingly relied on its groundwater sources. In coastal areas, these sources are replenished by rain each year, but the country also has vast non-renewable water reserves beneath the desert. For several years, demand for water has far exceeded supply. As a result, the country has experienced heavy overuse and depletion of aquifers, leading to significant problems such as aquifer depletion, deterioration in water quality, and saltwater intrusion [12]. The quality of groundwater includes its physical, chemical, and biological characteristics. Physical parameters such as temperature, turbidity, color, taste, and odor are part of the physical water quality assessment. Since most groundwater is colorless, odorless, and has no distinct taste, people usually focus more on its chemical and biological qualities [13]. Coliform bacteria are widely used as key microbial indicators for monitoring water quality. Contamination of water with human fecal matter poses a significant risk to public health, as it may contain enteric pathogens capable of causing disease in humans [14]. Protecting this vital resource from pollution is crucial. Bacterial pollution is an often unseen yet critical issue that poses significant risks to public health.

The biological quality of drinking water is primarily determined by the presence or concentration of microbes in the water. The main sources of infectious microbes are human and animal feces, which may be deposited either directly into water sources or in nearby areas. When feces are deposited away from the water source, the microbes must be transported—typically by surface runoff, infiltration, or other environmental processes—into the water [15]. During this transportation, microbes often undergo a dilution process. In some cases, they may be diluted to very low concentrations. However, even at low concentrations, certain microbes can still be present in numbers high enough to cause illness. Although water may contain undesirable chemicals, these chemicals pose a greater risk to human health due to fecal contamination. These chemicals can cause hazardous water contamination due to fecal contamination of groundwater. Therefore, determining whether fecal contamination is present is a crucial aspect of the analysis [16]. Accurate bacteriological analysis can be performed to determine whether it is a source of fecal-contaminated water. Fecal coliforms are the most common indicator of fecal contamination. The most valuable test for the routine quality control of water supplies is the *E. coli* count [16]. This study aims to detect the presence of *E. coli* in Tobruk groundwater. Where 17 samples were collected from wells in different areas of it. It covers the entire geographical area because repeating samples from the same area will lead to the same results. Also, the number of wells is relatively small compared to other cities because this source is new and was not common before the problem occurred at the desalination plant. Additionally, the cost of drilling these wells is high due to the rocky nature of the land.

Methods

Study Area and Sample Collection

This study was conducted in the city of Tobruk, eastern Libya, which is a coastal city located on the Mediterranean coast.



Figure 1. Wells from which samples were collected (Google Maps)

Sample collection

This study was conducted during the summer, when sample collection began on June 26, 2025 until 29 July of the same year, during which a representative sample of well water was collected at different depths and from different areas in the city of Tobruk. The samples were collected according to scientific methods and following the correct procedures, including wearing gloves, using sterile bottles, storing and transporting them in a healthy manner, not exposing them to heat or sunlight, and observing the timing so that they reach the laboratory within 20 minutes to ensure accurate results.

Table 1. Culture Media

MEDIA	COMPANY
Xylose-Lysine-Deoxycholate (XLD) agar	BD DIFCO™
Sorbitol MacConkey	BD DIFCO™
Bile esculin agar	BD DIFCO™
Macconkey broth	BD DIFCO™
EMB (Eosin Methylene Blue)	BD DIFCO™

Most probable number technique

The most probable number technique is a broth culture-based technique that involves replicated dilution of samples in suitable test media. It is a statistical method used to estimate the viable numbers of bacteria in a sample by inoculating broth in 10-fold dilutions, and is based on the principle of extinction dilution. Due to the convenience of the MPN method, it is frequently applicable in water analysis and quality testing and assurance of water and liquid products. Aliquot dilutions of the sample are evaluated for the presence of microorganisms. Bring glass tubes of different capacities: one set of 100ml, one set of 20ml, and others of 10ml or 15ml. First, they must be thoroughly sterilized to ensure they are free of any microorganisms. An autoclave is used for this purpose. Secondly, they must be filled with MacConkey Broth culture medium. The bottles are divided into three groups as follows: The first group consists of one bottle with a capacity of 100 ml; the second group consists of five bottles with a capacity of 20 ml; and the third group consists of five bottles with a capacity of 10 ml or 15 mL. Preparation method: The broth is prepared using the correct scientific method as explained in its dedicated section. The bottles are filled as follows: The first bottle is filled halfway with 50 mL of MacConkey broth, the second bottle is filled with 10 mL of MacConkey Broth, and the third bottle is filled with 5 mL of broth.

Table 2. Bottles of sterile MacConkey broth (purple): the following are required

Number of bottles	mL of broth	Strength of broth
1	50	double
5	10	double
5	5	single

- Label each bottle with the sample number.

- Mix the water sample thoroughly by turning the bottle several times.
- Remove the bottle cap, then light the bottle's opening. Next, add the water sample to be tested.
- Add 50 ml of water to the bottle containing 50 ml of broth. This is most easily done by pouring directly into the bottle of broth up to a 100 ml graduation mark scratched previously on the bottle.
- Add 10 ml of water to each of the five bottles containing 10 ml of broth.
- Add 1 ml of water into each of the five bottles containing 5 ml of broth.
- Incubate all tubes at 44° C for 24- hours.

Record results by observing gas production and collection of bubbles in the Durham tube, and the fermentation shown by a change in color of MacConkey broth from purple to yellow. Count the number of positive tubes.

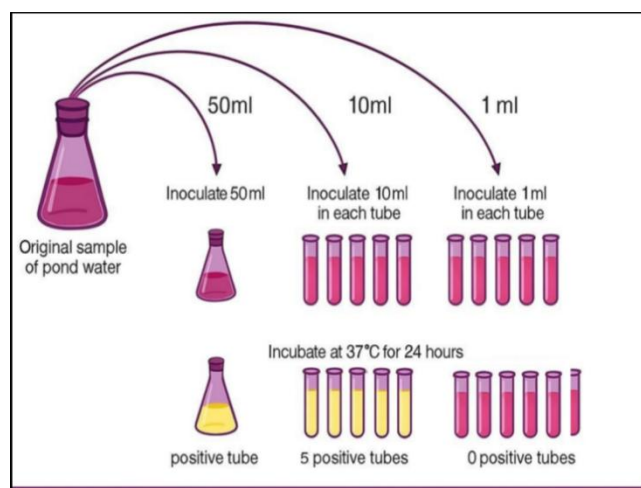


Figure 2. Multiple fermentation test tubes

Interpretation of results: the recorded results are then compared against a standard reference table known as the most probable number (MPN) table. This table provides a correlation between the pattern of positive and negative tubes and the concentration of bacteria in the sample. For instance, a result pattern of (1-5-0) corresponds to a specific MPN value that indicates the estimated number of bacterial cells in 100ml of the water sample. In this example, the MPN table would interpret a (1-5-0) result as an estimate of 25 bacterial cells per 100ml of the sample.

Statistical analysis

Data obtained from the bacteriological examination of groundwater samples were entered and organized using Microsoft Office Excel 2019. Descriptive statistical methods were applied to summarize the results. The findings were presented as frequencies, percentages, and bacterial counts in tables and figures to illustrate the distribution of microbial contamination among the investigated wells.

Results and discussion

E. coli Isolation

The results of isolating samples collected from several wells in the area (Al-khaleeg – Al-hurriya – Al-manara 1 – Al-manara 2 – Almatar alqadim – Aljamiea algharbia – Krum Alkhail – Al-Taslih – Abdul Fattah Younis Road – Al-nadhura – Masjid Aleatiq – Palestine Street – Masjid Fatima Al-Zahraa – Al-zuhur – Al-Hadaïq – Al-mukhtar – Bab Al-zaytoun) in the city of Tobruk showed that 13 bacterial samples were obtained from the total samples taken (17 samples), where 76.5 % of the areas taken from which bacteria appeared (Figure 3). This indicates that the water from the wells in these areas is highly polluted compared to the area. Of the 13 samples that contained bacterial growth, *E. coli* was isolated from seven different wells (Figure 4).

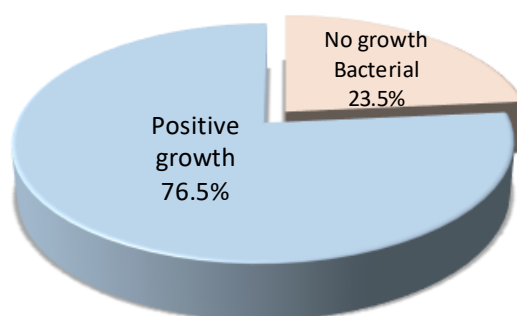


Figure 3. Percentage of samples showing bacterial growth from the total water samples collected

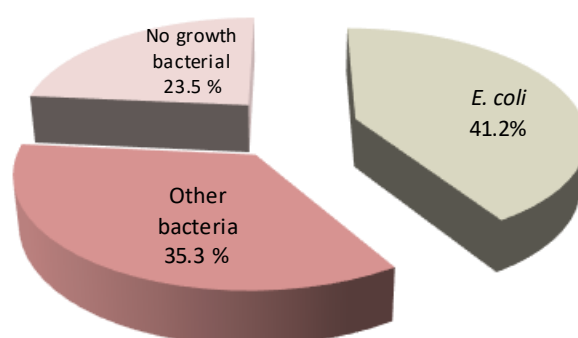


Figure 4. Percentage of *E. coli* from all positive growths and non-growth

Out of the seventeen analyzed samples, bacterial growth was detected in thirteen samples (76.5%), while four samples (23.5%) showed no detectable bacterial growth. *E. coli* was isolated from seven samples (41.2%), making it the most prevalent microorganism identified in the present study. This finding is consistent with the well-established role of *E. coli* as a primary indicator of fecal contamination in water systems, as reported by [17], and the World Health Organization [18]. The relatively high proportion of *E. coli*-positive samples suggests significant fecal input, potentially originating from sewage leakage, surface runoff, or improper waste disposal practices in the studied areas. Mixed bacterial growth was observed in six samples (35.3%), where *E. coli* co-occurred with Coliform bacteria, *Klebsiella spp.*, or *Proteus spp.* The presence of polymicrobial contamination is environmentally significant, as it reflects multiple contamination sources and prolonged exposure to organic pollution. Similar observations were reported by [19] and [20], who demonstrated that mixed microbial populations in water are commonly associated with deteriorated sanitation systems and biofilm development within water distribution or storage environments.

Single bacterial isolates were identified in seven samples (41.2%), including *Acinetobacter calcoaceticus*, *Serratia odorifera*, *Proteus spp.*, *Citrobacter freundii*, and *Pseudomonas spp.*. These organisms are widely recognized as environmental and opportunistic bacteria capable of surviving in aquatic ecosystems enriched with organic matter [26]. Their presence may indicate environmental contamination influenced by soil intrusion, stagnant water conditions, or anthropogenic activities rather than direct fecal pollution alone. Conversely, the absence of bacterial growth in four samples (23.5%) may reflect better water quality, greater protection from contamination sources, or bacterial concentrations below the detection limits of the applied methods. Comparable findings have been reported in groundwater studies where depth, soil composition, and natural filtration significantly reduced microbial infiltration [21]. Overall, the numerical distribution of bacterial isolates in this study aligns with previous research and highlights the combined impact of environmental conditions and human activities on microbial water quality, reinforcing the need for continuous monitoring and effective wastewater management to reduce public health risks.

Morphological Identification

E. coli was identified based on its physical characteristics. Positive tubes were cultured on methylene blue (EMB), a differential medium for *E. coli*, used to distinguish it from other enteric bacteria. Colonies exhibited a green metallic luster, indicating that the colonies produce organic acids as a result of glucose fermentation.



Figure 5. metallic green Sheen of *E. coli* on EMB Agar

Microscopic Examination

A smear was made from a colony grown on MacConkey agar, and it was stained with Gram's stain. The bacteria appeared as short rods, negative to Gram's stain, and not spore-forming, and this is what was mentioned in [22].

Biochemical Tests

Biochemical tests were carried out for all the isolates, and the results are shown in (Table 2). The bacterial isolates showed a positive result to the indole test; a red ring was observed on the surface of the medium as a result of tryptophan degradation by the enzyme tryptophanase. This is considered an important test for differentiating *E. coli* from other members of the Enterobacteriaceae family. Regarding the citrate test, the medium showed no color change from green to blue, indicating that the bacteria were unable to utilize citrate as the sole carbon source due to the absence of the citrate permease enzyme; since no citric acid was produced, there was no decrease in the medium's pH and thus no color change. The oxidase test was also done on the isolates, and all gave negative results, as there was no color shift to burgundy, which indicates the isolates don't have cytochrome oxidase as a hydrogen receptor. The urease test was also done, and all gave a negative result since the isolates don't have the urease enzyme and can't lyse urea (Table 2). And these results are in agreement with what's mentioned in [23,24, 25]. The triple sugar iron (TSI) medium is used as a culture medium named for its ability to test the microorganism's ability to ferment sugars, producing hydrogen sulfide and resulting in an acid/acid reaction (yellow slant/yellow butt), which indicates lactose fermentation (Table 2).

Table 2. Identification of *E. coli* by biochemical tests

Isolate	Biochemical tests				
	Oxidase	Urease	Indole	Citrate	TSI
<i>E. coli</i>	-	-	+	-	A/A+ gas

Distribution of Bacterial Species Isolated from Different Sampling Sites

(Table 3 and Figure 6). presents the distribution of bacterial species isolated from seventeen samples collected from different sampling sites in Tobruk city. The findings demonstrate a clear variation in both the presence and diversity of bacterial contamination among the investigated locations. Several Gram-negative bacteria were identified, including *E. coli*, Coliform bacteria, *Klebsiella spp.*, *Pseudomonas spp.*, *Proteus spp.*, *Citrobacter freundii*, *Serratia odorifera*, and *Acinetobacter calcoaceticus* (Table 3, Figure 6). *E. coli* was the most frequently isolated microorganism, occurring either as a single isolate, such as in Almatar alqadim samples, or in combination with *Coliforms*, *Klebsiella spp.*, and *Proteus spp.*, *E. coli* + *Coliform spp.* + *Proteus spp.*, which strongly suggests possible fecal contamination in several sampling sites. In addition, mixed bacterial growth was observed in some samples, where two or more bacterial species were simultaneously isolated, indicating a higher level of microbial pollution and potential environmental exposure to multiple contamination sources, As in Al-Taslih, Al-Nadhura, Al-Mukhtar, Aljamiea algharbia, and Bab Al-zaytoun. Conversely, no bacterial growth was detected in a number of samples, suggesting either the absence of microbial contamination or bacterial concentrations below the detection limit of the applied methods. Overall, these results reflect spatial variability in microbial water quality and emphasize the importance of continuous monitoring and effective management strategies to minimize microbial contamination and protect public health (Table 3).

Table 3. Bacterial Contamination Profile of Water Samples Collected from Different Areas

Sample No.	Name of the area	Sample Code	Type of bacterial isolates
1	Al-Khaleeg	KHJ	Acinetobacter calcoaceticus
2	Al-Hurriya	HAR	Serratia odorifera
3	Al-Manara 1	MAN1	Proteus spp.
4	Al-Manara 2	MAN2	Citrobacter freundii
5	Almatar alqadim	AMT	E. coli
6	Aljamiea algharbia	ALG	E. coli + Coliform spp.
7	Krum Al Khail	KUM	E. coli + Coliform spp. + Proteus spp.
8	Al-Taslih	ATS	E. coli + Klebsiella spp.
9	Abdul Fattah Younis Road	AFY	Pseudomonas spp.
10	Al-Nadhura	NZR	E. coli + Klebsiella spp.
11	Palestine Street	SHP	Pseudomonas spp.
12	Masjid Aleatiq	ALQ	-
13	Al-Zuhur	ZUR	-
14	Al-Hadhura	HDQ	-
15	Masjid Fatima Al-Zahraa	FZM	-
16	Al-Mukhtar	MKH	E. coli + Klebsiella spp.
17	Bab Al-zaytoun	BZT	E. coli + Coliform spp.

The predominance of *E. coli* and coliform bacteria observed in the present study is consistent with numerous previous investigations that identify these organisms as key indicators of fecal contamination in water sources [17, 18]. The frequent isolation of *E. coli*, either alone or in combination with other enteric bacteria such as *Klebsiella spp.* and Coliforms, strongly suggests contamination originating from human or animal waste, possibly due to sewage leakage, surface runoff, or inadequate sanitation infrastructure. The detection of mixed bacterial populations in several samples further supports findings reported by [19, 20], who demonstrated that polymicrobial contamination often reflects prolonged environmental exposure and multiple pollution sources. The presence of opportunistic pathogens such as *Pseudomonas spp.*, *Acinetobacter calcoaceticus*, and *Serratia odorifera* aligns with previous studies indicating that these organisms are commonly associated with aquatic environments, biofilm formation, and organic matter enrichment, particularly in polluted or stagnant waters [26]. Moreover, the isolation of *Proteus spp.* and *Citrobacter freundii* is environmentally significant, as these bacteria are frequently linked to soil, wastewater, and decaying organic material, and their presence in water systems has been reported as a marker of environmental degradation and anthropogenic impact [27]. Such findings highlight the role of environmental factors, including temperature, nutrient availability, and hydrological conditions, in shaping bacterial diversity and survival.

In contrast, the absence of detectable bacterial growth in some samples may indicate better water quality, effective natural filtration, or lower exposure to contamination sources. Similar observations have been reported in studies where groundwater depth, soil composition, and reduced human activity limited microbial intrusion into water systems [21]. Overall, the results of this study corroborate existing literature and underscore the influence of both environmental conditions and human activities on microbial water quality, emphasizing the need for continuous monitoring and improved wastewater management to mitigate public health risks.

The *E. coli* detection test in a water sample using the multiple test tube method

The results of this study showed the presence of *E. coli* bacteria in Krum Alkhail samples in all three groups as follows (1-5-4) (Table 4). *E. coli* bacteria reappeared in the Al-Nadhura samples, with the first 50 ml bottle positive, as well as all five tubes in the second group (10 ml) and three tubes in the third group (5 ml), as follows (1-5-3). The results of the Al-Taslih samples were as follows (1-4-0): the first 50 ml vial turned yellow, and four 10 ml tubes from the second group showed lactose fermentation. The third group (5 ml) did not show a positive result (Table 4). As for the Almatar alqadim samples, the results were as follows: The first 50 ml vial showed no lactose fermentation, nor did the second group of 10 ml tubes. However, in the third group of 5 ml tubes, two out of five tubes turned yellow, resulting in a score of (0-0-2). *E. coli* bacteria reappeared in the Aljamiea algharbia samples, with the first 50 ml vial and one tube in the second group (10 ml) turning yellow. The third group (5 ml) showed no fermentation, resulting in a score of (1-1-0). The results also showed fermentation in the first 50 ml vial of the Al-Mukhtar samples and in three tubes from

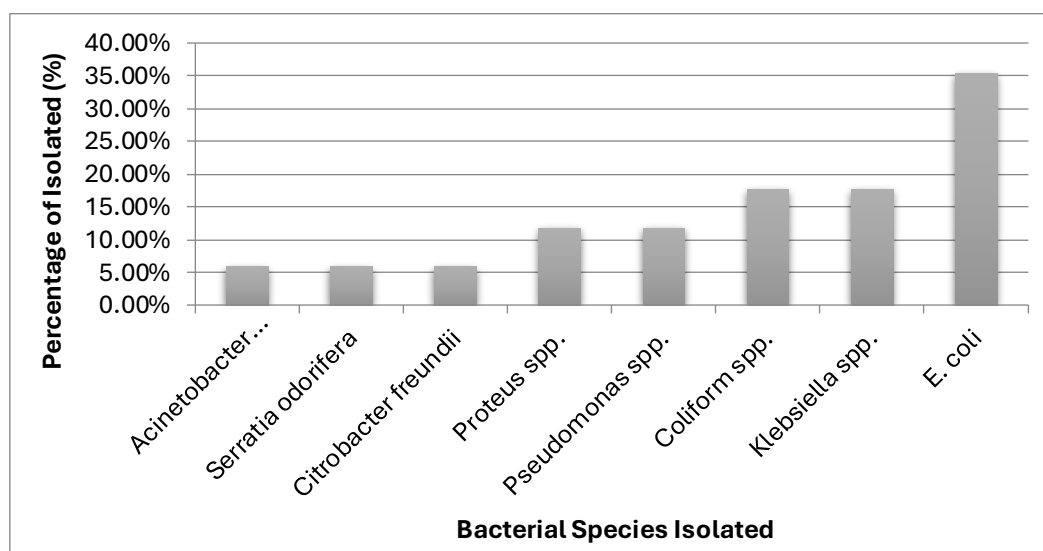


Figure 6. Distribution of bacterial species isolated from seventeen samples collected from different sampling sites in Tobruk city

the second group 10 ml. But no fermentation was observed in the third group of 5 ml tubes, resulting in a score of (1-3-0). As for the Bab al-Zaytoun sample, the first 50 ml vial and two tubes from the second group were positive, while no fermentation was observed in the third group, resulting in a score of (1-2-0). The results also showed that all the sample tubes from Al-Khaleej, Al-Hurriya, Palestine Street, Masjid Al-Ateeq, Al-Zuhour, Al-Hadaeq, and Masjid Fatima Al-Zahraa were negative (0-0-0), meaning that no lactose fermentation occurred in any of these samples due to the absence of *E. coli* bacteria. This also applies to the Al-Manara 1 and Al-Manara 2 samples. Also, the samples from the well on Abdel Fattah Younis Road yielded negative results.

Table 4. Results of Escherichia coli Analysis in Groundwater Samples Collected from Different Areas of Tobruk Using the Most Probable Number (MPN) Method

Volume of sample in each bottle			50ml	10 ml	1ml
Number of bottles used:			1	5	5
Sample No.	Name of the area	Sample Code			
1	Al-Khaleeg	KHJ	0	0	0
2	Al-Hurriya	HAR	0	0	0
3	Al-Manara 1	MAN1	0	0	0
4	Al-Manara 2	MAN2	0	0	0
5	Almatar alqadim	AMT	0	0	2
6	Aljamiea algharbia	ALG	1	1	0
7	Krum Alkhail	KUM	1	5	4
8	Al-Taslih	ATS	1	4	0
9	Abdul Fattah Younis Road	AFY	0	0	0
10	Al-Nadhura	NZR	1	5	3
11	Palestine Street	SHP	0	0	0
12	Masjid Aleatiq	ALQ	0	0	0
13	Al-Zuhur	ZUR	0	0	0
14	Al-Hadaeq	HDQ	0	0	0
15	Masjid Fatima Al-Zahraa	FZM	0	0	0
16	Al-Mukhtar	MKH	1	3	0
17	Bab Al-zaytoun	BZT	1	2	0

The enumeration of *E. coli* in groundwater samples collected from different locations using the Most Probable Number (MPN) technique

After counting the number of positive tubes in the previous table (Table 4), this table (Table 5) presents the enumeration of *E. coli* in groundwater samples collected from different locations using the Most Probable Number (MPN) technique based on positive tube combinations. The results reveal pronounced spatial variability in the microbiological quality of the investigated wells, reflecting differences in well depth, hydrogeological conditions, and potential contamination sources. Out of the 17 analyzed samples, 7 samples (41.2%) were positive for *E. coli*, while the remaining samples showed negative

results for this indicator organism. However, it is important to note that only samples 12, 13, 14, and 15 were completely free from any bacterial growth, indicating superior microbiological quality at these sites. In contrast, samples 1, 2, 3, 4, 9, and 11, although negative for *E. coli*, contained other bacterial species, suggesting general microbial contamination not directly associated with fecal pollution. The presence of non-*E. coli* bacteria may be attributed to naturally occurring environmental microorganisms or secondary contamination related to well maintenance and surrounding soil conditions. This observation supports previous findings indicating that the absence of *E. coli* does not necessarily guarantee overall microbiological safety of groundwater [17]. The highest *E. coli* concentration was recorded in Krum Al-Khail, reaching 160 cells/100 mL at a well depth of 85 m, indicating severe fecal contamination. This level far exceeds international drinking water standards, which require the complete absence of *E. coli* in 100 mL of potable water [18].

Similarly, Al-Nadhura exhibited a relatively high *E. coli* count of 90 cells/100 mL at a depth of 100 m, demonstrating that increased well depth alone does not necessarily protect groundwater from microbial intrusion. Moderate *E. coli* contamination was observed in wells with depths ranging from 70 to 115 m, including Al-Taslih (115 m, 13 cells/100 mL), Al-Mukhtar (70 m, 8 cells/100 mL), Al-Jamiaa Al-Gharbia (80 m, 3 cells/100 mL), Bab Al-Zaytoun (70 m, 3 cells/100 mL), and Al-Matar Al-Qadim (15 m, 2 cells/100 mL). Although these concentrations are relatively low, they still indicate fecal contamination and render the water unsuitable for direct human consumption. Previous studies have emphasized that even low *E. coli* counts represent recent fecal intrusion and may be associated with the presence of pathogenic microorganisms [20]. Interestingly, samples 12, 13, 14, and 15, which showed no bacterial growth, were associated with wells having depths between 15 and 75 m. This suggests that effective natural filtration, favorable geological formations, adequate well construction, and distance from contamination sources may play a more decisive role than depth alone in protecting groundwater quality. These findings are consistent with the conclusions of [21], who highlighted the importance of soil and aquifer characteristics in limiting bacterial transport. Overall, the results indicate that well depth is an important but insufficient factor on its own in determining groundwater microbiological safety. Structural integrity of wells, hydrogeological settings, proximity to pollution sources, and land-use practices collectively influence the occurrence and distribution of microbial contaminants.

The findings confirm that several groundwater sources in the study area are microbiologically compromised, with varying degrees of *E. coli* contamination. Moreover, the presence of other bacterial species in *E. coli*-negative samples highlights the necessity of comprehensive microbiological assessment rather than reliance on a single indicator organism. Continuous monitoring, improved sanitation management, and appropriate water treatment measures are essential to ensure public health protection.

Table 5. The table shows the estimated *E. coli* concentration in water samples expressed as MPN per 100 mL

Sample No.	Name of the area	Well depth	Combination of test tube	Estimated <i>E. coli</i> Count (MPN/100 mL)	Result
1	Al-Khaleeg	105	(0-0-0)	0	Negative
2	Al-Hurriya	70	(0-0-0)	0	Negative
3	Al-Manara 1	9	(0-0-0)	0	Negative
4	Al-Manara 2	11	(0-0-0)	0	Negative
5	Almatar alqadim	15	(0-0-2)	2	Positive
6	Aljamiea algharbia	80	(1-1-0)	3	Positive
7	Krum Alkhail	85	(1-5-4)	160	Positive
8	Al-Taslih	115	(1-4-0)	13	Positive
9	Abdul Fattah Younis Road	80	(0-0-0)	0	Negative
10	Al-Nadhura	100	(1-5-3)	90	Positive
11	Palestine Street	20	(0-0-0)	0	Negative
12	Masjid Aleatiq	18	(0-0-0)	0	Negative
13	Al-Zuhur	75	(0-0-0)	0	Negative
14	Al-Hadaiq	75	(0-0-0)	0	Negative
15	Masjid Fatima Al-Zahraa	18	(0-0-0)	0	Negative
16	Al-Mukhtar	70	(1-3-0)	8	Positive
17	Bab Al-zaytoun	70	(1-2-0)	5	Positive

In the present study, *E. coli* was detected in groundwater wells with depths ranging from 9 to 115 m, indicating fecal contamination of both shallow and deep aquifers. These findings are consistent with previous studies reporting the presence of *E. coli* across a wide range of groundwater well depths. [28], detected *E. coli* in groundwater wells exceeding 50 m in depth in Brazil, attributing contamination to inadequate well construction and sanitation practices. Similarly, [29]

reported *E. coli* contamination in tubewells with depths between 6 and 36 m in Bangladesh, particularly in areas affected by surface infiltration. Studies from rural Canada documented *E. coli* in untreated private wells with depths commonly ranging from 10 to 90 m, demonstrating the vulnerability of deeper groundwater sources to microbial contamination [30]. In arid environments, [31] reported detectable *E. coli* levels in groundwater wells exceeding 100 m in depth in Saudi Arabia, while local studies in Libya confirmed the presence of *E. coli* in wells with depths of approximately 20–100 m, linking contamination to sewage leakage and insufficient well protection [8]. Together, these studies support our results and confirm that *E. coli* contamination of groundwater is not restricted to shallow wells, but can extend to deeper aquifers under favourable hydrogeological and anthropogenic conditions.

Distribution of Bacterial Genera Isolated from Groundwater Samples Collected from Different Locations

Table [6] presents the distribution of the most common bacterial genera isolated from the analyzed water samples. A total of 17 water samples were examined for the presence of *Klebsiella spp.*, *Coliform spp.*, *Proteus spp.*, and *Pseudomonas spp.*. The results indicate that bacterial contamination was detected in several samples; *Klebsiella spp.* and *Coliform spp.* were the most frequently isolated organisms, each detected in three samples, whereas *Proteus spp.* and *Pseudomonas spp.* were isolated from two samples each, as shown in the total counts (Table 6). These results support what previous studies have indicated regarding the prevalence of bacterial contamination indicators, such as coliforms and enteric bacteria in general, in various water sources. Therefore, the presence of *Coliform spp.* in some samples is consistent with findings reported in river and well water analyses in other regions, which showed high levels of coliform indicators in surface water samples and well water samples. This reflects the risk of fecal or environmental contamination (*Coliforms* and *E. coli*) in drinking and irrigation water (for example, in Lebanon 2021, where fecal coliforms were detected in more than 96% of the samples [32]. The occurrence of *Coliform spp.* in several samples is of particular concern, as coliform bacteria are widely recognized as indicator organisms of fecal contamination, suggesting possible infiltration of sewage, animal waste, or surface runoff into the water sources.

Similarly, the detection of *Klebsiella spp.*, an opportunistic pathogen commonly associated with soil, vegetation, and fecal matter, further supports the likelihood of environmental or anthropogenic contamination (Table 6). Furthermore, the isolation of *Klebsiella spp.* in some samples is consistent with previous reports in which this bacterial genus was observed in drinking water and wells, and its presence has been linked to environmental contaminants and the intrusion of wastewater or sewage into surface and groundwater sources, suggesting that some samples were exposed to contaminants similar to those reported in water quality analysis studies in Africa and Asia [33].

The results also indicate the presence of *Proteus spp.* and *Pseudomonas spp.* in smaller quantities, consistent with previous studies showing that these species can be extracted from contaminated water sources in both human and rural environments. This reflects the potential microbial diversity in surface and groundwater and the complexity of sources of environmental pollution [34]. Overall, the findings demonstrate that the detection of opportunistic and indicator bacteria in specific samples highlights localized microbial contamination. This variability underscores the importance of continuous microbiological monitoring of water sources, particularly those used for drinking purposes. The isolation of fecal indicator bacteria emphasizes potential public health risks and indicates the need for improved protection of water sources and implementation of appropriate treatment strategies. Thus, the microbial distribution pattern in this study aligns with the scientific literature, which indicates that bacterial contamination indicators, especially *Coliforms* and *Klebsiella* species, are used as indicators of fecal or environmental water contamination, and that the presence of these species in drinking water and well samples is an indicator of partial deterioration in microbial quality at some of the studied locations [32].

Table 6. Occurrence of Common Bacterial Species Isolated from Water Samples.

Bacteria isolates Sample No.	<i>Klebsiella</i> <i>spp.</i>	<i>Coliform</i> <i>spp.</i>	<i>Proteus</i> <i>spp.</i>	<i>Pseudomonas</i> <i>spp.</i>
1	-	-	-	-
2	-	-	-	-
3	-	-	+	-
4	-	-	-	-
5	-	-	-	-
6	-	+	-	-
7	-	+	+	-
8	+	-	-	-
9	-	-	-	+
10	+	-	-	-

11	-	-	-	+
12	-	-	-	-
13	-	-	-	-
14	-	-	-	-
15	-	-	-	-
16	+	-	-	-
17	-	+	-	-
Total	3	3	2	2

The bacteriological analysis of the examined water samples revealed the presence of a limited number of less common Gram-negative bacterial species (Table 7). Among all analyzed samples, *Acinetobacter calcoaceticus*, *Serratia odorifera*, and *Citrobacter freundii* were each detected in only one sample, corresponding to a very low frequency of occurrence. *Acinetobacter calcoaceticus* was isolated exclusively from the Al-Khaleeg sample, while *Serratia odorifera* was detected only in the Al-Hurriya sample. Similarly, *Citrobacter freundii* was identified in the Al-Manara 2 sample (Table 3 and Table 7). No growth of these bacterial species was observed in the remaining samples, indicating that their distribution was sporadic rather than widespread across the investigated water sources. These findings indicate that the majority of water samples were free from these opportunistic Gram-negative bacteria, reflecting generally acceptable bacteriological quality. Nevertheless, their presence, even at low frequency, aligns with previous reports showing that environmental water sources may occasionally harbor opportunistic bacteria associated with aquatic ecosystems and localized contamination events [18,26]. The isolation pattern observed suggests spatial heterogeneity among sampling sites, as the bacteria were confined to specific samples rather than being uniformly distributed.

Similar findings have been reported in prior studies, documenting intermittent occurrence of *Acinetobacter*, *Serratia*, and *Citrobacter* species in water, especially in locations affected by anthropogenic activities or environmental stressors [35,27]. The results of this study indicate that the less common Gram-negative bacteria detected, *Acinetobacter calcoaceticus*, *Serratia odorifera*, and *Citrobacter freundii*, though present at very low frequencies, carry important environmental and public health implications. Species of *Acinetobacter* are known to persist in drinking water systems, surface water, and wastewater, with the potential to survive outside clinical settings and act as opportunistic pathogens under certain conditions [35]. Although *Serratia odorifera* is infrequently reported, members of the genus *Serratia* have been isolated from both clinical and environmental samples, indicating their ability to persist in diverse habitats. Their detection in this study suggests localized contamination, possibly related to anthropogenic activities or insufficient sanitary protection at the water source. *Citrobacter freundii* is a recognized opportunistic bacterium found in aquatic environments, wastewater, and the intestines of humans and animals. Even at low frequencies, its presence raises health concerns due to its ability to harbor antibiotic resistance genes and its potential role in opportunistic infections, particularly among vulnerable populations [27].

Collectively, the sporadic isolation of these bacteria indicates that water sources may serve as reservoirs for opportunistic Gram-negative organisms, potentially contributing to the horizontal transfer of antimicrobial resistance genes within environmental microbial communities. The uneven distribution observed across sampling sites underscores the need for targeted monitoring and water protection measures, particularly in areas close to sewage discharge or agricultural runoff. In summary, *Acinetobacter calcoaceticus*, *Serratia odorifera*, and *Citrobacter freundii* were detected in a limited number of water samples, highlighting their low overall prevalence in the studied sources. Nevertheless, their isolation represents an important environmental and epidemiological indicator due to their opportunistic nature and potential association with contaminated environments. These findings emphasize the need for continuous water quality monitoring, enhanced protection of water sources, and effective treatment strategies to minimize health risks. Future studies should increase the number of sampled sites, incorporate molecular detection techniques, and examine antimicrobial resistance profiles of the isolated bacteria to support public health programs and ensure compliance with national and international water quality standards [18,26]. The bacteriological analysis of the examined water samples revealed the presence of a limited number of less common Gram-negative bacterial species (Table 7).

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associated with aquatic ecosystems and localized contamination events [18,26]. The isolation pattern observed suggests spatial heterogeneity among sampling sites, as the bacteria were confined to specific samples rather than being uniformly distributed. Similar findings have been reported in prior studies, documenting intermittent occurrence of *Acinetobacter*, *Serratia*, and *Citrobacter* species in water, especially in locations affected by anthropogenic activities or environmental stressors [35,27].

The results of this study indicate that the less common Gram-negative bacteria detected, *Acinetobacter calcoaceticus*, *Serratia odorifera*, and *Citrobacter freundii*, though present at very low frequencies, carry important environmental and public health implications. Species of *Acinetobacter* are known to persist in drinking water systems, surface water, and wastewater, with the potential to survive outside clinical settings and act as opportunistic pathogens under certain conditions [35]. Although *Serratia odorifera* is infrequently reported, members of the genus *Serratia* have been isolated from both clinical and environmental samples, indicating their ability to persist in diverse habitats. Their detection in this study suggests localized contamination, possibly related to anthropogenic activities or insufficient sanitary protection at the water source. *Citrobacter freundii* is a recognized opportunistic bacterium found in aquatic environments, wastewater, and the intestines of humans and animals. Even at low frequencies, its presence raises health concerns due to its ability to harbor antibiotic resistance genes and its potential role in opportunistic infections, particularly among vulnerable populations [27].

Collectively, the sporadic isolation of these bacteria indicates that water sources may serve as reservoirs for opportunistic Gram-negative organisms, potentially contributing to the horizontal transfer of antimicrobial resistance genes within environmental microbial communities. The uneven distribution observed across sampling sites underscores the need for targeted monitoring and water protection measures, particularly in areas close to sewage discharge or agricultural runoff. In summary, *Acinetobacter calcoaceticus*, *Serratia odorifera*, and *Citrobacter freundii* were detected in a limited number of water samples, highlighting their low overall prevalence in the studied sources. Nevertheless, their isolation represents an important environmental and epidemiological indicator due to their opportunistic nature and potential association with contaminated environments. These findings emphasize the need for continuous water quality monitoring, enhanced protection of water sources, and effective treatment strategies to minimize health risks. Future studies should increase the number of sampled sites, incorporate molecular detection techniques, and examine antimicrobial resistance profiles of the isolated bacteria to support public health programs and ensure compliance with national and international water quality standards [18,26].

Table 7. Occurrence of Less Common Bacterial Species Isolated from Water Sample

Bacteria isolates Sample No.	<i>Acinetobacter calcoaceticus</i>	<i>Serratia odorifera</i>	<i>Citrobacter freundii</i>
1	+	-	-
2	-	+	-
3	-	-	-
4	-	-	+
5	-	-	-
6	-	-	-
7	-	-	-
8	-	-	-
9	-	-	-
10	-	-	-
11	-	-	-
12	-	-	-
13	-	-	-
14	-	-	-
15	-	-	-
16	-	-	-
17	-	-	-
Total	1	1	1

Suggested bacteriological criteria for drinking water

The results of comparing the samples with the proposed drinking water standards, as shown in this table, were as follows: The water samples from the wells of Masjid Fatima Al-Zahra, Al-Hadaiq, Masjid Aleatiq, and Al-Zuhur showed no bacterial

growth and were considered excellent and potable (Table 8). The water samples from Almatar alqadim, Aljamiea algharbia, Al-Mukhtar, and Bab Al-Zaytoun, which contained between (1 – 10) bacterial cells, were acceptable according to the proposed drinking water standards. However, periodic testing of the equipment is necessary (Table 8). The results of this study showed that the water sample from the Al-Taslih contained between (10 – 50) bacterial cells. This was deemed unacceptable, and it was determined that structural defects and poor maintenance of the pump and its base should be identified, addressed, and the equipment and source of contamination disinfected (Table 8). As for the water samples from both Krum Alkhail and Al-Nadhura, the number of bacterial cells in them has increased to (more than 50) bacterial cells, and this indicates grossly polluted in them look for an alternative source, carry out necessary repairs, and disinfect well (Table 8).

Table 8. Comparison of Sample Results with Proposed Drinking Water Standards

Mean count <i>E. coli</i> 100ml	The samples number	Comments
(0)	ALQ – ZUR – HDQ – FZM	Excellent
(1-10)	AMT – ALG – MKH – BZT	Acceptable, but make regular sanitary checks on equipment
(10-50)	ATS	Unacceptable: look for and correct structural faults and poor maintenance of pump and plinth, then disinfect equipment and source
More than 50	KUM – NZR	Cross-contaminated: look for an alternative source, or carry out necessary repairs, and disinfect well

Conclusion

The present study demonstrated considerable spatial variation in the microbiological quality of groundwater wells in Tobruk. Bacterial growth was detected in 76.5% of the samples, with *E. coli* detected in 41.2%, indicating fecal contamination in several wells. The highest *E. coli* levels were recorded in Krum Alkhail and Al-Nadhura, while several wells showed no detectable bacterial growth. Other environmental and opportunistic bacteria were also isolated, highlighting the complexity of groundwater contamination. Overall, the findings emphasize the need for regular microbiological monitoring, improved well protection and sanitation, and appropriate treatment of groundwater before human consumption.

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