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Prevalence of *Escherichia coli* and *Coliform* Contamination in Unbranded Refillable Drinking Water Depots in Rural South Lampung, Indonesia: A Case Study around ITERA

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Abstract

Drinking water is an important aspect for the body in sustaining life. In Indonesia, the refillable drinking water depot business is now growing rapidly and has an important meaning in providing drinking water that is easily accessible to the community. However, drinking water is a product that is susceptible to contamination, so public ignorance of the quality of refillable drinking water needs to be analyzed to avoid the risk of diseases that may be caused by refillable drinking water from depots such as diarrhea. The purpose of this study was to test the presence of *E. coli* and *Coliform* bacteria in refillable drinking water produced by depots in Rural Indonesia. The sampling method in this study used *simple random sampling* method. For the analysis of *E. coli* and *Coliform* bacteria, the *Most Probable Number* (MPN) method was used and identification of *Coliform* types based on biochemical tests (TSIA, SIM, citrate, urease). The results showed that five samples tested were positive for *Coliform* >0/100 mL and three samples contained *E. coli*. The hygiene of both operators and consumers is an important factor in producing quality drinking water. The five depots showed that none of the operators cared about their own hygiene.

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Introduction

Water is one of the important factors for living things to survive. The human body needs about one to two and a half liters of water or equal to 6-8 glasses per day. Each person's water needs vary depending on body weight, age, physical activity, temperature (climate), and diet (Kusumawardani *et al.*, 2020). If water needs are not met, it can cause the body to lack fluid or dehydration. Dehydration will damage cognitive function so that the body becomes unfocused (Suhestry *et al.*, 2022). Bottled drinking water is the community's choice because it is considered hygienic and practical, however, the community has switched to the existence of refillable drinking water which comes from depots by providing relatively cheaper prices than bottled drinking water. Bottled drinking water comes from mountain spring sources, while refillable drinking water from depots usually utilizes groundwater with drilled wells or dug wells.

Refillable drinking water depot is a medium-scale industrial business that manages raw water into drinking water in bulk form and packaged without a brand using an old container to sell directly to consumers. The principle of refillable drinking water treatment is filtration and disinfection. After the raw water is stored in a storage tank (made of food grade), the water is then filtered with the aim of filtering coarse to micro particles. First, the water will pass through a silica (SiO_2) filter to filter out coarse particles. Then, the water passes through a tube filled with activated carbon to absorb color, taste, smell, and other organic matter. After that, the water is filtered using another filter-to-filter fine particles to a maximum of 10 (ten) microns and 1 (one) microns to filter bacteria (Budiyono & Sumardiono, 2013). After the filtration stage, the water will be collected back in a special tank which will later be drained and irradiated using ultraviolet (UV) lamps to ensure that there are no germs and bacteria left (Sugriarta, 2025). With this refillable water filling depot, consumers can immediately drink water without the hassle of cooking it first.

The refillable drinking water Depot business is now growing rapidly and has an important role in providing drinking water that is easily accessible to the community. Good drinking water must fulfill microbiological, physical and chemical requirements (pH, chlor, Fe, sulfate, etc) (Pakpahan *et al.*, 2015). However, there are still many refillable drinking water depots that have not conducted regular inspections,

thereby not all of them are guaranteed to be clean (Rangga *et al.*, 2015).

According to the author's survey, there are 10 depots operating within a maximum distance of 2 km from the campus of Institut Teknologi Sumatera (ITERA). ITERA is one of the state universities in Lampung Province where every year there is a drastic increase in the number of students (ITERA Team, 2021). The increasing number of students shows that the higher population around ITERA also affects the need for drinking water. The ignorance of students or the community about the quality of refillable drinking water needs to be analyzed to avoid the risk of diseases that may be caused by refill drinking water from depots such as diarrhea which can hamper the activities of students or the community (Suhestry *et al.*, 2022). It is hoped that students or the community will not only consider the cheap price but also look at the quality aspects of the drinking water consumed, so as not to hamper student activities. Therefore, this study focused on analyzing the quality of refillable drinking water, especially on microbiological contamination.

Drinking water is a product that is susceptible to *E. coli* and *Coliform* microbial contamination. One group of *Coliform* bacteria that can be pathogenic is *E. Coli*. The presence of these bacteria in drinking water indicates water pollution or a low level of sanitation. Certain types of *Coliform* including *E. coli* produce enterotoxins that can cause diarrhea, meningitis, urinary tract infections, dysentery, and other diseases (Indrie & Wahyuni, 2020). In Indonesia, drinking water standards follow the World Health Organization standards which in some cases are adjusted to conditions in Indonesia. Drinking water must be tested for microbes and must not contain *E. coli* and *Coliform* bacteria in accordance with the Indonesian Minister of Health Regulation number 492 of 2010, which is 0 colonies/100 mL (Minister of Health, 2010).

Previous studies have shown that many refillable drinking water depots in Lampung contain *E. coli* and *Coliform* bacteria. 9 out of 32 depot samples in Bandar Lampung City were found to be positive for *Coliform* with MPN index >0/100 mL of sample (Ayu *et al.*, 2019). Another study reported that of the 8 depots examined in the Kampung Baru area of Bandar Lampung City, 7 depots were found with a *Coliform* value of 0/100 mL and 1 depot with a *Coliform* value of 3/100 mL (Suhestry *et al.*, 2022).

Based on the description above, this study was conducted to test the presence of *E. coli* and *Coliform bacteria* specifically in refillable drinking water, especially those produced by depots, as well as the effect of good manufacturing practice on the presence of bacteria in refillable drinking water.

Materials and Methods

Materials

The materials used were 5 refillable drinking water samples obtained from depots around ITERA, 70% alcohol, *lactose broth (*Oxoid), *brilliant green lactose bile broth* 2% (Oxoid), *eosin methylene blue agar (*Oxoid), *Kovach* reagent (Himedia), *triple sugar iron agar* (Himedia), *sulfide indole motility (Himedia), Simmon citrate agar (Himedia)*, and urea *base agar (Himedia)*. The tools used are sample bottles, beakers, Durham tubes, test tubes, measuring pipettes, *bulb* pipettes, micropipettes, microtips, test tube racks, analytical scales (Biobase), vortex (Thermoscientific), Bunsen, ose, incubator (Memmert IN 55), autoclave (GEA), *laminar airflow (*Biobase), petri dish, *hot plate*, *magnetic stirrer,* spray bottle, lighter, medical gloves, *tissue,* opaque paper, mattress thread, ice box, label, and stationery.

Research method

This study is a descriptive observational study with a *cross-sectional* design. The population in this study was 10 refillable drinking water depots around the ITERA campus. Samples were obtained in total of 5 samples from 5 depots selected using *simple random sampling* techniques. The data obtained as many as 5 data from the MPN test were analyzed using the MPN table according to the Thomas 333 series formula, then the bacterial biochemical test was analyzed descriptively with the aim of describing the characteristics of each object studied. The data collected was then adjusted to the maximum limit of microbial contamination in accordance with the Minister of Health Regulation number 492 of 2010 concerning quality requirements for refillable drinking water.

Research Stages

(1) Sampling

Samples were taken directly through purchase using refillable gallon containers that were produced water from the depot. The gallon containers were sealed and brought to the laboratory for analysis. Samples were analyzed by transferring water from gallons into 100 mL sample bottles.

(2) Sample testing

The sample testing carried out was a microbiological test with the parameters of *Escherichia coli* and *Coliform* content. The method used is *Most Probable Number* (MPN) with 333 tube series consisting of *presumptive test*, *confirmed test,* and *complete test*. Testing was then continued with biochemical tests including the TSIA (*Triple Sugar Iron Agar*) test, SIM (*Sulfite Indole Motility) test*, citrate test and urease test.

Research parameters

Testing of *Coliform* contamination using the *Most Probable Number* (MPN) method which refers to the procedure (SNI 01-2897-1992) and identification of *Escherichia coli* bacteria based on biochemical tests using the method (SNI 01-2897-1992) and (Irawan, 2020) with modifications.

Results and Discussion

Coliform analysis on MPN test

The principle of the MPN (*most probable number*) method is that the sample is diluted to a certain dilution level, the sample is positive if *E. coli* and *Coliform* ferment lactose which is characterized by the emergence of gas on the media used (Lestari *et al.*, 2018). The results of the presumptive test examination on refillable drinking water samples from depots around ITERA are presented in Table 1.

Table 1. Estimation test results after 24-48 hours incubation

No.	Sample code	Dilution			Description
		$3x10^{-1}$	$3x10^{-2}$	$3x10^{-3}$	
1	DEPOTS A	3	3	3	Next *confirmed test*
2	DEPOTS B	3	2	2	Next *confirmed test*
3	DEPOTS C	2	0	0	Next *confirmed test*
4	DEPOTS D	3	3	0	Next *confirmed test*
5	DEPOTS E	1	0	0	Next *confirmed test*

Description: 0 = 3 negative tubes; 1 = 1 positive tube; 2 = 2 positive tubes; 3 = 3 positive tubes

The data in Table 1 each sample gave positive test results characterized by the presence of gas trapped in the Durham tube and the media became cloudy in the presumptive test, most likely the growing bacteria are *Enterobacteriaceae* because they are able to ferment lactose (Chaniggia *et al.*, 2020). To strengthen the gas formed in the presumptive test produced by *Coliform* bacteria, all samples with positive test results continued at the *confirmed test* stage. The results of the confirmed test on refillable drinking water samples from depots around ITERA are presented in Table 2.

The data in Table 2 each sample gave positive test results characterized by the presence of gas trapped in the Durham tube as well as changes in the media to cloudy green. The approximate number of bacteria in the refillable drinking water samples was determined by looking at the combination of positive tubes according to Thomas' formula as shown in Table 3 below.

The results obtained in Table 3 all samples have an MPN index value> 0/100 mL and overall the sample that has the highest MPN/100 mL index value is sample A with a value of 95/100 mL, meaning that there are 95 bacterial colonies in 100 mL of sample, while the lowest MPN/100 mL value is sample C which is a value of 3/100 mL, meaning that there are 3 bacterial colonies in 100 mL of sample, so that all refillable drinking water samples tested have not met the drinking water quality requirements as regulated in Indonesian Minister of Health Regulation number 492 of 2010 where the maximum allowable *Coliform* bacteria contamination limit is 0/100 mL of sample (Minister of Health, 2010).

If the level of *Coliform* contamination in the sample is higher, the presence of pathogenic bacteria in it is also greater (Agrippina, 2019). *Coliform* bacteria are used as indicators of gastrointestinal bacterial contamination with pathogenic properties in drinking water that has been contaminated with fecal *Coliform* (e.g., *Escherichia coli*). The contamination can also come from non-fecal *Coliform* such as in soil or water (e.g., *Enterobacter, Citrobacter, Klebsiella*). Therefore, the presence of *Coliforms* in drinking water is not always indicated by fecal contamination, but also by inadequate treatment or sanitation (Agrippina, 2019).

All refillable drinking water depots sampled have different sanitation and hygiene conditions, allowing for differences in the amount of *Coliform* contamination in each refillable drinking water sample tested. The sample from DEPOTS A can be seen in Table 3 which provides the highest MPN value, in line with the observation data of refillable drinking water depots around ITERA in Table 6 where the roof conditions are not ceilinged with cement floors, the depot is on the side of the road, the gallon washing location is outside the depot, gallon cap storage outside the production cabinet, UV light is off, and operators do not pay attention to personal hygiene. In contrast to the sample from DEPOTS C which gave the lowest MPN value, although the roof condition had a ceiling with a white ceramic floor, the gallon washing location was inside the depot, the gallon cap storage was inside the UV room, the UV light was on, but the location of the depot was on the roadside and the lack of personal hygiene of the operators caused the presence of *Coliform* contamination although in small amounts.

The sterilization process in drinking water treatment is the most important way to remove or kill unwanted microbes. Bacterial contamination becomes ineffective if the sterilizer used is inadequate, such as the UV lamp is rarely replaced and the light intensity is not correct (Kasim *et al.*, 2016). The process of disinfecting with *ultraviolet* irradiation is emitted through a UV tube while the water is flowing with a wavelength of 254 nm (Budiyono & Sumardiono, 2013). The process produces photons that will be released and absorbed by microbial DNA, and makes microbial DNA damaged so that microbial cell metabolism is disrupted and then slowly dies (Putro *et al.*, 2013).

From the presumptive and confirmatory tests, it can be concluded that the bacteria contained in the drinking water samples are *Coliform bacteria*. Furthermore, it is necessary to conduct a complementary test to determine the type of *Coliform bacteria* contained in the five samples whether there are *Escherichia coli bacteria* or other types of *Coliforms. The* results of the complementary test examination on refillable drinking water samples from depots around ITERA are presented in Table 4.

Table 2. Results of the assay after 24-48 hours incubation

No.	Sample code	Dilution		
		$3x10^{-1}$	$3x10^{-2}$	$3x10^{-3}$
1	DEPOTS A	3	2	1
2	DEPOTS B	1	1	0
3	DEPOTS C	0	0	1
4	DEPOTS D	3	1	1
5	DEPOTS E	2	0	0

Description: 0 = 3 negative tubes; 1 = 1 positive tube; 2 = 2 positive tubes; 3 = 3 positive tubes

Table 3. MPN *Coliform* index in refillable drinking water samples around ITERA

No.	Sample Code	Combination of tubes that are positive in the *confirmed test*	MPN index/100 mL sample	Description
1	DEPOTS A	3-2-1	95	TMS
2	DEPOTS B	1-1-0	7	TMS
3	DEPOTS C	0-0-1	3	TMS
4	DEPOTS D	3-1-1	58	TMS
5	DEPOTS E	2-0-0	10	TMS

Description: TMS = not eligible

The results of the complementary test showed that there were three samples with the characteristics of *Escherichia coli* bacterial colonies, namely DEPOTS samples A, B, and D. The metallic green produced on EMBA media is an indicator that the bacteria can ferment lactose strongly, so that the acid produced will react with color indicators such as eosin Y and *methylene blue*, and produce a metallic green color with a purple-black core (Habibah, 2016). This property is owned by the type of *fecal Coliform* bacteria (Holt *et al.*, 1994). Bacteria that ferment lactose slowly will produce low acid so that the colonies are purplish red or pink (Habibah, 2016). This trait is owned by the type of *non-fecal Coliform bacteria* (Holt *et al.*, 1994). Therefore, the colonies produced in DEPOTS samples C and E gave negative results in the complementary test and it is suspected that the characteristics of the colonies are *non-fecal Coliform species* (Holt *et al.*, 1994).

Identification of bacteria in biochemical tests

Coliform bacteria colony forms that have been obtained in the complementary test are followed by bacterial identification tests based on the results of biochemical tests. The results of bacterial biochemical tests obtained from refillable drinking water samples around ITERA as a whole are listed in Table 5.

The observation results for the TSIA test on *Escherichia coli* and *Klebsiella pneumoniae* bacterial isolates from refillable drinking water samples are negative for sulfide, can ferment carbohydrates, and produce gas. If the media changes color from red to yellow both on the *slant* and *butt, it* indicates that the bacteria can ferment carbohydrates in the form of glucose, lactose, and sucrose in large quantities (Rahmiati, 2020). If only the *butt* is yellow, it indicates that the bacteria ferment carbohydrates in low amounts (Rahmiati, 2020). The fermentation process by the test bacteria will form gas, causing the test media to break or lift up (Rohadi *et al.*, 2016).

SIM media has peptone and thiosulfate components as sulfur sources and iron ammonium sulfate as H_2 S indicators. If the bacteria can produce H_2 S, a black precipitate will form on the media (Layne, 1971). The results in this test did not form a black precipitate in either *Escherichia* or *Klebsiella* isolates so that the results for the H_2 S test were said to be negative.

Indole production can be detected after adding *Kovach* reagent. *Kovach* reagent will react with indole and form a pink layer on the surface of the media. The observation results gave positive indole in *Escherichia* bacteria and negative indole in *Klebsiella* bacteria. These results are in accordance with previous research where *Escherichia bacteria are indole* positive while *Klebsiella are* indole negative because *Escherichia* bacteria have the enzyme *tryptophanase which* can hydrolyze *tryptophan* amino acids into indole compounds and pyruvic acid (Wardhana *et al.*, 2021).

The motility test is used to see that a bacterium is motile (can move) which is indicated by the presence of blurred spread in the media puncture area (Ayu *et al.*, 2019). The observation result of *Escherichia motility* test is positive; it means that the bacteria can move. *Klebsiella* motility test results are negative which is in accordance with the character of members of the genus *Klebsiella* which is not motile (Stiles & Lai-King, 1981).
The citrate test was conducted to determine the type of *Enterobacteriaceae* bacteria in its ability to use citrate as the only source of carbon and energy for bacteria. The results of observations on *Klebsiella* bacterial isolates were positive with a change in media color from green to blue, while *Escherichia coli* isolates remained green. *Klebsiella* bacteria will use citrate as a carbon source in its growth and energy formation, which will then give a positive reaction to the test (Bolla *et al.*, 2021). Citrates will be broken down by the enzyme *citratpermease* into *oxaloacetate* and *acetate*. During the enzymatic process, *oxaloacetate is* converted to pyruvate and CO_2. During the reaction, the media becomes alkaline as CO_2 binds with sodium (Na) and H_2 O to form sodium carbonate (Na_2CO_3). The sodium carbonate produced will change the pH indicator *bromothymol blue* in the media so that there is a color change in positive results from green to blue (Bambang *et al.*, 2014).

Table 4. Interpretation of complementation test results on EMBA media

No.	Sample code	Results	Description
1	DEPOTS A	Metallic green colonies, convex round shape, smooth with flat edges	Next biochemical test
2	DEPOTS B	Metallic green colonies, convex round shape, smooth with flat edges	Next biochemical test
3	DEPOTS C	Purplish red colony, convex round shape, slimy surface	Next biochemical test
4	DEPOTS D	Metallic green colonies, convex round shape, smooth with flat edges	Next biochemical test
5	DEPOTS E	Purplish red colony, convex round shape, slimy surface	Next biochemical test

Table 5. Bacterial biochemical test results from DEPOTS samples around ITERA

No.	Sample Code	Biochemical Test								Conclusion
		TSIA			SIM			Citrate	Urease	
		Slant	Butt	Gas	Sulfur	Indol	Motil			
1	DEPOTS A	K	K	+	-	+	+	-	-	*Escherichia coli*
2	DEPOTS B	K	K	+	-	+	+	-	-	*Escherichia coli*
3	DEPOTS C	K	K	+	-	-	-	+	+	*Klebsiella pneumoniae*
4	DEPOTS D	K	K	+	-	+	+	-	-	*Escherichia coli*
5	DEPOTS E	K	K	+	-	-	-	+	+	*Klebsiella pneumoniae*

Description: TSIA: *triple sugar iron agar*; SIM: *sulfid indole motility*; *Slant:* slope surface; *Butt*: base surface; K = yellow; (+) = change; (-) = no change.

The results of the urease test observations on *Klebsiella* bacterial isolates are positive with a change in media color from yellow to pink, while *Escherichia* bacterial isolates remain yellow. This is because *Klebsiella* is a bacterium that has a urease enzyme that can break down urea into ammonia (Elfidasari *et al.*, 2014). The enzyme can form ammonia by breaking carbon and nitrogen bonds. The ammonia formed gives the media an alkaline atmosphere so that with the *phenol red* indicator the media changes color from yellow to pink, but *Escherichia* does not have the urease enzyme, so the test gives negative results.

The data in Table 5 indicate that the bacteria present in DEPOTS A, DEPOTS B, and DEPOTS D samples are true *Escherichia coli,* while DEPOTS C and DEPOTS E samples are true *Klebsiella pneumoniae,* which has been confirmed by biochemical tests and compared with the characteristics of *Escherichia* genus members in *Bergey's Menual of Determinative Bacteriology* (Holt *et al.*, 1994).

Escherichia coli is a group of *Enterobacteriaceae* or enteric bacteria. These bacteria are commonly found in the human colon. However, under certain circumstances, if the bacteria enter other organs, it will cause infections in the human digestive tract such as abdominal pain, diarrhea, vomiting and nausea. *Escherichia coli* can infect humans when the body is experiencing a decrease in immunity or moves from its natural habitat. *Escherichia coli* is pathogenic when it is in other body tissues outside the intestine by attaching and invading tissues, then multiplying colonies and releasing toxins. There are several types of pathogenic *E. coli* that are distinguished based on their ability to cause disease, namely enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli (*EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli (*EIEC), enteroaggregative *E. coli (*EAEC), and diffusion adherent *E. coli (*DAEC) (Rahayu *et al.*, 2018).

Klebsiella pneumoniae bacteria are the main cause of pneumonia and urinary tract infection. Its transmission mostly occurs in patients during hospitalization through medical personnel (direct contact) and hospital facilities (breathing apparatus, urinary catheters, etc.) (Jasim & Farhan, 2020). Therefore, it is very rare to find cases of diarrhea caused by *Klebsiella* bacteria, however, the presence of these bacteria in drinking water is still considered as contamination because its quality decreases. The presence of *Klebsiella pneumoniae* in drinking water and food is usually associated with inadequate processing or sanitation. According to previous studies that detected the presence of enteropathogenic bacteria in water sources and drinking water, *Klebsiella pneumoniae* infection can enter the body through unwashed fruits or vegetables and contaminated water due to poor sanitation (Sihombing & Budiarso, 2017).

Risk factors for bacterial contamination in refillable drinking water

This study also observed the Good Manufacturing Practice (GMP) such as hygiene and sanitation of the depot to see deviations that occurred during the refillable drinking water treatment process with reference to the Minister of Health Regulation number 43 of 2014 concerning hygiene and sanitation of drinking water depots. Observations of the condition of the refillable drinking water depots are presented in Table 6.

According to previous research on risk factors for *Coliform* and *Escherichia coli* contamination in refillable drinking water, there are several factors that affect the number and type of bacteria found, namely raw water, handling of refill containers, processing, and cleanliness of the handler. (Amelia, 2019). Another study also added that depot conditions and equipment maintenance need to be considered in managing depots in order to produce quality drinking water (Amelia, 2019). With reference to the Minister of Health Regulation on the hygiene and sanitation of drinking water depots number 43 of 2014, most of the depots in the research location did not show a proper level of hygiene.

Depot conditions such as building construction including floors must be made of slippery materials for easy cleaning and the roof must be with a closed roof so that no insects or dirt enter (Rahayu *et al.*, 2013). Of the five depots sampled, only three met the requirements for floor conditions, and four had a covered roof. This is important to prevent dirt or insects from entering the production or filling area.

Table 6. Observation of refillable drinking water depots around ITERA

No.	Aspects	DEPOTS				
		A	B	C	D	E
1	Floor	Cement	Cement	White ceramic	White ceramic	White ceramic
2	Roof condition	No ceiling	Ceilinged	Ceilinged	Ceilinged	Ceilinged
3	Gallon cap storage	In plastic next to the production cabinet	Indoors (not production area)	Inside the UV chamber	Indoors (not production area)	Indoors (not production area)
4	Conditions around the depot	Inside stalls and on the roadside	Located on the roadside	Located on the roadside	Located on the roadside	Located on the roadside
5	UV light conditions during production	Die	Die	Live	Die	Live
6	Location of washing equipment	Outside the depot (not with soap)	Outside the depot (not with soap)	Inside the depot (using soap)	Outside the depot (no soap used)	Inside the depot (no soap)
7	Personal hygiene	Not wearing a mask and not washing hands	Not wearing a mask and not washing hands	Not wearing a mask and not washing hands	Not wearing a mask and not washing hands	Not wearing a mask and not washing hands

Before filling drinking water, washing gallons from consumers also needs to be done. Based on the technical requirements of drinking water depots and their trading, gallons must go through a washing stage using clean water and tara food detergent at a temperature of about 60°C to 85°C, after which they are rinsed with product water or drinking water. According to Table 6, out of the five sampled depots, only one depot (DEPOTS C) cleans gallons before filling using soap (dishwashing soap), while the other four depots only rinse and brush without using soap.

The cause of bacterial contamination at the stage of processing raw water into drinking water can also occur if the treatment process is not perfect. Table 6 shows three depots (DEPOTS A, B and D) with the UV lamp off. The sterilization process in drinking water treatment is the most important way to remove or kill unwanted microbes. Bacterial contamination becomes ineffective if the sterilization equipment used is inadequate, such as the UV lamp is rarely replaced and the light intensity is not correct (Kasim *et al.*, 2016).

In addition to building construction and equipment maintenance, the hygiene of both operators and consumers is an important factor in producing quality drinking water. The five depots showed that none of the operators cared about their own hygiene. This can be seen where none of the operators wash their hands and wear masks when refilling water. Previous research states that efforts that can be made to reduce contamination are washing hands with soap before receiving containers from consumers, dressing clean and neat, not smoking, and conducting regular health checks at least once every 2 years (Pakpahan *et al.*, 2015; Fadli *et al.*, 2021).

Conclusion

Based on the results of the analysis of *E. coli* and *Coliform* bacteria in refillable drinking water produced by depots, it was concluded that the five samples tested were positive for *Coliform* bacteria with an MPN index value of >0/100 mL and 3 samples were found to contain *Escherichia coli*. Five samples produced by depots around ITERA have not met the requirements of Indonesian Minister of Health Regulation number 492 of 2010 where the maximum allowable levels of *Coliform* and *Escherichia coli* bacteria are 0/100 mL of sample. If left untreated, it can trigger diarrhea outbreaks, especially in campus areas or densely populated places. The hygiene of both operators and consumers is an important factor in producing quality drinking water. The five depots showed that none of the operators cared about their own hygiene. This research still has limitations, namely the need to expand the area and multiply the sample of at least 30 depots for representatives, bacterial identification is based only on conventional cultures and biochemical tests.

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