

Bacteriological Assessment of Tap Water and Two Types Of Bottled Drinking Water Available At Basra City, South Of Iraq

Nidham M. Jamalludeen*

Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq

DOI:110.36348/SJPM.2019.v04i09.005

| Received: 05.09.2019 | Accepted: 12.09.2019 | Published: 20.09.2019

*Corresponding author: Nidham M. Jamalludeen

Abstract

Basra gets its water from the Shatt al-Arab, the waterway formed by the Euphrates and Tigris rivers. Then it takes its way to the community through the water treatment plant. The tap water samples were studied to assess their bacteriological characteristics and their suitability for drinking purposes. Ten different localities and two bottled drinking water companies at Basra city were selected in this study. Bacteriological examination of water samples included the most probable number of presumed coliforms and fecal coliform bacteria. The results showed that the total number of coliforms was detected in all water samples taken from the ten sites. These samples were also having a positive result using another methods EMB medium and Membrane Filtrations Technique. There was no evidence of coliforms presence in two samples of bottled drinking water. The most common group of organisms used in water quality control are coliforms. These organisms represent the bacteria usually present in the intestinal tract of animals and humans. Biological contamination of water may occur during transportation from the plant to the consumer or during storage in the tank. Improving and expanding existing water and wastewater treatment systems is likely to provide good, safe and sustainable sources of water in the long term.

Keywords: Basra, Tap water, MPN, coliforms.

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INTRODUCTION

Human population deserve clean and pure water empty from any kind of germs. It is undoubtedly, safe, germ-free water brings high standards of public health. There are several microbes that can affect water quality, as water containing a large number of harmless bacteria is not safe to drink, but it is a type of microorganism that is dangerous to humans [1, 2]. Bottled water that was prepared for drinking is generally regarded safe for usage by people. It serves the only reliable source of drinking water [3].

Coliform bacteria are living organisms in the environment and in the feces of all animals and warm-blooded humans. Coliform bacteria will not likely cause the disease. However, their presence in drinking water indicates that pathogens can be in the water system. Most pathogens that can contaminate water supplies come from human or animal feces. Testing of drinking water for all potential pathogens is complex, time-consuming, and costly. It is relatively easy and inexpensive to test coliform bacteria. If the coliform bacteria were found in the water sample; water system operators are working to find the source of pollution

and restore safe drinking water. On the other hand, pathogenic *Escherichia coli* as well as *Staphylococcus aureus* were found to be most prevalent species identified in the samples taken from restaurants and the food preparing spot using tap water in cleaning the surfaces. These organisms are very important foodborne pathogens and could be performed from contaminated tap water and might cause outbreak through the water born infections [4, 5].

Water pollution is a physical process that occurs in various water resources such as lakes, ground water, and rivers as a result of the natural activities [6, 7]. Utilization of poor-quality water causes waterborne diseases and might spread it around the local areas. In any way, drinking water should be free of color, sourness, odor and microbes. The term "indicator organism", as used in aquatic microbiology, means a vital organism whose presence is a sign of pollution (associated with fecal contamination from humans or other warm-blooded animals). Indicator organisms may be associated with pathogens, but they do not necessarily cause disease. Indicator organisms are used to assess water quality because their detection is more reliable and less time consuming. Pathogens appear in

fewer numbers of organisms in the index and are therefore less likely to be isolated. The organism used as an indicator must have several properties; and coliform group of organism fulfills most of these characters [8, 9, 10]. The World Health Organization

(WHO) [10] has developed new microbial guidelines for drinking water based exclusively on the presence of *E. coli* or coliform that should not be detected in any 100 ml sample collected from water either in the intervention or within the distribution system (Table 1).

Table-1: Values of Microbiological and Microbial Indicators in Drinking Water in the Community [10]

The parameter	values
*Part A Microbiological parameters	
<i>Escherichia coli</i>	0/100 ml
<i>Enterococci</i>	0/100 ml
*In water for sale in bottles or containers	
<i>Escherichia coli</i>	0/250 ml
<i>Enterococci</i>	0/250 ml
<i>Pseudomonas aeruginosa</i>	0/250 ml
Colony counts at 22 ° C	100/100 ml
Colony counts at 37 ° C	20/100 ml

The lactose-fermentation test became the prime diagnostic tool; *E. coli* ferments lactose with the formation of acid and gas; *Salmonella* spp. and *Shigella* spp. do not ferment lactose.

Coliform is determined by conducting coliform tests which involve several methods. The Most Probability Test (MPN) is a specific test to determine the presence of *E. coli* in a given sample. The coliforms are detected based on their characteristic capability of fermenting lactose with the production of gas [1]. *E. coli* (EC) medium is used to test water, milk, and other substances to demonstrate fecal contamination [1, 11, 12]. The membrane filter technique (MF) is another highly reproducible technique that can be used to test

relatively large quantities of the sample and produces more countable results faster than the multi-tube procedure. Some members of the coliform group can be cultured onto the Eosin Methylene Blue (EMB) medium and may produce glossy gloss a shiny metallic sheen [1, 13]. It had been also reported that rapid enzyme detection β-D-glucuronidase hydrolysis (Gluase-HR) is an alternative and rapid method to estimate *E. coli* contamination in fresh water [14].

Basra gets its water from the Shatt al-Arab, the waterway formed by the juncture of the Euphrates and Tigris rivers (Figure-1). Then it takes its way to the community through the water treatment plant.



Fig-1: Official map for Shatt Al-Arab that provide tap water for Basra city (<https://www.middleeasteye.net/news/how-locals-are-turning-basras-poisoned-rivers-drinking-water>)

The increase in salt water, the low water levels in rivers and the increase in chemical and biological contaminants in the Shatt al-Arab from sewage and industrial waste, exacerbated by the lack of suitable water treatment plants, led to the water crisis in Basra [15, 16]. "The province of Basra has suffered a number

of cases of diarrhea due to water contamination". That was approved by the head of the department of inspections at Basra Health Office who told the press conference that "the province had experienced a serious case of water contamination recently which leading to diarrhea and severe stomachache among people" [17].

Therefore, this study was conducted in aim to analyze the possible bacterial contamination and to measure the tap water quality for several different local areas within the Basra city, south of Iraq and two different types of bottled drinking water that have been commercially sold in the market of this city in an attempt of possible monitoring the source of contamination through three months repeated water samples examination.

MATERIALS AND METHODS

Collections of Samples

Ten different localities area within the Basra province were selected and labelled (Table-2). 200 ml of fresh tap water were collected in sterile container followed the procedure recommended by world health organization for sample collections [1]. Two other commercial bottled drinking water were also selected for test evaluation (Table-2). These samples were collected for a three-month interval from the same areas to follow up if there were any improvements or differences in the quality of water distribution in these areas.

Table-2: Name of the localities in Basra city that were the samples collected from in this study

No#	Sample ID	Name of the area (locality)& Bottled producer company
1	01	Al-Bradhaia
2	02	Al-Qibla
3	03	Abil-khasaib
4	04	Al-Maqal
5	05	Al-Tahsinia
6	06	Al-Jamhuria
7	07	Al-Tanumah
8	08	Al-Jazaer
9	09	Hai-Al-Hussain
10	10	Al-Jubaillah
11	11	Salsal. Co. (bottle)
12	12	Al Janaa en Al Mualaka. Co. (bottle)

Bacteriological Study

Detection of coliforms by Most Probable Number (MPN) test

A 5 tubes series arranged in three rows containing a MacConkey broth medium were inoculated with test portions (10 ml, 1 ml, and 0.1 ml) of a water sample. The tubes in the first row (F1) contain 10 ml of the presumptive medium of double force, while the tubes in the second and third rows (F2, F3) contain 10 ml of the presumptive average of single force. After a specific incubation period at 37° C, each

tube showing the composition of the gas was considered "presumptive positive" because the gas indicated the possibility of coliform. For the confirmatory test, an Eosin Methylene Blue (EMB) culture medium were inoculated with material taken from the positive tubes. The most probable number (MPN) of bacteria present were then estimated from the number of tubes inoculated and the number of positive tubes obtained Table-3. Is the standard MPN value per 100 ml of samples approved by world health organization [1].

Table-3: The value of MPN per 100 ml of the sample and 95% confidence limits for different combinations of positive and negative results (when five 10-ml, five 1-ml and five 0.1-ml test portions are used) [1]

Number of tubes giving a positive reaction			MPN/100 ml	95% confidence limits	
5 of 10 ml	5 of 1 ml	5 of 0.1 ml		Lower	Upper
0	0	0	<2	<1	7
0	1	0	2	<1	7
0	2	0	4	<1	11
1	0	0	2	<1	7
1	0	1	4	<1	11
1	1	0	4	<1	11
1	1	1	6	<1	15
2	0	0	5	<1	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25

3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	93
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	110
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	150
5	2	0	49	17	130
5	2	1	70	23	170
5	2	2	94	28	220
5	3	0	79	25	190
5	3	1	110	31	250
5	3	2	140	37	340
5	3	3	180	44	500
5	4	0	130	35	300
5	4	1	170	43	490
5	4	2	220	57	700
5	4	3	280	90	850
5	4	4	350	120	1000
5	5	0	240	68	750
5	5	1	350	120	1000
5	5	2	540	180	1400
5	5	3	920	300	3200
5	5	4	1600	640	5800
5	5	5	>1800	-	-

Detection of coliforms by Membrane Filtration Technique

The membrane-filtration method (MF) gives a direct count of total coliforms and thermotolerant coliforms present in a test sample of water. The method is based on filtration of a known amount of water through a membrane composed of a cellulose compound with a uniform pore diameter of 0.45 or 0.2µm; bacteria are kept on the surface of the membrane filter. When the membrane containing the bacteria is incubated on EMB at an appropriate temperature with selective differential culture, distinct colonies of thermocouples are developed, which can be counted directly. 100 mL of water sample was poured or pumped into the filter unit funnel. The water was then cleaned from the filter surface. Sterile forceps were used to remove the membrane from the rack and place it on the media surface, ensuring no air bubbles between the filter and media. Eosin Methylene Blue (EMB) was used in this test.

RESULTS

Three different methods, MPN, EMB medium, and MF Techniques were used in this study to analyze the quality of tap water and indicate the selected samples if they were fecally contaminated. The Most Probable Number Test (MPN) provided a virtual guide to the presence of coliforms. Pollinated coliform organisms have been detected (for three repeated water samples for each month for the same area) based on their characteristic ability to colonize. MPN was estimated by determining the number of tubes in each group that showed gas composition after appropriate incubation (Table 4-6; Fig-2). According to that standard MPN table (Table-3); result in this study showed that water samples collected from the above localities were not potable except the sample that were tested from bottled drinking water. The results were reported in Table 4-6.

Table-4: MPN index for the first month collection water sample by using MacConkey broth

Sample	Acid and Gas (AG)															Reading	MPN	Range 95% Probability	Potability
	10 ml					1 ml					0.1 ml								
Tubes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5				
01	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	5.4.3	280	90-850	No
02	-	-	-	+	+	-	+	+	-	-	-	-	-	-	-	2.2.0	9	2-21	No
03	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5.5.5	>1800	-	No
04	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	5.4.2	220	57-700	No
05	+	+	+	-	+	-	+	-	+	-	-	-	+	-	-	4.2.1	26	9-78	No
06	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	5.4.3	280	90-850	No
07	+	+	+	-	+	-	-	+	+	+	-	-	-	+	-	4.3.1	33	11-93	No
08	+	+	+	+	+	+	-	+	-	+	+	+	+	-	-	5.3.3	180	44-500	No
09	-	+	+	+	-	-	+	+	-	+	-	-	-	-	-	3.3.0	17	5-46	No
10	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+	5.3.3	180	44-500	No
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0.0	<2	<1-7	yes
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0.0	<2	<1-7	yes

Table-5: MPN index for the second month collection water sample by using MacConkey broth

Sample	Acid and Gas (AG)															Reading	MPN	Range 95% Probability	Potability
	10 ml					1 ml					0.1 ml								
Tubes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5				
01	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	5.5.1	350	120-1000	No
02	-	-	-	+	+	-	+	+	-	+	-	-	-	-	-	2.3.0	12	3-28	No
03	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5.5.5	>1800	-	No
04	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	5.3.3	180	44-500	No
05	-	+	+	+	+	-	-	-	-	+	+	-	+	-	-	4.1.2	26	9-78	No
06	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	5.5.3	920	300-3200	No
07	+	-	+	+	+	-	+	+	-	+	-	-	+	-	-	4.3.1	33	11-93	No
08	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	5.4.3	280	90-850	No
09	+	+	+	+	-	+	+	-	-	+	-	-	+	-	-	4.3.1	33	11-93	No
10	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	5.4.2	220	57-700	No
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0.0	<2	<1-7	yes
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0.0	<2	<1-7	yes

Table-6: MPN index for the third month collection water sample by using MacConkey broth

Sample	Acid and Gas (AG)															Reading	MPN	Range 95% Probability	Potability
	10 ml					1 ml					0.1 ml								
Tubes	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5				
01	+	+	-	+	-	-	+	+	+	-	-	-	-	-	-	3.3.0	17	5-46	No
02	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-	2.1.1	9	2-21	No
03	+	+	+	+	+	+	-	-	+	+	-	-	-	+	+	5.3.2	140	37-340	No
04	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	5.4.2	220	57-700	No
05	+	+	+	-	+	-	+	-	+	-	-	-	+	-	-	4.2.1	26	9-78	No
06	+	+	-	+	+	+	+	+	-	-	+	-	-	-	-	4.3.1	33	11-93	No
07	+	+	+	-	+	-	-	+	+	+	-	-	-	+	-	4.3.1	33	11-93	No
08	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-	4.1.2	26	9-78	No
09	+	+	+	+	-	-	+	+	-	+	-	-	-	-	-	4.3.0	27	9-80	No
10	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	5.2.0	49	17-130	No
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0.0	<2	<1-7	yes
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0.0	<2	<1-7	yes



Fig-2: Show the fermentation and gas production for the positive water sample with coliforms contamination

These MPN results showed an increase in the number of coliforms present in tap water. In the MacConkey broth all water samples showed a higher MPN than the limit except for samples taken from bottled drinking water that showed no evidence of coliform contamination. The average Eosin Methylene Blue also showed a high MPN indicator that did not conform to the standard and showed clear growth in *Escherichia coli* with a typical green metallic sheen

appearance (Fig-3). The development of turbidity in the fermentation tubes and the presence of gas in Durham tubes within 24 hours of incubation at 37° C were considered positive evidence of faecal coliforms in water samples. Growth results inoculated from positive tubes onto EMB medium have been shown in (Table-7 & Fig-3). All tap water samples showed positive EMB test results except for sample 11 and 12.

Table-7: Growth of *E. coli* on EMB medium

Sample	EMB medium		
	1 st month sample	2 nd month sample	3 rd month sample
01	+	+	+
02	+	+	+
03	+	+	+
04	+	+	+
05	+	+	+
06	+	+	+
07	+	+	+
08	+	+	+
09	+	+	+
10	+	+	+
11	-	-	-
12	-	-	-

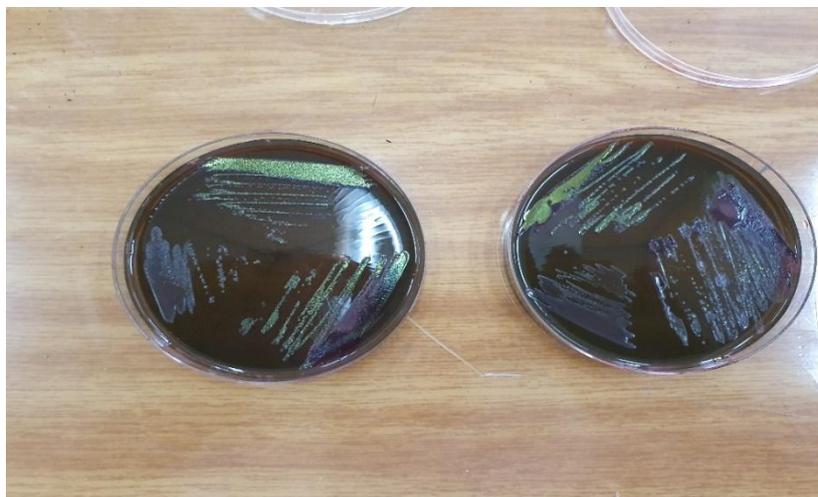


Fig-3: A typical green metallic sheen appearance for the *E. coli* isolated from contaminated tap water sample

In MF technique, the bacteria on the filter grew to a visible colony of bacteria on the EMB with a metallic green sheen. A bright green metallic sheen indicated the presence of *E. coli* in a water sample. All

tap water samples showed a positive result, resulting in growth with green metallic sheen accepting sample 11 and 12. Results have been shown in Table-8.

Table-8: Membrane filtration technique (MF)

Sample	Growth onto EMB medium		
	1 st month sample	2 nd month sample	3 rd month sample
01	+ Growth with green metallic sheen	+ Growth with green metallic sheen	+ Growth with green metallic sheen
02	+ Growth with green metallic sheen	+ Growth with green metallic sheen	+ Growth with green metallic sheen
03	+ Growth with green metallic sheen	+ Growth with green metallic sheen	+ Growth with green metallic sheen
04	+ Growth with green metallic sheen	+ Growth with green metallic sheen	+ Growth with green metallic sheen
05	+ Growth with green metallic sheen	+ Growth with green metallic sheen	+ Growth with green metallic sheen
06	+ Growth with green metallic sheen	+ Growth with green metallic sheen	+ Growth with green metallic sheen
07	+ Growth with green metallic sheen	+ Growth with green metallic sheen	+ Growth with green metallic sheen
08	+ Growth with green metallic sheen	+ Growth with green metallic sheen	+ Growth with green metallic sheen
09	+ Growth with green metallic sheen	+ Growth with green metallic sheen	+ Growth with green metallic sheen
10	+ Growth with green metallic sheen	+ Growth with green metallic sheen	+ Growth with green metallic sheen
11	- No growth	- No growth	- No growth
12	- No growth	- No growth	- No growth

DISCUSSION

The results of this study indicate that there may be serious water quality problems in Basra city. Bacteriological analysis of tap water samples indicated faecal coliforms in water except samples taken from commercially bottled water 11 and 12, which showed no evidence of coliform bacteria. Coliforms are the most common group of organisms used to control water quality. These organisms represent the bacteria usually present in the intestinal tract of animals and humans. Moreover, the presence of coliforms in drinking water can also indicate the collapse of the treatment process and that water transport does not contribute significantly [5, 18]. Proper maintenance of water distribution system and chlorination should be done according to the law and regulations to kill pathogens. Government should provide the newest and reliable instruments and trained staff responsible for the tap water quality analysis. The contamination that were noticed in this study might occur also during the storage in the house reservoirs. The source of tap water quality status and contamination might account for the sewage water it might be mixed with the distributed water as the dominant and primary contamination resulted from the poor sanitation and sewage treatment system in this area. Olds *et al.*, [19] had reported the same finding in their locality of study. The World Health Organization [10] describes coliform bacteria as a good test for the assessment water contamination but not necessarily an indicator of health risk. *E. coli* is the best indicator of fecal contamination. However, both bacterial tests are limited in their ability to detect the presence of other microbes such as viruses and protozoa [20]. The samples of tap water in this study were taken from the water distribution at the houses from different areas; odor and floating bodies were present in some samples, which considered again as an indicator of poor sanitation and poor storage procedure for this type of water. Same finding was reported also from study done in Nigeria [21]. In contrast, Similar study [22], which

was conducted in Telangana, India, reported clean water and good for consuming. However, the water, which is not suitable for drinking, may be usable for irrigation or for other domestic purposes.

Based on the above assessments conducted in this study for two different types of bottled water used for drinking in the Basra market. It seems that the use of a water bottle made from a reliable company is more reliable to drink in this area. Bottled water may be of good quality to people. However, a random series of monthly control and analysis should be performed to improve the continuity of such companies to provide water used for drinking. This study was just evaluating two kind of companies and a further study should be considered to evaluate many different other companies available in the city which might provide a bad quality drinking water. Several studies on bottled drinking water were done worldwide to evaluate their bottled water supplier. Some reported a bad quality [21]. Whereas, other reported a good quality product in their area [22].

Basra is facing a crisis of biological pollution as well as increasing the salinity of water and increasing the chemicals in the Shatt al-Arab from sewage and industrial waste. This study comes in agreement with recent evidence of biological pollution reported in this city [16].

CONCLUSION

This limited study was recognized a huge contamination with coliform bacteria at the tap water level reached to the community at their houses. This water is used from the majority people for drinking and other daily purposes. Two of the bottled water available on the market were potable for people. However, government authorities should consider a regular monitoring program to identify key sources of pollution to participate in improving health and reducing health

risks. In addition, they should make recommendations for the development of appropriate control measures to avoid any sudden public health hazard from such a vital water source.

ACKNOWLEDGMENT

The author is grateful to the Department of Microbiology in college of Medicine, University of Basrah, Iraq for their support. Also grateful to the technician Ms. Hanaa Agal Qallan for her kind assistant in preparing the equipment's and her help in media preparation. Special thanks for the students Sarah M. Abduljabar, Hadel A. Mohammed, and Sarah M. Kamil for their assistant in samples collections.

Financial Support and Sponsorship: Nil.

Conflicts of Interest: No conflicts of interest

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