

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 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 Subdistrict. The purpose of this study was to determine the contamination of *Escherichia coli* refill drinking water in drinking water depots in Malang. The samples taken were 20 refill drinking water depots in Malang. Sampling is done by simple random sampling. *Escherichia coli* examination using the Most Probable Number Test. Of the 20 samples tested, 2 samples met the requirements of drinking water quality according to PERMENKES and 18 other samples containing the number of coliform bacteria exceeding the maximum limit of 0 per 100 ml of water. There were *Escherichia coli* in 10 samples while 8 other samples contained other coliform bacteria.

Keywords: contamination; drinking water depot; *Escherichia coli*; refill drinking water

INTRODUCTION

Water is a chemical compound whose function is very important for the lives of human beings and other living things (Mukaromah & Yusrin, 2010). Water needed by humans includes clean and healthy water for use in cooking, washing, and bathing, also as suitable consumption for drinking purposes (Rahayu, 2010). Water can also act as a medium for disease transmission (Prüss *et al.*, 2002; Ashbolt, 2004). Water is a medium and environment which good for microorganisms habitat, both pathogenic and non-pathogenic microorganisms, therefore arises waterborne disease (Asfawi, 2004; Figueras & Borrego, 2010).

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, aesthetic and radioactive requirements (Szewyk *et al.*, 2000; Dietrich, 2006; Hijnen *et al.*, 2006). The mandatory parameters for microbiological drinking water quality determination are the total coliform bacteria, which are 0 per 100 ml of water 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. 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. Although 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 and the Food and Drug Supervisory Agency 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* sp., 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, 2013). 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, 2015). Thus, this study aims to determine the contamination of *Escherichia coli* refill drinking water at drinking water depots in Malang, East Java province.

MATERIALS AND METHODS

The research conducted is descriptive laboratory. The sample in this study were 20 refill drinking water depots in the city of Malang. Sampling is done by simple random sampling. Microbiology examination using the Most Probable Number Test consisting of presumptive test using Lactose Broth (LB) medium, confirmative test using Brilliant

Green Lactose Broth medium (BGLBB) and complete test using agar EMB.

RESULT 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 Index/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 coliform bacteria contamination in refill drinking water above shows that as many as 18 samples from 20 samples tested were contaminated with coliform bacteria.

Samples that gave positive results in the presumptive test were inoculated on agar EMB media and incubated at 35°C for 24 hours. EMB

agar test media is a differentiation medium for isolation of coliform bacteria. The presence of *Escherichia coli* is characterized by blackish purple colonies in the middle and metallic luster and 2-3 mm in diameter. Refill drinking water samples were tested, the results of inoculation on the EMB to provide varied results as in table 2.

Table 2. Results of inoculation on the agar EMB

No.	Sample	Colonies on EMB agar	Information
1	Sample 1	Colonies with metallic luster	<i>E.coli</i>
2	Sample 2	Colonies with metallic luster	<i>E.coli</i>
3	Sample 3	Colonies with metallic luster	<i>E.coli</i>
4	Sample 4	Colonies with metallic luster	<i>E.coli</i>
5	Sample 5	Colonies with metallic luster	<i>E.coli</i>
6	Sample 6	Pink colony	<i>Pseudomonas</i>
7	Sample 7	Blackish purple and mucoid pink colonies	<i>Pseudomonas / Enterobacter</i>
8	Sample 8	Colonies with metallic luster	<i>E.coli</i>
9	Sample 9	Pink colony	<i>Pseudomonas</i>
10	Sample 10	Pink colony	<i>Pseudomonas</i>
11	Sample 11	Colonies with metallic luster	<i>E.coli</i>
12	Sample 12	Colonies with metallic luster	<i>E.coli</i>
13	Sample 13	Pink colony	<i>Pseudomonas</i>
14	Sample 14	Pink-mucoid colony	<i>Pseudomonas / Enterobacter</i>
15	Sample 15	No inoculation	-
16	Sample 16	Blackish purple and mucoid pink colonies	<i>Enterobacter</i>
17	Sample 17	Colonies with metallic luster	<i>E.coli</i>
18	Sample 18	Pink-mucoid colony	<i>Pseudomonas / Enterobacter</i>
19	Sample 19	Colonies with metallic luster	<i>E.coli</i>
20	Sample 20	No inoculation	-

Samples of 15 and 20 were not inoculated to the EMB hence it showed negative results in the presumptive test, then as many as 18 samples were tested and inoculated in the EMB. The results of 10 samples of refill drinking water showed colonies with metallic luster. EMBs contain eosin and methylene blue which can inhibit the growth of gram-positive bacteria and support the growth of gram-negative bacteria. In addition, lactose contained and fermented by bacteria will produce colonies with dark nuclei which indicate acid production. *Escherichia coli* is a bacteria that can ferment lactose quickly and produce acid (Fong *et al.*, 2005; Edberg *et al.*, 2012; Rosales-Colunga *et al.*, 2012). Metallic luster produced by colonies due to deposits of metallic green pigments (Ranjbar *et al.*, 2014). The presence of *Escherichia coli* in refill drinking water indicates the possibility

of contamination as well as the presence of enteropathogenic microbes that can disrupt human health.

Factors that might cause positive results from the MPN presumptive test are the occurrence of contamination of refill drinking water in the processing process, including raw water storage, disinfection, and filtration (Dechesne & Soyeux, 2007). In addition, sanitation and hygiene from refill drinking water depots can affect the MPN test results. Poor sanitation and low hygiene cause contamination. Based on observations from refill drinking water depots, the samples of this study have poor sanitation and hygiene, also the disinfection process of containers did not meet the requirements. These can explain why the MPN results on samples of refill drinking water from the depot are high-positive result.

CONCLUSION

Based on the result of 20 samples tested, 2 samples met the requirements of drinking water quality according to PERMENKES and 18 other samples containing the number of coliform bacteria exceeding the maximum limit of 0 per 100 ml of water. There were *E. coli* in 10 samples while other samples contained coliform bacteria.

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