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The Findings of Escherichia Coli in Drinking Water with Reverse Transcriptase Polymerase Chain Reaction Method at 16S RNA Gene

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ABSTRACT

Drinking water is the fundamental needs of urban communities. Emerging drinking water refilled station (DWRS) causing decreased in water quality so required the existence of quality monitoring efforts. RT-PCR technique could detect the presence of Escherichia coli in drinking water. The RT-PCR method is superior in accuracy, efficiency, and specificity. This research aims to analyze the presence of Escherichia coli as an indicator of the quality of refilled drinking water with the technique of RT-PCR target 16s RNA. The sample in this study was ten drinking water refilled station with the total sample 30 samples measured in the inlet, outlet and output. The results of RT-PCR in Mariso district, obtained RNA Band in the gene 16S RNA at position 723-bp in the sample a. 13. While in Panakukkang district captured RNA Band in the gene 16S RNA at position 723-bp on sample B. 11; B. 12; B. 13. Conclusions, Genomic RNA template by RT-PCR can be used to detect bacteria Escherichia□ □

Keywords: *Escherichia Coli, 16S-RNA, drinking water, RT-PCR, Culture*

INTRODUCTION

Infection from drinking water caused 13 million people died annually, 2 million of them are infants and children. Consume water contaminated by pathogenic microorganisms may cause various gastrointestinal diseases.[1] Increasing for drinking water needs make growing drinking water refilled station (DWRS).[2]

Contamination in drinking water produced by a variety of physical hazards, chemical, biological, radioactive, equipment, poor sanitation, and hygiene.[3,4] Increased quality of water, waste disposal, and personal hygiene are essential to reduce contamination.[1] The number of drinking water refilled station in South Sulawesi province in 2015 were 1.017, qualified 591 and 426 unqualified. [5]

There are 28,908 diarrhea cases in Makassar city in 2013, *Incidence Rate* 21.3 ‰. One of the causes of diarrheal diseases were drinking water contaminated with bacteria.[5]

Coli's most probable number (MPN) is considered to be less accurate in detecting certain types of bacteria in the water. [6] RT-PCR techniques can be used to identify life bacteria. [7] RT-PCR have more accuracy, efficiency, and specificity.[8,9] Kandou et al., found 8.33% samples of bottled drinking water and 25% of drinking water samples polluted by *Escherichia coli* serotype O157: H7. The source of the contamination comes from unstandardized processing. [10]

RT-PCR can detect bacteria in different concentrations. Primary EF II applications decreased false-positive results compared to 16S primary rRNAs. The hydrophobic FHLP filter has a higher ability to absorb bacteria compared to HAWB hydrophilic filters. Hence the use of hydrophobic filters will increase the sensitivity of RT-PCR. [11,12] This research aims to analyze the presence of bacterial pathogens Escherichia coli as an indicator of the quality of drinking water refill with

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the technique of Reverse Transcriptase - PCR (RT-PCR) and target 16s RNA.

MATERIALS AND METHOD

This is an observational study to identify the presence of *Escherichia coli* as an indicator of the quality of refilled drinking water with RT-PCR 16SRNA target. Samples obtained from 5 drinking water refill station in Mariso and Panakukang district in Makassar city. Each example obtained from the inlet, outlet and drinking water at the level of the consumer (outputs), a total of 30 samples.

DNA Extraction

100 ml sample was added 900 ml solution of L6 (Lysis buffer) then incubated for 24 hours, put on a shaker for 2 hours then added 20 µ l suspension. This mix of vortices and placed on a shaker 10 rpm for 10 minutes. Vortex and centrifuge at 12,000 rpm for 15 minutes scoop out the supernatant. Reserving ten µl of supernatant fluid in the tube. Washed twice with 1 ml solution of L1 (Washing buffer), centrifuge and vortex for 15 seconds then discard the supernatant. Wash two times with 1 ml of 70% ethanol and once with acetone. Discard supernatant acetone, let the tube open and incubate at a temperature of 56 ^{0}C in the incubator for 10 minutes. Add 60 µl of TE-elution buffer, vortex well then the tube incubation at a temperature of 56^{0}C for 10 minutes. After that, the centrifuge for 30 seconds at 12,000 rpm. Move 50 µ l of supernatant into a new vial then keep at a temperature of 200C until ready to be processed by PCR technique.[13]

Amplification of DNA by PCR

Mixture PCR samples in PCR tubes. Every 16.9 µ l of sterile water, 2 µ l 10 mm *deoxynucleotide* triphosphate mixture 1 µ l 50 mM Mgso4, 2.5 µ L of 10 X amplification buffer 0.5 µ l 10 µ M Forward primer and 0.5 µ l 10 µ M reverse primer, 0.1 µ l (0.25 µ U/L) of Taq DNA polymerase and sterile water is added until the final volume was 22.5 µ l. The prepared vial that has filled each of the 2.5 µ l sample DNA. Each tube in a reaction mixture PCR content as much as 22.5 µ l. after that the tubes are filled by using PCR machines (hybrid, Ashford, UK) as many as 40 cycles each cycle consisted of denaturation at 94^{0}C for 1 min, annealing temperature 57^{0}C for 1 minute 15 seconds and the extension at a temperature of 72^{0}C for 30 seconds. The final extension at 72^{0}C for one night.[14]

Detection of PCR products

Each five µ l amplification products mixed with two µ l solution. Put in 1.5% agarose gel wells that are submerged in a tank containing a buffer Tris-EDTA acetic acid. Also included a marker (DNA λ/Hind III) into the wells of agarose to know the size of the PCR product, then DNA electrophoresis runs for 1 hour with the constant voltage temperatures 75 volts. After 1 hour, electrophoresis stopped and gels lifted and observed under ultraviolet light (UV). The results obtained in the form of a black ribbon pattern DNA (DNA bands) which shows the number and different patterns.

Da&

Results of detection of PCR with electrophoresis are analyzed based on whether or not there are pieces in DNA that are formed and data presented in a descriptive by using tables and images. Sequence and position of the Nucleotide Primer. 16SRNA Gene; Forward 5 ' CGA GCG GAC GTC GGG TGA GT3 ' (From 81) Reverse 5 ' ACA TCG TCG ACG GCG TTT TGG A3 ' (From 786). Size (bp) 723 Access number EF6209.

RESULTS

Analysis of a physical parameter DWRS In Mariso Makassar City including the temperature and TDS showed in table 1. Analysis of the Chemical parameters includes pH, iron, and chloride. Results on pH samples; A.11 (8.18), A.21 (8.01), A.31 7.09, A.41 (7.46), A.51 (7.7). Iron found in the samples; A.11 and A.51 about 0.1 mg/l. The highest chloride found in the sample A.51 100mg/l, while the lowest was on samples; A. 22, A. 23; A. 31; A. 32; A. 33 at 6 mg/l. Cultures found almost all of the sam

Table 1. Physical and chemical parameters DWRS In Mariso Makassar City

Code station	Sample Code	Source	The temperature °C	TDS (mg/l)	PH	Iron (mg/l)	Chloride (mg/l)	Culture MPNcoli
1	A. 1.1	Inlet	27.5	107.8	8.18	0.1	37	+
	A. 1.2	Outlet	27.8	102.3	8.22	0	10	+
	A. 1.3	Output	31.5	117.8	7.11	0	10	+
2	A. 2.1	Inlet	27.9	99.6	8.01	0	8	+
	A. 2.2	Outlet	27.5	96.8	8.18	0	6	+
	A. 2.3	Output	29.5	107.5	7.19	0	6	+
3	A. 3.1	Inlet	27.0	27.0	7.09	0	6	+
	A. 3.2	Outlet	27.5	111.2	8.18	0	6	+
	A. 3.3	Output	27.5	87.5	8.18	0	6	+
4	A. 4.1	Inlet	29.2	29.2	7.46	0	12	+
	A. 4.2	Outlet	28.6	38.4	7.64	0	10	+
	A. 4.3	Output	30.2	107.5	7.5	0	10	+
5	A. 5.1	Inlet	28.4	28.4	7.7	0.1	100	+
	A. 5.2	Outlet	32.2	45.5	7.11	0	95	+
	A.5.3	Output	29.2	260	7,77	0	95	+

Examination of a physical parameter in Panakukang Makassar City the temperature of the inlet, outlet and output of each sample shown in table 2. Analysis of the Chemical parameters includes pH, iron, and chloride. The results of the investigation of the PH at sample b. 1.1 (7.1), b. 2.1 (8.9), b. 3.1 (7.4), b. 4.1 (7.8), b. 5.1 (8). Iron and chloride were not found. Examination of Coli MPN method using culture retrieved sample code B. 1.1, B.1.2 and B. 1.3 a □

Tabel 2. Analysis of physical and chemical Parameters DWRS Station In Panakukang

Code station	Sample Code	Source	The temperature of the °C	TDS (mg/l)	Ph	Iron (mg/l)	Chloride (mg/l)	Coli MPN Culture
1	B. 1.1	Inlet	30,8	55,7	7,1	0	0	-
	B. 1.2	Outlet	30	30	7.7	0	0	-
	B. 1.3	Output	31	31	8.5	0	0	-
2	B. 2.1	Inlet	30,1	53,7	8.9	0	0	+
	B. 2.2	Outlet	29	29	9	0	0	+
	B. 2.3	Output	30,4	28.4	9	0	0	+
3	B. 3.1	Inlet	29,6	75,4	7,4	0	0	+
	B. 3.2	Outlet	29,4	29,4	7,6	0	0	+
	B. 3.3	Output	31,2	28,2	7,4	0	0	+
4	B. 4.1	Inlet	29,8	37,14	7,8	0	0	+
	B. 4.2	Outlet	27.5	27.5	7,8	0	0	+
	B. 4.3	Output	31,2	25,2	7.5	0	0	+
5	B. 5.1	Inlet	30,1	30,8	8	0	0	-
	B. 5.2	Outlet	29.2	29.2	8,5	0	0	-
	B. 5.3	Output	28	24	8,5	0	0	-

Analysis RT-PCR in the gene 16S RNA found in the samples a. 13 (positive Escherichia Coli) while the other samples undetected, as shown in table 3.

Table 3. Results of RT-PCR *Escherichia coli* 16S RNA-gene on DWRS in district Mariso

Slot	Sample Code	RT-PCR Results	NOTE
1	Marker	-	
2	A. 1.1	(-)	
3	A. 1.2	(-)	
4	A. 1.3	(+)	Detected
5	A. 2.1	(-)	
6	A. 2.2	(-)	
7	A. 2.3	(-)	
8	A. 3.1	(-)	
9	A. 3.2	(-)	
10	A. 3.3	(-)	
11	A. 4.1	(-)	
12	A. 4.2	(-)	
13	A. 4.3	(-)	
14	A. 5.1	(-)	
15	A. 5.2	(-)	
16	A.5.3	(-)	
17	Negative Control	(-)	

Analysis RT-PCR in the gene 16S RNA found in the samples B. 21, B. 22 and B. 33 (Positive *Escherichia Coli*) while the other samples undetected, as shown in table 4.

Table 4. Results of RT-PCR *Escherichia coli* 16S RNA-gene on DWRS in district Panakukakng

Slot	Sample Code	RT-PCR Results	NOTE
1	Marker	-	
2	B. 1.1	(-)	
3	B. 1.2	(-)	
4	B. 1.3	(-)	
5	B. 2.1	(+)	Detected
6	B. 2.2	(+)	Detected
7	B. 2.3	(+)	Detected
8	B. 3.1	(-)	
9	B. 3.2	(-)	
10	B. 3.3	(-)	
11	B. 4.1	(-)	
12	B. 4.2	(-)	
13	B. 4.3	(-)	
14	B. 5.1	(-)	
15	B. 5.2	(-)	
16	B. 5.3	(-)	
17	Positive Control	(-)	

Electrophoresis in Mariso district obtained RNA Band in the gene 16S RNA at position 723-bp in the sample A. 13. While in Panakukakng obtained on sample B. 11; B. 12; B. 13.

DISCUSSION

Escherichia coli contamination in drinking water is caused by the unstandardized process. Chlorine can kill Escherichia Coli by destructive process of transport and respiration of membrane cells. The Escherichia Coli serotype O157: H7 strain G can still survive on the low chlorine concentrations. [15]

The prolonged contact with the raw water the higher the chance microbes overgrowth. The contact time between water with UV light for at least four seconds, and the time of connection between the water and the ozone at least four minutes. The Faster water flows rate than the specified time, the effectiveness of UV as harmful bacteria exterminator will decrease. [16]

Observation using *electron microscopy scanning* indicated that *Escherichia coli* serotype O157: H7 sticking and multiply on the walls of the container and survive for more than 300 days. Poor hygiene of the bottles can make the formation of biofilms.[17] The sequence selected as targets for amplification, resulting in 234 bp and bp PCR product 115.[12] biofilm cells more durable against anti-microbial materials, the physical condition of such extreme heat, so the contamination by these cells can spread the disease through food and water. [10]

The hygiene dispenser is generally less noticed by the consumer. The method of a repeating dispenser reset without cleaning the inside of the container allowing the growth of microbes. The risk of microbial contamination can occur either in normal-temperature, cold or heat because germs can grow at the cold, regular or hot temperatures.[18] The impact of the microbial contamination in the dispenser can potentially cause diarrhea. Contamination of drinking water can occur at the level of the producers, sellers or consumers. Drinkable water should be qualified bacteriologically or chemically. One indicator for potable water is the amount of bacteria present. Health Director-General requirements limit bacterial impurities in food and drink is a number TPC < 100/ml sample.

Identification of Escherichia Coli conventionally using biochemical reactions test and inoculation, it requiring quite a long time, the biochemical tests is hard to do, and are not accurate. This is because the bacterial colony alleged *Escherichia coli* in selective media and deferential media is often not pure and mixed with other *Enterobacteriaceae* bacteria.

Identification of *Escherichia coli* using conventional methods requires 5-6 days, PCR method takes two days (48 hours). This is in line with the research conducted by the Infallible Radji et al. [19]; conventional methods take six days while the PCR method only takes 48 hours. The direct PCR methods can detect the presence of *Escherichia coli* in samples without isolation of colonies of bacteria first.[20] Thus the PCR method is more accurate and faster than conventional methods.

CONCLUSION

Genomic RNA template by RT-PCR can be used to detect bacteria Escherichia coli in drinking water refills more quickly and accurately than conventional methods.

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Conflict of Interest- The author declares no conflict interest regard this research

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