Detection of Escherichia coli

by Mufida Fathin Rofiqi

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Detection of Escherichia coli and Total Coliform in Refill Drinking Water at Jepara Regency

Mufida Fathin Rofiqi[a]*, Marijam Purwanta[b], Lilik Djuari[c]

*mufida.fathin.rofiqi-2019@fk.unair.ac.id
[a]Medical Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[b]Department of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[c]Department of Public Health and Preventive, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

Introduction: Water are important for human life. The Minister of Health of the Republic of Indonesia stipulates the Minister of Health Number 492 of 2010 about drinking water quality requirements to ensure drinking water are safe for consumption. One of the requirements are free from Escherichia coli or total Coliform. When these bacteria are found in drinking water, it can cause diarrhea. In Province of Central Java, diarrhea is the biggest cause of death for children under five. Therefore, one of the locations in Province of Central Java was chosen, namely Jepara Regency, more precisely, Jepara District.

Methods: This study used descriptive with a cross-sectional design. Data collection using total sampling technique and obtained 28 samples. Then the examination was carried out at the Regional Health Laboratory of Jepara Regency using the filter membrane method and Chromocult Coliform Agar Plate.

Results: From 28 samples, 11 samples (39.3%) were contaminated by Coliform with 8 samples (72.72%) contaminated by Escherichia coli.

Conclusion: Following up the finding of the study, it is hoped that further researchers will carry out researches on the hygiene sanitation of drinking water depot, so the cause of contaminations in drinking water refill are known.

Keywords: Escherichia coli; total Coliform; Membrane Filter; Refill Drinking Water

Introduction

Refill drinking water is water that can be consumed directly because it has been sterilized at potable water depot. Before sterilizing source water into potable water, the depots are required to pass the requirements for places, equipment, handlers, and source of water with evidence of a sanitation hygiene certificate issued by the district/city health office[1]. The poor hygiene sanitation has a potential to contaminated the produce of potable water that can bring on waterborne diseases[2]. Waterborne diseases are disease that affect people who consume beverages that contaminated by

pathogenic agents such as viruses, bacteria, and protozoa[3]. The most common waterborne disease is diarrhea. In 2019, 39% of under-five mortality in Province of Central Java was caused by diarrhea with incidence rate 843/1000 population[4]. In a study of children under five feces that has been done in South Sumatra, West Papua, and West Nusa Tenggara, the most common cause of diarrhea was Rotavirus strain equine-like G3[5].

Diarrhea can occur due to the contamination of Coliform bacteria such as Escherichia, Shigella, Salmonella, dan Enterobacter. From those Coliform bacteria, Escherichia coli alter to indicator faecal contamination because it does not have spores which makes the bacteria susceptible to die so it can be detected when the contamination was occurs[6][7]. Bacterial contamination can be caused by various ways, such as food falling on the floor or breast milk stored in the room temperature. In a study conducted in Surabaya, it was found that food that fell on the floor was directly contaminated with gram-negative bacilli even though it was only for 1 second[8]. In addition, there is a study that says that storing breast milk at room temperature for 2 hours or more is found to have bacterial contamination[9].

The Minister of Health of the Republic of Indonesia stipulates the Regulation of the Minister of Health of the Republic of Indonesia No. 492 of 2010 concerning the requirements for potable water quality to prevent bacterial contamination, which must be free from Escherichia coli and total Coliform bacteria[10]. The examination must be carried out at least every 6 months at an accredited Regional Health Laboratory[11].

In this study, one of the regencies in Province of Central Java was selected, namely Jepara Regency, more precisely, Jepara Subdistrict which is in the middle of the city and previously no bacteriological research has been conducted on refilled drinking water using the membrane filter method[12][13][14]. In addition, due to the Covid-19 pandemic, there were obstacles in checking potable water so that in the past 2 years the majority of them were not carried out bacteriological examination of potable water in laboratory. Necessarily during the Covid-19 pandemic, hygiene sanitation and body immunity should be improved because the manifestations of Covid-19 can be intestinal inflammation, vomiting, or decrease of appetite[15].

Research Elaboration

This study used descriptive with a cross-sectional design that held on 23-31 September 2021 after obtaining a permit letter from Faculty of Medicine Airlangga University and National Unity and Political Agency of Jepara Regency.

The variables of this study were potable water, Escherichia coli, and total Coliform. This study used potable water from potable water depot lcated in Jepara District as the research population. The data was collected using total sampling technique which means all of the population were include as a sample, total there were 28 samples.

The samples were collected on 23-24 September 2021 using bottles that have been sterilized by hot air oven at 160°C for 2 hours, directly in the same day the samples were brought to the Regional Health Laboratory of Jepara Regency and stored in the refrigerator at 4°C. Sample storage at 4°C does not affect the growth of Escherichia coli and total Coliform because the bacteria were in dormant state which means the bacteria did not grow nor die[16][17].

On 28-29 September 2021, samples were examined to detect Escherichia coli and total Coliform using filter membrane method, the brand was Pall Corporation with a diameter of 47 mm and a pore diameter of 0.22 μm and Chromocult Coliform Agar Plate. Both filter membrane (MF) and most probable number (MPN) method can be used for detection of Escherichia coli and total Coliform, but filter membrane method is faster, more practical, and able to detect Escherichia coli and total Coliform at once[18]. The examination was carried out in a laminar flow cabinet using a laboratory coat and handscoon and all equipment were sterilized using an autoclave at 121°C for 15 minutes to avoid environmental contamination[19].

Here is the process, the filter membrane is placed in the middle of the laboratory filtration system and 100 ml of potable water is poured into the laboratory filtration system. After the potable water has been filtered, the filter membrane is attached to the surface of the Chromocult Coliform Agar Plate (CCA Plate) and incubated at 37°C for 1x24 hours. Finally, bacterial colonies were counted using the bacteria colony counter. The way to count the bacterial colonies, Coliform type Escherichia coli shows blue colonies, other type of Coliform shows pink colonies, while the total Coliform is the sum of the blue colonies and pink colonies or the sum of only one colony.

Results

Total there are 28 samples in the District of Jepara that can be seen on table 1.

Table 1. Total Sample in the District of Jepara

Criteria		Percentage N (%)
District Health Office License	Have a license	17 (60.7)
	Does not have a license	11 (39.3)
Bacterial Contamination	Qualified	17 (60.7)
	Unqualified	11 (39.3)
TOTAL		28 (100.0)

Table 1 showed that from 28 samples potable water depot, 11 depots (39.3%) did not have a license from the Jepara District Health Office, while 17 depots (60.7%) already had a license. In addition, from 28 potable water depots, 11 depots (39.3%) were unqualified based on the Minister of Health of the Republic of Indonesia No. 492 of 2010 concerning the requirements for drinking water quality, while 17 depots (60.7%) were qualified.

In table 2, it can be seen the difference in contamination between potable water depots that already have a license and those that do not.

Tabel 2. Bacterial Contamination Between Licensed and Unlicensed Potable Water Depot

Category		Percentage N (%)
Does Not Have a License	Contaminated with bacteria	6 (54.5)
	Not contaminated with bacteria	5 (45.5)
Have a License	Contaminated with bacteria	5 (29.4)
	Not contaminated with bacteria	12 (70.6)

Based on the table 2 above, it can be seen that potable water depots that do not have license have greater bacterial contamination (54.5%) compared to potable water depots that have license (29.4%). In addition, potable water depots that have a license have a higher bacteria-free rate (70.6%) than potable water depots that do not have a license (45.5%).

To identification the bacterial contamination of the 11 samples can be seen in the table 3 below.

Table 3. Identification of Bacterial Colonies

Category		Percentage N (%)
Does Not Have a License	Escherichia coli and other Coliform	4 (66.7)
	Other Coliform	2 (33.3)
Have a License	Escherichia coli and other Coliform	4 (80.0)
	Other Coliform	1 (20.0)

Based on table 3, it can be seen that potable water depots those who do not have a license or those who have a license are found to be more contaminated with Escherichia coli and other types of Coliform (66.7%) and (80%) rather than contaminated only with other types of Coliform (33.3%) and (20%). This study can be concluded that 11 samples were contaminated by Coliform with 8 samples contaminated by Escherichia coli.

Discussion

In this study, there was a significant difference among bacterial contamination between potable water who have a license (29.4%) and those who do not have a license (54.5%). This happens because the potable water depots who do not have a license was not being registered to the District Health Office. So, those depots were not monitored well and they do not have a sanitation hygiene certificate which means the hygiene sanitation of equipment, place, handlers, and source of water can't be evaluated. According to the regulation of the Minister of Health of the Republic of Indonesia Number 43 of 2014, to get the sanitation hygiene certificate those potable water depots have to pass the requirements for place, equipment, handlers, and water sources with the minimum score is 70[1].

The result in this study, from 28 samples there were 11 samples that contaminated by Coliform with 8 samples contaminated by Escherichia coli. This result was similar with the research that has been done in South Tangerang, from 12 samples of potable water there were 6 samples that contaminated by Escherichia coli and Coliform[20]. Other research was also has been done in Manado, from 9 samples of potable water there were 7 samples that contaminated by Coliform with 7 samples were contaminated by Escherichia coli[21].

The contamination of Coliform bacteria and Escherichia coli can occur due to several factors such as source of water that has been contaminated[22][23], poor environmental hygiene sanitation of the potable water depot[24][25], lack of attention to hygiene and health of the handlers[26][27], and gallon washing process that does not appropriate to the regulations[28][29].

According to the observation in this study, it was found that the environmental hygiene of the

potable water depot was poor such as the lack of lighting and ventilation, the trash can was not closed, and there was no hand washing area. Some studies show that improperly managed and closed sewerage could increase the incidence of diarrhea[30][31]. In addition, the handlers paid less attention to the environmental hygiene such as smoking, the clothes were not clean, not wearing masks, and not washing hands before serving the consumers. A study shows that not washing hands and bad sanitation hygiene behavior could increase the occurrence of bacterial contamination[32][33]. Furthermore, source of water, source water processes into potable water, and gallon washing processes also need to be considered. The majority of the gallon washing process in this study was only done by brushing and rinsing the gallon and then immediately filled it with potable water, the gallon washing process should be done with food grade detergent and clean water with a temperature of 60-85°C then rinsed with potable water to remove the rest of the detergent[11]. In several studies, it was found that water sources and source of water processes into potable water have an impact on the incidence of waterborne disease[34][35].

Conclusion

In the research on refill drinking water in Jepara District, from 28 samples, there are 11 samples that did not pass the microbiological parameters of the Regulation of the Minister of Health of the Republic of Indonesia Number 492 of 2010 concerning the requirements for drinking water quality. From 11 samples that did not pass the regulation, 11 samples were contaminated by Coliform with 8 samples contaminated by Escherichia coli.

Based on the results of this study, microbiological examination of potable water needs to be carried out in the hope that potable water depot owners pay more attention to environmental health, equipment, and handlers at their potable water depots so it can increase public interest in the use of refilled drinking water. In addition, it is hoped that the owners of potable water depots will immediately register their potable water depot permits to the local Health Office so that it will be easier to monitor and the potable water produced is safe for consumption. For further researchers, it is expected to conduct research on water sources, the process of sterilizing tools and materials at drinking water depots, and environmental hygiene of potable water depot workers to find out the biggest factor causing contamination of potable water.

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