



## Detection Of Waterborne Pathogens in Drinking Water in Ajdabiya City

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الكشف عن مسببات الأمراض المنقولة بالمياه في مياه الشرب بمدينة أجدابيا

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### Abstract:

Waterborne pathogens pose a major health risk in both developed and developing countries. Providing quality domestic water for the community is crucial. **The aim of this study** was to examine various drinking water sources in several areas of Ajdabiya to ensure they are free of contamination and fit for drinking. Community health was evaluated to determine the level of water pollution. **Method:** Four water samples were collected: 21 from the public network (river water), 23 from bottled water (mineral water), 4 from well water, and 2 from rainwater. Total coliforms were analyzed, Escherichia coli and Streptococcus were analyzed in water samples. The household survey included questions about the city's residential area, the main source of drinking water at home, the type of drinking water tanks, the frequency of chlorine addition to drinking water, the frequency of cleaning drinking water tanks, and whether the drinking water had been tested. Has a family member become ill after drinking the water? What type of illness? Do you know the sources of water pollution? Are you aware of the risks of water pollution? In your opinion, what is the best solution to address the problem of drinking water pollution? **The results** of the tests were compared to the permissible limits set by the World Health Organization finding that coliform was 100%, Escherichia coli was 73.9%, and streptococcus was 23.9% of the water samples. E. coli concentration levels were high. The household survey results revealed that water pollution was highest in the eastern and northern parts of the city among residents using the public water network. Pollution levels were highest in underground tanks, followed by rooftop tanks. Pollution levels were lowest among those who cleaned their tanks twice a year. Chlorinated water contains a much lower number of colony-forming units (CFU) per 100 ml of water sample. **In conclusion**, improper sewage disposal is a primary factor contributing to water pollution in the city, followed by pollution from contaminated water tanks and a lack of awareness of water purification and sterilization. There is an urgent need to take emergency steps to stop further deterioration of water quality and to improve the existing water quality to protect the population from the spread of waterborne diseases.

**Keywords:** Detection, Waterborne Pathogens, Drinking Water .

### المخلص

تشكل مسببات الأمراض المنقولة بالمياه خطراً صحياً كبيراً في كل من البلدان المتقدمة والنامية. إن توفير مياه منزلية عالية الجودة للمجتمع أمر بالغ الأهمية. كان الهدف من هذه الدراسة هو فحص مصادر مياه الشرب المختلفة في العديد من مناطق أجدابيا للتأكد من خلوها من التلوث وصلاحياتها للشرب. تم تقييم صحة المجتمع لتحديد مستوى تلوث المياه. الطريقة: تم جمع أربع عينات مياه: 21 من الشبكة العامة (مياه النهر)، و 23 من المياه المعبأة (المياه المعدنية)، و 4 من مياه الآبار، و 2 من مياه الأمطار. تم تحليل إجمالي القولونيات، وتم تحليل الإشريكية القولونية والمكورات العنقودية في عينات المياه. تضمن المسح المنزلي أسئلة حول المنطقة السكنية في المدينة، والمصدر الرئيسي لمياه الشرب في المنزل، ونوع خزانات مياه الشرب، وتكرار إضافة الكلور إلى مياه الشرب، وتكرار تنظيف خزانات مياه الشرب، وما إذا كانت مياه الشرب قد تم فحصها. هل

مرض أحد أفراد الأسرة بعد شرب الماء؟ ما نوع المرض؟ هل تعرف مصادر تلوث المياه؟ هل أنت على دراية بمخاطر تلوث المياه؟ برأيك، ما هو الحل الأمثل لمعالجة مشكلة تلوث مياه الشرب؟ قورنت نتائج الاختبارات بالحدود المسموح بها التي وضعتها منظمة الصحة العالمية، ووجدت أن نسبة البكتيريا القولونية كانت 100%، والإشريكية القولونية 73.9%، والعقدية 23.9% من عينات المياه. كانت مستويات تركيز الإشريكية القولونية مرتفعة. كشفت نتائج المسح المنزلي أن تلوث المياه كان أعلى في الأجزاء الشرقية والشمالية من المدينة بين السكان الذين يستخدمون شبكة المياه العامة. كانت مستويات التلوث أعلى في الخزانات الجوفية، تليها خزانات الأسطح. كانت مستويات التلوث أقل بين أولئك الذين ينظفون خزاناتهم مرتين في السنة. تحتوي المياه المعالجة بالكلور على عدد أقل بكثير من وحدات تكوين المستعمرات (CFU) لكل 100 مل من عينة المياه. في الختام، يُعد التخلص غير السليم من مياه الصرف الصحي عاملاً رئيسياً يساهم في تلوث المياه في المدينة، يليه التلوث من خزانات المياه الملوثة وقلة الوعي بتنقية المياه وتعقيمها. هناك حاجة ملحة لاتخاذ خطوات طارئة لوقف المزيد من تدهور جودة المياه وتحسين جودة المياه الحالية لحماية السكان من انتشار الأمراض المنقولة بالمياه..

**الكلمات المفتاحية:** الكشف ، مسببات الأمراض المنقولة بالمياه ، مياه الشرب.

## Introduction

Around the world, water is exposed to many biological and other pollutants. According to the World Health Organization's 2019 report. <sup>(1)</sup> globally, more than a billion people do not have access to clean water sources. Waterborne diseases are a global burden, estimated to cause more than 2.2 million deaths annually; approximately 1.6 million annual deaths result from diseases caused by biological pollutants. <sup>(2)</sup> There are different pathways for pathogens to enter the human body, either directly, such as through drinking water, or indirectly, through food. Waterborne infection occurs by ingesting water contaminated with a variety of infectious agents or by contact with contaminated water. <sup>(3)</sup> In predominantly rural communities, about 85 percent of the population consumes unsafe water from sources such as reservoirs, rivers, and wells, this water rarely meets legal drinking standards. <sup>(4)</sup> Infectious diseases caused by pathogenic bacteria, viruses, and protozoa are common and widespread health risks associated with drinking water. Most waterborne pathogens are introduced into the drinking water supply via human or animal feces (enteric pathogens) but also can be found naturally in aquatic environments because native aquatic microorganisms. <sup>(5)</sup> Water quality must be continuously monitored to ensure it is free of pathogens. <sup>(6)</sup> The most commonly used indicator for determining the quality of drinking water is *E. coli*, as its presence is always associated with fecal contamination of the water. <sup>(7)</sup> The World Health Organization classifies fecal coliform data into the following risk categories: 0 CFU/100 mL (no risk); 1-10 CFU/100 mL (low risk); 10-100 CFU/100 mL (medium risk); 100-1000 CFU/100 mL (high risk); and  $\geq 1000$  CFU/100 mL (very high risk). Various intestinal infections resulting from water pollution are associated with symptoms; the most common of which are vomiting, nausea, and diarrhea. These symptoms can be serious, even fatal. <sup>(8)</sup> Water resources in the city of Ajdabiya face numerous problems, including scarcity of clean water and quality issues, like chemical, biological, or physical contamination of water sources, particularly in areas with increased human activity or those close to sewage outlets. <sup>(9)</sup> The problem of water pollution in Ajdabiya is extremely significant because of its environmental and health impacts on the population. This is due to the rising groundwater table, which causes sewage and rainwater to seep into drinking water reservoirs. The increased volume of sewage is attributed to the city's population growth, which places pressure on the dilapidated wastewater treatment plant, contributing to the seepage of contaminated water into the ground. Corrosion by water networks and damage to the rainwater drainage system have also led to water seepage through them, damaging groundwater, increasing health risks to residents, and threatening the recurrence of diseases such as the 1996 cholera outbreak. A study of Al-Kaseh et al. found the groundwater quality in Ajdabiya to be unfit for drinking. <sup>(10)</sup> **General Assessment of drinking water quality**

Water quality parameters Three main characteristics important to drinking water quality including; Microbiological - viruses, bacteria, protozoa and worms. Chemicals – minerals, metals and Physical - taste, color, and odor. characteristics for Safe drinking water: free from pathogens, free from impurities, colorless, tasteless and odorless. <sup>(6)</sup> To verify that drinking water is microbiologically contaminated, an analysis should be carried out for microorganisms indicating feces.

### Total coliform bacteria.

T.C. Bacteria. It is a Gram-negative, rod-shaped bacterium that does not form spores and lives in aerobic or anaerobic environments. It is found in water contaminated with human and animal feces. This bacterium can ferment lactose at a temperature of 35 degrees Celsius or higher. The presence of this bacterium in water indicates that water treatment is insufficient. <sup>(11)</sup>

### *Escherichia coli* (*E. coli*)

Many people are susceptible to *E. coli* infection, and the risk of infection increases in certain age groups, such as children and the elderly. The risk is also greater for people with weakened immune systems, digestive problems,

or low stomach acid. Eating undercooked foods or contaminated milk also increases the risk of infection. It is sometimes linked to seasonal changes and is more prevalent in the summer. <sup>(12)</sup>

### **Risk factors of E. coli infection**

While anyone can experience an E. coli infection, certain people are at higher risk than others. Risk factors include: Age: Older adults and young children are more likely to experience serious complications from E. coli. Weakened immune systems: People with weakened immune systems are more susceptible to E. coli infections. Season: E. coli infections are more likely to occur during the summer months, from June to September. Low stomach acid levels and certain foods, such as unpasteurized milk or juices, and eating undercooked meat can increase your risk of E. coli. <sup>(12)</sup>

### **Streptococcus faecalis**

Streptococcus faecalis tend to persist longer in the environment than thermotolerant or total coliforms and are highly resistant to drying. Streptococci, along with other microbial indicators has been used to assess the effectiveness of groundwater treatment and the removal of microbial contaminants. In another study, the amount of Streptococcus bacteria and other bacteria in dairy products was measured. <sup>(13)</sup>

### **Aim Of This Study**

Detection of waterborne pathogens present in drinking water in the city of Ajdabiya. 2- Isolation and identification of the main types of pathogenic organisms that cause water pollution in the city. 3- Prevent water-related outbreaks in the city and maintain the health of the population. Search for available solutions to the problem of water pollution and implementing control measures to prevent the problem from getting worse.

### **Literature Review**

Waterborne pathogens emerged in 1992; the main causes of waterborne infection are coliform bacteria, in addition to E. coli. (Park J. et al., 2018). <sup>(14)</sup> Improving water quality could reduce the global burden of disease by 4%. However, waterborne infections remain high. Numerous outbreaks have been recorded worldwide due to various pathogens, such as E. coli, Giardia, and Pseudomonas aeruginosa. These pathogens cause various types of liver and skin infections, as well as acute intestinal and respiratory diseases. (Karmali M.A. et al., 2018). <sup>(15)</sup> Twenty-one outbreaks and 507 cases of waterborne diseases were reported to 1996 and 2006. In the United States, 1,870 outbreaks were recorded due to water pollutants. (Van Nevel S. et al., 2017). <sup>(16)</sup> Enteric bacteria have a minimal infectious dose (MID), in the range of 10<sup>7</sup> to 10<sup>8</sup> cells, but it is much lower with some species, such as Shigella spp. (10<sup>1</sup>–10<sup>2</sup>), Campylobacter spp., E. coli (10<sup>6</sup>–10<sup>8</sup>), and V. cholera (10<sup>3</sup>). Moreover, protozoa only need a few oocysts to cause the disease, approximately (10<sup>1</sup>–10<sup>2</sup>). (Gruenberg J. et al., 2006). <sup>(17)</sup> The environment is home to a wide variety of bacteria, some of which are mildly. Pathogens are highly contagious. Poor sanitation and contaminated drinking water are the main causes of their spread. Cholera, typhoid fever, and dysentery are among the most common diseases and are widespread in developing and poor countries, especially among children under 5 years of age. These diseases affect their growth, lead to a decrease in their intelligence quotient, and sometimes leads to death. A study reported that 9% of the 6.5 million children expected to die annually, and diarrhea is the main cause (Evans et al., 2014; Dora et al., 2015). <sup>(18, 19)</sup> Diarrheal diseases also negatively affect academic and social development, affecting children's learning process (Evans et al., 2014). <sup>(18)</sup> In a previous study in Palestine, 1520 water samples were tested in schools for the biological properties of drinking water; of these, approximately 299 water samples were contaminated with different types of pathogens. Approximately 19.7% of school water tanks were contaminated (UNICEF Survey Results, 2012). <sup>(20)</sup> According to a global report by UNICEF and the World Health Organization The quality of drinking water is important to human health. He explained that few governments care about water quality and conduct regular water testing. Water quality monitoring is more common in urban areas than in rural areas (WHO and UNICEF, 2016). <sup>(21)</sup> Measurements of E. coli in Lebanon's drinking water showed that compatibility with national drinking water health standards varies widely (UNICEF and WHO, 2018). <sup>(22)</sup>

### **Material and methods**

#### **Data collection and questionnaire**

Family homes were visited in four areas of Ajdabiya. To understand the water situation in the city and analyze the microbial content of the water. Families in the study area were asked for permission to take water samples and to distribute a questionnaire. Fifty houses were selected to assess the quality of drinking water in the city. The selected houses cover the different geographical areas of the city: east, west, north and south. A considerable number of houses were selected in each part of the study area, and each student collected ten samples. A questionnaire was administered to the head of the household or a family member. We collected information about water in homes using pre-prepared questions in a questionnaire, the house location, the source of drinking water, and water treatment methods. SPSS version 19 was used to analyze the data and find the relationship between the relevant variables in the questionnaire.

### Field visits and collection of drinking water samples

Selected homes were visited during the period from June 28, 2021, to July 10, 2021 to collect drinking water samples and complete the questionnaire. Also, 50 drinking water samples were collected— one sample (250 ml) from each home—for laboratory analysis. The samples were collected following all health protocols and the water sample collection protocol used by water laboratories and includes the following steps:

1. Use containers designated for water collection. These sterile, closed containers are opened only during the collection of samples obtained from a laboratory specialized in water analysis.
  - 2- Wash and sterilize hands, wear gloves and sterile towels before collecting a sample
  - 3- Samples are collected from the faucet after opening it for five minutes, then passing a flame source outside the tap. Then, the take the sample after several minutes.
  - 4- In the designated containers, the water is collected, tightly closed, and the sample number and date are marked on them.
  - 5- Samples are sent to the water laboratory in containers containing ice bags. The time required to send the sample should not exceed 6-24 hours as a maximum to prevent damage to the sample
  - 6- Samples are received then sent for analysis in the water laboratory of Al Nahr Company, Benghazi, where they are examined for microbiological properties (T.C.F, E.C, Streptococcus)
- Tests and methods for analyzing the quality of domestic drinking water, total microbiological titration, colonic membrane, and fecal colonic membrane filtration.

### The multiple tube fermentation technique

Detect total coliform (D. T. C.) Media and reagents:

#### Isolation media

Lauryl tryptose broth; MacConkey broth. Lactose broth

#### Confirmatory media

Brilliant green lactose broth (BGLB). Eosin methylene blue agar (EMB)

Nutrient agar slant. EC medium broth

Tryptone water broth

#### Equipment

Autoclave

Incubators were set at 36 °C, and 44 or 44.5 °C.

Pipettes (0.1, 1, 10) ml), test – tubes, and racks

Durham tubes

Loop with a 3 mm diameter

Sodium thiosulfate solution, required when chlorinated supplies are tested.

Laboratory disinfectant

Safety equipment: mask, gloves, coat and safety glasses

#### Procedure

##### Presumptive stage

- 1 – 10 ml of sample to each of three tubes containing 10 ml of double-strength medium
- 2 – 1.0 ml of sample to each of three tubes containing 5 ml of single-strength medium
- 3 – Add 1.0 ml of sample to each of three tubes containing 5 ml of single-strength medium.
- 4 – Examine the tubes for gas formation after incubation for 24 hours at 35°C, gently shaking them before examination.

##### Total colony counts

Media and reagents: Nutrient agar. TGE agar; Standard plate count agar; Ethanol 70 %

#### Equipment

- Petri dishes, pipette, 0.1 ml, air incubator set at 37 °C, colony counter
- Laboratory disinfectant
- Safety equipment: mask, gloves, coat & safety glasses

#### Procedure

1 – Swirl the sample bottles to mix

2 – Aseptically pipette 0.1–1 ml into a petri dish placed on a level bench

Sterilize with 70% ethanol and repeat for the other samples

3 – Use Petri dishes and pour 20 ml of molten agar into them, taking care to sterilize the dishes.

4 – Mix the sample immediately with the agar using a gentle swirling motion for 05 – 10 seconds, then keeping petri dish flat throughout, allow the agar to set before inverting the dish

5 – Incubate the sample at 37 °C for 24 to 28 h

6 – Count and record all visible colonies after incubation using the colony counter, If the count exceeds 300, record it as < 300 colonies/ml.

Gas formation in the secondary tube of lauryl tryptose broth over 48 hours, along with the appearance of non-spore-forming Gram-negative rod-shaped bacteria in the agar culture, indicates the presence of a member of the coliform group.

#### **Alternative confirmation stage**

The coliform test (using EC medium or isolated media) is applicable to the investigation of drinkable water. (pp9-52)

1 – Shake or rotate primary fermentation tube showing gas, and using a sterile loop, 3mm in diameter, transfer one loopful of culture to two tubes containing brilliant green lactose bile broth and EC broth.

2 – Incubate the inoculated brilliant green lactose bile broth tube for 48 hr at 35 °C , and EC broth for 24 hr at 44.5°C Formation of gas or turbidity in any amount in the inverted vial of the brilliant green lactose bile broth fermentation at any time within ( 24 / 48 hrs ) constitutes a positive for total coliform bacteria and formation of gas / turbidity in any amount in the vial of EC broth after 24h constitutes a positive for thermotolerant fecal coliform bacteria If no gas formation occurs after 24 hr, it constitutes a non-fecal coliform ( pp95 , 9-47 , 9-52 )

To detect E coli, incubate each tube showing formation of gas / turbidity in the inverted vial of EC broth to tryptone water at 44°C for 24 hr, growth with gas production confirms the presence of thermotolerant coliforms. Add 0.2-0.3 ml of Kovacs reagent and color of red color indicates the synthesis of indole from tryptophan and confirming the presence of E coli.

**Note:** growth and gas production in the absence of indole confirm thermotolerant coliforms

3 – Based on the number of positive tubes in each dilution of the confirmed or completed test, the calculated density of coliform bacteria in the sample can be obtained from Table 1 for MPN indices and 95% confidence limits for drinking water testing.

#### **Media and reagents**

Isolation media

- Azide dextrose broth

Or Ethyl violet azide broth (EVA)

#### **Confirmatory media**

Bile esculin azide agar

#### **Equipments**

- Autoclave incubators were set at 36 °C and either 44 or 44.5 °C
- Pipettes (0.1, 1, 10 ml) ml
- Test tubes and racks
- Loop with a 3 mm diameter
- Sodium thiosulfate solution is required when chlorinated supplies are tested
- Laboratory disinfectant
- Safety equipment: mask, gloves, coat and safety glasses

#### **Procedure**

##### **Presumptive stage**

1 – Add 10 ml of the sample to each of the three tubes containing 10 ml of double-strength medium

2 – 1.0 ml of sample to each of three tubes containing 5ml of single strength medium

3 – 1.0ml of sample to each of three tubes containing 5ml of single-strength medium

4 – incubate inoculated fermentation tubes at 35 + 0.5°C after 24 hours shake each tube gently and examine it and if absent turbidity has formed and reincubate and reexamine at the end of 48 + 3 hours, formation of turbidity in tubes or vials within 48 + 3 hr. constitutes a positive presumptive test.

5 – the absence of turbidity formation at the end of 48 + 3 hr. of incubation constitutes a negative test.

##### **Confirmed stage**

1 – Submit all turbidity tubes within 24–28 hours of incubation to the confirmed test transfer to the confirmatory medium. Shake or rotate primary tube showing turbidity and do a sterile loop, (3mm in diameter), transfer one loopful of culture to Bile Esculin Azide Agar.

2 – Incubate the petri dish for 24 hours at 44.5 °C. Formation of black color within 24 hours constitutes a positive confirmed test.



3 – The calculated density of fecal streptococcus bacteria in a sample can be obtained from the MBN table based on the number of positive tubes in each dilution of the confirmed or completed test. Table 1 shows MBN indices and 95% confidence limits for potable water testing.

### **Detect total coliform (T.C.F.)**

Media and reagents:

- Isolation media:
- m-Endo broth
- or m-FC medium
- or lauryl sulfate broth

### **Confirmatory media**

- Brilliant green lactose bile (BGLB)
- Lauryl tryptose broth
- EC Medium broth
- Tryptone Water broth

### **Equipment**

- Membrane filter
- Absorbent pads
- Filtration units
- Containers for culture medium
- Autoclave
- Incubators were set at 36 °C, and 44 or 44.5 °C.
- Test-tubes and racks
- Durham tubes
- Loop with a 3mm diameter
- Sodium thiosulfate solution: is required when chlorinated supplies are tested.
- Laboratory disinfectant safety equipment includes a mask, gloves, coat, and safety glasses.

### **Procedure:**

#### **Presumptive stage:**

1 – Place a sterile absorbent pad in a sterile petri dish using sterilized forceps. replace the lid, and do not touch the pad or the inside of the petri dish.

2 – Pour m-Endo broth medium into petri dish over the absorbent pad. replace the petri dish lid.

Set up the membrane filter assembly. Use sterilized forceps to place a membrane filter.

Pour 100 ml of sample into the funnel, apply vacuum, and filter the sample.

Turn off the vacuum and lift off the funnel top. Using sterilized forceps, transfer the filter immediately to the previously prepared Petri dish.

Invert the Petri dish and incubate at  $35 \pm 0.5$  °C for 22–24 hours.

After incubation, use a 10-15x microscope to count the red colonies with a greenish-gold metallic sheen and record the results.

#### **Confirmation stage**

Confirm total coliforms using lauryl tryptose and brilliant green bile broth.

1 – Touch the needle or swab the coliform (sheen) colony grown on m-Endo plate and transfer it to a single-strength lauryl tryptose (LT) broth tube; then touch the same coliform colony with the needle or swab and transfer to a brilliant green bile (BGB) broth tube.

2 – Invert both tubes to eliminate any air bubbles trapped in the inner vials. Incubate the tubes at  $35 \pm 0.5$  °C. After one hour, invert the tubes to remove trapped air in the inner vial, then continue incubation.

3 – after 24 hours, check the inner vials for growth and gas growth (turbidity) and gas bubbles in both the LT and BGB broth tubes. verify that the colonies are coliforms. If one or both tubes do not show gas, reincubate both tubes for an additional 24 hours. If no gas is present in the LT broth tube after 48 hours, the colony is not a coliform, and additional testing is not necessary.

4 – Confirm positive results. If growth and gas are produced in the LT broth tube but not in the BGB broth tube, inoculate another BGB tube from the gas-positive LT broth tube, incubate this BGB broth tube and check for growth and gas after 24 hours and/or after 48 hours. If growth and gas are produced within  $48 \pm 3$  hours, the colony is confirmed as a coliform.

### Confirmation of fecal coliforms (EC medium)

Analyze total coliform-positive potable water samples for the presence of fecal coliform or *E. coli*. Confirm fecal coliform from a membrane filter positive for total coliforms by swabbing the membrane with a sterile cotton swab and inoculating a tube of EC medium broth. Growth and gas production in the EC medium confirms the presence of fecal coliforms.

Incubate the tube at  $44.5 \pm 0.2$  °C and after 24 hours, check growth and gas bubbles in the EC medium broth tube confirm the presence of fecal coliforms

### Calculation of coliform density

Total coliforms/100 mL = (coliform colonies counted x 100)/(mL sample filtered).

If no coliform colonies are observed, report the coliform colonies counted as "< 1 coliform/100 mL."

If growth covers the entire filtration area of the membrane, or a portion of it, and colonies are not discrete, report results as "confluent with or without coliforms."

If the total number of colonies (coliforms plus non-coliforms) exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as "too numerous to count" (TNTC)

**Table1:** Use the Most Probable Number (MPN) by the three-tube method, or use the detection of total coliform bacteria (TCF)

3 of 18ml each	3 of 1ml each	3 of 0.1ml each	Per 10ml	Lower	Upper
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1,300
3	3	1	460	71	2,400
3	3	2	1,100	150	4,800



**Figure1:** The multiple tube fermentation technique equipment

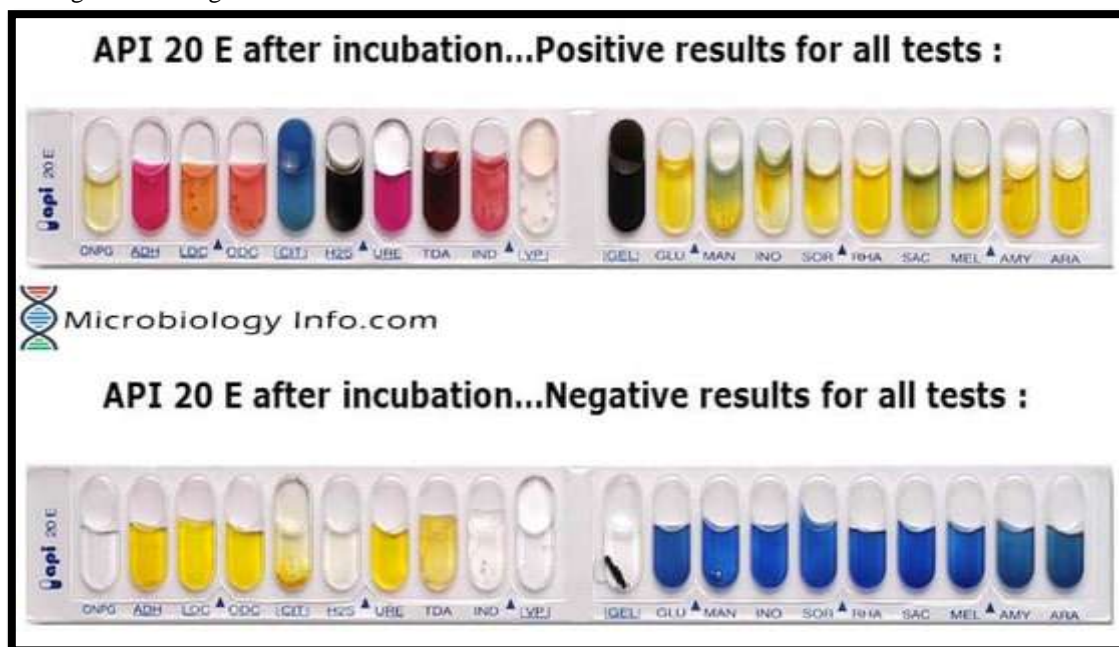
## Methods

### The API (Analytical Profile Index) 20E Test. <sup>(23)</sup>

API 20E is a biochemical panel for identification and differentiation of members of the family Enterobacteriaceae. quick, safe and easy to perform.

### Principle

The API test consists of a plastic strip, located in 20 chambers, containing dry media for different types of bacteria. When the sample is added to each chamber, an enzymatic reaction occurs resulting from either the catabolism of proteins or amino acids, or the fermentation of carbohydrates, depending on the type of bacteria. The results are read using various reagents.



**Figure 2:** API (Analytical Profile Index) 20E Test

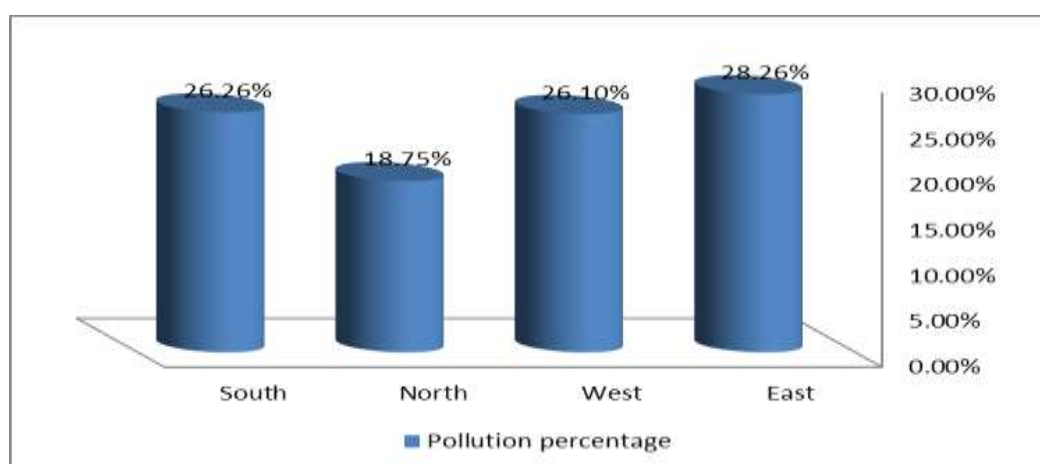
## Statistical Analysis

The statistical analysis of the data obtained from the questionnaire was conducted using SPSS version 19, yielding the following results:



**Table 2:** Percentage of Pollution in homes by residential area in the city

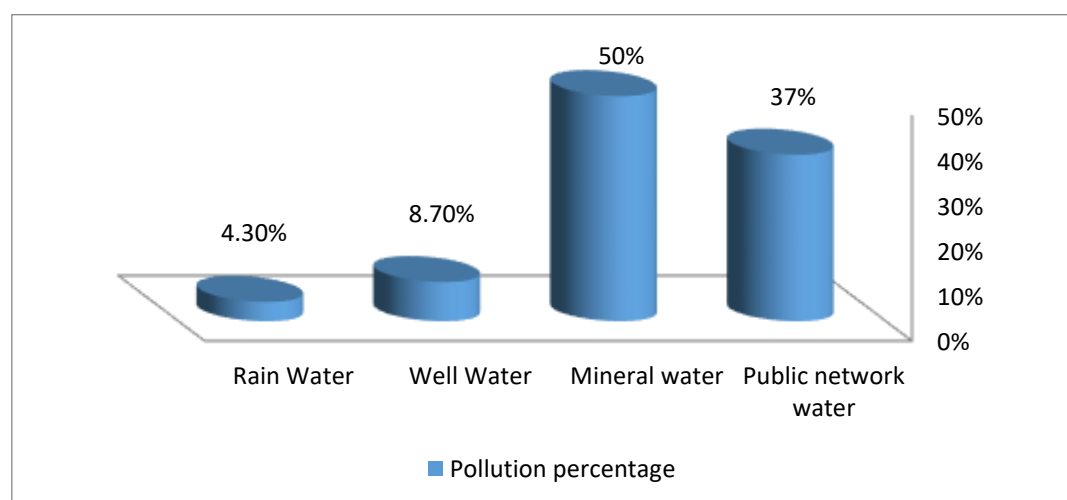
Residential area	House Number	Number of houses with polluted water	Pollution percentage
East	13	13	28.2 %
West	12	12	26.1%
North	13	9	18.75 %
South	12	12	26.1%
Total	50	46	100%

**Figure 3:** The Percentage of pollution in homes by residential area in the city

Detection of microbes in relation to the housing site in the city which includes 13 houses in the eastern region, showed a contamination percentage of 28.26%. The pollution rate of 12 houses in the western region is 26.0%. Twelve houses in the northern region have a pollution rate of 18.75%. And Thirteen homes in the southern region had a pollution rate of 26.26%.

**Table 3:** The percentage of pollution depends on the type of drinking water in the house.

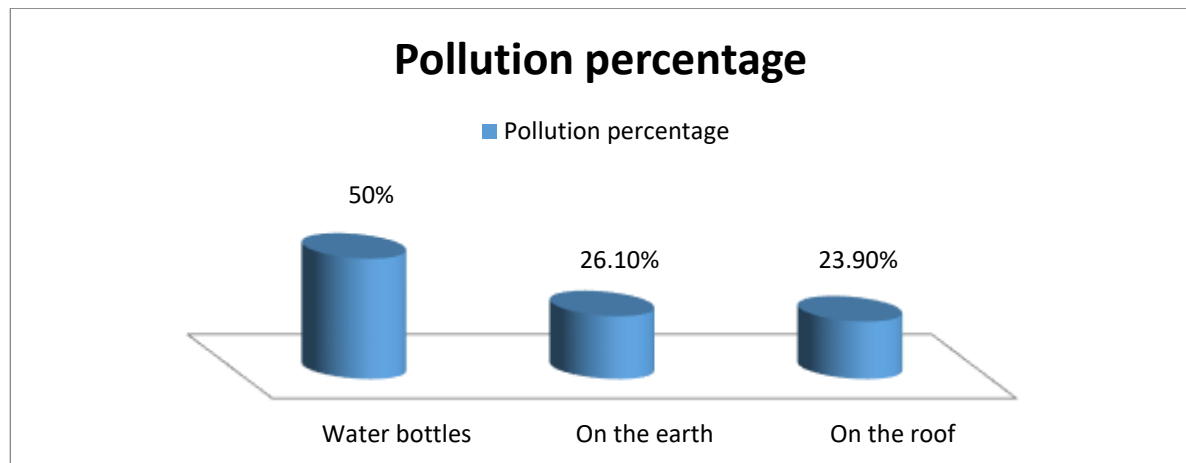
Home drinking water source	House number	Number of houses with polluted water	Pollution percentage
Public network water	21	17	37 %
Mineral water	23	23	50 %
Well Water	4	4	8.70 %
Rain Water	2	2	4.30 %
Total	50	46	100 %

**Figure 4:** The percentage of pollution depends on the type of drinking water in the house.

The analysis conducted on the source of drinking water in fifty houses in Ajdabiya showed that twenty-one houses representing 37%, use public network water, while twenty-three houses representing 50%, buy mineral water from shops, and four houses (8.7%) use well water. Additionally, 2 houses (4.3%) get their water from rainwater.

**Table 4:** Pollution rate based on the type of water storage tank

The type of water tank used	House Number	The amount of polluted houses	Pollution percentage
On the roof	11	11	23.90%
On the earth	16	12	26.1 %
Water bottles	23	23	50 %
Total	50	46	100

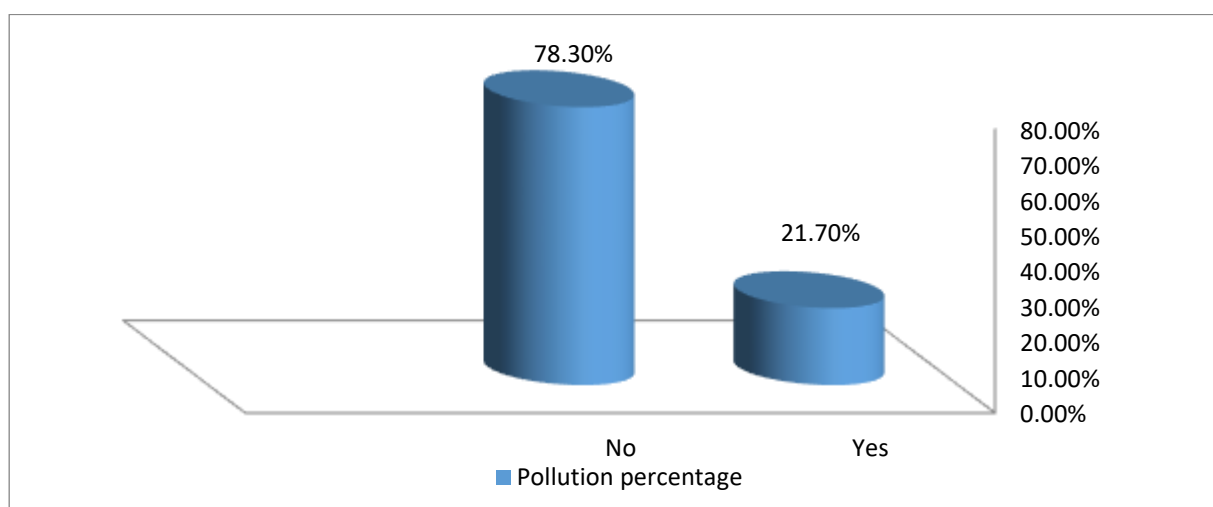


**Figure 5:** Pollution rate based on the type of water storage tank

Causes of drinking water contamination drinking water in homes: A statistical analysis was conducted and the risk factors for bacterial contamination and their proportions. Surface-stored water was found to be 23.9% polluted; water stored underground in 12 homes was 26.1% polluted; and mineral water bought by 23 homes was 50% polluted

**Table 5:** The relationship between the periodic addition of chlorine to the household water and the percentage of water pollution.

Is chlorine added to the water periodically?	House Number	Pollution percentage
Yes	10	21.70 %
No	36	78.30 %
Total	46	100 %

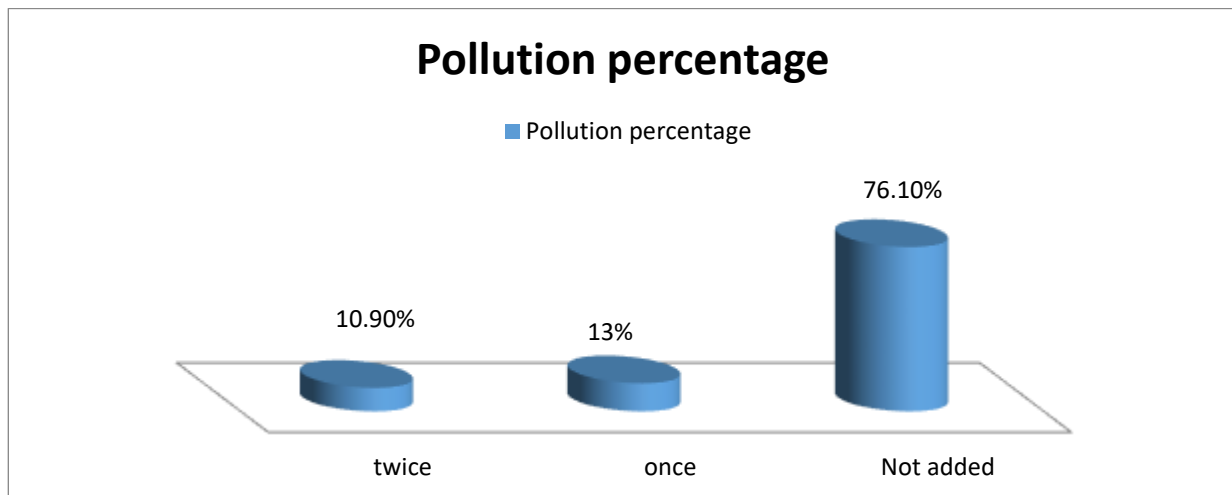


**Figure 6:** The relationship between the periodic addition of chlorine to the house water and the percentage of water pollution.

When people were asked about the periodic use of chlorine to disinfect drinking water, the answers were as follows: Of the 46 homes with drinking water, 10 homes (21.7%) used chlorine to disinfect water, and 36 homes (78.3%) did not use chlorine.

**Table 6:** The relationship between the frequency of cleaning the water tank at home and pollution.

Cleaning the water tank at home.	House Number	Pollution percentage
Not added	35	76.10 %
Once	6	13 %
Twice	5	10.90 %
Total	46	100 %

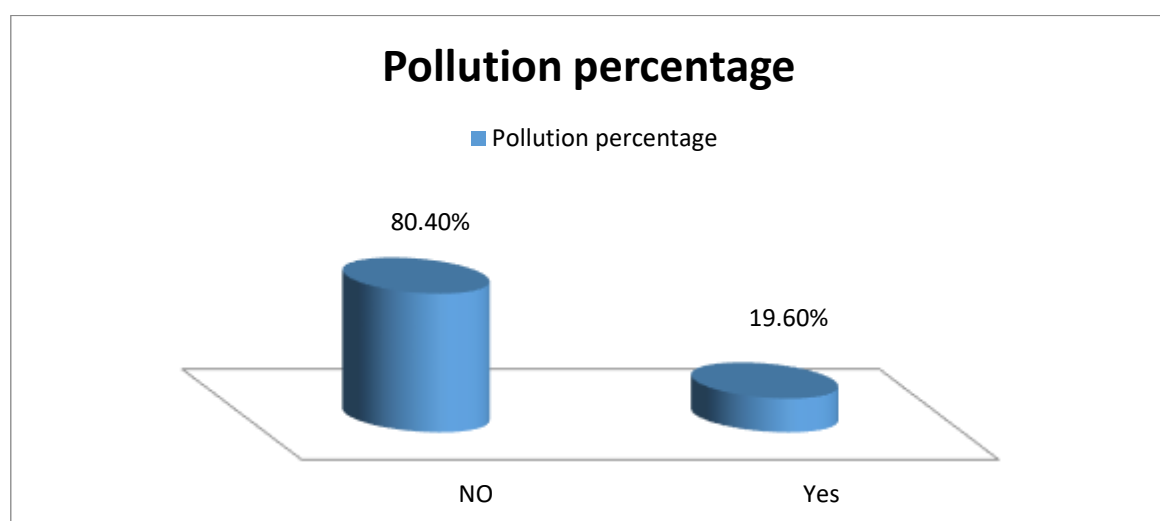


**Figure7:** The relationship between the frequency of cleaning the water tank at home and pollution.

When people were asked about the frequency of water tank cleaning at home, the answers were as follows: Of the 46 houses where drinking water was polluted, 35 houses (76.1%) did not clean the tanks, and 6 houses (13%) cleaned the tanks once a year. While 5 houses representing 10.9% cleaned the tanks twice a year.

**Table 7:** The relationship between drinking water testing and the percentage of pollution.

Is drinking water tested at home before	House Number	Pollution percentage
Yes	9	19.6 %
NO	37	80.4 %
Total	46	100 %

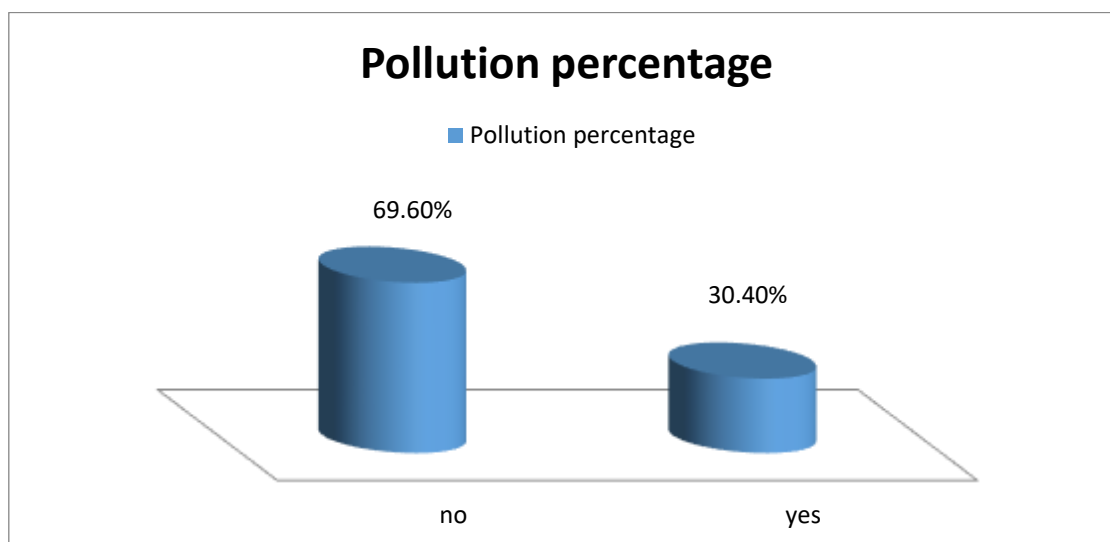


**Figure 8:** The relationship between drinking water testing and the percentage of pollution

The following table examines the relationship between the drinking water test and the percentage of contamination. Of the 46 houses with polluted water, only 9 houses (19.6% contamination rate) had previously conducted a drinking water analysis at home, while 37 houses (80.4% pollution rate) had not.

**Table 8:** The relationship between water pollution and disease in the home

Has anyone in the house fallen ill due to water	House Number	Pollution percentage
Yes	14	30.40 %
No	32	69.60 %
Total	46	100 %

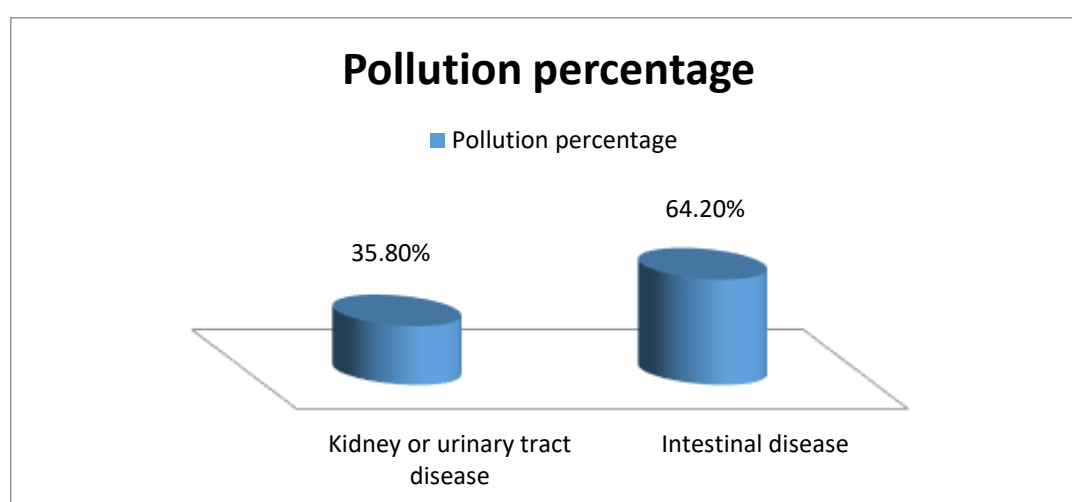


**Figure 9:** The relationship between water pollution and disease in the home

The water pollution rate at 14 homes was 30.4%, while it was 69.6% at 32 homes

**Table 9:** Types of diseases caused by water

The type of disease	House Number	Pollution percentage
Intestinal disease	9	64.2 %
Kidney or urinary tract disease	5	35.8 %
Other diseases	0	0 %
Total	14	100 %

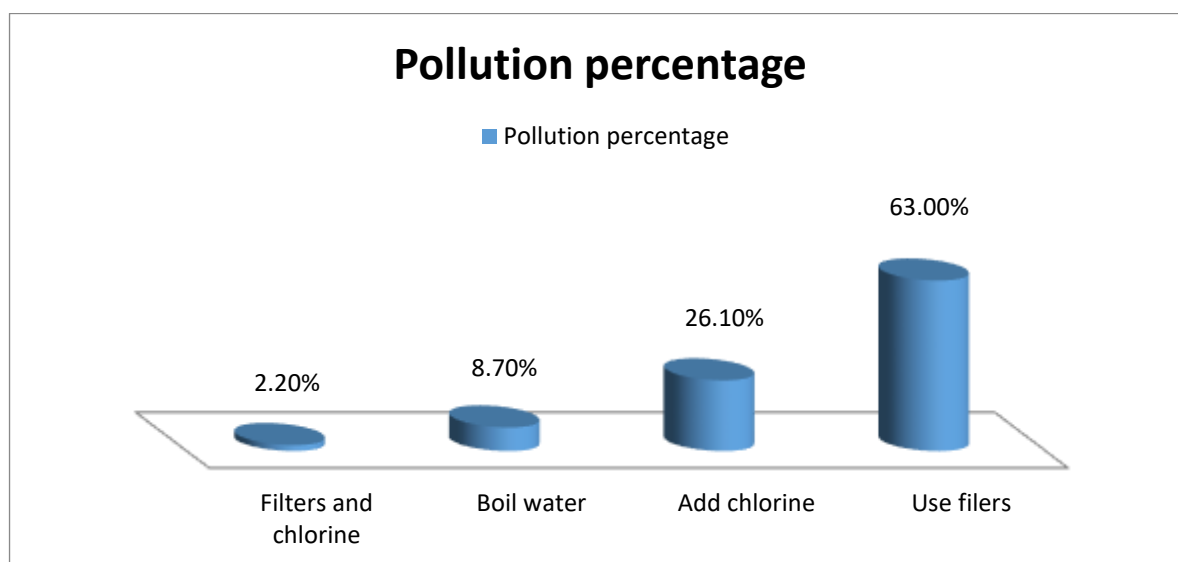


**Figure 10:** Types of diseases caused by water

In the current research, 9 people, representing a pollution rate of 64.2% confirmed the occurrence of symptoms. Additionally, 5 homes, representing 35.8%, had suffered from symptoms. Additionally, no other diseases were observed.

**Table 10:** The relationship between the use of different methods of water sterilization and the percentage of pollution.

Type of drinking water sterilization method	House Number	Pollution percentage
Use filers	29	63 %
Add chlorine	12	26.1 %
Boil water	4	8.7 %
Filters and chlorine	1	2.2 %
Total	46	100 %

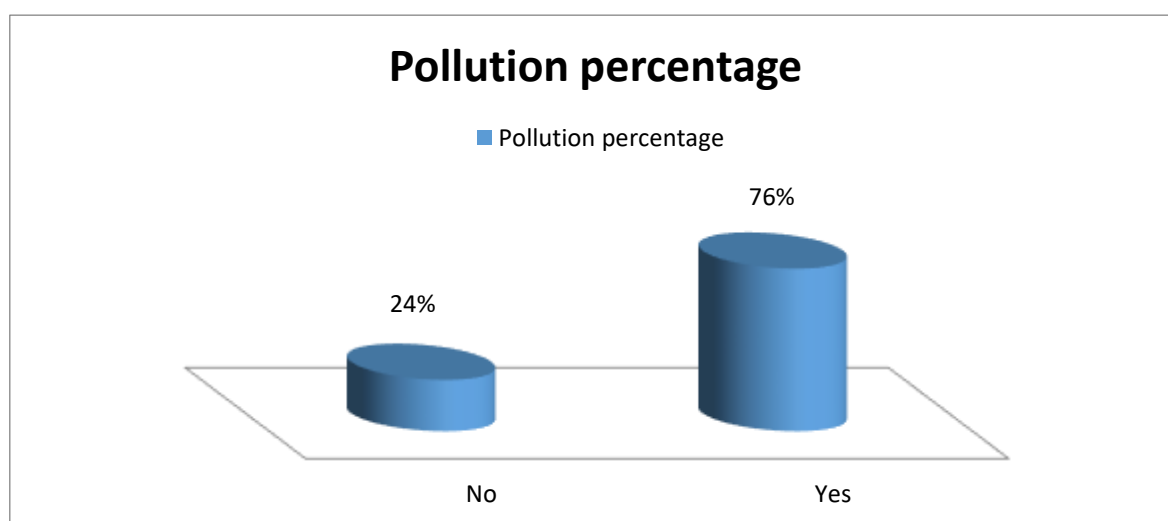


**Figure 11:** The relationship between the use of different methods of water sterilization and the percentage of pollution.

Of the houses, 29 houses, representing 63% of the pollution rate, out of 46 houses, used filters to sterilize the polluted water. It was found that 12 houses representing a pollution rate of 26.1% used chlorine; 4 houses a pollution rate of 8.7%; while one house represents 2.2% used both filters and chlorine

**Table 11:** Do you know the sources of water pollution?

Do you know the sources of water pollution?	House Number	Pollution percentage
Yes	38	76 %
NO	12	24 %
Total	50	100 %



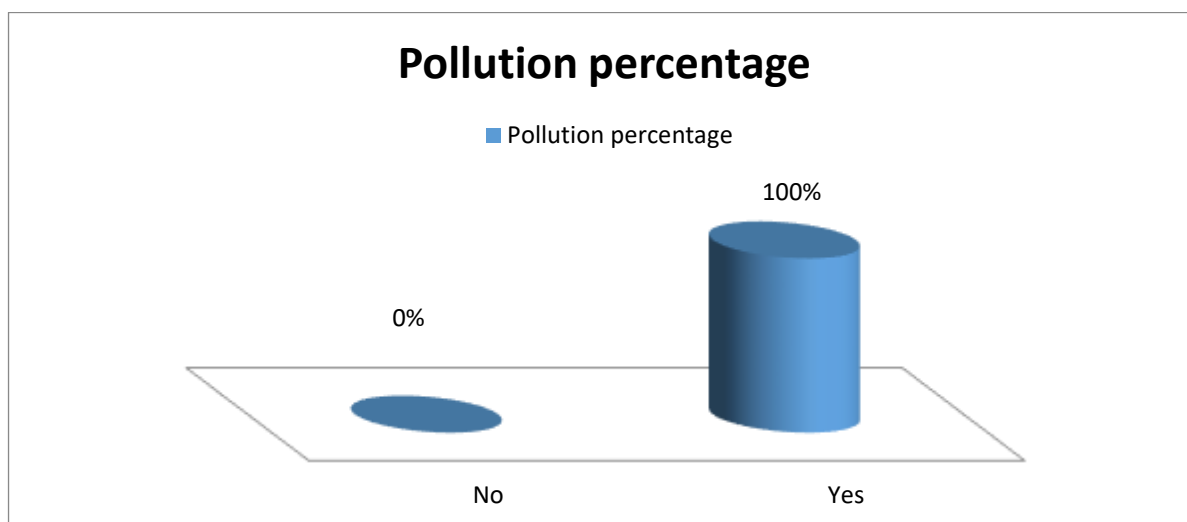
**Figure 12:** Sources of water pollution knowledge



When asked about their knowledge of the sources of water pollution, the vast majority of respondents were familiar with the sources. Specifically, 38 respondents (76% of 50) answered 'yes,', and 12 answered 'no'. They represented 24% with a 'no' response.

**Table 12;** Do you understand the dangers of water pollution?

Do you understand the dangers of water pollution?	House Number	Pollution percentage
Yes	50	100%
No	0	0 %
Total	50	100 %

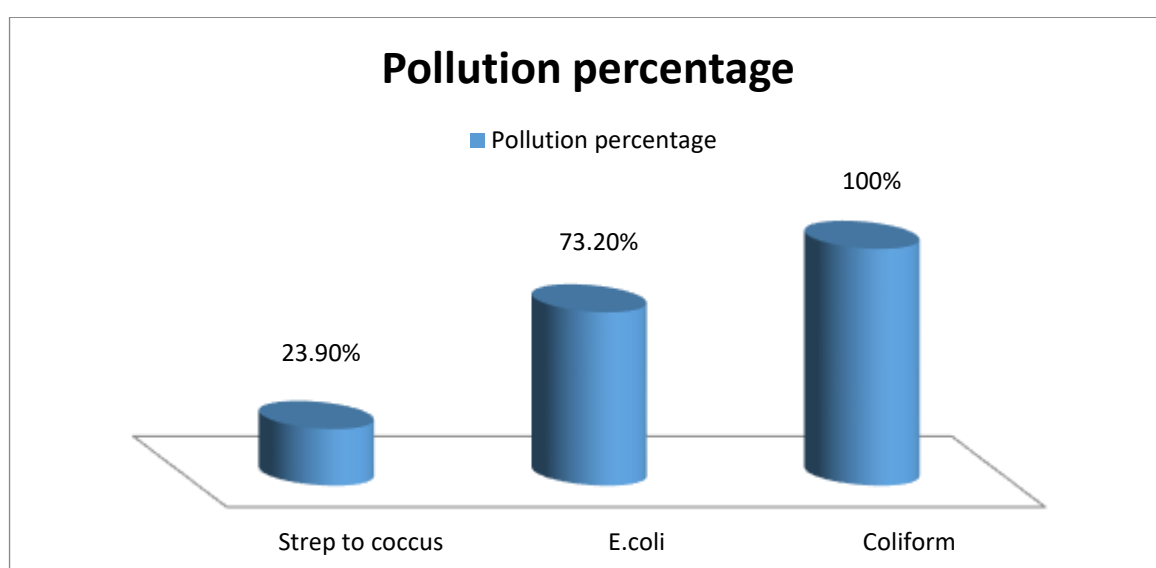


**Figure 13:** Do you understand the dangers of water pollution?

When people were asked about their knowledge of the danger of water pollution in 50 questionnaires, all answers were affirmative, representing 100%.

**Table 13:** Comparison of contamination with different types of bacteria

The percentage of contamination with different types of bacteria	House Number	Pollution percentage
Coliform (t, c, f)	46	100 %
E. coli	34	73.9 %
Streptococcus	11	23.9 %



**Figure 14:** Comparison of contamination with different types of bacteria

Upon detecting total fecal coliform bacteria in the 46 contaminated houses, total coliforms were found in 100% of them, with 34 houses (73.2%) containing *E. coli* bacteria. Additionally, 11 homes contained *Streptococcus faecalis*, representing 23.9%.

## Discussion

In this research, a questionnaire was conducted, and water samples were analysed to assess the quality of drinking water in homes. Drinking water samples were taken from 50 houses from where the family members drink water. The study included houses located in the eastern, western, northern, and southern regions of the city, covering all areas. Fifty questionnaires were submitted to fifty families. It includes 13 homes in the eastern and southern regions and 12 homes in each of the western and northern regions. **Table 2:** shows that the proportion of dwellings, according to the level of detected pollution by geographical area, indicates similar pollution risks across all geographical areas. However, the north of the city achieved the best results and the lowest pollution rate, while eastern Ajdabiya recorded the worst results regarding the pollution rate. **Table 3:** shows drinking water sources in Ajdabiya's houses. From a study conducted in academic institutions in Abbottabad (Pakistan,) to assess the quality of drinking water and its effects on health. **Ahmed (2015)**<sup>(24)</sup> evaluated and analysed 63 water samples. 54% of the facilities use government water supplies, 53% use underground wells, and 44% use wells. The most common water source was a well. **From Tables 2 and 3,** we conclude that the similar pollution percentages in different areas of the city are because 50% of people buy bottled water (mineral water) or water from filter shops, which are often anonymous and may contain many Pathogens. The incomplete sewage networks in most areas of the city and the dependence of many people on underground tanks called cesspits to dispose of waste have led many residents to fear the contamination of drinking water as a result of mixing it with black water, which led to less use of well water and even rainwater. **Table 4:** Due to the lack of interest in sterilization of tanks by people, high rates of pollution were found in both the ground tanks and on the roofs of houses, and because of the large number of mineral waters used by the population, which are highly polluted, was the reason for the presence of the highest pollution rate which results from low water quality, (WHO, 2011).<sup>(25)</sup> **Table 5:** The relationship between the regular addition of chlorine to household water and the level of water pollution is shown. It was found that the highest level of pollution was in unchlorinated water. According to the World Health Organization's recommendations (2006), the permissible chlorine level ranges between 0.6-1.0 mg/L. **In a similar study,** Sanchez (2015).<sup>(26)</sup> studied the chemical and microbiological quality of water in Uberaba. Water samples were collected from fountains and kitchen taps of eight government institutions. Faecal coliform and total coliform tests were above values acceptable by legislation in more than 50% of the samples, and chlorine concentration was below the minimum allowed by law in approximately half of the samples. **Table 6:** Relationship between the frequency of cleaning the water tank at home and pollution. It is clear that the lowest percentage of houses with polluted water cleaned their tanks twice a year, which limited the proliferation of pathogens. **Table 7:** The relationship between drinking water testing and the percentage of pollution. It is clear that the majority of the population showed no interest in analysing their household water to determine its suitability for health and the absence of pathogens. **Table 8:** The relationship between water pollution and disease in the home. It turns out that a significant percentage of people who have been exposed to some during their lives types of diseases and experienced contamination of drinking water. **Table 9:** Types of diseases caused by water, which water causes various diseases and symptoms ranging from mild to severe, Depending on the type of pathogen transmitted to the person, the occurrence of gastrointestinal disease symptoms varies. In the current research, symptoms of gastrointestinal diseases due to water ranged from vomiting, colic, diarrhea, and bloating, resulting from drinking water at home. Additionally, some individuals suffered from urinary tract infections, experiencing pain and burning during urination. **Similar studies** on the relationship between drinking water quality and human health have been conducted locally in Palestine. A study by **Arafat H. (2011)**.<sup>(27)</sup> in Tubas Governorate examined this relationship and showed that diarrhea was the most common health problem, followed by eye diseases and skin diseases. **Table 10:** The relationship between the use of different methods of water sterilization and the percentage of pollution showed that the pollution rate was high with the use of chlorine-neutral filters, while water boiled successively had lower pollution. From the above, it is clear that the population has more confidence in filters. Then in all other means of sterilization. **Table 11:** Do you know the sources of water pollution? In the previous table, the residents were asked about their knowledge of the sources of water pollution. The vast majority of them were familiar with these sources. **Table 12:** Do you understand the dangers of water pollution? When people were asked about their knowledge of the dangers of water pollution in 50 questionnaires, all answers were "yes" in the 50 households, representing 100%. In both Tables 11 and 12, the people were aware and well-informed about the dangers that polluted water may cause to their health. **Table 13:** Comparison of contamination with different types of bacteria. For full detection of coliform bacteria among 50 houses, 46 were contaminated with various forms of the bacteria. Another study in Lebanon, conducted by UNICEF in cooperation with the World Health Organization (2016).<sup>(28)</sup> conducted a water quality survey. The survey showed that 53 percent of the population in Lebanon does not drink safe water and that the water is contaminated with *E. coli*.

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## Conclusion

Many residents of Ajdabiya face challenges related to the quality of drinking water. In this research, we collected drinking water samples to examine this risk. The study included a cross-sectional census and data collection on the types of drinking water used in the city. Samples were collected and analyzed to detect the presence of *Escherichia coli*, total coliform, and streptococcus. Results from the 50 water samples examined showed that 100% of the samples in the city were contaminated with total coliform; *Escherichia coli* was present in 73.9%, fecal coliform bacteria in 73.9%, and 23.9% were contaminated with streptococcus. Drinking water pollution accounts for 64.2% of all Intestinal related diseases in the city. Although public network water is available in most homes, 50% of households buy mineral or filtered water from stores, and some have alternative sources such as rainwater storage or well water; most of these sources tested positive for total coliform bacteria. The presence of these risk factors is due to the prevalence of a wide range of pathogens in the drinking water stored in homes, and the use of contaminated water was the main reason associated with the increase in total coliform bacteria in the water.

## Recommendations:

Safe drinking water must be provided in homes, its quality continuously monitored, and testing conducted in all areas of the city.

Educate the population about the importance of testing drinking water at home. Contaminated homes should be monitored to ensure water tanks are cleaned periodically. Raise awareness about the dangers of drinking contaminated water.

Develop a strategy in the city so that houses obtaining drinking water from sources other than the public water network, such as wells and rainwater, add chlorine to the water.

Construct septic tanks in accordance with appropriate health, environmental, and construction standards to prevent groundwater contamination.

Stop consuming domestic water and inform the competent authorities if there is any change in the color, taste, or smell of the water, and promptly report any disease symptoms after consuming it.

Use available sterilization methods, such as boiling drinking water or using multi-stage filters, and periodically add chlorine to domestic water tanks.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

The author(s) declare that they have no conflict of interest.

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## Appendix

### The Questionnaire

Detection of microbial contaminants in drinking water in the city of Ajdabiya

Family Name: ..... Date: .....

#### 1. Residential Area in the City

East

West

North

South

#### 2. Main Source of Drinking Water at Home

Public Network Water (River)

Well Water

Rain Water

Mineral Water)

#### 3. Type of Drinking Water Tanks

Ground

Rooftop

Mineral Water Bottles

#### 4. Is chlorine added to drinking water regularly?

Yes

No

#### 5. How often is chlorine added?

Once a year

Twice a year - Not once

#### 6. Are drinking water tanks cleaned regularly? 6

Yes

No

#### 7. How often are drinking water tanks cleaned at home? 7

Once a year

Twice a year - Less than that

#### 8. Is Has your home's drinking water been tested?

Yes

No

#### 9- Has anyone in the household ever become ill after drinking the water?

Yes

No

#### 10. What type of illness?

Intestinal disease

Kidney disease

Other

#### 11. Do you know the sources of water pollution?

Yes

No

#### 12. Are you aware of the risks of water pollution?

Yes

No

#### 13. In your opinion, what is the best solution to address the problem of drinking water pollution?

Use filters

Use chlorine

Boil water