Pharmaceutical and Biomedical Sciences Journal
Volume 4(1), 2022, 45-52

Analysis of *Escherichia coli* Microbial Contamination and Total *Coliform* Bacteria in Refill Drinking Water in Pondok Cabe Ilir Village, South Tangerang City

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Received: 27 February 2022; Accepted: 19 May 2022

Abstract: Water is an essential nutrient for human health. It is important to maintain adequate drinking water intake to prevent dehydration, which can cause hypothermia, dizziness, constipation, and kidney stones. Currently, water-filling stations are an alternative source of drinking water because of limited access to clean water at affordable prices. The purpose of this study was to determine bacterial contamination at a drinking water station in Pondok Cabe Ilir, South Tangerang, Banten, based on Permenkes RI No. 492/Menkes/Per/IV/2010. The study was conducted using the Most Probable Number (MPN) method and the IMViC test, Triple Sugar Iron test, H_2S production test, and motility test to identify *Escherichia coli*. The results showed that one out of five refilled drinking water samples contained *Coliform* bacteria above the threshold according to Minister of Health Regulation No. 492/Menkes/Per/IV/2010.

Keywords: *Coliform bacteria, E. coli, Most Probable Number (MPN), refilled drinking water*

1. INTRODUCTION

Water is a primary requirement for humans; therefore, it is needed in sufficient quantities and with guaranteed safety for consumption by all residents. Unfortunately, the community's high demand for safe drinking water is not matched by the ease of access to safe drinking water. This encourages the presence of bottled drinking water industries (AMDK) and refilled drinking water depots (DAMIU). Many people prefer to use refilled drinking water because it is less expensive than bottled drinking water. There were 283 refilled drinking water depots in South Tangerang City in 2012. However, the presence of depots is not matched by permits, guidance, supervision, and circulation, resulting in low drinking water quality guarantees (Radji et.al. 2008).

Supervision of the quality of refilled drinking water has been regulated by the government through Minister of Health Regulation No.492/Menkes/Per/IV/2010 concerning Drinking Water Quality Requirements. Among the mandatory parameters contained in the Minister of Health Regulation are microbiological parameter requirements, where the levels of *E. coli* and total *Coliform* bacteria allowed in refilled drinking water are 0/100 mL of sample (Ministry of Health, 2010).

E. coli is a common bacterial flora found in the intestines of humans and animals. Pathogenic serotypes, on the other hand, can cause diarrhea via a variety of mechanisms, including Enterotoxigenic *E. coli* (ETEC), which can cause traveler's diarrhea, and Enteropathogenic *E. coli* (EPEC), which can cause diarrhea in infants (Edberg, 2000).

Diarrhea plays a role in 31% of deaths in children aged 1 month to 1 year and plays a role in 25% of deaths in children aged 1 to 4 years in Indonesia (Kemenkes RI, 2008). Banten has one of the highest diarrhea rates in the country, at 8.0% (Ministry of Health of the Republic of Indonesia, 2013). According to BPS data

for the City of South Tangerang in 2015, diarrhea was the 9th most common cause of hospitalization in RSU Kota Tangerang Selatan, with 92 cases.

The researchers were interested in examining *E. coli* contamination and total *Coliform* bacteria in Refill Drinking Water Depots (DAMIU) in the area based on the description above and because one of the researchers lives in Pondok Cabe Ilir Village, South Tangerang City.

2. RESEARCH METHOD

The research design used was descriptive analytic with a cross-sectional approach referring to the method used by Bambang *et al.* (2013) with modifications.

2.1 Sampling and Observation

The process of taking refilled drinking water samples began by surveying the amount, location, and sampling permits from DAIMU in the Pondok Cabe Ilir sub-district. The sample was then brought to the Microbiology Laboratory of the Pharmacy Department at the Syarif Hidayatullah State Islamic University Jakarta to carry out a predictive test which included a confirmation test and an identification test.

2.2 Predictive Test

In a Durham tube containing Lactose Broth (LB) media, each sample was diluted from 5 depots (D1, D2, D3, D4, D5) to a concentration of 10^{-3}. The samples were then incubated at 36 ± 1ºC for 24 and 48 hours. The Durham tube-forming gas was recorded as a positive sample.

2.2.1 Confirmation Test

From a positive Durham tube in the predictive test, 1 ose was taken and inoculated in 2% Brilliant Green Lactose Bile Broth (BGLB 2%) media, then the samples were incubated at 36 ± 1°C for 24-48 hours.

Table 1. The results of the suspected incubation period of 24 and 48 hours

Sample	Predictive Test Result (After 48 hours of incubation)								
	Dilution 10^{-1}			Dilution 10^{-2}			Dilution 10^{-3}		
	1	2	3	1	2	3	1	2	3
D1	-	-	-	-	-	-	-	-	-
D2	-	+	-	-	-	-	-	-	-
D3	-	-	-	-	-	-	-	-	-
D4	-	-	-	-	-	-	-	-	-
D5	-	-	-	-	-	-	-	-	-
BM	-	-	-	-	-	-	-	-	-
$B10^{-1}$	-	-	-	-	-	-	-	-	-
$B10^{-2}$	-	-	-	-	-	-	-	-	-
$B10^{-3}$	-	-	-	-	-	-	-	-	-

Note: 1= Tube replication 1; 2= Tube replication 2; 3= Tube replication 3; BM = Negative control media; $B10^{-1}$= Negative control of dilution 10^{-1};$B10^{-2}$= Negative control of dilution 10^{-2} ; $B10^{-3}$= Negative control of dilution 10^{-3}; (-): No bubbles formed in the Durham tube;(+): Bubbles formed in the Durham tube.

The Durham tube that produces gas was recorded as a positive sample.

2.2.2 Identification Test of *E. coli* Bacteria

Identification tests were conducted on selected colonies that had been rejuvenated previously using media (Eosin Methylene Blue Agar (EMBA) and Nutrient Agar (NA). Identification of *E. coli* bacteria was carried out by Gram staining, Indole, Methyl Red, Voges Proskauer, Citrate (IMViC) test, and the Triple Sugar Iron test. The testing technique refers to the method used by Bambang et al (2013).

3. RESULTS AND DISCUSSION

3.1 Predictive Test Results

Table 1 shows the results of the predictive test. During the replication of the second tube, only sample D2 at 10^{-1} dilution showed positive results, whereas samples D1, D3, D4, and D5 only showed media turbidity. Meanwhile, in the negative control, there was no turbidity in the media or gas bubbles in the Durham tube.

The presence of gas bubbles in the Durham tube and turbidity in the LB media indicated that the sample contained *Enterobacteriaceae* bacteria. This is because these bacteria can ferment sugar through the fermentation of a mixture of acids and butandiol (Müller, 2001).

3.2 Affirmation Test Results

The results of the affirmation test are shown in Figure 1. Based on the results of the presumptive test, only samples from the 2nd Depot (D2) advanced to the assertion test, where gas bubbles were observed after a 48-hour incubation period.The Most Likely Number Value (APM) for each sample was processed from the data from the presumptive test results using the Hopkins table as presented in table 2.

Figure 1. Formation of gas bubbles in the assertion test.

The data in table 2 shows that in samples D1, D3, D4, and D5 the APM/mL value was <3/mL. This indicated that no *Coliform* bacteria were detected in the sample (Suprihatin, 2008), while the APM/mL value in the D2 sample was 4/mL. The quality of drinking water was determined by the level of contamination by microbes. The more the number of *Coliform* bacteria contained in drinking water, the worse the quality of drinking water. Drinking water quality requirements must meet the content of *E. coli* bacteria in drinking water, number 0/100 ml (Mirza, 2014).

Table 2. APM/mL values from samples D1-D5

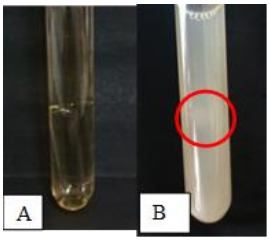
Sample Name	Positive tube combination	APM/mL values
D1	0-0-0	<3
D2	1-0-0	4
D3	0-0-0	<3
D4	0-0-0	<3
D5	0-0-0	<3

Sample D2 contaminated by *Coliform* bacteria can be caused by several factors, including contaminated raw material water, pollution during transportation of raw material water to the depot, unsterile water containers, buildings and equipment that are not kept clean, or depot conditions that are not eligible (Walangitan et. al., 2016). Besides that, contamination by *Coliform* bacteria in sample D2 can also be caused by the long

shelf life of raw water in the holding tank (Rahayu *et al.*, 2013; Violita *et al.*, 2010) considering that raw water in D2 only comes every 2-3 week.

E. coli Identification Test Results

E. coli identification test on sample D2 was carried out after the presumption test and confirmation test. Identification of *E. coli* needs to be conducted because this bacterium is considered the best indicator of fecal contamination or contamination in drinking water samples (Edberg, 2000).



Note: A: Culture media before incubation;
B: The culture medium becomes cloudy and there are gas bubbles in the Durham tube after 48 hours of incubation.

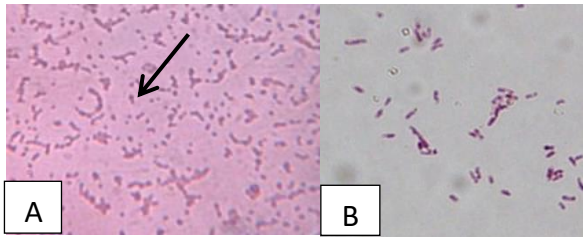
Figure 2. *E. coli* identification test results

The presence of bubbles and the occurrence of turbidity in the culture media as shown in Figure 2 showed that sample D2 was positive for *E. coli* according to the American Public Health Association (APHA). The results of the bacterial culture from the D2 sample inoculated on EMBA media showed the form of a single shiny metallic green colony which was an indicator that lactose and/or sucrose had fermented by faecal *Coliform*, namely from *E. coli* bacteria (Figure 3) (Leboffe et. al., 2010).

Note : Green metallic luster colonies were formed

Figure 3. Identification test results on EMBA media

The results of the Gram stain test observed using a microscope showed that the bacteria in sample D2 showed the character of *E. coli* that is in the form of short bacilli and red in color (Figure 4).



Note: A: Observation of samples with a 100x magnification microscope; B: Control observation with a 1000x magnification microscope

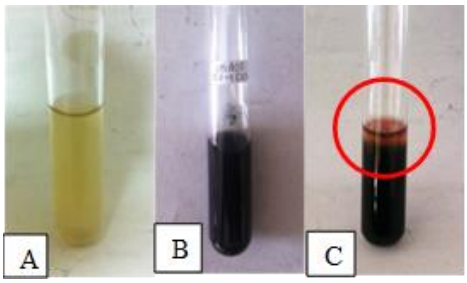
Figure 4. Gram staining results of sample D2

IMViC test results which include the Indole, Methyl Red, Voges Proskauer, and Citrate tests are presented in table 4. This test was conducted to identify the presence of bacteria from the Klebsiella, Enterobacter, and *E. coli* groups (Zahera et. al., 2011).

Table 3. IMViC Test Results

Indol Test	Positive in producing indole
Methyl Red Test	Positive in producing acid
Voges Proskauer Test	Negative results in acetyl methylcarbinol.
Citrat Test	Negative in forming $NaCO_3$

In the Indole test results, a red ring was formed when sample D2 was dropped by KOVAC reagent, indicating that the bacteria contained in it produced indole (Figure 5).

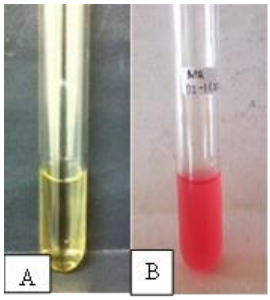


Note: A: SIM media without bacteria; B: The media turned black after being inoculated with bacteria; C: A red ring was formed on the media that had been dripped with KOVAC reagent

Figure 5. Indole test results

The results of the methyl red test on sample D2 showed that there was a color change in the medium

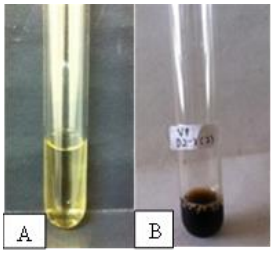
from light yellow to red after adding methyl red (Figure 6). This color change occurs due to the acidic atmosphere formed in the media (Hemraj *et al.*, 2013) as a result of glucose fermentation through the process of glycolysis into a mixture of acids namely acetic, lactic, and formic acids; CO_2, and ethanol. The presence of this acid formation can be detected using methyl red by changing the color of the medium to red. (Bambang *et al.*, 2014; Müller, 2011).



Note : A: Media contains bacteria before the incubation period; B: The media contains bacteria after incubation and has been dripping with methyl red which turns red.

Figure 6. Methyl Red Test Results

The results of the Voges Proskauer test from sample D2 using Barritt A and Barritt B reagents showed a change in the color of the media to dark brown (Figure 7).

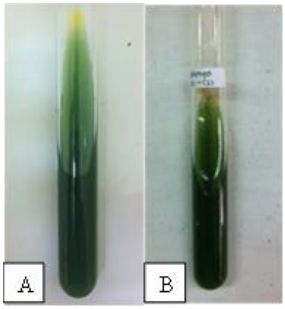


Note A: Methyl Red-Voges Proskauer (MR-VP) media contains bacteria before incubation (light yellow color); B: MR-VP media that has been inoculated and dripped with Barritt A and Baritt B reagents (the color of the culture medium changes to brown).

Figure 7. Voges Proskauer Test Results

In the Voges Proskauer (VP) test, *E. coli* plays a role in the fermentation process of glucose into a mixture of acids, ethanol and carbon dioxide (Müller, 2001). In the presence of peptones in the MR-VP media, the acetoin formed from glucose fermentation will undergo an oxidation process with the addition of KOH and produce a red color in the media. In this reaction, *E. coli* will give a negative response since it shows a brown-yellow color (Hemraj, *et al.*, 2013).

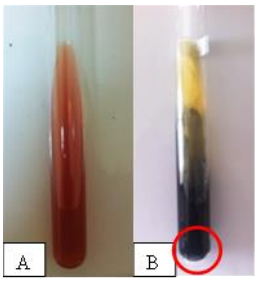
The results of the citrate test on sample D2 showed positive results because there was no color change in the Simmons citrate medium (Figure 8) which indicates that the bacteria inoculated in the media are enteric bacteria, such as *E. coli* (Müller, 2001).



Note: A: Citrate media contains bacteria before the incubation period;B: Simmon Citrate media contains bacteria after an incubation period of 96 hours (no change in media color).

Figure 8. Citrate Test Results

The results of the Triple Sugar Iron test on sample D2 showed a change in the color of the medium to yellow on the surface of the agar and the base of the agar to black color, indicating the nature of *E. coli* (Figure 9).



Note: A: TSIA media that has been inoculated with bacteria before incubation; B: TSIA media that has been inoculated with bacteria after an incubation period of 48 hours (a change in the color of the media to yellow on the agar surface, black on the agar base, and slightly raised agar).

Figure 9. Triple Sugar Iron Test Results

The occurrence of a yellow color change on the surface of the agar in the citrate test is due to the acidic nature that arises in the media as a result of the fermentation of sugars (lactose, sucrose, and glucose). In the results of the H_2S production test from sample D2, there was a change in the color of the TSIA media and SIM media to black (Figure 10). This is due to the production of H_2S by bacteria in the D2 sample. Several strains of *E. coli* have been reported to produce H_2S, although *E. coli* itself does not typically produce H_2S (Park et al, 2015).

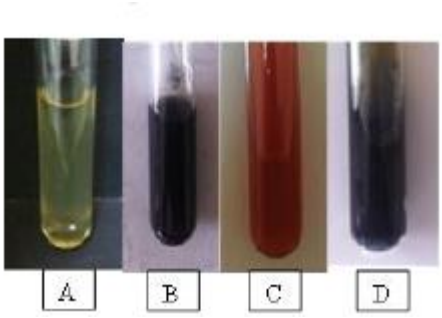
The results of the motility test from the D2 sample showed changes that occurred in the SIM media after 24 hours of incubation, where turbidity occurred in the media. This indicated growth in the puncture area which indicates that the bacteria in sample D2 are motile (Leboffe *et al.*, 2011).

The overall test results of the D2 sample referring to SNI 01-2897-1992 are summarized in Table 5. When compared with the IMViC test which refers to SNI 01-2897-1992, the bacteria present in sample D2 are typical of *E. coli*. This was confirmed by other tests such as the TSI test, motility, H_2S production which

Table 4. The test of entire sample D1-D5

	D1	D2	D3	D4	D5	Typical *E.Coli*	Atypical *E. Coli*
Predictive Test	0-0-0	1-0-0	0-0-0	0-0-0	0-0-0		
Confirmation Test	Not conducted	1-0-0	Not conducted	Not conducted	Not conducted		
Identification of *E. coli* APM/mL	<3	4	<3	<3	<3		
Test on *E. coli* broth media	Not conducted	Bubbles are formed	Not conducted	Not conducted	Not conducted		
Test on Eosin Methylene Blue Agar media	Not conducted	Colonies are green with a metallic sheen	Not conducted	Not conducted	Not conducted		
Gram Stain Test	Not conducted	Red in color, rod shape	Not conducted	Not conducted	Not conducted		
Indol Test	Not conducted	+	Not conducted	Not conducted	Not conducted	+	-
Methyl Red Test	Not conducted	+	Not conducted	Not conducted	Not conducted	+	+
Voges Proskauer Test	Not conducted	-	Not conducted	Not conducted	Not conducted	-	-
Sitrat Test	Not conducted	-	Not conducted	Not conducted	Not conducted	-	-
Triple Sugar Iron	Not conducted	A/A, G, H_2S	Not conducted	Not conducted	Not conducted		
Motilitas	Not conducted	+	Not conducted	Not conducted	Not conducted		
H_2S Production	Not	+	Not	Not	Not		

showed that the bacteria contained in sample D2 were *E. coli*.



Note: A: SIM media that has been inoculated with bacteria before incubation;B: SIM media that has been inoculated and incubated (media color turns black);C: TSIA media that has been inoculated with bacteria; D: TSIA media that has been inoculated with bacteria and incubated (the color of the media at the bottom of the tube turns black)

Figure10. H_2S Production Test Results

4. CONCLUSION AND SUGGESTIONS

From the five Refill Drinking Water Depots in Pondok Cabe Ilir Village, South Tangerang City, which was tested for contamination from *E. coli* using the Most Likely Number (APM) method, it was shown that the D2 sample was contaminated with *Coliform* bacteria with a value of 4 *Coliform*/mL while the other samples showed a negative response with the value of 3 *Coliform*/mL.

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