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Research Article

Analysis of coliform bacteria contamination in drinking water refill in Ambon City

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ABSTRACT

In recent years, refillable drinking water businesses have grown rapidly in several cities in Indonesia, including Ambon City. On the one hand, Refillable Drinking Water Depots (DAMIU) support efforts to realize a healthy society by expanding the reach of clean water consumption, On the other hand, DAMIU tend to be problematic when faced with business interests. Feces contain coliform bacteria which is an indicator of water contamination. This study aims to determine the presence of coliform bacteria in Ambon City. The samples used in the study came from three sub-districts of refill drinking water depot sources, namely the Teluk Dalam sub-district, Nusaniwe sub-district, and Baguala sub-district. Microbiological testing of refill drinking water using MPN (most probable number) method. The MPN test results showed that three refill drinking water samples were not contaminated with coliform bacteria because there was no turbidity or gas formation in the Durham tube for gas-forming bacteria. This indicates that the three refill drinking water samples tested microbiologically have good quality and are safe for consumption, because in 100 ml of water no coliform bacteria were found in accordance with Permenkes No. 492/Menkes/Per/IX/2010 regulations on the requirements and supervision of water quality, which states that the microbiological requirements for drinking water are MPN Coliform /100 ml sample = 0. There are no coliform bacteria in the refillable drinking water depot in Ambon City so the water is suitable for consumption.

Keywords: Refillable drinking water depot, refillable drinking water, coliform bacteria, most probable number

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INTRODUCTION

Water is one of the most abundant natural resource. Water is a colorless, odorless and tasteless chemical compound (Susana, 2003). The entire metabolism is disrupted by the fulfillment of this requirement. In addition to supporting 70% of the human body, water regulates food substances in the body, facilitating digestion, metabolism, and balance. The need for fresh water in Ambon City in 2010 was 15,894,849 m3, while the water supply in the dry season was only 8,267,275 m3 and 9,244,885 in the rainy season (Latuihamallo and Putuhena, 2016).

The consumption of refillable drinking water is higher than that of bottled drinking water, because the price of refillable drinking water is relatively cheaper than bottled water, which is one-third to one-quarter the price of bottled water. The price of refillable drinking water is cheaper because there are no packaging and shipping costs required to open a Refillable Drinking Water Depot (DAMIU), and no large capital is required to open this business. In recent years, the refillable drinking water business has grown rapidly in several cities in Indonesia, including Ambon City (Latuconsina and de lima, 2020). Refillable drinking water depots are currently spread across all sub-districts of

Ambon City. On the one hand, the presence of Refillable Drinking Water Depots (DAMIU) supports efforts to realize a healthy society by expanding the reach of clean water consumption, but on the other hand, refillable drinking water depots (DAMIU) tend to be problematic when faced with business interests (Sampulawa and Tumanan, 2016) Moreover, the competition between depots is quite strong, and result of which it is not uncommon for the quality of drinking water to be neglected (Natalia et al., 2014).

Drinking water that is healthy and safe for consumption must meet requirements that include physical, chemical and bacteriological requirements. According to Sutrisno and Suciastuti (2002), in Byna et al. (2009), physical requirements include color, odor, taste, temperature, and turbidity. Water turbidity can be caused by the presence of organic and inorganic materials in the water, such as mud and materials derived from disposal. Chemical quality is related to the ions of harmful compounds and metals, such as Hg, Pb, Ag, Cu, and Zn. Residues of other toxic compounds are pesticide, which can cause changes in the smell, taste and color of water (Pratiwi, 2007).

Bacteriological requirements for drinking water According to the regulation of the Minister of Health of the Republic of Indonesia Number 907/Menkes/SK/VII/2002, drinking water should not contain pathogenic bacteria, which can cause disease, especially those of the digestive tract, namely coliform bacteria. The standard for coliform bacteria content in drinking water is 0 per 100 ml (Bambang et al., 2014) If the quality of drinking water does not meet the requirements, especially bacteriological quality, it will cause health problems such the onset of diseases such as diarrhea, cholera, typhoid, hepatitis, dysentery and gastroenteritis (Pirsaheb et al., 2017). The pollution of drinking water sources by bacteria and other contaminants can endanger public health. This research was conducted to prevent diseases transmitted through drinking water contaminated by coliform bacteria The purpose of this study was to determine the presence of coliform bacteria in refill water circulating in Ambon City using the most probable number (MPN) method.

METHODS

The type of research was descriptive. This study was conducted from September 22, 2022, to October 23, 2022. The sampling site was filled with drinking water DEPOTS spread across Baguala sub-district (1 depot), Teluk Ambon sub district (1 depot), and Nusaniwe sub district (1 depot).

The Most Problable Number (MPN) method is used to test the bacteriological quality of the refill drinking water. The MPN method consists of three stages: presumptive test, confirmed test, and completed test. For the refill drinking water test, the MPN method is carried out up to the confirmation test method, because this method is strong enough to be used as a test for the presence or absence of Coliform bacteria in refill drinking water samples (Shodikin, 2007). The calculation is based on positive tubes, that is tubes showing microbial growth after incubation at a certain temperature and time and can be determined from the gas bubbles produced in Durham tubes. The MPN value is determined by a combination of the number of positive tubes (acid and gas) per series after incubation (Waluyo, 2009). The MPN method was used with the 3 series tube method, namely with 3 x10 ml, 3 x 1 ml, and 3 x 0.1 ml samples, and data analysis was carried out descriptively using tables adjusted to the results of the examination.

Data collection and retrieval procedures

1. Observation of the refill drinking water depot that has been selected as a sample.
2. Direct sample collection was conducted by buying refill drinking water at refill drinking water depots located in three sub-districts in Ambon City.

Sterilization of tools

The tools used for the test such as Petri dishes and spatulas/stirrers, are first wrapped in paper. The test tubes, Erlenmeyers flaks, and glass beakers were covered with cotton and wrapped in aluminum foil. Then, all the tools were placed in an autoclave and sterilized at 121 ºC within 15 minutes with a total cooling of up to 2 hours and a pressure of 1 atm. Subsequently workspace such as the work table, incubation room, and Laminar Air Flow (LAF) were cleaned by pouring 70% alcohol on cotton or sprayed directly and then applied to the entire workspace. This aims to keep the space and tools sterile before use and to prevent contamination (Misan and Diana, 2016).

Sampling

Sampling was adapted from the stages of Sudiana and Sudirgayasa (2020) with minor modifications as follows:

1. Aseptic hands first before taking water samples using 70% alcohol to prevent contamination.
2. Wear handscoon on both palms
3. Prepare tools for water collection in the form of a place to store water samples, namely Erlenmeyer which has been sterilized and closed.
4. Fill the Erlenmeyer with water, then close it tightly and label it.
5. The sample was placed in a cool or ice box, and the samples were taken to the laboratory for examination.

Preparation of Lactose Broth (LB) media

The calculation method for LB media

The calculation method for LB media can be calculated as follows (Muchlis et al., 2019):

$$\frac{V1}{W1} = \frac{V2}{W2}$$

1. LB media, as much as 1.17 grams, and the powder was dissolved in 90 ml of water in an Erlenmeyer flask and then heated on a Hot Plate until homogeneous.
2. Each LB as much as 10 ml into a test tube containing a Durham tube in an inverted position by being placed upside down and labeled (marked by writing the sample location, and followed by writing 10^{-1}, 10^{-2}, 10^{-3}).
3. The test tubes were then closed using cotton plugs and, sterilized using an autoclave at 121 °C for 30 minutes.
4. The journey to the laboratory for two hours.

Testing stages/presumtive tes

The Estimation Test is carried out using the following procedure:

1. Nine-test tubes for 1 drinking water sample divided into three groups with each group consisting of three tubes.
2. 10 ml of sample was pipetted and then put into a test tube containing LB solution.
3. Then 1 ml of the sample was pipetted into a test tube containing LB solution.
4. 0.1 ml of sample was pipetted then put into a test tube that has contained LB solution.
5. Shake the tube gently so that the water sample evenly spreads throughout the media.
6. All the test tubes were incubated at 37°C for 24-48 hours. ~~e~~ach tube was observed to see the presence or absence of gas, the presence of gas indicates a positive prediction. ~~i~~ncubation results were then observed in the presence or absence of gas.

Positive results were characterized by the formation of gas and bubbles in the Durham tube.

Data Analysis

The results of laboratory examinations are processed manually by presenting the data that has been processed by presenting the data using tables. The stages of analysis are shown with the data, namely the Coliform presumptive test using the MPN method of a series of 3 tubes, then the value in the MPN table is multiplied by 1/factor dilution in the middle.

RESULTS AND DISCUSSION

The total number of coliform in drinking water was-obtained from the results of laboratory tests using the MPN test with reference to the standard Permenkes RI No.492/MENKES/PER/IV / 2010. The maximum allowable content in drinking water is zero-per 100 ml of drinking water. An overview of the number of coliform bacteria found in the refill drinking water in the three districts of Ambon City can be seen in the following table:

Table 1. Test results of coliform test results on refill water in Ambon City

Sample	Number of Tubes									Positive tube combination
	10 ml			1 ml			0,1			
	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	
Depot of Teluk Ambon Sub District	-	-	-	-	-	-	-	-	-	0 0 0
Depot of Baguala Sub District	-	-	-	-	-	-	-	-	-	0 0 0
Depot of Nusaniwe Sub district	-	-	-	-	-	-	-	-	-	0 0 0

Description: coliform bacteria test results refill drinking water samples in Ambon City
(+) = Formed gas /discoloration of the tube
(-) = No gas is formed/no color change in the tube

The most probable number is the standard value for Safe Drinking Water Quality and has been regulated by several health standards. Based on Permenkes Regulation (2010) on the requirements and supervision of water quality, the microbiological requirements for drinking water are MPN coliform / 100 ml sample = 0. This proves that lactose fermentation does not occur in bacteria in the coliform group. From the observations when sampling the drinking water refill, information was obtained that could be a factor affecting the quality of the drinking water refill produced. Some factors that affect the quality of drinking water produced are raw water, operator hygiene, handling of the buyer's container, and depot conditions (Trisnaini et al., 2018).

According to Askrening et al. (2017) factors that can affect the high quality of the MPN value and the level of contamination of drinking water products produced are the raw water used, cleanliness around the depot, handling of the buyer's container, and condition of the depot. This Drinking Water Depot is likely to be very susceptible to

coliform bacteria contamination-. Another factor contributing to the high level of pollution in refilled drinking water depots is the cleanliness of the operators who handle and fill the containers brought by consumers.

The same thing is stated in (Rosita, 2014), which explains that the factors that can affect the quality of poor water products produced are raw materials, the length of time the water is stored in the shelter, handling the container buyer, operator hygiene, environmental cleanliness around the depot, and depot conditions. Product quality testing that has been done cannot guarantee that the water produced is free from pollution and safe for public health. Strict supervision of the operation of the Drinking Water Depot business needs to be improved considering that the number of depots that do not check the quality of water products are still operating and serving consumers.

CONCLUSION

Based on the results of microbiological testing of drinking water requirements that have been carried out, using the Most Probable Number method on drinking water refills, it was concluded that the drinking water of three districts in Ambon City is suitable for consumption because it is not contaminated with coliform bacteria that meet standards according to the regulations of the Ministry of Health of the Republic of Indonesia 492/Menkes/Per/IV/2010 the maximum allowable level for coliforms in drinking water is 0.

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