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**CONTAMINATION OF *Escherichia coli* DRINKING WATER REFILLS ON DRINKING WATER DEPOTS IN MALANG CITY**

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**ABSTRACT**

Data from the Malang City Health Office (2012) shows the increasing number of refill drinking water depots in the city of Malang, until 2013 there were 163 drinking water depots scattered in each Malang City Subdistrict. The purpose of this study was to determine the contamination of Escherichia coli refill drinking water in drinking water depots in the city of Malang.

Research is a descriptive laboratory. The samples taken were 20 refill drinking water depots in the city of Malang. Samples taken that have fulfilled the inclusion criteria are refill drinking water depots registered with Malang City Health Office. Examination of Escherichia coli using the Most Probable Number Test consisting of presumptive test using Lactose Broth (LB) medium and confirmative test using Brilliant Green Lactose Broth (BGLB) medium. Escherichia coli was found as many as 18 samples from 20 samples tested.

Keywords : Escherichia coli, refill drinking waterTop of Form

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**INTRODUCTION**

Water is a chemical compound whose function is very important for the lives of human beings and other living things (Mukaromah and Yusrin, 2010). Water needed by humans includes clean and healthy water for use in cooking, washing and bathing and water that is suitable for consumption for drinking purposes (Rahayu, 2010). Water can also act as a medium for disease transmission. Water is a medium and environment that is good for the life of microorganisms, both pathogenic and non-pathogenic microorganisms, therefore arises what is called water borne disease (Asfawi, 2004).
Drinking water is water that goes through processing or without processing that meets health requirements and can be drunk directly. Drinking water is safe for health if it meets physical, microbiological, chemical and radioactive requirements. The mandatory parameters for microbiological drinking water quality determination are total coliform bacteria and Escherichia coli.

Meeting the community's drinking water needs varies greatly. In big cities, in terms of fulfilling drinking water needs, the community also consumes refill drinking water depots. Refill drinking water depots is a business entity that manages drinking water for community needs in bulk and unpackaged forms. Judging from the price of refill drinking water is cheaper than bottled drinking water, some even set prices up to 1/4 of the price of bottled drinking water. But in terms of quality, people still doubt because there is no clear information in terms of the process and regulations regarding circulation and supervision.

Various previous studies in various major cities in Indonesia, showed refill drinking water was less safe or contaminated with bacteria that could harm human health. Research conducted by the Bogor Agricultural Institute (IPB) and the Food and Drug Supervisory Agency (BPOM) stated that most of the drinking water products produced by refill drinking water depots did not meet the bottled drinking water industry standards. The research was conducted in several major cities such as Jakarta, Bandung, Medan and Surabaya. The results of these two institutions showed that refill drinking water was contaminated with coliform bacteria, Escherichia coli, Salmonella, and even detected water samples containing heavy metal cadmium (Rubaiah, 2005).

One of the diseases caused by drinking water with poor microbiological quality is diarrhea (Wandrivel et al., 2012). Diarrhea is a prominent disease in the city of Malang with 34,322 cases in 2012 (East Java Provincial Health Office, 2012).
Data from the Malang City Health Office shows the increasing number of refill drinking water depots in Malang City, until 2013 there were 163 drinking water depots scattered in each Kota Malang District (Malang City Health Office, 2012). So this study aims to determine the contamination of Escherichia coli refill drinking water (AMIU) at drinking water depots in the city of Malang, East Java province.

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**METHOD**

**Research design**

The research conducted is descriptive laboratory. The sample in this study were 20 refill drinking water depots in the city of Malang. Samples taken that have fulfilled the inclusion criteria are refill drinking water depots registered with the Health Office. Microbiology examination using the Most Probable Number Test consisting of presumptive test using Lactose Broth (LB) medium and confirmative test using Brilliant Green Lactose Broth (BGLBB) medium.

**Sampling**

1. Bottles of sterile neck samples are brought close to the bunsen fire while rotating.
2. Quickly enter the sample into a sample bottle that has been given a specific name.
3. After filling 90% of the sample bottle again.

**Sterilization of tools and materials**

1. Fill the autoclave with water to near sarangan.
2. Enter materials (medium) or equipment to be sterilized.
3. Let the steam valve open, turn on the autoclave.
4. If the steam valve has dripping liquid, it means that the space in the autoclave is saturated with moisture. Close the steam valve.
5. Allowing the autoclave to light up to a temperature of 1210C and a pressure of 15 lbs. then maintain for 15-20 minutes.
6. After 15-20 minutes at vapor pressure lbs, turn off the autoclave, waiting for the pressure to decrease for 5-10 minutes.
7. Open the valve slowly, release steam until the pressure returns to zero.

**Making Lactose Broth (LB) Media**

1. Weighed 3.25 grams of Lactose Broth media, put into an erlenmeyer measuring 500 ml.
2. Dissolved with 250 ml distilled water and measured pH 6.9.
3. Then heated to boiling, pour each into a test tube as much as 5 ml (use the durham tube in the upside position).
4. Cover with cotton coated with aluminum foil.
5. Sterilize in an autoclave for 15 minutes with a temperature of 121 ° C.
6. If the ingredients have been sterile, it is marked with a brown color change.
7. Lift the material from the autoclave, allow it to cool to room temperature (15-30 ° C).
8. Stored in a refrigerator with a temperature of 2-8 ° C.

**Making BGLB Media (Bile Green Lactosa Broth)**

1. Cleaned the workbench, then sterilized with alcohol.

2. Balance sheet balance is balanced first at zero position.

3. Then a test tube is prepared which is already filled with a durham tube.

4. Weighed 32 grams of BGLB media, put it into an erlenmeyer flask, then dissolved with 800 ml distilled water, stirring until homogeneous.

5. Then poured into a test tube as much as 5 ml, cover with sterile cotton coated with aluminum foil.

6. Put in a basket, tie and cover with brown paper or aluminum foil, on paper written with BGLB, date and month of manufacture.

7. Sterilization by autoclaving at a temperature of 1210C for 15 minutes, after completion then cool.

**Presumptive Test (Presumtive Test)**

Pre-test test using the MPN (Most Probable Number) (3: 3: 3) 9 tube method, as follows:

1. Prepared 9 tubes of LB (Lactose Broth) which inside were filled with durham tubes in the upside position.
2. The test sample is shaken until homogeneous.
3. Then 3 LB tubes were inoculated with 10 ml samples, 3 LB tubes inoculated with 1 ml sample and 3 LB tubes inoculated with 0.1 ml sample.
4. Then all LB tubes containing samples were incubated at 37 ° C for 24 - 48 hours.

**Confirmation Test (Confirmative Test)**

This test uses BGLB (Bile Green Lactose Broth) media.
This test is done to confirm the positive results of the test estimates.

1. From each tube that shows positive gas at the presumtive test, it is shaken and 1-2 oz is taken.
2. Then inoculated on the BGLB tube after that the BGLB tube was incubated at 37 ° C and 44 ° C for 24 - 48 hours.
3. Observed the formation of gas in each tube the number of positive BGLB gas cylinders was recorded and the results were referred to MPN table 1.
4. Figures obtained from the table show MPN Coliform and Fecal Coli per 100 ml sample test samples.
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**RESULTS AND DISCUSSION**

Refill drinking water samples obtained from refill drinking water depots in Malang city were 20 samples. Microbiology examination using the Most Probable Number Test. The first stage is presumptive test using Lactose Broth (LB) medium because it is a medium to detect the presence of coliform bacteria. A positive result is indicated by the formation of gas in a durham tube and is acidic when the media turns yellow. In the medium of Lactose Broth (LB) contains peptone and meat extracts which provide important nutrients for bacterial metabolism. Lactose contained also provides a source of carbohydrates that can be fermented by coliform bacteria.
Samples that showed positive results in the presumption test were followed by a confirmation test to determine the total Escherichia coli using BGLB media. Total Escherichia coli in refill drinking water using Brilliant Green Lactose Broth (BGLB) media can be seen in table 1 as follows:

Table 1 Total Escherichia coli in Refillable Drinking Water using Brilliant Green Lactose Broth (BGLB) media

|  |  |  |
| --- | --- | --- |
| **Sample** | **Dilution Level** | **MPN****(per 100 ml)** |
| **10 ml** | **1 ml** | **0,1 ml** |
| 1 | 3 | 2 | 0 | 93 |
| 2 | 3 | 0 | 1 | 39 |
| 3 | 3 | 0 | 2 | 64 |
| 4 | 2 | 1 | 0 | 15 |
| 5 | 3 | 0 | 0 | 23 |
| 6 | 3 | 0 | 2 | 64 |
| 7 | 2 | 2 | 1 | 28 |
| 8 | 3 | 0 | 1 | 39 |
| 9 | 2 | 1 | 1 | 20 |
| 10 | 3 | 0 | 1 | 39 |
| 11 | 2 | 2 | 1 | 28 |
| 12 | 3 | 0 | 1 | 39 |
| 13 | 2 | 1 | 1 | 20 |
| 14 | 3 | 0 | 1 | 39 |
| 15 | 0 | 0 | 0 | 0 |
| 16 | 2 | 1 | 1 | 20 |
| 17 | 3 | 0 | 2 | 64 |
| 18 | 2 | 2 | 1 | 28 |
| 19 | 2 | 1 | 0 | 15 |
| 20 | 0 | 0 | 0 | 0 |

Based on the examination of the number of Escherichia coli bacteria contamination in refill drinking water above shows that as many as 18 samples from 20 samples tested were contaminated with Escherichia coli bacteria.

**CONCLUSION**

There were 18 samples of Escherichia coli contamination from 20 samples produced from 20 refill drinking water depots in Malang.

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